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Review

# Extranuclear-initiated estrogenic actions of endocrine disrupting chemicals: Is there toxicology beyond paracelsus?

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ABSTRACT

Endocrine Disrupting Chemicals (EDCs), including bisphenol-A (BPA) do not act as traditional toxic chemicals inducing massive cell damage or death in an unspecific manner. EDCs can work upon binding to hormone receptors, acting as agonists, antagonists or modulators. Bisphenol-A displays estrogenic activity and, for many years it has been classified as a weak estrogen, based on the classic transcriptional action of estrogen receptors serving as transcription factors. However, during the last two decades our knowledge about estrogen signaling has advanced considerably. It is now accepted that estrogen receptors ERα and ERβ activate signaling pathways outside the nucleus which may or may not involve transcription. In addition, a new membrane estrogen receptor, GPER, has been proposed. Pharmacological and molecular evidence, along with results obtained in genetically modified mice, demonstrated that BPA, and its substitute BPS, are potent estrogens acting at nanomolar concentrations via extranuclear ERα, ERβ, and GPER. The different signaling pathways activated by BPA and BPS explain the well-known estrogenic effects of low doses of EDCs as well as non-monotonic dose-response relationships. These signaling pathways may help to explain the actions of EDCs with estrogenic activity in the etiology of different pathologies, including type-2 diabetes and obesity.



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The world produced 311 million tons of plastic in 2014 [1]. From an environmental perspective, the accidental release and the indiscriminate discard of this long-lasting material represents a global problem. Plastics are now common litter in waters and land [2,3]. Accumulation is not the only trouble raised by plastics: migration and contamination of the environment may affect

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human's health [4,5]. Relatively small plastic items such as baby bottles are composed of more than 100 chemicals of unknown identity. Worryingly, it has been verified, that most chemical components of plastic parts have estrogenic activity [6].

Polycarbonate plastic is a polymer built by ester bonds between bisphenol-A (BPA) molecules. It is present in many daily products, including, CDs, DVDs, automobile equipment, construction glazing, sports safety equipment and medical devices. Moreover, polycarbonate is commonly used in tableware, reusable bottles and food storage containers. These items are in contact with food and drinks and they are repeatedly heated and reused.

The polymer forming polycarbonate plastic is biologically inactive, yet the monomer is not. The plastic industry claims that polycarbonate plastic is durable, shatter-resistant and heat-resistant [7]. However, there is data demonstrating that ester bonds between BPA molecules are hydrolyzed by high temperature, acidic or basic media, leading to BPA release to the environment [8–11]. Furthermore, BPA is not only used in polycarbonate plastic but also as an additive in polyvinyl chloride (PVC), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyethylene terephthalate (PET) and polystyrene (PS). These plastics are also used in household appliances like water supply, water bottles, stretch film used for food packaging and other common items. Migration from these appliances has been measured to water and food [12,13].

Although the main source of exposure to BPA is assumed to be via oral ingestion [14], new significant sources are now being considered [15]. Transdermal route may be particularly important in the case of BPA, commonly used in thermal paper for cash receipts [16,17]. This multitude sources of exposure likely contributed to observe detectable levels of BPA in the urine of a 93% of US citizens, demonstrating its widespread incidence [18]. Its concentration in human serum is in the low picogram to low nanogram per milliliter range [14].

Exposure to a variety of plastic pollutants may contribute to the etiology of different diseases [19,20]. These include type-2 diabetes, obesity, male and female reproduction, alterations in brain development and cancer among others [14,21–24]. In this short review, we will focus in the molecular mechanisms that EDCs with estrogenic activity, particularly BPA and its alternative BPS, may use to initiate its cellular actions. We will omit the important role that gender differences, aging, timing and duration of exposure have in EDCs action as well as their possible role as metabolic disruptors. For interested readers, this information has recently been compiled and discussed in an exhaustive manner in the second scientific statement on EDCs of The Endocrine Society [20].

## 1. The estrogenic action of BPA

BPA can act on cells after binding to many different targets, depending on its concentration [25]. However, traditionally, it has been classified as a weak estrogen. This is because the classical definition of estrogenicity of an exogenous chemical is based on the property of these compounds to bind ERα and ERβ, and to act subsequently as transcription factors when binding to the estrogen response elements (EREs) in the DNA [26]. Estrogen receptors ERα and ERβ are located in the cytosol or in the cell nucleus. When ligands bind to them, they dimerize and bind to the EREs in the DNA and incorporate co-regulators to control transcriptional activity. The selectivity of a particular steroid hormone depends on the ligand, the receptor and the effector site [27,28]. Therefore, the same ligand binding to the same receptor in different target tissues or cells can produce different responses. This will depend on how that particular cell metabolize the ligand and the different isoforms, amount, variants and phosphorylation state of the receptors expressed. In addition, the metabolic state of the cell also affects the expression of co-regulators, which will alter the final transcriptional activity [29,30]. In summary, the final transcriptional activity depends on many different factors that vary depending on the specific combinations of ligands, tissues and cell types. Assays based on measuring gene transcription by luciferase activity in transfected cells or yeast are of great utility to identify EDCs with estrogenic transcriptional activity [31]. However, for the reasons exposed above, we must be cautious when assuming that a xenoestrogen has a weak or strong transcriptional activity based only on these measurements. In any case, it was demonstrated that when transfecting cell lines with ERα and ERβ, BPA behaved as a weak estrogen acting at the transcriptional level within the micromolar range [32]. The binding of BPA to ERα and ERβ was low, with an affinity 10,000-fold lower than 17β-estradiol (E2) to both ERs subtypes. The estrogenic potency of BPA *in vitro* was between 1000 and 5000 fold lower than that of E2 [32]. These results indicated that BPA acted as a weak estrogen and implied that doses within the micromolar range were necessary to produce an estrogenic effect. However in the last years increasing number of studies has demonstrated that BPA can elicit estrogen-like effects with the same potency as E2, challenging the concept of BPA as a weak estrogen with no effects at low doses. Of note, hundreds of published studies describe BPA effects within the nanomolar range to which humans are exposed [33,34].

## 2. A new definition for estrogenicity

To understand how BPA can exert effects at low doses we should note that the classic definition of estrogenicity cited in the above paragraph is not wrong, but is somehow incomplete. Estrogens not only act by binding to ERα and ERβ, moving to the nucleus and acting as transcription factors binding to EREs. The scenario is much more complex. Classical ERs can also interact with other transcription factors such as Sp1, Ap1 or NFkB and indirectly regulate transcription [35]. Additionally, it is known since the 1970's that estradiol produces rapid effects that cannot be explained by the classic mechanism of ERs acting as transcription factors [36–38]. In 1995, Pappas et al., [39] revealed that ERα was located out of the nucleus, associated with the plasma membrane. In 1999, Levin's laboratory [40] demonstrated that both ERα and ERβ initiated responses outside the nucleus, a concept widely accepted nowadays. The molecular mechanism of ERs acting extranuclearly is now better understood [41–43]. Experiments performed during the 1980′s and 1990′s indicated that estrogen receptors other than ERα and ERβ may exist [38,44–47]. In 2005, the orphan receptor GPR30 was characterized as a G-protein-coupled estrogen receptor that signaled from either, the endoplasmic reticulum or the plasma membrane [48,49]. GPR30 is currently known as G-protein-coupled estrogen receptor (GPER). Whether E2 is the endogenous ligand for GPER is still controversial, but it seems undeniable that this receptor binds E2 and initiates signaling cascades [50–52]. Recently, it was proposed that, in addition to estrogen receptors, many active binding sites at the membrane may exist that may explain the plethora of different extranuclear-initiated estrogenic responses [53].

Extranuclear-initiated signaling regulates many different cellular pathways, including, kinase, and ion channel activity [54,55] and gene transcription [56,57]. Therefore, we should define the estrogenicity of exogenous chemicals based on the ability of these compounds to bind to any estrogen receptors, ERα, ERβ or GPER, and act subsequently inside or outside the nucleus as signaling molecules, independently of whether the final cellular response is the regulation of transcriptional activity or any other cellular event.