CHAPTER 1

Classical and Non-classical Estrogen Receptor Effects of Bisphenol A

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1.1 Introduction

The word estrogen is commonly used to refer to 17β-estradiol (E2) due to its physiological relevance and predominance during reproductive growth. Estrogens are sex steroid hormones primarily synthesized in the ovaries and the adrenal glands, adipose tissue, brain, and testis. Estrogen displays a broad spectrum of physiological functions, including regulation of the menstrual cycle and reproduction, bone density, brain function, cholesterol mobilization, control of inflammation, and development of breast tissue and sexual organs.[1] While estrogens play diverse and similar physiological roles in both sexes,[2] they control primary and secondary sexual characteristics in females. In puberty, estradiol (E2) promotes epithelial cell proliferation and mammary glands, whereas estrogen helps prepare the mammary gland for milk production during pregnancy.[2–5] The lower levels of estrogens produced in men are essential for sperm maturation, erectile function, and healthy libido.[6] Besides this sexual and reproductive role, E2 exerts many actions in other systems such as the adipose tissue, bone, brain,

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cardiovascular system, endocrine system, pancreas, liver, and skeletal muscle.[7,8] It is important to note that any synthetic or semi-synthetic steroid that mimics the effects of natural estrogens is considered an estrogen.

1.2 Mechanism of Estrogen Signaling

All the physiological functions of estrogen are mediated *via* estrogen receptors (ERs). In 1958, Elwood Jensen discovered ERs by showing that female reproductive tissues could uptake estrogen from the circulation by binding to proteins. Later, those estrogen-bound receptors migrated to the nucleus, stimulating the transcription of various genes.[9,10] To date, several mechanisms for ER transcriptional regulation have been described in the literature. A classical mechanism is where E2 interacts with intracellular estrogen receptor (ERα or ERβ) resulting in receptor dimerization. This complex is then translocated to the nucleus, where it binds to estrogen response element (ERE) sequences through their DNA-binding domains.[11]

Estrogens are also shown to regulate transcription of several genes that do not contain EREs in their promoter regions, the mechanisms known as ''indirect genomic signaling'' or ''transcriptional cross-talk.'' Here, estrogen indirect signaling influences activation or suppression of target gene expression by acting through protein–protein interactions with other transcription factors such as stimulating protein-1 (Sp-1), activating transcription factor (ATF)-2, Fos/c-jun, the ATF-1/cAMP (cyclic adenosine monophosphate) response element binding protein (ATF-1/CREB), and the nuclear transcription factor-Y (NF-Y).[12–16]

Yet, not all estrogen effects fit under the transcriptional regulation of steroid action. The observation of extremely fast estrogen-mediated biological responses led to the hypothesis that estrogen could be acting through mechanisms not involving the direct target gene of G Protein-Coupled Estrogen Receptor 1 (GPER1).[17] In addition, both ERα and ERβ have been identified outside the nucleus, *i.e.* in the cytoplasm, mitochondria, and associated with the plasma membrane,[18] from where they can rapidly activate other signaling cascades. Both the GPER1 and some variants of ERα or ERβ are associated with non-genomic estrogen signaling.[19,20] Non-genomic actions of the ERα or ERβ could be induced *via* a sub-population of receptors located at the cell membrane which activate intracellular signaling cascades such as the phospholipase C (PLC)/protein kinase C (PKCs) pathways,[21] the Ras/Raf/MAPK (mitogen-activated protein kinase) cascade,[22] the phosphatidyl inositol 3 kinase (PI3K)/Akt kinase cascade,[23] and the cAMP/protein kinase A (PKA) signaling pathway.[24,25]

ER can also be activated in the absence of estrogens or other receptor agonists, an interesting phenomenon observed in many cells and is known as ''ligand-independent signaling''.[26–28] This ligand-independent ER activation requires the action of regulatory molecules necessary for phosphorylation, such as PKA, PKC, MAPK phosphorylation cascade, inflammatory cytokines (interleukin (IL)-2), cell cycle regulators (RAS p21

protein activator cyclins A and D1), and peptide growth factors (epidermal growth factor (EGF), insulin, insulin-like growth factor-1, and transforming growth factor-β).[29]

These various mechanisms of action highlight the complex multifactorial processes induced by estrogen, estrogen-like molecules, and their cellular receptors. Several studies have shown the existence of additional convergent pathways involving both genomic and non-genomic factors that result in the regulation of gene transcription.[28,30]

1.3 Estrogenic Assessment of Xenoestrogens, and the Case of Bisphenol A

Xenoestrogens are man-made chemicals that disrupt the endocrine system by mimicking or interfering with the actions of estrogen. These disruptions can lead to estrogen dominance as well as developmental, reproductive, neurological, and immune effects. Xenoestrogens encompass a variety of chemicals, which may be of either synthetic or natural origin. Natural xenoestrogens are represented by phytoestrogens (derived from plants) and mycoestrogens (substances produced by fungi). Synthetic xenoestrogens are molecules produced by chemical synthesis, which are widely used in agricultural chemicals (pesticides) and industrial by-products (certain plastics or detergents), along with pharmaceutical estrogens.[31] Because of the ability of xenoestrogens to interfere with the endocrine system, they are also classified as endocrine-disrupting chemicals (EDCs). The estrogenic activity of some xenoestrogens such as octyl-phenol and bisphenol A (BPA) was accidentally discovered when they disrupted the experiments that studied the effects of natural estrogens.[32,33] Throughout the years, the appearance of adverse developmental and reproductive effects in aquatic and wildlife species living within or near areas contaminated with xenoestrogens was reported.[34–38] However, substantial evidence has pointed to the fact that these chemicals can mimic the action of the natural estrogen, although they do not exhibit a similar structure to that of estrogens. Thus, it became important to develop accurate assays that could evaluate the risk of xenoestrogens.

Commonly used *in vitro* screening methods are based on the classical concept of estrogenicity, such as competitive ER-binding assays and yeast-based reporter assays. However, as described earlier, E2 can mediate its estrogenic activity by many other signaling pathways, and not all of them are evaluated by these receptor-based assays. *In vitro* assays cannot detect pro-estrogens metabolized *in vivo* to estrogen, which further underestimates the potency of pro-estrogens and their metabolites.[39]

Bisphenol A, also known as BPA, is a synthetic man-made chemical with a molecular weight of 228.29 $g\,mol^{-1}$ with the chemical formula $(CH_3)_2C(C_6H_4OH)_2$. This monomer was first synthesized by Dianin in 1891 and reported to be a synthetic estrogen in the 1930s.[40] In the 1950s, BPA was

rediscovered as a compound that could be used to synthesize the first epoxy resins as protective coatings and later polymerized to make hard plastic called polycarbonate, which is strong enough to replace steel and clear enough to replace glass.[41] Since then, BPA has been used widely in industrial production and has become one of the highest volume chemicals produced worldwide. More than 6 billion pounds of BPA are produced each year, and >100 tons are released into the atmosphere by yearly production.[42]

Like other chemicals, BPA can be released (or leached) from these materials under heat stress or through acidic and basic conditions, which accelerates the hydrolysis of the ester bond linking BPA monomers and leads to human exposure.[43–45] It is estimated that BPA-contaminated food contributes to >90% of overall BPA exposure, whereas exposure through dental surgery, dermal absorption, and dust ingestion remains <5% in normal situations.[46] Overall, human exposure to BPA is consistent and widespread, and biomonitoring studies have reported that >90% of individuals have detectable amounts of BPA in urine samples in the United States, Germany, and Canada.[47–49] Exposure to BPA is a major health concern due to its ability to disrupt the endocrine system,[50,51] and, in many ways, it has become a model EDC. BPA has deleterious effects on the cardiovascular system, alters metabolism, contributes to cancer, and changes immune and reproductive systems. Exposure to BPA is associated with several human diseases.[52] In contrast with these reports, plastic manufacturers have started to release ''BPA-free'' plastic material, and the scientific community continues to report the risk of BPA for human beings and wildlife health, highlighting the demand for screening BPA exposures as a research priority.[53–55]

The dispute over the safety of the use of BPA has resulted in a deep divide between regulatory toxicologists working for federal agencies or chemical industries and scientists trained in the principles of endocrinology. These principles include the understanding of ''low-dose'' effects, non-monotonic dose responses, and co-exposure effects, as well as the presence of sex-specific and tissue-specific effects of BPA.[56–59] Another subject of significant debate surrounding BPA exposure is the possible mechanisms by which BPA is thought to exert endocrine-disrupting properties. BPA was initially thought to exert EDC actions primarily by disrupting the activity of the classical estrogen signaling pathways, using ERs as transcription factors binding to the ERE site in the DNA.[60,61] Nowadays, increasing BPA research shows that BPA can also trigger non-classical estrogen-activated pathways *via* binding to membrane ERs.[39,62] Moreover, many other signaling systems such as thyroid function,[63] androgen signaling,[64] hormone biosynthesis and/or metabolism and numerous other mechanisms that converge upon endocrine and reproductive systems have been proposed to explain BPA actions.[52,65] Far from addressing concerns about the safety of BPA doses accepted by government agencies, the incidence and prevalence of health problems associated with BPA exposure have increased worldwide. Numerous reviews are available regarding sources of BPA exposure, biomonitoring studies, mechanisms of action, and exposure effects, both *in vitro* and

in vivo.[42,45] Furthermore, there are extensive reviews of BPA's low-dose effects and molecular mechanisms related to toxicity on human health.[51,52,65–67] Here, we examine the estrogenic activity of BPA when acting through the classical and non-classical ER-activated pathways to examine whether these mechanisms are at the root of the effect of BPA exposure on the endocrine system.

1.4 BPA-mediated Effects *via* Classical Estrogen Receptors

Like estrogens, BPA regulates different physiological processes such as growth, development, and homeostasis of numerous tissues through the binding and activation of the classical estrogen receptors, ERα and ERβ[51] (see Figure 1.1). These estrogen receptors are encoded by two separate genes located on human chromosomes 6 and 14, respectively.[68,69] In 1993, BPA's estrogenic activity was rediscovered while looking for an estrogen-binding protein in yeast. Krishnan *et al.* showed that BPA leached from polycarbonate flasks during autoclaving, rather than coming from yeast, as initially thought. Leaching was confirmed by performing different competitive binding assays such as ER and reversal estrogen action by tamoxifen, with the lowest effective dose being 10–20 nM.[32] The BPA molecule has structural features that confer the ability to bind to ERα and ERβ subtypes, although it displays 1000 to 10 000 times weak binding affinity to these receptors compared to the natural hormone E2.[35,65,70] The weaker affinity of BPA for ERs could be accounted for at least in part by the 42 van der Waals interactions within the BPA–ERα complex, in contrast to of the 51 interactions involved in the binding of E2–ERα.[71] Surprisingly, BPA could exert a stronger estrogen-like activity at nanomolar doses than at micromolar doses.[67,72]

Mechanisms for ER-mediated gene regulation are complex and depend on the recruitment of tissue-specific co-regulators that differentially affect ER interaction with EREs of different target genes.[73,74] BPA selectively binds to ERα and ERβ, but has a higher binding affinity relative to E2 at ERβ than at ERα in target cells.[75,76] A recent study in a rat model showed that BPA treatment decreased ERα and increased ERβ mRNA expression.[77] Similar effects were observed in female mice hearts exposed to 5 μg BPA/kg body weight during myocarditis, showing significant decreased ERα and increased ERβ mRNA expression levels.[78] Gould *et al.* previously emphasized that BPA is not merely a weak estrogen; rather, it exhibits characteristics of a distinct molecular mechanism of action *via* ERα.[79] An extensive uterotrophic analysis of BPA confirmed that it could induce proliferative and stimulatory changes in estrogen targets in concentrations ranging from 0.1 to 100 mg kg^{-1}.[80] Another study confirmed that BPA at a dose of 750 μg per mouse stimulated uterine proliferation in an ERα-dependent manner, which was highly correlated to that of E2.[81] In Ishikawa cells, BPA exposure decreased the protein expression

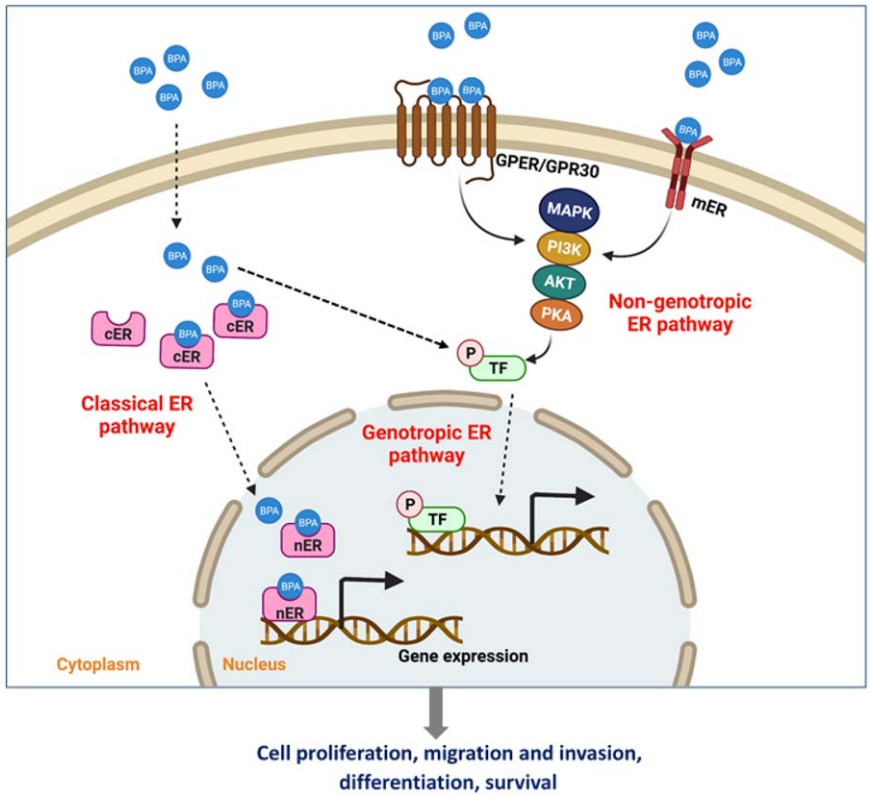

Figure 1.1 Mechanisms of bisphenol A (BPA) action *via* classical and non-classical estrogen receptor (ER) pathways. In the classical ER pathway, BPA binds with the ERs located in the cytoplasm (cER) or in the nucleus (nER) and regulates the transcription/translation of genes/proteins. In the non-genotropic pathway, BPA binds to membrane ER (mERs) and G-protein coupled ERs (GPER/GPR30), which triggers rapid estrogenic signaling *via* activation of cellular kinase systems such as mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), protein kinase A (PKA). In the non-classical genotropic ER pathway, BPA regulates gene expression by increasing or decreasing the expression of several transcription factors (TFs). P: phosphorylation. Created with BioRender.com.

levels of glucocorticoid-regulated kinase 1 (SGK1) and epithelial Na^+ channel α-subunit (ENaCα) *via* an ER pathway, causing damage in embryo implantation and uterus decidualization in mice.[82] During adulthood, BPA has been shown to bind with ERs located in the testis and causes harmful effects on germ cell differentiation, sperm production, and levels of male reproductive hormones.[83]

BPA induced the migration of normal human colon mucosal epithelial (NCM460) cells by increasing the expressions of integrin β1 and matrix metalloproteinase (MMP) mediated by ERβ.[84] In addition, BPA was shown to

induce the key transcription factor of epithelial–mesenchymal transition (EMT) and alter the expression and nuclear localization of the zinc-finger transcription factor *Snai1* and in hemangioma cells *via* an $ERα-mediated signaling pathway, resulting in the migration and invasion of hemangioma cells.[85] Such BPA-exposed induction of ER-dependent EMT was also observed in human breast cancer cells (MCF-7) by increasing the expression of C-X-C chemokine receptor type 4 (CXCR4), a receptor of CXCL12, thereby enhancing migration and invasion capacity of cells.[86] Up-regulation of CXCL12 by BPA exposure *via* ER activation was also reported in human ovarian carcinoma cells.[87] More recently, HepG2 cells exposed to BPA increased the transcriptional activity of *CYP2C9* by forming a BPA–ERα complex binding with ERE in the promoter region of *CYP2C9* gene.[88]

1.5 BPA-mediated Effects *via* Non-classical ER

Beyond the classical ER-modulator effect, BPA can also act *via* genomic and non-genomic pathways (see Figure 1.1). In the genomic pathway, BPA binds with the ERs located in the cytoplasm. The binding modifies overall receptor signaling that affects nuclear chromatin function and regulates the transcription and translation of genes and proteins, affecting cell proliferation, differentiation, and survival.[8] Studies have shown that BPA can facilitate estrogen-like activities similar to or stronger than E2.[89–91] These effects observed at low BPA doses can be explained at least partially by rapid responses *via* non-classical estrogen-triggered pathways.[39,52,65,66] BPA interacts differently within the ligand domain of ERs compared to E2.[79] BPA also has different co-activator recruitment, as highlighted by the fact that the BPA–ERb complex showed 500-fold greater potency than BPA–ERα in recruiting the co-activator TIF2.[76] Therefore, the adverse effects of BPA exposure on human health could be mediated by rapid activation of non-ER-dependent signaling pathways, which produce fast biological responses on specific cellular targets.

1.5.1 Non-genotropic ER Actions

Adverse effects of BPA exposure on human health are also mediated *via* non-genomic-dependent signaling pathways involving membrane estrogen receptor (mER).[92] Activation of mERs by BPA triggers rapid estrogenic signaling *via* activation of cellular kinase systems such as PKA, PKC, PI3K, MAPK, changes in levels of cAMP, and intracellular calcium as demonstrated in several human cancers.[93] High-dose BPA tends to acts as an ER antagonist by directly regulating the genomic transcription. In contrast, BPA at low doses is generally thought to disrupt the biological function in a non-genomic manner mediated by mERs.[94] BPA has been shown to promote cell proliferation or apoptosis by binding to mERs and rapid activation of downstream pathways.[95,96]

G-protein coupled estrogen receptor (GPER/GPR30) is one of the most common mERs that plays an important role in the toxic effects of BPA.[66,97] GPR30 mRNA is expressed in numerous tissues with different expression patterns.[98] GPR30 is a seven-transmembrane-domain receptor that binds BPA at a half maximal inhibitory concentration of 630 nM and its relative binding affinity when compared to E2 equals 2.83 nM in stably transfected ER-negative HEK293 cells.[92] Interestingly, studies have demonstrated that the binding affinity of E2 to GPR30 is 10-fold lower than ERα, whereas BPA affinity to GPR30 is about 50-fold higher than ERα.[92,99] In GC-2 cells, BPA-induced GPR30-mediated activation of the epidermal growth factor receptor (EGFR)-MAPK signaling pathway, along with activating the c-Fos gene and cell-cycle gene Cyclin D1 inhibition.[100] Similarly, BPA induced GPER-mediated activation of EGFR and MAPK signaling, leading to meiotic arrest in a zebrafish model.[101]

MAPK, PI3K/AKT, nuclear factor (NF)κB, JNK, and Ca^{2+} homeostasis are the most widely studied pathways associated with BPA and cancer.[93,102–104] BPA has been shown to activate the GPER1 (GPER/EGFR/extracellular signal-regulated kinase (ERK)1/2) signaling pathway in cancer cells by inducing the expression of the proto-oncogene c-fos (a significant gene in the early estrogen response) and activator protein-1 (AP1) target genes.[105,106] Similar signaling cascade activation was also reported by low dose of BPA, resulting in the progression of male germ-cell cancer.[107] In triple-negative breast cancer (TNBC) cells, BPA proliferative and pro-survival effects depend on ERK1/2 and AKT activation.[108] Constant BPA exposure to breast cancer cells induced resistance to an EGFR-targeted anti-cancer drug *via* EGFR/ERK1/2 pathway activation, and increased protein levels of BCL2 (anti-apoptotic) and SOD1 (anti-oxidant).[109,110] BPA-mediated resistance to anti-cancer drugs also activated pro-survival signaling pathways, such as PI3K/AKT/mTOR pathways.[111,112] Wang *et al.*[113] have demonstrated that BPA could stimulate the growth, invasion, and migration of RL95-2 cells *via* the MAPK pathway, which may upregulate cyclo-oxygenase-2 expression. BPA exposure was shown to induce migration and invasion of lung cancer cells and increased proliferation and migration of laryngeal squamous cell carcinoma (LSCC) cells mediated *via* the GPER-dependent pathway.[114,115] Interestingly, the BPA–GRP30 complex also induced testicular seminoma cell proliferation *in vitro*, the effect of which was reverted using a GPR30 antagonist, G15.[95] Castillo Sanchez *et al.*[116] found that BPA activates the GPER-dependent pathway and the kinases (*i.e.* focal adhesion kinase (FAK), proto-oncogene tyrosine-protein kinase (SRC), and ERK2) required for migration by increasing the activity of AP-1 and NFκB-DNA binding. In cervical cancer, BPA activated NFκB signaling *via* IKKβ resulting in cell migration and upregulation of fibronectin and metalloproteinase-9.[117] BPA also increased levels of leptin receptors, which induced proliferation by STAT3, ERK1/2, and AKT phosphorylation.[118] Additionally, BPA-induced Ca^{2+} signaling initiated at the plasma membrane activated the transcription factor CREB in the nucleus within 15 minutes of exposure.[119] Yaguchi[120] reported that BPA

activated the EGFR/ERK pathway by facilitating Ca^{2+} influx in Ishikawa cells and EGF secretion to the extracellular space. BPA also induced apoptosis of KGN cells (a human granulosa-like tumor cell line) through GPER-dependent activation of the ROS/Ca^{2+}–ASK1–JNK signaling pathway.[121] BPA was shown to impair glucose tolerance, increase body weight, and reduce insulin secretion in mice,[122,123] effects that were protected in GPR30 knockout female mice.[124] Exposure to low-dose BPA increases GPR30 and produces specific inflammatory proteins, including IL8, IL6, and monocyte chemoattractant protein-1α, both in cultured mature adipocytes and in stromal-vascular fraction cells isolated from mammary human adipose tissue biopsies.[125]

Another membrane-associated ER, named ERα36, was reported to promote BPA-induced leiomyoma cell proliferation by activating SRC, EGFR, and MAPK p44/42, increase expression of growth factor receptor-bound protein 2 (*Grb2*), son of sevenless homolog 1 (*Sos1*) and Ras.[126] Integrins are transmembrane receptors that can regulate cellular signals. BPA was found to increase the expressions of MMP-2, MMP-9, integrin β1, and integrin α5 in the placenta, inducing a greater proportion of the labyrinth and spongiotrophoblast layers in mice.[127] Environmental doses of BPA induced cancer cell migration *via* a rapid direct activation of integrin β1.[128]

1.5.2 Genotropic ER Actions

Several nuclear transcription factors (TFs) are associated with BPA action *via* non-classical ER signaling (see Figure 1.1). In particular, studies have shown that induction of adipogenic TFs, such as PPARγ, C/EBPs, and Nrf2 play an important role in the ''obesogenic effect'' of BPA. Other evidence suggests a critical role of HAND2 protein and HOX family members in BPA-mediated detrimental effects.

1.5.2.1 PPARγ

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily known to exert a wide range of biological effects on adipogenesis, cell proliferation, differentiation, immune response, and metabolism. The PPAR family comprises three different subtypes (PPARα, β/δ, γ), all of which are important regulators of glucose and lipid metabolism.[52] The activity of PPARγ is governed by the binding of small lipophilic ligands, chiefly fatty acids, derived from nutrition or metabolism. Perinatal BPA exposure *via* milk during lactation modified early adipogenesis by modulating adipocyte hypertrophy and overexpression of pro-adipogenic transcription factors, PPARγ, increasing the body weight of female pups, but not male pups.[129] Similarly, gestational BPA exposure increases PPARγ expression level in pre-adipocytes isolated from female sheep progeny, but not from male progeny.[130]

It has been reported that BPA effects mediated *via* PPARγ are not only observed in adipose tissue, but also in the liver. Subcutaneous injection of BPA in mice increases the PPARγ gene expression levels in the liver, resulting in fasting hyperglycemia, glucose intolerance, and high levels of non-esterified fatty acids.[122] Hepatic PPARγ expression, along with increased fat mass and total body weight, were also observed in male mice exposed to BPA.[131] Several conflicting data on PPARγ-mediated BPA effects have been obtained in culture cells. Increased PPARγ levels were observed in murine 3T3-L1 cells exposed to both low and high doses of BPA.[131,132] Conversely, no differences in PPARγ 1 and PPARγ 2 expression levels were observed in the same cells in response to BPA exposure.[133]

BPA has also been shown to significantly upregulate PPARγ expression in adult human pre-adipocytes and freshly cultured omental adipose tissue from child donors.[134,135] In contrast, BPA effects in human adipose-derived stem cells was not shown to be mediated by PPARγ.[134]

1.5.2.2 C/EBP

Similar to that of PPARγ, the role of CCAAT/enhancer-binding proteins (C/EBPs) as mediators of BPA effects is presently disputed. C/EBPs encompasses a family of six transcription factors with structural and functional homologies, but with different transactivating abilities and tissue specificities.

While BPA-exposed female rats have reported an increase of C/EBP expression in adipocytes,[129] no effect of BPA was observed on this transcription factor in 3T3-L1 cells.[133] A recent study highlighted the involvement of other members of the C/EBP family in the metabolic damage caused by BPA, where triglyceride accumulation in human adipose-derived mesenchymal stem cells by BPA exposure is associated not only to the upregulation of PPARγ and C/EBPα, but also to the increase of C/EBPβ gene expression.[136] C/EBP-mediated BPA effects have also been linked with liver dysfunction and disease. Female mice fed a diet supplemented with BPA showed a decreased level of C/EBPα in fetal livers compared to the control animals, an effect not observed in male mice. This highlights BPA-mediated disruption of fetal liver maturation in a sex-specific manner and may further alter the expression level of albumin, alpha-fetoprotein, and glycogen synthase.[137]

1.5.2.3 Nrf2

Nuclear factor eythroid-2-related factor 2 (Nrf2) is a basic leucine zipper transcription factor regulating the expression of antioxidant proteins and protects against oxidative damage.[138] BPA administered orally to lupus-prone MRL/lpr mice has been shown to decrease Nrf2 expression in renal tissue exacerbating lupus nephritis, highlighting a protective role of Nrf2 in BPA-induced renal damage.[139] Unlike the kidneys, Nrf2 impairs liver function in leptin-deficient mice by decreasing Kelch-like ECH-associated protein 1 (Keap1) and increasing lipid accumulation associated with

constitutive activation of Nrf2.[140] Such BPA-induced Nrf2 upregulation *via* Keap1 inactivation has been reported in a human hepatoma cell line.[141] Furthermore, 25 $\mu g\,kg^{-1}$ per day of BPA administration to pregnant CD-1 mice increased Nrf2 expression and its recruitment to the Srebp-1c promoter, causing hepatic lipid deposition.[142]

1.5.2.4 HAND2

Heart- and neural crest derivatives-expressed protein 2 (HAND2) is a basic helix-loop-helix transcription factor, which plays an important role in establishing proper implantation for pregnancy. Chronic BPA exposure to female mice decreases HAND2 expression in the uterine stroma, affecting embryo implantation and formation of the decidua during the early phases of pregnancy.[143] HAND2 overexpression is also associated with increased proliferation of cardiac progenitor cells,[144] and emerging evidence indicates an association between cardiovascular diseases and BPA.[145] Interestingly, BPA significantly upregulates ERβ expression and H3K9 and H4K12 histone acetylation, which could be responsible for HAND2 upregulation and the increased percentage of heart malformations.[146] Thus, HAND2 represents a key regulator of several organs such as the uterus and heart, and impairment of its expression following BPA exposure may lead to reproductive and cardiac disorders *via* genetic and epigenetic mechanisms.

1.5.2.5 HOX

The term ''HOX'' was originally termed as a group of related HOX genes that encode for transcription factors characterized by a well-conserved DNA sequence known as the homeobox. HOX genes are found in humans and rodents and are expressed during embryogenesis and early development, where they act as master transcriptional regulators.[147,148] Among the HOX genes, HOXA10 is expressed in the female reproductive system and is important for normal decidualization and pregnancy, while HOXB9 and HOXC6 are involved in the development of the mammary gland.[149–151] Female pups exposed in utero to 0.5–1.0 $mg\,kg^{-1}$ BPA showed an elevated level of HOXA10 in uterine stromal cells, which may mediate the decidualization defects.[151] HOXB9 is also known to activate in response to BPA exposure in breast cancer cells.[102] Studies have also shown that BPA increases HOXB9 and HOXC6 expression in the mammary glands of ovariectomized rats and culture human breast cancer cells (MCF7), highlighting that BPA harmful effects in breast tumors are mediated *via* these transcription factors.[152,153]

1.6 Conclusion

Most of the world's population is widely exposed to BPA, as it is largely used to manufacture polycarbonate plastic and is released into foods and beverages. A growing body of evidence indicates that BPA exposure has been

linked to numerous adverse perinatal, childhood, and adult health outcomes in both animals and humans, such as reproductive effects, developmental effects, metabolic disease, and cancer.[93,154,155] However, several contrasting results about the adverse effects of BPA have been described, which may be due to the use of different doses, experimental models or conditions.[42] In parallel, increasing efforts have been taken to elucidate the molecular mechanisms through which BPA acts. The integration of the knowledge about the molecular pathways of BPA with epidemiology could certainly improve the understanding of the toxic effects of BPA on human health.

As summarized in this chapter, there is no doubt that BPA is an estrogenic compound that can initiate the classical ER pathway through binding to relatively specific ERs and regulating gene expression. Additionally, BPA-estrogenic action also acts through non-classical estrogen-activated pathways. Rapid non-genomic and genomic actions may be mediated by BPA's interactions with the membrane-associated ERs and/or GPERs and a suite of important transcription factors. BPA exposure significantly impacts growth, survival, proliferation, invasion, migration, and apoptosis in cell- and tissue-specific manners through these mechanisms. In addition, exposure to BPA may facilitate chemotherapy resistance to anticancer drugs and also interacts with several receptors and causes aberrant changes in numerous pathways such as GPER/EGFR/ERK1/2, JAK/STAT, PI3K/AKT/mTOR, SRC1-3, phosphorylation of cyclin D1, AKT, PPARγ, and Ca^{2+} homeostasis. BPA probably exerts these adverse effects with rapid signaling, showing the presence of extracellularly accessible binding sites that act to regulate intracellular signaling. This rapid signaling may elicit signal transduction pathways responsible for growth and differentiation and in energy and nutrient metabolism. It has become evident that BPA can activate transduction signaling pathways that vary across cell types, and the total disruption effect arises from the combination of rapid mechanisms and longer signaling effects, as highlighted in this chapter. The mechanisms at the root of these multiple effects are numerous and are activated at BPA concentrations below the concentration range in which ''pharmaceutical'' effects are detected by classical toxicology and create non-monotonic dose–response curves.[156] It is also interesting to note that all the upstream pathways may contribute to stable and inheritable modifications by regulating epigenetic enzymes, which may also sustain initial exposure to BPA.[157] Nevertheless, these mechanisms are only applicable and reported in some cell types and animal models. More mechanistic and comprehensive insights into other biological systems are needed to unravel the classical and non-classical ER effects of BPA. Identifying BPA effects in different model systems may help raise awareness within the scientific community and the manufacturing industry of the need to seek alternatives to BPA to reduce its harmful effects. Therefore, to date, the best practice is still the precaution of limiting the use of plastic materials and promoting BPA-free products.

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References

1. J. Liang and Y. Shang, Estrogen and cancer, *Annu. Rev. Physiol.*, 2013, **75**, 225–240.
2. E. R. Simpson, M. Misso, K. N. Hewitt, R. A. Hill, W. C. Boon, M. E. Jones, A. Kovacic, J. Zhou and C. D. Clyne, Estrogen–the good, the bad, and the unexpected, *Endocr. Rev.*, 2005, **26**, 322–330.
3. C. J. Gruber, W. Tschugguel, C. Schneeberger and J. C. Huber, Production and actions of estrogens, *N. Engl. J. Med.*, 2002, **346**, 340–352.
4. R. D. Koos, Minireview: Putting physiology back into estrogens' mechanism of action, *Endocrinology*, 2011, **152**, 4481–4488.
5. J. L. Voogt, Control of hormone release during lactation, *Clin. Obstet. Gynecol.*, 1978, **5**, 435–455.
6. M. Schulster, A. M. Bernie and R. Ramasamy, The role of estradiol in male reproductive function, *Asian J. Androl.*, 2016, **18**, 435–440.
7. J.-Å. Gustafsson, What pharmacologists can learn from recent advances in estrogen signalling, *Trends Pharmacol. Sci.*, 2003, **24**, 479–485.
8. N. Heldring, A. Pike, S. Andersson, J. Matthews, G. Cheng, J. Hartman, M. Tujague, A. Ström, E. Treuter, M. Warner and J.-Å. Gustafsson, Estrogen Receptors: How Do They Signal and What Are Their Targets, *Physiol. Rev.*, 2007, **87**, 905–931.
9. E. V. Jensen, E. R. Desombre, T. Kawashima, T. Suzuki, K. Kyser and P. W. Jungblut, Estrogen-binding substances of target tissues, *Science*, 1967, **158**, 529–530.
10. E. V. Jensen, T. Suzuki, T. Kawashima, W. E. Stumpf, P. W. Jungblut and E. R. DeSombre, A two-step mechanism for the interaction of estradiol with rat uterus, *Proc. Natl. Acad. Sci. U. S. A.*, 1968, **59**, 632–638.
11. N. Fuentes and P. Silveyra, Estrogen receptor signaling mechanisms, *Adv. Protein Chem. Struct. Biol.*, 2019, **116**, 135–170.
12. A. Aranda and A. Pascual, Nuclear hormone receptors and gene expression, *Physiol. Rev.*, 2001, **81**, 1269–1304.
13. M. Göttlicher, S. Heck and P. Herrlich, Transcriptional cross-talk, the second mode of steroid hormone receptor action, *J. Mol. Med.*, 1998, **76**, 480–489.
14. R. O'Lone, M. C. Frith, E. K. Karlsson and U. Hansen, Genomic targets of nuclear estrogen receptors, *Mol. Endocrinol.*, 2004, **18**, 1859–1875.
15. S. Safe and K. Kim, Nuclear receptor-mediated transactivation through interaction with Sp proteins, *Prog. Nucleic Acid Res. Mol. Biol.*, 2004, **77**, 1–36.
16. B. Saville, M. Wormke, F. Wang, T. Nguyen, E. Enmark, G. Kuiper, J. A. Gustafsson and S. Safe, Ligand-, cell-, and estrogen receptor

subtype (alpha/beta)-dependent activation at GC-rich (Sp1) promoter elements, *J. Biol. Chem.*, 2000, **275**, 5379–5387.
17. E. R. Prossnitz and M. Barton, The G-protein-coupled estrogen receptor GPER in health and disease, *Nat. Rev. Endocrinol.*, 2011, **7**, 715–726.
18. S. R. Hammes and E. R. Levin, Extranuclear steroid receptors: nature and actions, *Endocr. Rev.*, 2007, **28**, 726–741.
19. M. Barton, E. J. Filardo, S. J. Lolait, P. Thomas, M. Maggiolini and E. R. Prossnitz, Twenty years of the G protein-coupled estrogen receptor GPER: Historical and personal perspectives, *J. Steroid Biochem. Mol. Biol.*, 2018, **176**, 4–15.
20. E. J. Filardo and P. Thomas, Minireview: G protein-coupled estrogen receptor-1, GPER-1: its mechanism of action and role in female reproductive cancer, renal and vascular physiology, *Endocrinology*, 2012, **153**, 2953–2962.
21. M. J. Marino, S. T. Rouse, A. I. Levey, L. T. Potter and P. J. Conn, Activation of the genetically defined m1 muscarinic receptor potentiates N-methyl-D-aspartate (NMDA) receptor currents in hippocampal pyramidal cells, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 11465–11470.
22. E. G. Dos Santos, M. N. Dieudonne, R. Pecquery, V. Le Moal, Y. Giudicelli and D. Lacasa, Rapid nongenomic E2 effects on p42/p44 MAPK, activator protein-1, and cAMP response element binding protein in rat white adipocytes, *Endocrinology*, 2002, **143**, 930–940.
23. M. Marino, F. Acconcia and A. Trentalance, Biphasic estradiol-induced AKT phosphorylation is modulated by PTEN via MAP kinase in HepG2 cells, *Mol. Biol. Cell*, 2003, **14**, 2583–2591.
24. Q. Gu and R. L. Moss, 17 beta-Estradiol potentiates kainate-induced currents via activation of the cAMP cascade, *J. Neurosci.*, 1996, **16**, 3620–3629.
25. G. Picotto, V. Massheimer and R. Boland, Acute stimulation of intestinal cell calcium influx induced by 17β-estradiol via the cAMP messenger system, *Mol. Cell. Endocrinol.*, 1996, **119**, 129–134.
26. M. A. Bennesch and D. Picard, Minireview: Tipping the balance: ligand-independent activation of steroid receptors, *Mol. Endocrinol.*, 2015, **29**, 349–363.
27. A. Maggi, Liganded and unliganded activation of estrogen receptor and hormone replacement therapies, *Biochim Biophys Acta*, 2011, **1812**, 1054–1060.
28. P. Vrtačnik, B. Ostanek, S. Mencej-Bedrač and J. Marc, The many faces of estrogen signaling, *Biochem. Med.*, 2014, **24**, 329–342.
29. S. Nilsson, S. Mäkelä, E. Treuter, M. Tujague, J. Thomsen, G. Andersson, E. Enmark, K. Pettersson, M. Warner and J. A. Gustafsson, Mechanisms of estrogen action, *Physiol. Rev.*, 2001, **81**, 1535–1565.
30. L. Björnström and M. Sjöberg, Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes, *Mol. Endocrinol.*, 2005, **19**, 833–842.

31. I. Paterni, C. Granchi and F. Minutolo, Risks and benefits related to alimentary exposure to xenoestrogens, *Crit. Rev. Food Sci. Nutr.*, 2017, **57**, 3384–3404.
32. A. V. Krishnan, P. Stathis, S. F. Permuth, L. Tokes and D. Feldman, Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving, *Endocrinology*, 1993, **132**, 2279–2286.
33. A. M. Soto, H. Justicia, J. W. Wray and C. Sonnenschein, *p*-Nonylphenol: an estrogenic xenobiotic released from ''modified'' polystyrene, *Environ. Health Perspect.*, 1991, **92**, 167–173.
34. L. J. Guillette, Jr., T. S. Gross, G. R. Masson, J. M. Matter, H. F. Percival and A. R. Woodward, Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida, *Environ. Health Perspect.*, 1994, **102**, 680–688.
35. M. Sonavane, N. Creusot, E. Maillot-Maréchal, A. Péry, F. Brion and S. Aït-Aïssa, Zebrafish-based reporter gene assays reveal different estrogenic activities in river waters compared to a conventional human-derived assay, *Sci. Total Environ.*, 2016, **550**, 934–939.
36. M. Sonavane, J. E. Schollée, A. O. Hidasi, N. Creusot, F. Brion, M. J. Suter, J. Hollender and S. Aït-Aïssa, An integrative approach combining passive sampling, bioassays, and effect-directed analysis to assess the impact of wastewater effluent, *Environ. Toxicol. Chem.*, 2018, **37**, 2079–2088.
37. C. Sonnenschein and A. M. Soto, *The Society of Cells – Cancer and Control of Cell Proliferation*, Bios Scientific, Oxford, 1999, 154.
38. J. P. Sumpter and S. Jobling, Male sexual development in ''a sea of oestrogen'', *Lancet*, 1993, **342**, 124–125.
39. P. Alonso-Magdalena, A. B. Ropero, S. Soriano, M. García-Arévalo, C. Ripoll, E. Fuentes, I. Quesada and Á. Nadal, Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways, *Mol. Cell. Endocrinol.*, 2012, **355**, 201–207.
40. E. C. Dodds and W. Lawson, Synthetic strogenic Agents without the Phenanthrene Nucleus, *Nature*, 1936, **137**, 996.
41. S. A. Vogel, The politics of plastics: the making and unmaking of bisphenol a ''safety'', *Am. J. Public Health*, 2009, **99**(Suppl 3), S559–S566.
42. L. N. Vandenberg, M. V. Maffini, C. Sonnenschein, B. S. Rubin and A. M. Soto, Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption, *Endocr. Rev.*, 2009, **30**, 75–95.
43. A. M. Calafat, Z. Kuklenyik, J. A. Reidy, S. P. Caudill, J. Ekong and L. L. Needham, Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population, *Environ. Health Perspect.*, 2005, **113**, 391–395.
44. J. H. Kang, F. Kondo and Y. Katayama, Human exposure to bisphenol A, *Toxicology*, 2006, **226**, 79–89.

45. C. A. Richter, L. S. Birnbaum, F. Farabollini, R. R. Newbold, B. S. Rubin, C. E. Talsness, J. G. Vandenbergh, D. R. Walser-Kuntz and F. S. vom Saal, In vivo effects of bisphenol A in laboratory rodent studies, *Reprod. Toxicol.*, 2007, **24**, 199–224.
46. T. Geens, D. Aerts, C. Berthot, J. P. Bourguignon, L. Goeyens, P. Lecomte, G. Maghuin-Rogister, A. M. Pironnet, L. Pussemier, M. L. Scippo, J. Van Loco and A. Covaci, A review of dietary and non-dietary exposure to bisphenol-A, *Food Chem. Toxicol.*, 2012, **50**, 3725–3740.
47. T. Bushnik, D. Haines, P. Levallois, J. Levesque, J. Van Oostdam and C. Viau, Lead and bisphenol A concentrations in the Canadian population, *Health Rep.*, 2010, **21**, 7–18.
48. H. M. Koch, M. Kolossa-Gehring, C. Schröter-Kermani, J. Angerer and T. Brüning, Bisphenol A in 24 h urine and plasma samples of the German Environmental Specimen Bank from 1995 to 2009: a retrospective exposure evaluation, *J. Exposure Sci. Environ. Epidemiol.*, 2012, **22**, 610–616.
49. J. S. LaKind, J. Levesque, P. Dumas, S. Bryan, J. Clarke and D. Q. Naiman, Comparing United States and Canadian population exposures from National Biomonitoring Surveys: Bisphenol A intake as a case study, *J. Exposure Sci. Environ. Epidemiol.*, 2012, **22**, 219–226.
50. B. S. Rubin, Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects, *J. Steroid Biochem. Mol. Biol.*, 2011, **127**, 27–34.
51. Y. B. Wetherill, B. T. Akingbemi, J. Kanno, J. A. McLachlan, A. Nadal, C. Sonnenschein, C. S. Watson, R. T. Zoeller and S. M. Belcher, In vitro molecular mechanisms of bisphenol A action, *Reprod. Toxicol.*, 2007, **24**, 178–198.
52. I. Cimmino, F. Fiory, G. Perruolo, C. Miele, F. Beguinot, P. Formisano and F. Oriente, Potential Mechanisms of Bisphenol A (BPA) Contributing to Human Disease, *Int. J. Mol. Sci.*, 2020, **21**, 5761.
53. A. Bergman, J. J. Heindel, T. Kasten, K. A. Kidd, S. Jobling, M. Neira, R. T. Zoeller, G. Becher, P. Bjerregaard, R. Bornman, I. Brandt, A. Kortenkamp, D. Muir, M.-N. B. Drisse, R. Ochieng, N. E. Skakkebaek, A. S. Byléhn, T. Iguchi, J. Toppari and T. J. Woodruff, The impact of endocrine disruption: a consensus statement on the state of the science, *Environ. Health Perspect.*, 2013, **121**, A104–A106.
54. V. Le Fol, S. Aït-Aïssa, M. Sonavane, J. M. Porcher, P. Balaguer, J. P. Cravedi, D. Zalko and F. Brion, In vitro and in vivo estrogenic activity of BPA, BPF and BPS in zebrafish-specific assays, *Ecotoxicol. Environ. Saf.*, 2017, **142**, 150–156.
55. R. T. Zoeller, T. R. Brown, L. L. Doan, A. C. Gore, N. E. Skakkebaek, A. M. Soto, T. J. Woodruff and F. S. Vom Saal, Endocrine-disrupting chemicals and public health protection: a statement of principles from The Endocrine Society, *Endocrinology*, 2012, **153**, 4097–4110.
56. B. F. Healy, K. R. English, P. Jagals and P. D. Sly, Bisphenol A exposure pathways in early childhood: Reviewing the need for improved risk

assessment models, *J. Exposure Sci. Environ. Epidemiol.*, 2015, **25**, 544–556.
57. M. Sonavane and N. R. Gassman, Bisphenol A co-exposure effects: a key factor in understanding BPA's complex mechanism and health outcomes, *Crit. Rev. Toxicol.*, 2019, **49**, 371–386.
58. R. Valentino, V. D'Esposito, F. Ariemma, I. Cimmino, F. Beguinot and P. Formisano, Bisphenol A environmental exposure and the detrimental effects on human metabolic health: is it necessary to revise the risk assessment in vulnerable population?, *J. Endocrinol. Invest.*, 2016, **39**, 259–263.
59. F. S. vom Saal and C. Hughes, An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment, *Environ. Health Perspect.*, 2005, **113**, 926–933.
60. G. G. Kuiper, J. G. Lemmen, B. Carlsson, J. C. Corton, S. H. Safe, P. T. van der Saag, B. van der Burg and J. A. Gustafsson, Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta, *Endocrinology*, 1998, **139**, 4252–4263.
61. C. Q. Sheeler, M. W. Dudley and S. A. Khan, Environmental estrogens induce transcriptionally active estrogen receptor dimers in yeast: activity potentiated by the coactivator RIP140, *Environ. Health Perspect.*, 2000, **108**, 97–103.
62. A. L. Wozniak, N. N. Bulayeva and C. S. Watson, Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-alpha-mediated Ca^{2+} fluxes and prolactin release in GH3/B6 pituitary tumor cells, *Environ. Health Perspect.*, 2005, **113**, 431–439.
63. R. T. Zoeller, Environmental chemicals as thyroid hormone analogues: new studies indicate that thyroid hormone receptors are targets of industrial chemicals?, *Mol. Cell. Endocrinol.*, 2005, **242**, 10–15.
64. H. J. Lee, S. Chattopadhyay, E.-Y. Gong, R. S. Ahn and K. Lee, Antiandrogenic Effects of Bisphenol A and Nonylphenol on the Function of Androgen Receptor, *Toxicol. Sci.*, 2003, **75**, 40–46.
65. F. Acconcia, V. Pallottini and M. Marino, Molecular Mechanisms of Action of BPA, *Dose-Response*, 2015, **13**, 1559325815610582.
66. Y. Ma, H. Liu, J. Wu, L. Yuan, Y. Wang, X. Du, R. Wang, P. W. Marwa, P. Petlulu, X. Chen and H. Zhang, The adverse health effects of bisphenol A and related toxicity mechanisms, *Environ. Res.*, 2019, **176**, 108575.
67. F. S. Vom Saal and L. N. Vandenberg, Update on the Health Effects of Bisphenol A: Overwhelming Evidence of Harm, *Endocrinology*, 2021, **162**, bqaa171.
68. E. Enmark, M. Pelto-Huikko, K. Grandien, S. Lagercrantz, J. Lagercrantz, G. Fried, M. Nordenskjöld and J. A. Gustafsson, Human estrogen receptor beta-gene structure, chromosomal localization, and expression pattern, *J. Clin. Endocrinol. Metab.*, 1997, **82**, 4258–4265.
69. L. P. Menasce, G. R. White, C. J. Harrison and J. M. Boyle, Localization of the estrogen receptor locus (ESR) to chromosome 6q25.1 by

FISH and a simple post-FISH banding technique, *Genomics*, 1993, **17**, 263–265.
70. H. Fang, W. Tong, R. Perkins, A. M. Soto, N. V. Prechtl and D. M. Sheehan, Quantitative comparisons of in vitro assays for estrogenic activities, *Environ. Health Perspect.*, 2000, **108**, 723–729.
71. V. Delfosse, M. Grimaldi, J. L. Pons, A. Boulahtouf, A. le Maire, V. Cavailles, G. Labesse, W. Bourguet and P. Balaguer, Structural and mechanistic insights into bisphenols action provide guidelines for risk assessment and discovery of bisphenol A substitutes, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 14930–14935.
72. W. V. Welshons, K. A. Thayer, B. M. Judy, J. A. Taylor, E. M. Curran and F. S. Vom Saal, Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity, *Environ. Health Perspect.*, 2003, **111**, 994–1006.
73. C. M. Klinge, Estrogen receptor interaction with co-activators and co-repressors, *Steroids*, 2000, **65**, 227–251.
74. C. M. Klinge, Estrogen receptor interaction with estrogen response elements, *Nucleic Acids Res.*, 2001, **29**, 2905–2919.
75. G. G. Kuiper, B. Carlsson, K. Grandien, E. Enmark, J. Häggblad, S. Nilsson and J. A. Gustafsson, Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta, *Endocrinology*, 1997, **138**, 863–870.
76. E. J. Routledge, R. White, M. G. Parker and J. P. Sumpter, Differential effects of xenoestrogens on coactivator recruitment by estrogen receptor (ER) alpha and ERbeta, *J. Biol. Chem.*, 2000, **275**, 35986–35993.
77. S. S. Mohamad Zaid, N. M. Kassim and S. Othman, Tualang Honey Protects against BPA-Induced Morphological Abnormalities and Disruption of ERα, ERβ, and C3 mRNA and Protein Expressions in the Uterus of Rats, *Evidence-Based Complementary Altern. Med.*, 2015, **2015**, 202874.
78. K. A. Bruno, J. E. Mathews, A. L. Yang, J. A. Frisancho, A. J. Scott, H. D. Greyner, F. A. Molina, M. S. Greenaway, G. M. Cooper, A. Bucek, A. C. Morales-Lara, A. R. Hill, A. A. Mease, D. N. Di Florio, J. M. Sousou, A. C. Coronado, A. R. Stafford and D. Fairweather, BPA Alters Estrogen Receptor Expression in the Heart After Viral Infection Activating Cardiac Mast Cells and T Cells Leading to Perimyocarditis and Fibrosis, *Front. Endocrinol.*, 2019, **10**, 598.
79. J. C. Gould, L. S. Leonard, S. C. Maness, B. L. Wagner, K. Conner, T. Zacharewski, S. Safe, D. P. McDonnell and K. W. Gaido, Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol, *Mol. Cell. Endocrinol.*, 1998, **142**, 203–214.
80. C. M. Markey, E. H. Luque, M. Munoz De Toro, C. Sonnenschein and A. M. Soto, In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland, *Biol. Reprod.*, 2001, **65**, 1215–1223.

81. S. C. Hewitt and K. S. Korach, Estrogenic activity of bisphenol A and 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) demonstrated in mouse uterine gene profiles, *Environ. Health Perspect.*, 2011, **119**, 63–70.
82. M. Yuan, M. Hu, Y. Lou, Q. Wang, L. Mao, Q. Zhan and F. Jin, Environmentally relevant levels of bisphenol A affect uterine decidualization and embryo implantation through the estrogen receptor/serum and glucocorticoid-regulated kinase 1/epithelial sodium ion channel α-subunit pathway in a mouse model, *Fertil. Steril.*, 2018, **109**, 735–744, .e731.
83. C. Williams, M. Bondesson, D. N. Krementsov and C. Teuscher, Gestational bisphenol A exposure and testis development, *Endocr. Disruptors*, 2014, **2**, e29088.
84. T. Shi, C. Zhao, Z. Li, Q. Zhang and X. Jin, Bisphenol a exposure promotes the migration of NCM460 cells via estrogen receptor-mediated integrin β1/MMP-9 pathway, *Environ. Toxicol.*, 2016, **31**, 799–807.
85. D. Zhai, J. He, X. Li, L. Gong and Y. Ouyang, Bisphenol A regulates Snail-mediated epithelial-mesenchymal transition in hemangioma cells, *Cell Biochem. Funct.*, 2016, **34**, 441–448.
86. G. A. Lee, K. A. Hwang and K. C. Choi, Inhibitory effects of 3,3′-diindolylmethane on epithelial-mesenchymal transition induced by endocrine disrupting chemicals in cellular and xenograft mouse models of breast cancer, *Food Chem. Toxicol.*, 2017, **109**, 284–295.
87. J. M. Hall and K. S. Korach, Endocrine disrupting chemicals promote the growth of ovarian cancer cells via the ER-CXCL12-CXCR4 signaling axis, *Mol. Carcinog.*, 2013, **52**, 715–725.
88. J.-Y. Xu, L. Wu, Z. Shi, X.-J. Zhang, N. A. Englert and S.-Y. Zhang, Up-regulation of human CYP2C9 expression by Bisphenol A via estrogen receptor alpha (ERα) and Med25, *Environ. Toxicol.*, 2017, **32**, 970–978.
89. P. Alonso-Magdalena, S. Morimoto, C. Ripoll, E. Fuentes and A. Nadal, The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance, *Environ. Health Perspect.*, 2006, **114**, 106–112.
90. E. R. Hugo, T. D. Brandebourg, J. G. Woo, J. Loftus, J. W. Alexander and N. Ben-Jonathan, Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes, *Environ. Health Perspect.*, 2008, **116**, 1642–1647.
91. A. Zsarnovszky, H. H. Le, H. S. Wang and S. M. Belcher, Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex: potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A, *Endocrinology*, 2005, **146**, 5388–5396.
92. P. Thomas and J. Dong, Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption, *J. Steroid Biochem. Mol. Biol.*, 2006, **102**, 175–179.

93. N. G. Khan, J. Correia, D. Adiga, P. S. Rai, H. S. Dsouza, S. Chakrabarty and S. P. Kabekkodu, A comprehensive review on the carcinogenic potential of bisphenol A: clues and evidence, *Environ. Sci. Pollut. Res. Int.*, 2021, **28**, 19643–19663.
94. A. Nadal, E. Fuentes, C. Ripoll, S. Villar-Pazos, M. Castellano-Muñoz, S. Soriano, J. Martinez-Pinna, I. Quesada and P. Alonso-Magdalena, Extranuclear-initiated estrogenic actions of endocrine disrupting chemicals: Is there toxicology beyond paracelsus?, *J. Steroid Biochem. Mol. Biol.*, 2018, **176**, 16–22.
95. N. Chevalier, A. Bouskine and P. Fenichel, Bisphenol A promotes testicular seminoma cell proliferation through GPER/GPR30, *Int. J. Cancer*, 2012, **130**, 241–242.
96. S. González-Rojo, M. Lombó, C. Fernández-Díez and M. P. Herráez, Male exposure to bisphenol a impairs spermatogenesis and triggers histone hyperacetylation in zebrafish testes, *Environ. Pollut.*, 2019, **248**, 368–379.
97. Y. Qie, W. Qin, K. Zhao, C. Liu, L. Zhao and L. H. Guo, Environmental Estrogens and Their Biological Effects through GPER Mediated Signal Pathways, *Environ. Pollut.*, 2021, **278**, 116826.
98. S. Xu, S. Yu, D. Dong and L. T. O. Lee, G Protein-Coupled Estrogen Receptor: A Potential Therapeutic Target in Cancer, *Front. Endocrinol.*, 2019, **10**, 725.
99. C. M. Revankar, H. D. Mitchell, A. S. Field, R. Burai, C. Corona, C. Ramesh, L. A. Sklar, J. B. Arterburn and E. R. Prossnitz, Synthetic Estrogen Derivatives Demonstrate the Functionality of Intracellular GPR30, *ACS Chem. Biol.*, 2007, **2**, 536–544.
100. C. Wang, J. Zhang, Q. Li, T. Zhang, Z. Deng, J. Lian, D. Jia, R. Li, T. Zheng, X. Ding, F. Yang, C. Ma, R. Wang, W. Zhang and J. Guo Wen, Low concentration of BPA induces mice spermatocytes apoptosis via GPR30, *Oncotarget*, 2017, **8**, 49005–49015.
101. P. Thomas, Role of G-protein-coupled estrogen receptor (GPER/GPR30) in maintenance of meiotic arrest in fish oocytes, *J. Steroid Biochem. Mol. Biol.*, 2017, **167**, 153–161.
102. S. A. Hafezi and W. M. Abdel-Rahman, The Endocrine Disruptor Bisphenol A (BPA) Exerts a Wide Range of Effects in Carcinogenesis and Response to Therapy, *Curr. Mol. Pharmacol.*, 2019, **12**, 230–238.
103. M. Murata and J. H. Kang, Bisphenol A (BPA) and cell signaling pathways, *Biotechnol. Adv.*, 2018, **36**, 311–327.
104. W. Qu, Z. Zhao, S. Chen, L. Zhang, D. Wu and Z. Chen, Bisphenol A suppresses proliferation and induces apoptosis in colonic epithelial cells through mitochondrial and MAPK/AKT pathways, *Life Sci.*, 2018, **208**, 167–174.
105. S. Dong, S. Terasaka and R. Kiyama, Bisphenol A induces a rapid activation of Erk1/2 through GPR30 in human breast cancer cells, *Environ. Pollut.*, 2011, **159**, 212–218.

106. M. Pupo, A. Pisano, R. Lappano, M. F. Santolla, E. M. De Francesco, S. Abonante, C. Rosano and M. Maggiolini, Bisphenol A induces gene expression changes and proliferative effects through GPER in breast cancer cells and cancer-associated fibroblasts, *Environ. Health Perspect.*, 2012, **120**, 1177–1182.
107. Z. G. Sheng, W. Huang, Y. X. Liu and B. Z. Zhu, Bisphenol A at a low concentration boosts mouse spermatogonial cell proliferation by inducing the G protein-coupled receptor 30 expression, *Toxicol. Appl. Pharmacol.*, 2013, **267**, 88–94.
108. X. L. Zhang, N. Liu, S. F. Weng and H. S. Wang, Bisphenol A Increases the Migration and Invasion of Triple-Negative Breast Cancer Cells via Oestrogen-related Receptor Gamma, *Basic Clin. Pharmacol. Toxicol.*, 2016, **119**, 389–395.
109. I. S. Okon and M. H. Zou, Mitochondrial ROS and cancer drug resistance: Implications for therapy, *Pharmacol. Res.*, 2015, **100**, 170–174.
110. S. J. Sauer, M. Tarpley, I. Shah, A. V. Save, H. K. Lyerly, S. R. Patierno, K. P. Williams and G. R. Devi, Bisphenol A activates EGFR and ERK promoting proliferation, tumor spheroid formation and resistance to EGFR pathway inhibition in estrogen receptor-negative inflammatory breast cancer cells, *Carcinogenesis*, 2017, **38**, 252–260.
111. S. H. Dairkee, M. G. Luciani-Torres, D. H. Moore and W. H. Goodson, 3rd, Bisphenol-A-induced inactivation of the p53 axis underlying deregulation of proliferation kinetics, and cell death in non-malignant human breast epithelial cells, *Carcinogenesis*, 2013, **34**, 703–712.
112. W. H. Goodson 3rd, M. G. Luciani, S. A. Sayeed and I. M. Jaffee, D. H. Moore, 2nd and S. H. Dairkee, Activation of the mTOR pathway by low levels of xenoestrogens in breast epithelial cells from high-risk women, *Carcinogenesis*, 2011, **32**, 1724–1733.
113. K. H. Wang, A. P. Kao, C. C. Chang, T. C. Lin and T. C. Kuo, Bisphenol A-induced epithelial to mesenchymal transition is mediated by cyclooxygenase-2 up-regulation in human endometrial carcinoma cells, *Reprod. Toxicol.*, 2015, **58**, 229–233.
114. S. Li, B. Wang, Q. Tang, J. Liu and X. Yang, Bisphenol A triggers proliferation and migration of laryngeal squamous cell carcinoma via GPER mediated upregulation of IL-6, *Cell Biochem. Funct.*, 2017, **35**, 209–216.
115. K.-S. Zhang, H.-Q. Chen, Y.-S. Chen, K.-F. Qiu, X.-B. Zheng, G.-C. Li, H.-D. Yang and C.-J. Wen, Bisphenol A stimulates human lung cancer cell migration via upregulation of matrix metalloproteinases by GPER/EGFR/ERK1/2 signal pathway, *Biomed. Pharmacother.*, 2014, **68**, 1037–1043.
116. R. Castillo Sanchez, R. Gomez and E. Perez, Salazar, Bisphenol A Induces Migration through a GPER-, FAK-, Src-, and ERK2-Dependent Pathway in MDA-MB-231 Breast Cancer Cells, *Chem. Res. Toxicol.*, 2016, **29**, 285–295.

117. X. F. Ma, J. Zhang, H. L. Shuai, B. Z. Guan, X. Luo and R. L. Yan, IKKβ/NF-κB mediated the low doses of bisphenol A induced migration of cervical cancer cells, *Arch. Biochem. Biophys.*, 2015, **573**, 52–58.
118. A. Ptak and E. L. Gregoraszczuk, Bisphenol A induces leptin receptor expression, creating more binding sites for leptin, and activates the JAK/Stat, MAPK/ERK and PI3K/Akt signalling pathways in human ovarian cancer cell, *Toxicol. Lett.*, 2012, **210**, 332–337.
119. I. Quesada, E. Fuentes, M. C. Viso-León, B. Soria, C. Ripoll and A. Nadal, Low doses of the endocrine disruptor bisphenol-A and the native hormone 17beta-estradiol rapidly activate transcription factor CREB, *FASEB J.*, 2002, **16**, 1671–1673.
120. T. Yaguchi, The endocrine disruptor bisphenol A promotes nuclear ERRγ translocation, facilitating cell proliferation of Grade I endometrial cancer cells via EGF-dependent and EGF-independent pathways, *Mol. Cell. Biochem.*, 2019, **452**, 41–50.
121. M. Huang, M. Huang, X. Li, S. Liu, L. Fu, X. Jiang and M. Yang, Bisphenol A induces apoptosis through GPER-dependent activation of the ROS/Ca(2+)-ASK1-JNK pathway in human granulosa cell line KGN, *Ecotoxicol. Environ. Saf.*, 2021, **208**, 111429.
122. M. García-Arevalo, P. Alonso-Magdalena, J. Rebelo Dos Santos, I. Quesada, E. M. Carneiro and A. Nadal, Exposure to bisphenol-A during pregnancy partially mimics the effects of a high-fat diet altering glucose homeostasis and gene expression in adult male mice, *PLoS One*, 2014, **9**, e100214.
123. M. K. Moon, I. K. Jeong, H. Y. Ahn, H. H. Kim, Y. J. Park, H. C. Jang and K. S. Park, Long-term oral exposure to bisphenol A induces glucose intolerance and insulin resistance, *J. Endocrinol.*, 2015, **226**, 35–42.
124. A. Wang, J. Luo, W. Moore, H. Alkhalidy, L. Wu, J. Zhang, W. Zhen, Y. Wang, D. J. Clegg, X. Bin, Z. Cheng, R. P. McMillan, M. W. Hulver and D. Liu, GPR30 regulates diet-induced adiposity in female mice and adipogenesis in vitro, *Sci. Rep.*, 2016, **6**, 34302.
125. I. Cimmino, F. Oriente, V. D'Esposito, D. Liguoro, P. Liguoro, M. R. Ambrosio, S. Cabaro, F. D'Andrea, F. Beguinot, P. Formisano and R. Valentino, Low-dose Bisphenol-A regulates inflammatory cytokines through GPR30 in mammary adipose cells, *J. Mol. Endocrinol.*, 2019, **63**, 273–283.
126. L. Yu, P. Das, A. J. Vall, Y. Yan, X. Gao, M. I. Sifre, C. D. Bortner, L. Castro, G. E. Kissling, A. B. Moore and D. Dixon, Bisphenol A induces human uterine leiomyoma cell proliferation through membrane-associated ERα36 via nongenomic signaling pathways, *Mol. Cell. Endocrinol.*, 2019, **484**, 59–68.
127. X. Lan, L. J. Fu, J. Zhang, X. Q. Liu, H. J. Zhang, X. Zhang, M. F. Ma, X. M. Chen, J. L. He, L. B. Li, Y. X. Wang and Y. B. Ding, Bisphenol A exposure promotes HTR-8/SVneo cell migration and impairs mouse placentation involving upregulation of integrin-β1 and MMP-9 and

stimulation of MAPK and PI3K signaling pathways, *Oncotarget*, 2017, **8**, 51507–51521.
128. B. Jia, T. Shi, Z. Li, S. Shan, P. Ji and Z. Li, Toxicological effects of bisphenol A exposure-induced cancer cells migration via activating directly integrin β1, *Chemosphere*, 2019, **220**, 783–792.
129. E. Somm, V. M. Schwitzgebel, A. Toulotte, C. R. Cederroth, C. Combescure, S. Nef, M. L. Aubert and P. S. Hüppi, Perinatal exposure to bisphenol a alters early adipogenesis in the rat, *Environ. Health Perspect.*, 2009, **117**, 1549–1555.
130. Y. Pu, J. D. Gingrich, J. P. Steibel and A. Veiga-Lopez, Sex-Specific Modulation of Fetal Adipogenesis by Gestational Bisphenol A and Bisphenol S Exposure, *Endocrinology*, 2017, **158**, 3844–3858.
131. G. Biasiotto, I. Zanella, A. Masserdotti, R. Pedrazzani, M. Papa, L. Caimi and D. Di, Lorenzo, Municipal wastewater affects adipose deposition in male mice and increases 3T3-L1 cell differentiation, *Toxicol. Appl. Pharmacol.*, 2016, **297**, 32–40.
132. F. Ariemma, V. D'Esposito, D. Liguoro, F. Oriente, S. Cabaro, A. Liotti, I. Cimmino, M. Longo, F. Beguinot, P. Formisano and R. Valentino, Low-Dose Bisphenol-A Impairs Adipogenesis and Generates Dysfunctional 3T3-L1 Adipocytes, *PLoS One*, 2016, **11**, e0150762.
133. E. Atlas, L. Pope, M. G. Wade, A. Kawata, A. Boudreau and J. G. Boucher, Bisphenol A increases aP2 expression in 3T3L1 by enhancing the transcriptional activity of nuclear receptors at the promoter, *Adipocyte*, 2014, **3**, 170–179.
134. J. G. Boucher, R. Gagné, A. Rowan-Carroll, A. Boudreau, C. L. Yauk and E. Atlas, Bisphenol A and Bisphenol S Induce Distinct Transcriptional Profiles in Differentiating Human Primary Preadipocytes, *PLoS One*, 2016, **11**, e0163318.
135. J. Wang, B. Sun, M. Hou, X. Pan and X. Li, The environmental obesogen bisphenol A promotes adipogenesis by increasing the amount of 11β-hydroxysteroid dehydrogenase type 1 in the adipose tissue of children, *Int. J. Obes.*, 2013, **37**, 999–1005.
136. A. Salehpour, F. Shidfar, M. Hedayati, A. Neshatbini Tehrani, A. A. Farshad and S. Mohammadi, Bisphenol A enhances adipogenic signaling pathways in human mesenchymal stem cells, *Genes Environ.*, 2020, **42**, 13.
137. B. DeBenedictis, H. Guan and K. Yang, Prenatal Exposure to Bisphenol A Disrupts Mouse Fetal Liver Maturation in a Sex-Specific Manner, *J. Cell. Biochem.*, 2016, **117**, 344–350.
138. Q. Ma, Role of nrf2 in oxidative stress and toxicity, *Annu. Rev. Pharmacol. Toxicol.*, 2013, **53**, 401–426.
139. Y. Dong, Z. Zhang, H. Liu, L. Jia, M. Qin and X. Wang, Exacerbating lupus nephritis following BPA exposure is associated with abnormal autophagy in MRL/lpr mice, *Am. J. Transl. Res.*, 2020, **12**, 649–659.
140. J. Xu, A. C. Donepudi, V. R. More, S. R. Kulkarni, L. Li, L. Guo, B. Yan, T. Chatterjee, N. Weintraub and A. L. Slitt, Deficiency in Nrf2

transcription factor decreases adipose tissue mass and hepatic lipid accumulation in leptin-deficient mice, *Obesity*, 2015, **23**, 335–344.
141. M. Nakamura, H. Yamanaka, A. Oguro and S. Imaoka, Bisphenol A induces Nrf2-dependent drug-metabolizing enzymes through nitrosylation of Keap1, *Drug Metab. Pharmacokinet.*, 2018, **33**, 194–202.
142. P. C. Shimpi, V. R. More, M. Paranjpe, A. C. Donepudi, J. M. Goodrich, D. C. Dolinoy, B. Rubin and A. L. Slitt, Hepatic Lipid Accumulation and Nrf2 Expression following Perinatal and Peripubertal Exposure to Bisphenol A in a Mouse Model of Nonalcoholic Liver Disease, *Environ. Health Perspect.*, 2017, **125**, 087005.
143. Q. Li, J. Davila, A. Kannan, J. A. Flaws, M. K. Bagchi and I. C. Bagchi, Chronic Exposure to Bisphenol A Affects Uterine Function During Early Pregnancy in Mice, *Endocrinology*, 2016, **157**, 1764–1774.
144. Y. L. Schindler, K. M. Garske, J. Wang, B. A. Firulli, A. B. Firulli, K. D. Poss and D. Yelon, Hand2 elevates cardiomyocyte production during zebrafish heart development and regeneration, *Development*, 2014, **141**, 3112–3122.
145. J. Moreman, O. Lee, M. Trznadel, A. David, T. Kudoh and C. R. Tyler, Acute Toxicity, Teratogenic, and Estrogenic Effects of Bisphenol A and Its Alternative Replacements Bisphenol S, Bisphenol F, and Bisphenol AF in Zebrafish Embryo-Larvae, *Environ. Sci. Technol.*, 2017, **51**, 12796–12805.
146. M. Lombó, S. González-Rojo, C. Fernández-Díez and M. P. Herráez, Cardiogenesis impairment promoted by bisphenol A exposure is successfully counteracted by epigallocatechin gallate, *Environ. Pollut.*, 2019, **246**, 1008–1019.
147. M. Featherstone, in *Advances in Developmental Biology and Biochemistry*, Elsevier, 2003, vol. 13, pp. 1–42.
148. T. R. Lappin, D. G. Grier, A. Thompson and H. L. Halliday, HOX genes: seductive science, mysterious mechanisms, *Ulster Med. J.*, 2006, **75**, 23–31.
149. B. Bodey, B. Bodey, Jr., S. E. Siegel and H. E. Kaiser, Immunocytochemical detection of the homeobox B3, B4, and C6 gene products in breast carcinomas, *Anticancer Res.*, 2000, **20**, 3281–3286.
150. T. Hayashida, F. Takahashi, N. Chiba, E. Brachtel, M. Takahashi, N. Godin-Heymann, K. W. Gross, M. d. M. Vivanco, V. Wijendran, T. Shioda, D. Sgroi, P. K. Donahoe and S. Maheswaran, HOXB9, a gene overexpressed in breast cancer, promotes tumorigenicity and lung metastasis, *Proceedings of the National Academy of Sciences*, 2010, **107**, 1100.
151. C. C. Smith and H. S. Taylor, Xenoestrogen exposure imprints expression of genes (Hoxa10) required for normal uterine development, *FASEB J.*, 2007, **21**, 239–246.
152. P. Deb, A. Bhan, I. Hussain, K. I. Ansari, S. A. Bobzean, T. K. Pandita, L. I. Perrotti and S. S. Mandal, Endocrine disrupting chemical, bisphenol-A, induces breast cancer associated gene HOXB9 expression in vitro and in vivo, *Gene*, 2016, **590**, 234–243.

153. I. Hussain, A. Bhan, K. I. Ansari, P. Deb, S. A. Bobzean, L. I. Perrotti and S. S. Mandal, Bisphenol-A induces expression of HOXC6, an estrogen-regulated homeobox-containing gene associated with breast cancer, *Biochim Biophys Acta*, 2015, **1849**, 697–708.
154. J. R. Rochester, Bisphenol A and human health: a review of the literature, *Reprod. Toxicol.*, 2013, **42**, 132–155.
155. H. H. You and G. Song, Review of endocrine disruptors on male and female reproductive systems, *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.*, 2021, **244**, 109002.
156. F. S. vom Saal, B. T. Akingbemi, S. M. Belcher, L. S. Birnbaum, D. A. Crain, M. Eriksen, F. Farabollini, L. J. Guillette, Jr., R. Hauser, J. J. Heindel, S.-M. Ho, P. A. Hunt, T. Iguchi, S. Jobling, J. Kanno, R. A. Keri, K. E. Knudsen, H. Laufer, G. A. LeBlanc, M. Marcus, J. A. McLachlan, J. P. Myers, A. Nadal, R. R. Newbold, N. Olea, G. S. Prins, C. A. Richter, B. S. Rubin, C. Sonnenschein, A. M. Soto, C. E. Talsness, J. G. Vandenbergh, L. N. Vandenberg, D. R. Walser-Kuntz, C. S. Watson, W. V. Welshons, Y. Wetherill and R. T. Zoeller, Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure, *Reprod. Toxicol.*, 2007, **24**, 131–138.
157. S. Singh and S. S.-L. Li, Epigenetic Effects of Environmental Chemicals Bisphenol A and Phthalates, *Int. J. Mol. Sci.*, 2012, **13**, 10143–10153.