

Molecular Mechanisms of Action of BPA

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Abstract

Bisphenol A (BPA) exposure has been associated with serious endocrine-disrupting effects in humans and wildlife. Toxicological and epidemiological studies evidenced that BPA increases body mass index and disrupts normal cardiovascular physiology by interfering with endogenous hormones in rodents, nonhuman primates, and cell culture test systems. The BPA concentration derived from these experiments were used by government regulatory agencies to determine the safe exposure levels of BPA in humans. However, accumulating literature in vivo and in vitro indicate that at concentrations lower than that reported in toxicological studies, BPA could elicit a different endocrine-disrupting capacity. To further complicate this picture, BPA effects rely on several and diverse mechanisms that converge upon endocrine and reproductive systems. If all or just few of these mechanisms concur to the endocrine-disrupting potential of low doses of BPA is at present still unclear. Thus, taking into account that the incidence and/or prevalence of health problems associated with endocrine disruption have increased worldwide, the goal of the present review is to give an overview of the many mechanisms of BPA action in order to decipher whether different mechanisms are at the root of the effect of low dose of BPA on endocrine system.

Keywords

bisphenol A, endocrine disruptors, low doses, nuclear receptors, signal transduction pathways

Introduction

Bisphenol A (BPA; 4,4-dihydroxy-2,2-diphenylpropane; CAS 80-05-7) has a long story in science. This monomer was first developed as a synthetic estrogen in the 1890s, but only in the 1930s, the estrogenic properties of BPA were reported in the reproductive system of female rats.¹ Successively, chemical industries used BPA as a monomer in the manufacturing of polymers (eg, polycarbonate and epoxy resins), as an antioxidant and inhibitor of end of polyvinyl chloride polymerization, and as a precursor for the synthesis of a flame retardant.² In turn, these materials are currently used as components of many consumer products including reusable plastic bottles, feeding bottles, internal coating of food and beverage cans, thermal paper, medical devices, dental materials, and so on.² Unfortunately, like other chemical substances, BPA can be released from these materials in dependence of temperature and pH to migrate in food,^{3,4} air,^{5,6} skin,⁷ saliva,⁸ and blood.⁶ To make this picture more alarming, considerable amounts of BPA (ranging from 0.25 to 1.11 mg/kg) have been found in randomly selected fresh food samples from an area of Southern Italy, probably deriving from plastic irrigation pipes.⁹ Consequently, it is estimated that food contributes to more than 90% of the overall BPA exposure, while exposure through dust ingestion, dental surgery, and dermal absorption remains below 5% in normal situations.¹⁰

Overall, human exposure to BPA is frequent and widespread, and more than 90% of individuals have detectable amounts of BPA in urine as reported by biomonitoring studies conducted in the United States, Germany, and Canada.^{5,11-13}

However, the US Food and Drug Administration and the European Food Safety Authority have determined that human exposure to BPA is below safe exposure levels (from 50 to 4 µg/Kg weight/day).^{14,15} In marked contrast with these reassuring reports, France has banned BPA in food contact materials (LOI no 2010-729 du 30 juin 2010, <http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000022414734>), plastic manufacturers are starting to release “BPA-Free” plastic material, and the scientific community continues to publish discouraging statements on the risk of BPA for human beings and wildlife health, renewing the demand for making the screening for exposures a research priority.¹⁶⁻¹⁹

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These discrepancies derive from the serious endocrine-disrupting effects associated with BPA exposure.^{18,20} Toxicological *in vivo* studies indicate that BPA doses of 20 to 400 $\mu\text{g}/\text{kg}/\text{d}$ can disrupt normal physiology by interfering with endogenous hormones in rodents, nonhuman primates, and cell culture test systems.²¹ Epidemiological studies showed relationships between BPA exposure and increased body mass index,²²⁻²⁴ cardiovascular disease,²⁵ behavior,⁷ and other endocrine-disrupting effects, although other studies have been unable to reproduce these results.¹²

In contrast to this toxicological approach used by to predict the possibility of BPA effect, a new paradigm has emerged in the *in vivo* BPA research in which much lower, environmentally relevant doses of BPA are used to directly assess the hazards posed by this compound. From these experiments emerged a curious feature typical of the BPA actions: its oscillating nonmonotonic dose response. Biphasic U- or inverted U-shaped dose-response curves that show ascending and descending phases were reported in relation to the BPA dose used.^{26,27} After these experiments, endocrinologists, who were well familiar with the hormone nonmonotonic curves, began to challenge the high doses used by toxicological studies and well accepted by government regulatory agencies in the absence of other data, and they started to apply the basic approaches used for decades to determine physiologically relevant doses for hormones. In a very elegant study, the group of vom Saal et al²⁸ observed that the fetal serum estradiol concentration during the development of the male murine reproductive tract was 0.21 pg/mL of plasma. An increase in this estradiol level to 0.31 pg/mL of plasma resulted in permanent developmental changes (eg, enlargement of the prostate).²⁹ Therefore, an estrogenic endocrine disruptor, like BPA, would be biologically active in the fetus if its estrogenic activity in blood was equivalent to an increase in estradiol of only 0.1 pg/mL of plasma. These pioneering experiments opened a new avenue in the field of BPA effects. Nowadays, accumulating literature is available on BPA endocrine-disrupting capability at nanomolar to micromolar serum concentrations.²⁶⁻³² Nonetheless, a clear definition of low doses is not yet available. Vandenberg et al reported that low dose of a chemical could be evidenced by biological changes occurring in the range of typical human exposures or by any biological changes obtained at doses lower than those tested in toxicology assessments. Furthermore, low dose is any dose of a chemical below the lowest observed adverse effect level or a dose administered to an animal that produces blood concentrations of that chemical present in the general human population (ie, environmentally relevant dose).^{31,32} In this review, we will consider the BPA effects occurring at doses lower than those tested in toxicological studies and well accepted by government regulatory agencies (ie, lower than 50-4 $\mu\text{g}/\text{kg}$ weight/d in exposed animals and lower than 10^{-5} mol/L in cell lines).

Another subject of significant debate in the field of BPA action is the possible mechanisms at the root of BPA low-dose effects. Indeed, BPA triggers several action mechanisms including the interference of the activity of nuclear receptors,

noncanonical steroid hormone receptors, and orphan receptors (eg, aryl hydrocarbon receptor, AhR).^{33,34} Moreover, enzymatic pathways involved in the steroid biosynthesis and/or metabolism and numerous other mechanisms that converge upon endocrine and reproductive systems have been proposed to explain BPA actions.^{33,34} If all or just few of these mechanisms concur to the nonmonotonic curves of endocrine-disrupting potential of BPA is still unclear.

Thus, far from solving the contradiction on the safety of BPA doses accepted by government regulatory agencies, but considering that the incidence and/or prevalence of health problems associated with endocrine disruption increased worldwide, the goal of the present review is to give an overview of the many mechanisms of BPA action in order to decipher whether different mechanisms are at the root of the effect of low dose of BPA on endocrine system.

Mechanisms Leading to Activation of Nuclear Receptors

Estrogen Receptors

The BPA molecule has structural features that confer the ability to bind to the 2 estrogen receptor (ER) subtypes (ie, ER α and ER β), although BPA displays 1000- to 2000-fold less affinity to the ERs than 17 β -estradiol (E2), the most active estrogen.^{4,35} Both ER α and ER β , as well as the other 46 members of nuclear receptor superfamily, are ligand-activated transcription factors that, upon E2 binding, change conformation and migrate into the nucleus. Nuclear ligand-bound ERs interact with coactivators and corepressors and with estrogen responsive elements (EREs) in the promoters of target genes to regulate E2-target gene expression.^{35,36} However, the change in ER conformation differs as a function of the ligand,^{36,37} rendering ER more or less prone to the transcriptional coactivators or corepressors recognition.

Exogenous ligands produce a displacement of α -helices forming the ligand-binding domain (LBD) of ERs^{38,39} due to their chemical structure that does not allow a proper accommodation in the confines of the hormone-binding site, contrary to what happens when E2 binds to ERs. A different LBD displacement between the 2 ERs is also possible; indeed, BPA acts as an ER α agonist, producing the same displacement as that of E2, while BPA does not allow the ER β LBD to assume the right conformation, thus acting as an antagonist.³⁶ A proper LBD displacement is necessary for a transcriptional competent conformation, thus the BPA as well as other estrogen-like compounds binding to the ERs may lead to coactivators or corepressors recruitment depending on their agonist or antagonist action. As a consequence, differences in the regulatory activity on gene expression are expected. Distinct gene expression patterns following exposure to E2 or BPA have been reported in the uterus of immature rats.⁴⁰

Until recently, the majority of the studies on BPA focused on these nuclear mechanisms of estrogen responses that rely on ER action into the nucleus. On these bases, BPA has been and is still

defined as a “weak estrogen.”⁴¹ However, estrogens, as well as other steroid hormones, can also induce rapid (seconds to minutes) extranuclear responses based on ER localized at the plasma membrane. A small pool of ER α and ER β is palmitoylated and localized at the plasma membrane in association with caveolin 1.^{36,42-44} At the plasma membrane, ligand-activated ERs interact with other signaling proteins (eg, growth factor receptors; cellular-Rous sarcoma oncogene [c-Src]), thus forming multimolecular complexes mediating the rapid signal transduction events.^{36,45} However, to date, the ER α -mediated extracellular regulated kinase/mitogen-activated protein kinase (ERK/MAPK) and phosphatidylinositol-3-kinase/AKT (PI3K/AKT) pathways, as well as the ER β -mediated p38/MAPK signaling, appear to be the unique molecular circuitries activated by E2 in different cell contexts.⁴⁶ Several data support that BPA-dependent estrogenic activity flows through the ER α -mediated extranuclear signals activation that results in the ERK/MAPK and AKT phosphorylation.^{4,37} Therefore, BPA acts as an E2-mimetic by binding to ER α leading to the activation of rapid extranuclear pathways (or nongenomic mechanism).^{4,37} Another example of BPA action by ER α extranuclear mechanisms is the Ca⁺⁺ release from intracellular stores that can lead to changes in cell motility, signaling processes, and exocytosis.⁴⁷ On the other hand, BPA behaves as an E2 antagonist preventing ER β to signal to its downstream targets (ie, p38/MAPK).^{35,37}

The physiological consequence of these BPA-induced modulations of E2 action mechanisms is diverse in dependence of the tissue examined; however, the E2 modulation of cell proliferation represents a good example of BPA impact on E2 effects. In fact, the balance between ER α /ER β signaling and levels is at the root of E2-regulated cell death and proliferation equilibrium, since ER α rapid signals drive cells to proliferation (eg, mammary ductal cells during lactation), while E2-induced ER β rapid signals inhibit cell proliferation (eg, mammary ductal cells after ovulation or lactation).^{46,48} Moreover, E2 exerts a fine regulation on ER α and ER β levels inducing the ligand-dependent reduction in the total ER α content and increasing ER β levels.⁴⁹ BPA exposure (from nmol/L to μ mol/L) mimics E2 in the presence of ER α acting as a proliferative agent⁴ while in the presence of ER β BPA acts as a complete antagonist of E2-ER β complex.^{35,37} All these data indicate that BPA modifies E2-regulated cell death and proliferation equilibrium by promoting only cell proliferation that, without the balance of ER β activities, could drive cells to cancer transformation.

A relatively recent discovery is the 7-transmembrane estrogen receptor, GPR30, that has been recognized as an estrogen receptor,^{50,51} although this notion continues to be seriously disputed.^{52,53} The BPA and other endocrine disruptors show high binding affinities for GPR30. In particular, in cells isolated from pancreatic islets, concentration of 10^{-9} mol/L BPA is able to influence oscillations of cytosolic Ca⁺⁺ concentration.⁵⁴ As GPR30 is expressed in a broad range of tissues, BPA could even activate other signals in all these tissues.⁵⁵

As a whole, the pleiotropic effects elicited by E2 are obtained by the synergy of different signal transduction pathways (ie, nuclear and extranuclear) and are mediated by different

receptors (ie, ER α , ER β , and GPR30) whose activation depends on the cellular context of target cells, on the receptor subtype and location within cells (ie, membrane, cytosol, and nucleus) as well as on the chemical nature of the ligand itself.^{36,37,46} The synergy between these mechanisms illustrates the complexity of BPA-induced endocrine disruption that should be taken into consideration when screening for environmental estrogens. In addition, in light of the effects of low doses of BPA on extranuclear mechanisms, it is now becoming clear that “weak” activity via one pathway (ie, nuclear mechanism) does not necessarily predict the potency of an endocrine disruptor or mimetic acting via another signaling pathway.

Androgen Receptor

Exposure to BPA has been associated with a reduced proportion of male births in the populations of a number of countries and increased the risk of cryptorchidism, hypospadias, and reduced semen quality in males,⁵⁶ suggesting a possible BPA interference with the male reproductive function. The androgen receptor (AR), expressed in all male and female organs, shares similar cellular localization⁴⁴ and action mechanisms of ERs. However, very few data are available on the effects of BPA on AR transcriptional activity, while a lack of knowledge is still present on the ability of these compounds to interfere with androgen-dependent extranuclear signals.⁵⁷⁻⁵⁹ In our laboratory, we evaluated BPA effects on mouse satellite cell differentiation, male rat vascular smooth muscle cells motility, and AR levels and transcriptional activity in human prostate cancer cells. All the cell models used expressed the AR wild type (ie, 110 kDa), while prostate cancer cells were positive for several AR splicing forms (eg, AR Δ LBD and AR 75-80 kDa). Surprisingly, BPA did not impair androgen effects in normal cell lines,^{60,61} but it acted as an antiandrogen in cancer cells when the AR splicing forms were expressed.⁶⁰ These data have recently been confirmed in HeLa cells transiently transfected with AR wild type (110 kDa) or AR mutants (ie, AR \sim 80 kDa and AR \sim 28 kDa; Marino M, unpublished data) and have also been established by other authors with different AR mutants.⁶² Thus, androgen signaling seems to be less prone to BPA interference, but BPA could interfere with the therapy in patients with advanced prostate cancer via mutant ARs.^{62,63} However, taking into account that ERs, principally ER β , are mainly expressed in male reproductive system (eg, testis, spermatozoa, and prostate),⁶⁴ it is worth considering whether some, or even all, of the above-reported endocrine effects of BPA are due not to their androgenicity but rather to their abilities to interfere with the action of estrogen receptors.^{57,60}

Estrogen-Related Receptors

Estrogen-related receptors (ERRs) are a subfamily of orphan nuclear receptors closely related to ER α and ER β . Three of these ERRs are known: ERR α , ERR β , and ERR γ . Although ERRs do not bind to estrogens, they share a significant and remarkable homology with ERs, particularly in DNA binding

domain and LBD. However, because ERR can bind to estrogen response elements, a possible overlap between ER and ERR action may exist.³⁶

The ERRs possess a constitutive transcriptional activity, which is known to be repressed by a few chemicals (ie, Desametasone [DES], 4-hydroxytamoxifen [4-OHT]). BPA strongly binds to human ERR with a half maximal inhibitory concentration (IC₅₀) value of 13.1 nmol/L preserving ERR γ constitutive activity even in the presence of 4-OHT.⁶⁵ As ERRs and ER α have the potential to interfere or collaborate with each other in regulating common target genes and ERR γ is highly expressed in the mammalian brain during development as well as in the brain, lung, and other tissues of adult, it could be possible that the effects of low-dose BPA could be mediated through this nuclear receptor.

Thyroid Hormone Receptor

Thyroid hormones (THs), including thyroxine (T4) and triiodothyronine (T3), are essential for normal brain development, and its mild or transient insufficiency produces different cognitive deficits in both humans and animals.⁶⁶ These effects are mediated by thyroid hormone receptor (THR), another member of nuclear receptor superfamily, localized into the nucleus where it acts as a repressor of transcription in strict association with DNA and corepressors (ie, NCoR and SMRT). In the genomic mechanism, T3 accesses to the cell nucleus to bind THR, which disengages corepressors in favor of coactivators to thyroid hormone responsive element. In turn, hormone responsive gene transcription occurs. Extranuclear actions of THR are, at least in part, dependent on integrin $\alpha v \beta 3$, which activates MAPK/c-Src pathway responsible for phosphorylation and activation of nuclear THR.⁶⁷ It has been reported that low doses of BPA impair T3 induction of THR in *Xenopus laevis* tail tissue resulting in antimetamorphic effect.⁶⁸ In addition, 10⁻⁹ to 10⁻⁷ mol/L BPA concentrations directly interfere with $\beta 3$ integrin/c-Src/MAPK/TR- $\beta 1$ pathway suppressing THR-mediated transcription.³⁰ As a whole, although these and other data suggest that THR is not a direct target of BPA action, perinatal BPA exposure at a very low level may influence TH effects in brain development presumably by extranuclear mechanisms.

Pregnane X Receptor

Constitutive androstane receptor (CAR) and pregnane X receptor (PXR) function as sensors of toxic by-products derived from endogenous metabolites and of exogenous chemicals in order to enhance their elimination. This unique function of CAR and PXR, members of nuclear receptor superfamily, sets them apart from the steroid hormone receptors. The broad response profile established that CAR and PXR are xenobiotic sensors that coordinately regulate xenobiotic clearance in the liver and intestine, inducing the transcription of genes involved in xenobiotic/drug metabolism and transport.⁶⁹

Pregnane X receptor is activated by many drugs and environmental pollutants including BPA and several analogues,

which act as potent agonists for human PXR (hPXR).⁷⁰ Although the BPA concentrations activating hPXR are relatively high, combinations of BPA and other endocrine disruptors could additively or synergistically activate hPXR in vivo. Thus, additional in vivo studies are required to establish the influence of such synergistic or additive effects in risk assessment because exposure to mixtures of chemicals is much more representative of real-world scenarios.

Peroxisome Proliferator-Activated Receptors

One of the great concerns raised against BPA is the putative effect of early exposure to BPA in the onset of obesity and metabolic syndromes.⁷¹ In particular, rat perinatal exposure to BPA modified early adipogenesis by modulating adipocyte hypertrophy and overexpression of lipogenic genes including PPAR γ (a nuclear receptor which dysregulation is involved in the onset of diabetes and obesity), sterol regulatory element binding protein 1C (*SREBP-1C*), lipoprotein lipase (*LPL*), and fatty acid synthase (*FAS*).^{72,73} Intriguingly, although BPA failed to directly bind to and activate PPAR γ -dependent gene transcription, lower brominated BPA analogs, which are also released in the environment, bind to the receptor displaying the highest transactivation efficiency at nmol/L to μ mol/L concentrations.⁷⁴

This discovery supports the idea that BPA could be involved in the disruption of energy balance in humans and wildlife.⁷⁵ Again, perinatal exposure could play a critical role being these BPA derivatives present in human cord blood (200 pg/g fresh weight) and maternal milk (0.1-37.4 ng/g lipid weight).⁷⁶ Furthermore, as the main transcriptional active form of PPAR γ is in association with RXR (an orphan nuclear receptor, which binds to other environmental disruptors), additive (acting only through PPAR γ) or synergistic (acting through both RXR and PPAR γ) effects could occur, increasing the risk of metabolic diseases.

Mechanisms Leading to Activation of Other Receptors

Aryl Hydrocarbon Receptor

Aryl hydrocarbon receptor is member of basic helix-loop-helix/PAS family transcription factors which mediates the effects of various environmental chemicals, including its most potent ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin. Upon ligand binding, cytoplasmic AhR associates with its translocator (AHR nuclear translocator; ARNT) and enters into the nucleus, where it binds to specific DNA response elements, leading to transcription of several genes involved in xenobiotic metabolism including cytochrome P450 family 1. Successively, the AhR repressor (AhRR) heterodimerizes with ARNT to terminate the activation of the AhR signaling pathway.⁷⁷

BPA treatment in utero (0.02-20 000 μ g/kg/d) upregulated the expression of AhRR, impairing the AhR expression and

function in embryos.⁷⁸ Notably, BPA did not dramatically alter genes in the AhR signaling pathway in adults.⁷⁹

Remarkably, it has been showed that the agonist-activated AhR/ARNT heterodimer directly associates with ER α and ER β . This association resulted in the activation of transcription and estrogenic effects.⁸⁰ However, AhR-induced activation of ER seems to be dependent on AhR ligand structure and, on the basis of the previously cited literature, does not necessarily occur for BPA.

Mechanisms Involving Hormone Metabolism

17 β -Estradiol, cortisol, progesterone, dihydrotestosterone, and aldosterone are the terminal steroid hormones produced in the steroid biosynthesis pathway. In this pathway, cholesterol is transported into the mitochondria by steroidogenic acute regulatory protein (StAR), where it is metabolized into pregnenolone by cytochrome P450 side chain cleavage (Cyp11a1). The activities of both StAR and Cyp11a1 provide the necessary precursors for the synthesis of other hormones, thus they are considered the rate-limiting factors for steroidogenesis.^{33,81} Exposure to BPA (10 and 100 μ g/mL) significantly decreased expression of Cyp11a1 and StAR; as a consequence, the levels of androstenedione, testosterone, and estradiol decreased. However, these effects were completely reversed when BPA exposure was removed.⁸¹ Moreover, BPA could increase or prevent the catabolism of steroid hormones.⁸² All together, these mechanisms leading to the modification of steroid hormone balance and availability into target cells could contribute to some of the reported BPA effects.⁵⁸

Mechanisms Involving Genetic and Epigenetic Regulation

Mammalian embryos undergo extensive germ line reprogramming, which demethylates the DNA. However, some epigenetic marks can be retained across generations (transgenerational epigenetic inheritance). Although the mechanisms are still unclear, it appears that maternal or paternal phenotypes can be passed to offspring and that these stable epigenetic modifications, including DNA methylation and histone modifications, can be influenced by environmental stimuli.⁸³ Thus, the environment can trigger epigenetic changes in parents, which can then be passed on to the offspring. In addition, direct epigenetic deregulation has been recently involved in the mechanisms of endocrine disruptors such as BPA.^{84,85} Intriguingly, early life exposure to BPA increased susceptibility to prostate carcinogenesis through changes in DNA methylation⁸⁶ and may alter gene expression in hypothalamic nuclei via DNA methylation and histone acetylation.⁸⁷ Mouse offspring perinatally exposed to low doses of BPA (ie, 5 ng/kg) showed hypermethylation in tail tissue.⁸⁸ Thus, the epigenetic deregulation of specific genes (eg, hormone receptors) during development may alter their expression or subsequent activity later in life, predisposing the organism to disease in adulthood.⁸⁹

Conclusion

There is substantial evidence indicating that BPA contributes to the risk of cancer, developmental problems, diabetes, obesity, metabolic syndrome, and possibly also contributes to infertility and subfertility.^{20,21} The mechanisms at the root of these multiple effects are numerous and involve BPA binding to membrane and nuclear estrogen receptors, interference with other nuclear and nonnuclear receptors, alterations in the synthesis or in the metabolism of hormone, and epigenetic deregulation. These mechanisms are activated at BPA concentrations below the concentration range in which “pharmaceutical” effects are detected by classical toxicology and generate nonmonotonic dose–response curves such as inverted U- or U-shaped curves.⁹⁰ These nonlinear dose–response curves complicate the effects obtained for low- and high-dose BPA administration. For instance, BPA shows a greater effect on prostate tumor cell proliferation at concentration of 1 nmol/L than it does at 100 nmol/L.⁹¹ Low-dose BPA (1 μ g/mL in drinking water, corresponding to 150 ng BPA/g body weight) increases weight of adipose tissue, while high-dose BPA (10 μ g/mL in drinking water, corresponding to 1.5 μ g BPA/g body weight) has no effect in female mice, and the opposite is true in male mice.⁹² In cerebellar neurons, BPA increased the phosphorylation of ERK (via ER-dependent extranuclear mechanisms) at low (10^{-10} – 10^{-12} mol/L) and high (10^{-7} – 10^{-6} mol/L) concentrations, but BPA did not affect ERK signaling at intermediate concentrations. Notably, the coadministration of E2 (10^{-10} mol/L) and BPA (10^{-12} – 10^{-10} mol/L) inhibited ERK activation.⁹³ These results highlight that BPA effects could be very different between genders and sustain that low- and high-dose effects are not constant between physiological outcomes.

Thus, even if initially considered to be a “weak” estrogen or androgen based on a low affinity for binding to ER α or AR or to activate their transcriptional activity, BPA stimulates extranuclear physiological responses and modulates the epigenetic regulation at low concentrations being equipotent with E2. These effects at low concentrations of BPA have been explained by the existence of an additional high-affinity BPA binding site for nuclear receptors with inhibitory activity.⁹³ However, as far as we know, no evidence for another BPA binding site has been reported, at least for ERs.³⁶ On the other hand, the reported changes in the ER conformation as a function of the ligand (ie, BPA vs E2) described earlier (see Estrogen Receptors section) could also be active in dependence on ligand concentrations. It is possible that at low concentrations of BPA induce specific receptor conformations that allow recruitment of just some, specific proteins but not others. Further experiments with BPA and E2 are required to demonstrate this hypothesis.

The data reported in this article strongly indicate that the safe level determined for BPA by classical toxicological studies does not protect against low-dose effects.⁹⁰ In addition, the large number of endocrine-disrupting compounds present in the environment raises questions about the possible additive, synergic effects of simultaneous exposure to multiple compounds.^{94,95} Finally, it is important to remember that the

coexistence of nuclear receptors with multiple splicing forms could influence the formation of homo- and heterodimers, receptor posttranslational modifications, and localization at the membrane, which are all parameters that can likely contribute to the complex properties of BPA.

As a whole, it is very difficult to determine at which point in time and at which concentrations BPA increases the risk of pathologies. As correctly stated by Watson et al⁹⁶ “extrapolation of results from very high doses to predict lowest effective doses is no longer acceptable”. Thus, an increase in information about responses and action mechanisms of low concentration BPA is expected in the near future. Until that time, the application of the precaution principle in use of BPA is, in this context, pivotal.

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