

Endocrine disruptors and reproductive health: The case of bisphenol-A

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Abstract

Epidemiological studies have reported that during the last 60 years the quantity and quality of human sperm has decreased and the incidence of male genital tract defects, testicular, prostate and breast cancer has increased. During the same time period, developmental, reproductive and endocrine effects have also been documented in wildlife species. The last six decades have witnessed a massive introduction of hormonally active synthetic chemicals into the environment leading some to postulate that the diverse outcomes documented in human and wildlife populations might be the result of extemporaneous exposure to xenoestrogens during development.

The estrogen-mimic bisphenol-A (BPA) is used as a model agent for endocrine disruption. BPA is used in the manufacture of polycarbonate plastics and epoxy resins from which food and beverage containers and dental materials are made. Perinatal exposure to environmentally relevant BPA doses results in morphological and functional alterations of the male and female genital tract and mammary glands that may predispose the tissue to earlier onset of disease, reduced fertility and mammary and prostate cancer.

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In recent months, the issue of endocrine disruptors has attracted considerable public attention in the US. Large readership newspapers like *USA Today*, *The Wall Street Journal*, and *The New York Times* among others, published some of the latest results found in experimental animals and, more importantly, in humans regarding the deleterious effects of fetal exposure to endocrine disruptors. Although the weight of experimental data obtained from wildlife populations and from controlled studies in animal models has grown extensively during the last decade, the issue of its relevance to human health has continued to trigger controversy. For some, it is difficult to acknowledge that humans may be as vulnerable to endocrine disruption as alligators, bald eagles or mice. Understandably, because many of the chemicals found to be endocrine disruptors are important in the manufacturing industry, there has been controversy about whether low, environmentally relevant doses produce deleterious effects in humans.

1. The controversy about low dose effects

At the request of the US Environmental Protection Agency, the National Toxicology Program (NTP) convened a meeting in

October 2000 on the low-dose issue. The final *NTP Endocrine Disruptors Low-Dose Peer Review* was published in 2001 (NTP, 2001). The report stated that there was “credible evidence for low-dose effects” and that “discrepancies in experimental outcome among studies showing positive and negative effects of BPA may have been due to different diets with differing background levels of phytoestrogens, differences in strains of animals that were used, differences in dosing regimen, and differences in housing of animals (singly versus group). Although some studies attempted to replicate previous findings, body weights and prostate weights of controls differed between these studies.”

Although the low dose issue is applicable to all endocrine disruptors, the industry’s reaction to this report focused on only one chemical, the ubiquitous BPA. A report funded by the American Plastics Council, written by a panel convened by the Harvard Center for Risk Analysis (HCRA), reviewed only a small number of studies, yet concluding that “the weight of the evidence for low-dose effects is very weak” (Gray et al., 2004). It is noteworthy that one of the panel members, Claude Hughes, in partnership with Fred vom Saal, published a separate, detailed and comprehensive analysis of all publications on BPA to date (vom Saal and Hughes, 2005). Readers are encouraged to consult this exhaustive article. Interestingly, Hughes and vom Saal found two predictors of negative results: animal strain and source of funding. First, Sprague–Dawley rats from Charles River are extremely insensitive to BPA. This strain was used in several

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studies that found no effect of BPA (Kato et al., 2005; Masutomi et al., 2004; Tyl et al., 2002). Second, 90% of the government-funded publications reported significant effects of low dose BPA while none of the industry-funded studies reported significant effects at similar doses. As succinctly put by vom Saal and Hughes: “It is thus reasonable to pose two questions: (a) Are government-funded scientists under real or perceived pressure to find or publish only data suggesting adverse outcomes? (b) Are industry-funded scientists under real or perceived pressure to find or publish only data suggesting negative outcomes?”

2. The human connection

An increase in the incidence of several cancers and reproductive system dysfunctions has been observed in the US and several European countries. During the period spanning 1973–1999, the US National Cancer Institute reported a cumulative increase in the incidence of breast cancer in all age and ethnic groups (34.8% among African–American and 27.9% among Caucasian women); an even higher increase was observed in prostate cancer incidence (82.5% among African–American and 74.4% among Caucasian men). Testicular cancer incidence has also been rising (49.4% among African–American and 51.8% among Caucasian men), and brain tumor incidence increased by 50.2% in children under the age of 14 years (Ries et al., 2002). A decline in the quantity and quality of sperm and an increase in the incidence of hypospadias and cryptorchidism has also been reported (Carlsen et al., 1992; Giwercman et al., 1993).

More recently, studies have shown the extent of human exposure to endocrine disruptors. For instance BPA, one of the most ubiquitous endocrine disruptors, has been measured in the serum of adult men and women with mean concentration of 1.49 ± 0.11 and 0.64 ± 0.10 ng/ml, respectively (Takeuchi and Tsutsumi, 2002). Its presence was also reported in maternal and fetal plasma and in placental tissue in humans (Ikezuki et al., 2002; Schonfelder et al., 2002) and in the milk of nursing mothers (Sun et al., 2004). Remarkably, the concentration of BPA in amniotic fluid was approximately five-fold higher than levels measured in maternal plasma. The most recently published study, the first involving a reference human population (394 samples analyzed), reported that BPA was found in 95% of urine samples (Calafat et al., 2005). In a smaller study, Arakawa et al. (2004) reported a median daily urinary excretion of BPA of 1.2 μ g/day and a maximum daily intake of BPA per body weight to be 0.23 μ g/kg/day.

3. The importance of time of exposure

Several studies have reported the consequences of accidental exposures to endocrine disruptors. Men exposed to polychlorinated biphenyls (PCBs) under the age of 20 years were found to have a lower chance of fathering a male offspring compared to non-exposed age-matched men (del Rio Gomez et al., 2002). Few studies have examined the fate of the offspring of women who were pregnant at the time of exposure. One such study reported that the sperm of Taiwanese men born to those women exposed to polychlorinated biphenyls (PCBs) and their combustion products, the polychlorinated dibenzofurans, had abnormal

morphology, reduced motility and strength (del Rio Gomez et al., 2002). Another study reported that breast-fed girls exposed *in utero* to high levels of polybrominated biphenyls (PBBs) due to an accidental contamination of the food chain in Michigan, experienced menarche at an earlier age compared to control population (Blank et al., 2000).

The true impact of endocrine disruptors on human health is difficult to assess because specific end points may be differentially affected at different ages (have different windows of vulnerability). For example, some studies found a direct correlation between the plasma levels of DDT metabolites and breast cancer risk, while other studies did not. Most of these studies were case–control studies; exposure was measured once the disease was diagnosed. Others were cohort studies that measured exposure during adult life, sometimes several years before diagnosis. Most of these studies measured dichlorodiphenyldichloroethene (DDE), a metabolite of DDT, as a surrogate, in specimens obtained long after DDT was banned.

A study by Cohn et al. used a different strategy. DDT was measured in samples taken before DDT was banned, and this study tested the hypothesis that DDT is a stronger breast cancer risk factor for women who were exposed during childhood and adolescence, when the mammary gland is undergoing prepubertal and/or pubertal development. They found that breast cancer risk increased with increasing concentrations of serum DDT for women exposed in childhood or adolescence. The association between high plasma levels of DDT and breast cancer was significantly stronger for women exposed to DDT before age 15 years than for women exposed after age 15 years (Cohn et al., 2002). This finding correlates with the observations that the mammary gland is most sensitive to developing cancer when irradiated at this age (Land et al., 2003). It may be concluded that there is no strong correlation between breast cancer and DDT exposure when DDT or DDE measurements are restricted to the time of onset or just before the onset of breast cancer. In contrast, breast cancer levels appear highly correlated with exposure to DDT prior to age 15 years.

Recently, Swan et al. (2005) showed for the first time a statistically significant link between prenatal exposure to phthalates and anogenital distance in infant boys. Anogenital distance is considered a sensitive marker of antiandrogen action during development in toxicologic studies in rodents. Anogenital distance is sexually dimorphic and androgen dependent, and in males is typically twice that of females (this holds true for humans and rodents). These investigators also observed that boys born to mothers with phthalate metabolites present in their urine are at higher risk of impaired testicular descent. A similar effect has been observed in experimental animals, and a “phthalates syndrome”, which also includes testicular, epididymal and gubernacular cord agenesis, has been described by Gray and Foster (2003).

Another limitation inherent in epidemiological studies is that humans are not exposed exclusively to the chemical being investigated, but instead to a mixture of chemicals, some of them acting through common pathways. In addition, no single compound can act as a surrogate or marker for the others because the contaminant profile varies among individuals. Moreover, different

chemicals have different toxicokinetics. Taking these considerations into account, we developed the concept of a marker of *total xenoestrogen exposure* (Sonnenschein et al., 1995; Soto et al., 1997). Using this marker, Ibarluzea et al. (2004) have reported a direct and significant correlation between total xenoestrogen levels in fat and breast cancer.

Epidemiological studies have thus far provided evidence supporting the suspicion that endocrine disruptors are affecting human health. Because of the limitations inherent in studies of human populations, it is important to test the effect of controlled exposure to endocrine disruptors in animal models. It is also important to examine exposure at different developmental periods in order to reveal specific windows of vulnerability to these compounds. These experiments should lead to testable hypotheses about the mechanisms underlying the observed effects. Below, we will explore the results of some animal studies with particular emphasis on the effects of exposure to the widespread estrogen-mimic, BPA. Our review of the literature concentrates on “low dose” effects, namely those that may be relevant to actual human exposure levels.

4. Prenatal exposure to BPA as a model of endocrine disruption

BPA is a monomer used in the manufacture of polycarbonate plastics and epoxy resins; about 100 tons of BPA are released into the atmosphere each year during production (Markey et al., 2001b). BPA has been found in aerosols and in dust particles

(Berkner et al., 2004; Matsumoto et al., 2005), and in surface and drinking water (Rodrigues-Mozaz et al., 2005). BPA plastics and resins are used in the manufacture of milk and food containers, baby formula bottles, water carboys (Biles et al., 1997), the interior lining of food cans (Brotons et al., 1994), and dental resins and composites (Olea et al., 1996). BPA has been shown to leach from these materials due to incomplete polymerization and to degradation of the polymers by exposure to high temperatures, occurring under normal conditions of use (Biles et al., 1997). BPA binds both nuclear estrogen receptor (ER) α and β (Krishnan et al., 1993; Kuiper et al., 1998; Soto et al., 1997) and plasma membrane-bound ERs (Wozniak et al., 2005). Because of the ubiquity of exposure, BPA has been chosen as a chemical model for xenoestrogen action. Several organs have been found to be affected by prenatal exposure to BPA. We will review some of the most recent reports published on the effects of BPA on the reproductive system and the mammary gland (see Fig. 1).

4.1. The ovaries and the oocytes

Prenatal exposure to either 25 or 250 ng BPA/kg/day induced changes in the gross anatomy of the mouse ovaries (Markey et al., 2003). There was an increase in the percentage of ovarian tissue occupied by antral follicles as well as a trend toward a decrease in the percentage of corpora lutea in the 3-month-old BPA-treated groups compared to the controls. This is suggestive of a reduced number of ovulated oocytes. Prenatal exposure to BPA also resulted in a significant increase of either unilateral

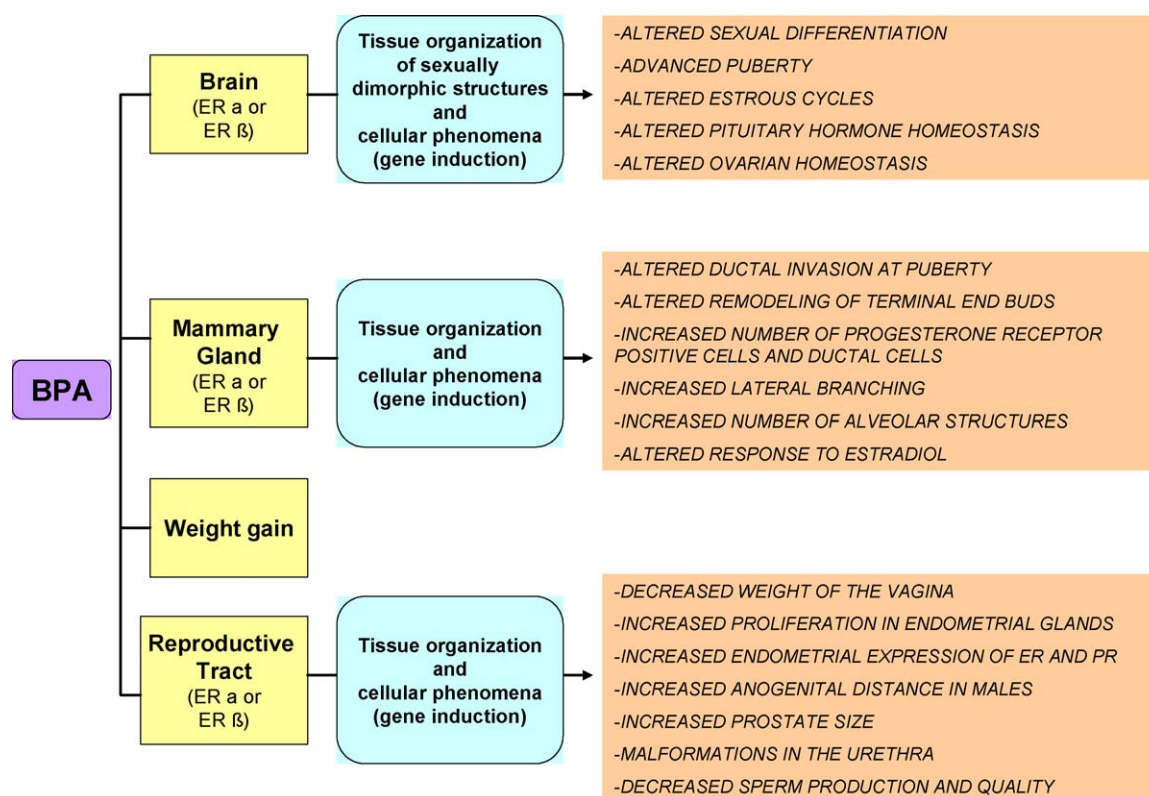


Fig. 1. Summary of the effects of perinatal exposure to BPA and their underlying mechanisms. It has been hypothesized that these effects may lead to altered fertility and fecundity, altered lactation and earlier onset/higher incidence of breast, testicular and prostate cancer.

or bilateral blood-filled ovarian bursae, a sign of reproductive aging in 6-month-old CD-1 mice.

After an accidental exposure to BPA, [Hunt et al. \(2003\)](#) reported an increase in meiotic disturbances including aneuploidy in oocytes collected from their mouse colony. They identified the source of BPA as the animals' cages as well as the plastic water bottles. Both were damaged during washing with the wrong detergent. The dose of BPA these juvenile female mice were exposed to was estimated to be 14–72 ng/g of body weight. When the experiment was repeated using a planned exposure of 20, 40 or 100 ng/g for 6–8 days, the congression failure (gross disturbances in chromosome alignment on the meiotic spindle) increased and a dose-related increase in the level of abnormalities was observed among the treated animals ([Hunt et al., 2003](#)).

Aneuploidy is considered one of the leading causes of miscarriages in humans ([Hassold and Hunt, 2001](#)). Recently, [Sugiura-Ogasawara et al. \(2005\)](#) have suggested a link between BPA exposure and recurrent miscarriages in humans. They observed that the BPA levels in the blood of women who miscarried were higher than in women who carried their pregnancies to term. Moreover, examination of tissues from women who lost their pregnancies revealed abnormal embryonal karyotypes that appeared to coincide with exposure to the highest BPA levels.

4.2. Body weight

The incidence of obesity is increasing in the Western world. Can the changes in food consumption habits and a lack of exercise be the only factors to blame? Studies by [Rubin et al. \(2001\)](#) have shown that female offspring born to rat dams that were exposed to drinking water containing either 1 mg/l of BPA (resulting in exposure of the dams to approximately 100 µg/kg/day) during pregnancy and lactation exhibited a significant increase in body weight that was sustained during adulthood. More specifically, female mice born to mothers exposed to the lower BPA dose (1 mg/l water, about 100 µg/kg/day) exhibited higher body weights than both control and high-dose exposed females. Although the male offspring of BPA exposed mothers exhibited significant increases in body weight through day 54, these results were less striking than those observed in their female siblings. Also using mice as an experimental model, [Howdeshell et al. \(1999\)](#) reported significant weight gain in female offspring exposed prenatally to BPA relative to controls. These experimental data suggest that the effect of BPA on body weight does not require the constant presence of a stimulus.

Are humans also at risk? Evidence suggests that humans may experience chronic exposure to BPA beginning before birth. BPA has been measured in the serum and urine of adult men and women ([Calafat et al., 2005](#); [Takeuchi and Tsutsumi, 2002](#)) and in amniotic fluid, fetal plasma and placental tissue ([Ikezuki et al., 2002](#); [Schonfelder et al., 2002](#)). Exposure during fetal development is considered to be more deleterious than adult exposure, since it may produce irreversible effects by interfering with organogenesis and histogenesis. However, this does not preclude that additional deleterious effects may result during postnatal exposure. In the case of persistent chemicals that bioaccumulate in fat, exposure during adult life may

also expose the next generation because these compounds traverse the placenta and are excreted in the milk. BPA has recently been measured in breast milk of lactating mothers ([Sun et al., 2004](#)).

4.3. Puberty and cyclicity

The onset of puberty correlates with nutritional state as well as with age. A sharp decrease in the onset of puberty has been observed since the 19th century (up to 12 months per decade) mostly due to nutritional changes. Although it seemed to have reached a plateau in the 1960s, an advance in the age of thelarche and menarche has been reported in recent years. However, there is a controversy in terms of the correlation between changes in pubertal maturation, childhood body size, rate of weight gain, over-nutrition, childhood obesity and early onset of puberty ([Ong et al., 2005](#)). In addition to the above mentioned weight gain, experimental data obtained from prenatally exposed mice to low dose BPA showed that the exposed animals displayed first estrus at a significantly earlier age than the non-exposed controls ([Howdeshell et al., 1999](#)). In addition, mice and rats exposed perinatally to BPA showed altered patterns of estrous cyclicity in adulthood ([Markey et al., 2003](#); [Rubin et al., 2001](#)).

[Lemos-Marini et al. \(2005\)](#) recently presented clinical data from two young females (a 7 and 2-year-old) that showed premature puberty onset (bilateral breast enlargement, enlarged ovaries and uterus). The 7-year-old had been exposed to “a strong smelling floor polishing wax and its solvent” for 3 months, and the 2-year-old drank milk from hormonally treated cows. Both cases of premature puberty regressed a few months after the exposure ceased. Results of a study in Denmark revealed that foreign adopted girls reached menarche at an earlier age than females born and raised in their country of adoption ([Teilmann et al., 2005](#)). When [Teilmann et al. \(2005\)](#) matched the girls by age and corrected the data by body mass index, the mean age at breast development and at menarche was significantly lower in adoptees. The authors suggested that the early timing of puberty might be explained by exposure to exogenous environmental factors earlier in development.

Laboratory rodents exposed perinatally to BPA had lower plasma LH concentrations than control animals after long-term ovariectomy ([Rubin et al., 2001](#)). This observation together with the disruption of estrous cyclicity indicates an altered function of the hypothalamic–pituitary axis in BPA-exposed females. However, the site(s) of the disturbance remains unclear.

The developing brain is exquisitely sensitive to estradiol. Various mechanisms facilitate precise temporal and spatial control of estrogen action during critical periods of brain development ([McEwen and Alves, 1999](#)). In rodents and other animal models, it has been documented that testosterone from the testes in the developing male is converted to estradiol *in situ* by aromatase present in specific brain regions during critical periods of perinatal development. The conversion of testosterone to estradiol plays an important role in the sexual differentiation of the rodent brain ([De Vries and Simerly, 2002](#); [McEwen and Alves, 1999](#)). Is it likely that perinatal exposure to environmental estrogen mimics might affect this developmental process?

To date, anatomical evidence of alterations in the sexual dimorphism of two brain regions has been reported in rats exposed perinatally to BPA (Funabashi et al., 2004; Kubo et al., 2003). Other sexually dimorphic regions known to be important for estrous cyclicity and estrogen positive feedback, such as the rostral preoptic area (Petersen and Barraclough, 1989), are currently being examined in BPA exposed offspring. These regions contain ERs and aromatase and are known to be “masculinized” by the *in situ* conversion of testosterone to estrogen in males or by the administration of testosterone to females during the critical period of sexual differentiation (McEwen and Alves, 1999). Therefore, it is likely that they could be influenced by exposure to exogenous estrogenic compounds like BPA during development. Neurons in these sexually dimorphic regions project to gonadotropin releasing hormone (GnRH) neurons (Petersen et al., 2003; Simonian et al., 1999) which provide the primary hypothalamic signal for gonadotropin synthesis and secretion, and the drive for the preovulatory LH surge required for ovulation. Xenoestrogens may therefore exert both indirect and direct effects on GnRH neurons as ERs are present in neurons that project to GnRH neurons, and have been localized within GnRH neurons themselves (Petersen et al., 2003; Skynner et al., 2000). Bourguignon et al. (2005) reported intriguing data obtained from female rats exposed for 5 days, at ages 6–10 days, to 10 mg/kg/day of DDT or 0.01 mg/kg/day of estradiol. They studied the process of hypothalamic maturation and observed an accelerated GnRH pulse frequency with reduced serum LH levels at 15 days of age in treated animals relative to controls. These data suggest a coexistence of increased hypothalamic stimulation and pituitary inhibition. They also observed that these treated animals experienced early vaginal opening and early first estrus compared to control animals suggesting long-term consequences of the accelerated hypothalamic maturation that occurred early in life.

In light of the above mentioned observations, one might conclude that endocrine disruptors contribute to the alterations in sexual maturity, estrous cyclicity and reproductive health observed during adulthood and that this is due to disturbances in perinatal gene expression as well as interference in processes involved in sexual differentiation of the brain.

4.4. The mammary gland

The development of the mammary gland represents a useful model to evaluate the effects of endocrine disruptors because this organ undergoes dramatic morphological and functional changes throughout the lifetime of the animal. Exposure to estrogens throughout life is the main known risk factor for breast cancer. In the 1940s a woman's lifetime risk of being diagnosed with breast cancer was 1 in 22; this number has now increased dramatically to 1 in 8 according to the 2002 report published by The National Cancer Institute's (NCI) Surveillance, Epidemiology, and End Results (SEER) Program {http://seer.cancer.gov/csr/1973_1999/}. Coincidentally, the last 60 years has been a period defined by the massive development, manufacture and the introduction of man-made chemicals

into the environment, many of which have been shown to be xenoestrogens.

The development of the mouse mammary gland is affected by perinatal exposure to environmentally relevant levels of BPA (Markey et al., 2001a). In 10-day-old mice, the mammary gland is relatively quiescent such that the ductal tree does not spread beyond the vicinity of the nipple. Perinatal exposure to BPA decreases DNA synthesis in mammary gland epithelial cells at 10 days of age. At 1 month of age, corresponding to the initiation of puberty in CD-1 mice, a direct correlation was recorded between ductal length (measured from the center of the lymph node to the leading edge) and the age at first proestrus. The slope was steeper in the controls and was reduced as the BPA dose was increased, suggesting that BPA exposure slows ductal invasion of the stroma. The area and the number of terminal end buds (the club-shaped structures that invade the stroma and mediate the longitudinal growth of the ducts) increased relative to the gland's ductal area. This was accompanied by a significant decrease of apoptotic activity in the cells of the terminal end buds in perinatally exposed animals. Apoptosis is believed to result in lumen formation of the subtending duct. These results suggest that inhibition of apoptotic activity may be the driving force of the observed impaired duct elongation (Munoz de Toro et al., 2005). The number of progesterone receptor (PR) positive epithelial cells was also increased, and these cells were frequently seen in clusters that are believed to be future branching points. Coincidentally, a significant increase in the number of lateral branches was observed in the BPA exposed animals. By 6 months of age, the mammary glands showed a dramatic expansion of the ductal network with a significant increase in terminal ducts and alveolar structures relative to the control. The appearance of the mammary glands of these virgin mice resembled that of early pregnancy (Markey et al., 2001a).

Animals exposed *in utero* to BPA have a significantly higher sensitivity to estradiol (Munoz de Toro et al., 2005) suggesting that prenatal exposure may increase the likelihood of neoplastic development, since estrogen exposure is the main risk factor for breast cancer. In addition, the increased number of epithelial ductal cells expressing PR may be attributed to the increased sensitivity to estrogen, since estrogens induce PR expression. Moreover, this increased expression of PR may explain the increased lateral branching, and thus, the increased ductal density. In this regard, it is noteworthy that increased mammographic density is a risk factor for breast cancer in humans. Furthermore, terminal end buds are the structures in which mammary cancer originates in both rodents and humans (Russo and Russo, 1978). The increase in the number of these structures is also consistent with an increased risk of cancer. Finally, the overall epithelial structures are significantly increased at 6 months of age. All these data support the hypothesis that perinatal exposure to estrogenic compounds in general and to BPA in particular, increases a woman's susceptibility to breast cancer.

4.5. Female reproductive tract

Estrogens exert a powerful influence on the development, regulation and endocrine control of the female genital tract.

These effects range from organizational ones, as evidenced by the abnormal shape of the uterus in animals and humans exposed perinatally to the synthetic estrogen diethylstilbestrol, to activation effects such as control of gene expression and proliferation of stromal and epithelial cells.

In utero exposure to environmentally relevant doses of BPA induced morphological and functional alterations in the reproductive tract in mice that were revealed during adulthood (at 3 months of age). These findings included decreased weight of the vagina and endometrial lamina propria, increased proliferative activity of epithelial cells in the endometrial glands, and increased expression of ER α and PR in the luminal epithelium of the endometrium and subepithelial stroma (Markey et al., 2005). One method for determining the estrogenicity of a chemical is the rodent uterotrophic assay, which measures an increase in wet weight of the uterus in sexually immature animals exposed for 3 days to the test compound. BPA induces a uterotrophic response at a minimum dose of 100 mg/kg/day or higher (estradiol induces uterotrophic response at 5 μ g/kg/day). However, when administered perinatally, BPA produces measurable and long lasting effects at doses that are 10^6 -fold lower than that required to induce the uterotrophic effect in prepubertal mice. Once again, this finding highlights the critical sensitivity of the developing organism to environmental estrogen exposure. The consequence of the above mentioned changes may be (1) to alter the responsiveness of the uterus to endogenous hormones imposed under different physiological conditions, such as pregnancy, or (2) to predispose the tissue to disease and carcinogenesis. In this regard, transgenic mouse models in which ER α expression is up-regulated in the uterus exhibited an earlier onset and a higher incidence of uterine adenocarcinoma following exposure to diethylstilbestrol (DES) at postnatal days 1–5 than wild-type mice (Couse et al., 1997).

4.6. Male reproductive organs

Male mice exposed to BPA *in utero* during gestational days 16–18 showed increased anogenital distance, increased prostatic size and decreased epididymal weight (Gupta, 2000). These changes persisted during adulthood. Male fetuses also exhibited an increase in the number of prostatic glandular buds (Timms et al., 2005). In adulthood, these animals exhibited decreased daily sperm production and enlarged prostates (vom Saal et al., 1998). Exposure of fetal prostates to BPA *in vitro* also resulted in prostate enlargement, which was shown to be mediated through the ERs present in the stroma, and this effect was blocked by antiestrogens (Gupta, 2000). BPA has been shown to increase the expression of androgen receptor in the prostate stroma of mice, while fetal exposure of rats to BPA induced alterations in the differentiation pattern of the peritubular stroma in this same organ (Ramos et al., 2001). A recent study also revealed that the increase in the number and size of dorsolateral prostate ducts and overall increase in prostate duct volume observed in male mouse fetuses is due to an increase in the proliferation of basal epithelial cells (Timms et al., 2005). Malformations in the urethra were also observed; its connection to the bladder was significantly constricted (Timms et al., 2005). Taken together,

these results indicate that prenatal BPA exposure results in permanent alterations of the morphology, histoarchitecture, and cell proliferation control in the prostate and other androgen-target tissues predisposing the affected individual to disease in adult life.

5. Future directions

There is a clear need for chemical and biochemical approaches aimed at a better understanding of the mechanism of action of xenoestrogens with regard to the low-dose effects revealed during developmental exposure. These approaches encompass several areas of study, such as signal transduction via membrane and nuclear ER, and analytical chemistry to measure these chemicals and their metabolites in tissues. More vexing is the fact that humans are exposed to a variety of endocrine disruptors acting through many different pathways at different times during their development. This poses two problems to be considered: interactions among chemicals acting through a common pathway and a single chemical affecting different pathways. The former is exemplified by the additive effect of xenoestrogens acting through ER α . Regarding the latter, phytoestrogens not only act through binding to the two ERs, but they also interfere with iodine metabolism in the thyroid gland (Doerge and Sheehan, 2002). Moreover, there is now evidence that in addition to binding to ERs, BPA also binds to the thyroid hormone receptor (Zoeller et al., 2005).

6. Conclusions

The organizational and functional changes reported to date provide important pieces of evidence for the understanding of how BPA exposure affects male and female reproduction in mammals. This ongoing research has both practical and theoretical implications. The former is the realization that wildlife and humans are affected by environmental exposure to hormonally active chemicals at levels previously considered to be irrelevant. The latter is that the prevalent view of development as the mere unfolding of a genetically determined program is incorrect, and that a reductionistic, bottom-up approach is not adequate to explain this complex problem. The complexity of developmental phenomena requires the use of both bottom-up and top-down approaches, as well as a new epistemology that would take into account the existence of emergent phenomena (Soto and Sonnenschein, 2005). Developmental biology, guided by organicist thinking, now has the tools to successfully revisit the old tradition of ecological regulation of development (phenotype plasticity) (Gilbert and Sarkar, 2000; Markey et al., 2003; Soto and Sonnenschein, 2005).

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