REVIEW

Endocrine Disruptors and Breast Cancer Risk – Time to Consider the Environment

Wael M Abdel-Rahman[1*&], Yasser M Moustafa[2], Bassamat O Ahmed[3], Randa M Mostafa[4&]

Abstract

The term endocrine disruptors is used to describe a variety of natural and manmade substances that have the capacity to potentially interfere with and modify the normal physiology of endocrine system either by mimicking, blocking or modulating the actions of natural endogenous hormones. The rising incidence of breast cancer over the last 50 years and the documented higher incidence in urban as compared to rural areas suggest a relationship to the introduction and increased use of xenoestrogens in our environment. The literature has developed over the last decades where initial experiments on endocrine disruptors did not support an involvement in breast cancer, and then evidence mounted implicating various environmental factors including hormones, endocrine disrupting chemicals and non-endocrine disrupting environmental carcinogens in the pathogenesis of breast cancer. Available data support the hypothesis that exposure to endocrine disruptors in utero leaves a signature on mammary gland morphogenesis so that the resulting dysgenic gland becomes more predisposed to develop tumors upon exposures to additional insults later on during life. Exceptionally, exposure to phytoestrogens could be beneficial to human health. Most of the available data are from well developed countries while the developing countries are still understudied regarding these issues. Here, we raise a note of caution about potential role of environmental toxins including endocrine disruptors in breast cancer development and call for serious measures to be taken by all involved parties in the developing world.

Keywords: Bisphenol A - breast cancer - endocrine disruptors - estrogen - phytoestrogens - soy

Introduction

In Europe, endocrine disruptors or endocrine disrupting chemicals (EDCs) have been defined as "substances foreign to the body that have deleterious effects on the individuals or their descendants, due to changes in endocrine function". In the United States, EDCs have been described as "exogenous agents that interfere with the production, release, transport, metabolism, binding, action or elimination of the natural ligands responsible for maintaining homeostasis, reproduction and/or regulating body development". Taken together, these definitions indicate that the effects induced by EDCs mostly involve disturbances, in some way, to hormonal homeostasis and action. Thus, endocrine disrupting effects of environmental contaminants and commercial products have the potential to cause adverse effects on sexual differentiation, growth, and development and may affect the fertility of males and females or increase the risk of hormone-dependent cancers, (Cravedi et al., 2007;

Sanderson, 2011). Many EDCs accumulate in the various compartments of the environment including air, water and soil and may be present in foods of plant and animal origin. Examples of common exposures include, but not limited to, synthetic hormones in pharmaceuticals, cosmetics and meat; pesticides in food; solvents in cleaning products; Bisphenol A (BPA) in food containers; and various chemicals in combustion products, plant health treatments, detergents and the chemical industry in general (Mostafa et al., 2007). The United States Environmental Protection Agency (EPA) has identified hundreds of compounds that are typical EDCs and thousands of others which are suspected of having similar properties (Toppari et al., 1996; Crisp et al., 1998). Interestingly, the majority of the typical ones were not designed to affect or modulate the endocrine system in the target species: some, such as dichlorodiphenyltrichloroethane (DDT) or the pyrethrins were used to kill mosquitoes and or other pests that could spread fatal diseases such as malaria. Many others were designed for industrial purposes as flame retardants or

*[1]Department of Medical Laboratory Sciences, [3]Department of Nursing, College of Health Sciences, [4]Department of Basic Medical Sciences, College of Medicine, University of Sharjah, P.O.Box 27272, Sharjah, United Arab Emirates, [2]Department of Pharmacology, College of Pharmacy, Suez Canal University, Egypt &Equal contributors *For correspondence: whassan@sharjah.ac.ae*

to improve the characteristics of plastics (Bisphenol-A) (Patisaul and Adewale, 2009). Surprisingly, some natural compounds secreted by plants (Phytoestrogens) or fungi have an endocrine disrupting effects in some animal species possibly as a natural defence to introduce male infertility to the herbivore animals (Yurino et al., 2004; Patisaul and Adewale, 2009). This review will focus on the most common compounds in our environment which have endocrine disrupting potential and the relationship of these to the increased risk of breast cancer.

EDCs and Breast Cancer

Breast cancer affects more than a million new cases every year and is the most common cause of cancer-related death in females worldwide (Parkin and Fernandez, 2006). Breast cancer incidence has been generally rising over the last 50 years (Parkin and Fernandez, 2006) with rapid increases observed particularly in developing countries (Bray et al., 2004), including those in the Middle-East (El Saghir et al., 2007). Interestingly, developing countries show a higher incidence of breast cancer in urban compared to rural areas (Dey et al., 2010). Furthermore, studies showed that women who migrate from low incidence to high incidence environment tend to develop breast cancer at higher rate than before supporting the hypothesis that breast cancer risk is strongly associated with environmental factors (Ziegler et al., 1993).

Epidemiological and animal studies suggested that the major risk factor for breast cancer is overexposure to ovarian hormones supported by the increased risk associated with early age of menarche, nulli parity, delayed pregnancy and/or late age of menopause (Pike et al., 1993). Dizygotic twins exposed to high estrogen levels are at higher risks while females born from pre-eclampsia associated pregnancy, a hypoestrogenic condition, are at lower risk (Ekbom et al., 1992). Synthetic estrogens could also have the same impact on breast cancer risk such as the case with diethylstilbestrol (DES; see later). Many studies have demonstrated that various EDCs that could act as xenoestrogens are more abundant in urban areas (Harner et al., 2004; Gray et al., 2009). In particular, exposures to plastics containing BPA and phthalates are major concern in urban areas (Ndahi, 2000). These compounds are being detected in the urine of people in developed countries (Wittassek et al., 2007; Calafat et al., 2008; Becker et al., 2009) universally across the population most probably as a result of increased plastic usage worldwide. Short-acting xenoestrogens are also seen in other categories of products, such as food preservatives, cosmetics, and detergents (Maizlish and Moses, 1990; Byford et al., 2002; Darbre et al., 2002). Even exposures to some heavy metals such as cadmium (Kortenkamp, 2011) or compounds that are not structurally similar to estrogen, such as dioxins (Viel et al., 2008; Taylor et al., 2009), were reported to increase breast cancer risk in humans and to produce some estrogenic effects in animal models. Therefore, it is not surprising that some authors hypothesized that the current increase of breast cancer incidence particularly in the Western world may be due to environmental exposure to a variety of hormonally active, estrogenic, chemicals

(Davis et al., 1993).

Characteristics of EDCs carcinogenesis

Breast cancer development may be related to overexposure to estrogen as discussed above. The mammary stroma appears to play an important role in response to hormonal exposure. The primary role of mammary stroma in modulating the proliferative effects of estrogen is widely recognized to the extent that some experts hypothesized that cancer, in general, is a fault in tissue organization (Soto et al., 2008). Additionally, the carcinogenic mechanisms of EDCs appear to deviate from those of classical carcinogens in many ways (Diamanti-Kandarakis et al., 2009). Exposure of a developing foetus or infant to an EDC may cause more serious, long term or delayed effects in contrast to exposure of an adult to the same chemical. Researchers in the field hypothesized that the environment of a developing organism interacts with the individual's genes to determine the propensity of that individual to develop a disease or dysfunction later in life and introduced the term "the fetal basis of adult disease" to describe this phenomenon (Barker et al., 2002; Diamanti-Kandarakis et al., 2009). This, obviously, implies that there is a lag between the time of exposure and the manifestation of a disorder.

An additional important feature of carcinogenesis related to EDC is that individuals and populations are usually exposed to a mixture of EDCs, which might have additive or synergistic effects (Crews et al., 2000). Studies which addressed this issue through measuring 16 organochlorine pesticides as well as the total effective xenoestrogen burden (TEXB-alpha) in adipose tissue of 198 women at the time of breast cancer diagnosis found positive correlation of some of these measures with increased breast cancer risk (Ibarluzea et al., 2004). Later on, it became clear that the concentration of any single EDC does not need to be high to exert its effect. Low doses may even exert more potent effects than higher doses and EDCs may exert non-traditional dose-response curves, such as inverted-U or U-shaped curves (Vom et al., 2007).

Finally, some EDCs may not change the DNA nucleotide sequence, but can alter the epigenome, thus controlling many genes through epigenetic mechanism. Epigenetic changes can have wide range of effects on various processes including growth and development as well as carcinogenesis similar to other established forms of the genetic instability (Abdel-Rahman, 2008). Moreover, some EDCs have the ability to modify the epigenome of the germ line permanently so that the resulting disease can become transgenerationaly transmitted to subsequent progeny. The acquisition of transgenerational phenotypes by EDCs is discussed in more detail in specialized reviews (Skinner et al., 2010; 2011).

Critical periods of exposures to EDCs

Epidemiological and experimental data indicates that excessive estrogen exposure, be it natural, synthetic or EDC with estrogenic effect, during development may increase the risk of developing breast cancer later on during life. This theory is best supported by the observed increase in the incidence of breast cancer in females

exposed to DES in utero after they reached the age of 40 years and that the risk increased in a dose-dependent manner (see later discussion) (Palmer et al., 2002; 2006; Hoover, 2011). Similar pattern was reproduced in the rats exposed prenatally to DES and then challenged with the chemical carcinogen dimethylbenzanthracene (DMBA) at puberty. These rats had a significantly greater incidence of mammary tumors at 10 months of age than animals exposed prenatally to vehicle (Boylan and Calhoon, 1979).

Experiments based on exposing animals to the BPA during gestation or early postnatal period showed that histopathological changes are induced in target organs that persist lifelong and dictates additional changes upon further exposures to toxic chemicals later on during the animal's life (Richter et al., 2007). BPA acts via ERα and ERβ or the membrane bound forms of ERα present in estrogen-target organs and may induce complex and varied phenotypes depending on which receptor is responding. In developing rat, BPA likely binds to ERα and ERβ of the stromal cells from embryonic day 12.5 through 18 and disturb the normal stromal-epithelial interactions in the mammary gland which are essential for the proper formation, growth, and hormone responsiveness of the fetal mammary gland. BPA induces enlarged fat pad and accelerated maturation of fat cells, which alter development of the epithelial ductal tree, cell shape, size, and organization (Vandenberg et al., 2007). These changes could explain the predisposition of these animals to develop tumors later on.

Exposure to some EDCs during early adulthood was also associated with increased breast cancer risk after long periods. A prospective study that measured exposure to DDT several years before cancer diagnosis (median time to diagnosis was 17 years) showed that high levels of serum DDT was associated with 5-fold increased risk of breast cancer among women who were under 20 years when DDT use reached its peaked. Women who were not exposed to DDT before 14 years of age did not show any positive association (Cohn et al., 2007).

Common EDCs

Synthetic estrogens: the tragedy of diethylstilbestrol (DES)

DES is a synthetic nonsteroidal estrogen that was first synthesized in 1938 at the University of Oxford through research funded by the Medical Research Council (MRC) public funds and, according to the MRC policy, it was not patented. DES was approved by the United States Food and Drug Administration (FDA) as estrogen-replacement therapy (from the 1940s until the late 1980s) and for treatment of other conditions such as prostate cancer in men. It was used extensively, even over-the-counter, to prevent spontaneous abortion and other pregnancy complications in women with a history of miscarriage. The DES dogma spread rapidly so that it was given to pregnant women in general to produce "stronger babies" and even administered to newborns to enhance weight gain (Yoonessi et al., 1981). More scientific evaluation of the drug in 1950s showed that it lacks efficacy altogether. Finally, it was taken off the market in 1971 subsequent to the elegant report by Herbset and coworkers who observed

that in utero DES exposure has predisposed these girls "known as DES daughters" to develop an extremely rare type of cervicovaginal clear-cell adenocarcinoma (CCAC) at a young age (Herbst et al., 1971). In addition to a more than 100-fold increased risk of CCAC, DES daughters also suffered from increased incidences of vaginal dysplasia, vaginal and cervical adenosis, malformations of the cervix, vagina, and uterus, infertility and pregnancy complications (Rubin, 2007).

Unfortunately, DES was used extensively for other, non-medical, purposes including use as growth promoter in chicken and cattle as well as in commercial products such in cosmetics and skin care products. These sources could have contributed to a low-dose human exposure. DES implants in poultry were outlawed in 1959 but it continued in cattle until it was eventually banned in 1979 (Metzler, 1981).

DES and breast cancer risk: Several million pregnant women and their offspring were prescribed DES between the early 1940s and the 1970s. Of these, around three millions were from the USA while others were in Canada, the UK, Europe, Australia, and New Zealand. The exposed mothers (first generation) showed a modest increased risk of breast cancer (Greenberg et al., 1984).

The USA National Cancer Institute (NCI) coordinated large prospective studies to analyze the effects of perinatal DES exposure; the results gradually implicated DES in breast cancer development in DES-daughters (second generation). In 2002 Palmer et al showed notorious evidence of a 2.5-fold increased risk of breast cancer among women 40 years or older (Palmer et al., 2002). As the second generation grew older, and more cases became available for the NCI project, the study included over 4,000 women with confirmed DES exposure. The 2006 report confirmed that the increased risk of breast cancer among the second generation was discernible after the age of 40 and found out that the risk was then increased with age. Furthermore, a significant dose–response was also deduced (Palmer et al., 2006). The 2011 follow up report on the same NCI cohort supported these findings especially the dose-response relationship which was established through its surrogate marker ,vaginal epithelial changes, in the 2011 report as compared to the less robust calculations of the 2006 report (Hoover et al., 2011). Collectively, the available data support a causal relationship between DES exposure during pregnancy and breast cancer risk among daughters and should have clinical implications for these women as the authors suggested (Palmer et al., 2006; Hoover et al., 2011). Moreover, studies of the third generation might be justified to find out whether or not DES exposure could exert transgenerational epigenetic effect (Skinner et al., 2011).

Phytoestrogens

Phytoestrogens, also called 'dietary estrogens', are a diverse group of plant-derived nonsteroidal compounds that have the ability to cause estrogenic effects because of their ability to bind to ERs; and some of them show structural similarity with estradiol. Chemically, they include a large group of substituted natural phenolic compounds that could be broadly divided into flavonoids

and non-flavonoids. The isoflavonoids are flavonoid phytoestrogens that could exert a potent estrogenic activity. These are most prevalent in legumes, especially soybeans, they are present in many types of fruits and vegetables and they are also a common dietary supplement in the Western diet (Xiao, 2008). Genistein is naturally occurring organic compound found in a number of plants including soybeans. It belongs to one of the most active phytoestrogen groups known as isoflavones which is chemically related to the isoflavonoids. The major component of non-flavonoids phytoestrogens are the lignans which could be found in flaxseed (linseed) products, pumpkin seeds, green tea, coffee, and other fiber-rich foods. Fewer research data are available on the lignans compared to the isoflavonoids.

The endocrine disrupting potential of phytoestrogens was first reported in 1940s when it was noticed that red clover pastures had caused remarkable decline in fertility of grazing sheep. This effect was attributed to the high concentration of a phytoestrogens in red clover (Adams, 1995). Later on, another report was published on similar effect caused by a soy-based diet in captive cheetahs (Setchell et al., 1987). These incidents have raised concerns over the potential risk of flavonoid phytoestrogens to human health. To the contrary, the utilization of soy in the western diet and the public acceptance of the ideas that soy rich foods have a protective affects against cardiovascular disease and cancers has initiated scientific debate on the validity of these ideas.

Estrogenic activity of isoflavones: Genistein can activate both of the ERα and ERβ at physiological doses with more preferential binding to ERβ (Paech et al., 1997; Chang et al., 2008). Important observations relevant to genistein action are that ERβ is assumed to bind to ERα and inhibits its proliferative action and that the levels of these receptors varies during different stages of the breast development with ERβ expressed at higher levels during early development and in normal adult breast, while ERα is higher than ERβ in breast cancer cells (Speirs and Walker, 2007). Recent data showed that phytoestrogens could induce the formation of heterodimers of ERα and ERβ in cells expressing the two receptors and that these heterodimers inhibit the growth of mammary cells (Powell et al., 2012). The level of circulating estradiol was shown to dictates whether isoflavones acts as estrogen agonist or anatagonist. Hwang and coworkers found that the test isoflavonoids act like estrogen antagonists with the premenopausal dose of E2 and thus inhibit estrogenic actions by E2, whereas they exert estrogen agonist activity with the lower dose of estrogen close to the serum levels of postmenopausal women (Hwang et al., 2006).

Soy, isoflavones and breast cancer risk: Many epidemiologic studies have addressed the association between soy intake or dietary isoflavones in general, and risk of breast cancer. The results were inconsistent most likely because of the different populations studied or because of various ethnicities within the same population. Meta-analyses of these conflicting results supported a conclusion that soy isoflavones intake may be associated with a reduced risk of breast cancer incidence in Asian populations, but not in Western populations (Dong and

Qin, 2011). Moreover, soy intake during childhood and adolescence was associated with a greater risk reduction for breast cancer than adult intake (Korde et al., 2009). This finding is consistent with the significance of the critical periods of exposures to EDCs in cancer predisposition or cancer prevention.

A recent short-term intervention study in high-risk Western women showed that mixed soy isoflavones did not reduce breast epithelial proliferation, but to the contrary, exerted stimulatory effect on breast epithelial cell proliferation in premenopausal women (Khan et al., 2011). These stimulatory effects of soy isoflavones of epithelial cells proliferation, which in theory could be associated with increased risk of breast cancer, have been noted by other authors (Petrakis et al., 1996; Santell et al., 1997). Similarly, case-control studies from the U.K. reported a positive association between exposure to isoflavones, as estimated by its serum and urinary metabolites daidzein and equol, and the breast cancer risk (Grace et al., 2004; Ward et al., 2008). A major critisim to these findings was that, the median of isoflavone serum levels was very low (2 ng/ml [8 nM] for daidzein and 0.2 ng/ml [0.8 nM] for equol) (Grace et al., 2004). In contrast, a Chinese case-control study showed inverse associations between the plasma genistein (>76.95 ng/ml or 285 nM) and the risk of breast cancer or even benign conditions (Lampe et al., 2007). Similar inverse associations were obtained in a nested Japanese case-control study (Iwasaki et al., 2008) and in postmenopausal women in a multiethnic American cohort study (Goodman et al., 2009).

Since the Women's Health Initiative declared in 2002 that the risks of long-term classical hormone replacement therapy for menopausal symptoms exceed the benefits, many women shifted to alternative treatments, including botanical dietary supplements (Canderelli et al., 2007), and almost half of these women were using soy products (Mahady et al., 2003). Given that some animal and cell line studies showed stimulatory effects of isoflavones on the growth of already formed breast cancer (see below), researchers set out to evaluate the potential risk associated with using isoflavones as hormone replacement therapy in postmenopausal women. Contrary to these expectations, trends for a reduced risk of cancer recurrence were observed in the United States, with increasing quintiles of isoflavones intake (at levels comparable to those in Asian populations) among postmenopausal women and tamoxifen users (Guha et al., 2009). Similarly, a Chinese study reported an inverse association between soy food intake and total mortality or cancer occurrence among breast cancer survivors, with a daily isoflavone intake of 62.7 mg and more, regardless of ER status in the breast cancers or tamoxifen use (Shu et al., 2009). Even though these studies are somewhat reassuring, experts believe that the literature is still missing data on the use of isolated isoflavones and supplements as well as the longterm safety of isoflavone supplements and soy extracts in breast tissue, especially among breast cancer patients and survivors (Andres et al., 2011).

In vitro studies of the effect of soy isoflavones on breast cancer cell lines reported some beneficial effects such as reactivation of tumor suppressor genes BRCA1 and

BRCA2 silenced by promoter hypermethylation (Bosviel et al., 2012), inhibition of the breast cancer progenitor cell proliferation (Montales et al., 2012), induction of apoptosis and inhibiting the proliferative signals mediated through NFϰB (Seo et al., 2011). Conversely, some others have noticed opposite, stimulatory, effects on tumor cell growth (van Duursen et al., 2011). This dichotomy could be related to various factors such as the isoflavones dose used as well as the type of the estrogen receptor expressed in the cell line model. Klein and King concluded that isoflavones could exert proliferative effects at concentrations below 10 uM and antiproliferative effects at higher concentrations (Klein and King, 2007). Regarding the ER status, genistein caused growth suppression in ERβ expressing human breast cancer regardless of the dose used, while in cells expressing more ERα than ERβ, this isoflavone has exerted biphasic effects with growth stimulation at low concentrations and growth inhibition at high concentrations (Rajah et al., 2009). Finally, genistein exerted additive effect when combined with trastuzumab on HER2-overexpressing human breast cancer cells and ERα/β positive breast cancer cells (Lattrich et al., 2011).

Several ovarictomized animal models, recapitulating postmenopausal conditions, in which breast cancer cell lines were xenografted subcutaneously demonstrated that various soy products could exert stimulatory effect on the growth of these cancer cells (Allred et al., 2001a; 2001b; Ju et al., 2006). Contrasting results were obtained in non-ovarictomized severe combined immunedeficient (SCID) mice (Mai et al., 2007). Studies in laboratory animals have also reported protective effects of soy isoflavones if administered perinataly (Molzberger et al., 2012) or later on prior to carcinogen exposure (Sahin et al., 2011). Such data further highlight the significance of the critical periods of exposures to EDCs in cancer predisposition or cancer prevention. Furthermore, some *in vivo* data suggest that soy isoflavones could interfere with the therapeutic effects of tamoxifen and the aromatase inhibitors such as letrazole on breast cancer cells (Ju et al., 2002; 2008). A recent study re-warns breast cancer patients of consuming dietary genistein while on tamoxifen treatment since low dose dietary genistein was found to interfere with tamoxifen and negates its therapeutic effects in a preclinical mice model (Du et al., 2012). The metabolism of soy isoflavones is different between rodents and humans with much higher peripheral blood concentrations of biologically active genistein in mice, hence, the animal data have to be interpreted with caution (Setchell et al., 2011).

In summary, the available data from cell lines, animal models or human studies do not provide clear evidence to advice clinicians, breast cancer patients, or postmenopausal women on phytoestrogen use. These data rather raise concerns over the possibility of encountering adverse effects in some cases including postmenopausal women, breast cancer patients on tamoxifen or letrazole medication or breast cancer survivors.

Bisphenol A (BPA)

BPA is a synthetically produced chemical plasticizer that is commonly used in the production of polycarbonate plastics, to increase clarity and resilience, and in the production of epoxy resins used to line metal cans and various containers for foods, beverages, drinks, baby bottles and water tanks. It is also a component of many office, laboratory and hospital supplies and materials. Human exposure to BPA occurs mainly through consumption of canned food and drinks because BPA migrates from the containers into the contents when heated (Brede et al., 2003) or even under normal conditions of use due to degradation of the weak ester bonds that link the BPA monomers. Thus, some vulnerable groups such as babies or hospital patients could be exposed to high concentrations of BPA that could exert uncertain risk. BPA is also a ubiquitous contaminant of water supplies which could engender the wildlife and the observed effects of BPA on vertebrate wildlife species were attributed to its action as an estrogenic EDC (Crain et al., 2007).

The most likely explanation for BPA endocrine disrupting potential is its weak binding affinity for the estrogen receptors, ERα and ERβ, even though this affinity is around 2000-fold weaker than that of estradiol with some studies showing that it has grater affinity to ERβ than ERα (Gould et al., 1998; Kuiper et al., 1998; Kim et al., 2001). However, the Interaction of BPA with ERα was entirely unique and different from that of weak estrogens (estrone and estriol), partial ERα agonists (raloxifene or 4-OH-tamoxifen), or a pure ERα antagonists (Gould et al., 1998). Furthermore, BPA rapidly initiates complex set of signaling which could not be explained by its binding to estrogen receptors and it exerts many of these effects at low doses even lower than the current "safe" exposure limit for humans (Krishnan et al., 1993; Takeshita et al., 2001; Quesada et al., 2002). Current research is focused on understanding the full spectrum and the mechanisms of BPA endocrine disrupting actions (Watson et al., 2007a; 2007b).

Carcinogenic effects of BPA in vitro: Multiple actions have been attributed to BPA *in vitro* all of which could potentially lead to enhanced carcinogenicity. Some reports have indicated that BPA can induce point mutations, double stranded DNA breaks, DNA adducts, or aneuploidy as detailed below.

BPA has been shown to induce KRAS mutations in transformed human embryo fibroblast cells and mutations that lead to ouabain resistance at concentrations of 10^{-7}-10^{-5}M but the majority of studies have suggested that BPA is not directly mutagenic (Takahashi et al., 2001).

Bisphenol A has been shown to induce DNA double strand breaks that could be the precursors of various genomic alterations in MCF-7 breast cancer cells (Iso et al., 2006). DNA adduct is a piece of DNA covalently bonded to a 'cancer-causing' chemical which could be the start of a cancerous cell. Although cells carrying DNA adducts do not necessarily evolve into tumors, the formation of these molecular lesions in target mammary cells may bear relevance for the potential involvement of BPA in breast carcinogenesis. Adduct formation was also observed with aneuploidy and morphological transformation in SHE cells treated with BPA (Tsutsui et al., 1998). More recently, formation of DNA adducts in both liver (3.4-fold higher than in controls) and mammary

cells (4.7-fold higher than in controls) of female CD-1 mice receiving BPA in their drinking water (200 mg/kg body weight/day) were reported (Izzotti et al., 2009).

BPA-induced activation of Erk1/2 in human breast cancer cell lines (MCF-7, MDA-MB-231, SKBR3) through activation of the novel estrogen receptor G protein-coupled receptor 30 (GPR30) (Dong et al., 2011).

Finally, BPA can antagonize the cytotoxicity of chemotherapeutic agents doxorubicin, cisplatin, and vinblastine *in vitro* independent of classical estrogen receptors, thus it may affect the outcomes of chemotherapy (LaPensee et al., 2009; 2010).

Carcinogenicity of perinatal BPA exposure in vivo: There is a strong evidence to implicate perinatal BPA exposure and mammary cancer in rodents. Experiments on Wistar-Furth rats exposed to low concentrations of BPA from embryonic day 9 through postnatal day (PND) 1 (2.5, 25, 250, or 1,000 μg BPA/kg body weight/day), showed that rats exposed to any of the above BPA doses had a 3- to 4-fold increase in the number of hyperplastic ducts that were Ki67-positive (i.e. proliferating) and ER-positive compared to controls at PND 50 (Murray et al., 2007). Surprisingly, at PND 90, only those animals exposed to the lowest dose of BPA had a significant increase in the number of these structures compared with controls while animals exposed to the two highest doses developed severe dysplastic changes (Murray et al., 2007). Invasive cancers were observed in those animals exposed to low doses of BPA when they were further challenged by a sub-carcinogenic dose of the chemical carcinogen N-nitroso-N-methylurea at puberty (Durando et al., 2007). BPA was also reported to increase the number of terminal end buds lateral branching and epithelial density, the presence of secretory products within the alveoli and increased stromal cell nuclear density (Markey et al., 2001; 2003; Munoz-de-Toro et al., 2005; Durando et al., 2007). Furthermore, BPA has been shown to cross the placenta in rodents and increase the bioavailability of estrogens at the fetal circulation (Richter et al., 2007).

A recent study has shown that prenatal exposure of Wistar rats to 250 μg BPA/kg body weight/day has increased mammary ERα and decreased the steroid receptor co-regulator SRC-3 expression at PND 50 and PND 110. At PND 50, an increased vascular area associated with higher VEGF expression was also observed in these rats. At PND 110, the vascular area was still increased, but VEGF expression was similar to that of control rats. The altered endocrine environment of the mammary gland and the increased angiogenesis supply additional mechanism to explain the higher frequency of pre-neoplastic lesions found in these animals later in life (Durando et al., 2011). A global analysis of cellular proteins by antibody microarray showed that there are at least 13 proteins in mammary tissue dysregulated by BPA. These changes included upregulation of ERβ and proteins involved in cell proliferation and inhibition of apoptosis, tissue remodelling, inflammation, stress response, and glutathione synthesis (Izzotti et al., 2010). BPA has also been reported to induce aneuploidy in Syrian Hamster Embryo (SHE) cells that correlated with morphological cellular transformation (Tsutsui et al., 1998; 2000).

Conclusion

Well established risk factors for breast cancer such as history of excess estrogenic exposure, lifestyle factors and/or genetic predisposition do not seem to account for the increasing risk for the disease. The latest Breast Cancer Fund report entitled 'State of the Evidence: The Connection Between Breast Cancer and the Environment' released in October 2010 stressed upon the growing evidence linking breast cancer to a wide range of environmental exposures including BPA in food containers. The report proposed initiatives to develop a US national breast cancer prevention plan more focused on the most vulnerable populations including African-American women and workers in addition to pregnant women and infants.

The scale of the problem is mystified in many developing countries due to multiple factors including paucity of research data and the general trend to underestimating the magnitude of the environmental problems. Moreover, the low economic standards usually push people to use unhealthy products and ignore the safety issues. Few publications attempted to dissect the relation between EDCs or other environmental toxins and breast or other cancers in Africa, Asia and South America. However, the observed Western-Eastern as well as urban-rural differences in the patterns of breast and other cancers in these geographical areas was addressed in a few studies which suggested a major role of environmental exposures (Dey et al., 2010; Nieminen et al., 2012). These data justify further studies to tackle the problem at epidemiological, clinical and molecular aspects. Simultaneously, it is imperative to enforce regulatory and legislative changes to limit potential exposures through industry, food or water and to raise the public awareness of these issues. We propose the following measures as guidelines for interested parties:

Non-governmental organizations and advocates representing environmental health/justice should play an active role to: A) Improve environmental and health regulations and help set priorities for legislative and regulatory action to protect public health. B) Convince health authorities to initiate 'Endocrine Disruptor Screening Program' especially for young adults residing in potentially hazards areas. C) Support legislation that ban the manufacture, distribution and sale of consumer products containing toxic levels of EDCs and other products with long-term health effect (e.g., BPA and Phthalates) especially in children toys and child care articles or, at least require labeling adequate to allow consumer to make informed and safe purchases. D) Support legislation that ban the use of hormones in meat and milk or require labeling of these additives so consumers can make informed decisions. E) Advice people to avoid canned food and plastic containing BPA altogether, if possible, or to avoid leaching of these chemicals through microwaving food in plastic container, putting plastic in the dish-washer, or using hard detergents on plastic.,

Introducing legislation to require manufactures to: A) Provide health and safety information to government

agencies prior to releasing any chemical into market. B) Enforce premarket health safety testing of all cosmetics and personal care products. C) Recall of products containing ingredients that have not been proven safe through scientific testing and/or do not bear appropriate labels warning consumers that the products ingredients have not been tested for safety. D) Analyzing the resulting adverse health outcomes among high risk groups such as workers being exposed to certain chemicals. E) Find safe alternatives to toxic chemicals through green chemistry research on bio-based plastics that can be composted after use. F) Establish and/or expand national and regional bio-monitoring laboratories.

Academic institutions, and fund raising agents should support research to: A) Develop methods for identifying sources and routes of exposure for chemicals in the local environment. B) Help in screening chemicals for hormonal activity, analyzing the resulting adverse health outcomes among high risk groups, and finding safe alternatives to toxic chemicals.

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