Review

Bisphenol A: An endocrine and metabolic disruptor

Bisphénol A : un perturbateur endocrinien et métabolique

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Abstract

Bisphenol A (BPA), initially designed, like diethylstilbestrol, as a synthetic estrogen, has been rapidly and widely used for its cross-linking properties in the manufacture of polycarbonate plastics and epoxy resins. Because of incomplete polymerization and degradation of the polymers by exposure to higher than usual temperatures, BPA leaches out from food and beverage containers, as well as from dental sealants. In humans, free active unconjugated BPA is metabolized by rapid glucuronoro- or sulfo-conjugation and eliminated via renal clearance. However, exposure to environmental nanomolar concentrations of BPA is ubiquitous and continuous via different routes: oral, air, skin. In rodents, fetal and perinatal exposure to such environmentally relevant doses of BPA has been shown to affect the brain, liver, gut, adipose tissue, endocrine pancreas, mammary gland and reproductive tract and function. Similar concentrations are also able in vitro to impact human malignant breast, prostate, male germ or adipocyte cell lines (with a promoting effect and by interfering with chemotherapy drugs), or to stimulate pancreatic β cell insulin secretion. High levels of BPA have recently been correlated with obesity, diabetes, cardiovascular diseases, polycystic ovarian disease or low sperm count. However, before the real impact of BPA on human health can be clearly assessed, prospective longitudinal epidemiological studies are needed as well as characterization of selective biomarkers to verify long-term exposure and selective imprinting.

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Résumé

Le bisphénol A (BPA) est une substance chimique très ubiquitaire présente dans la plupart des plastiques polycarbonés et les résines époxy, qui est relargué sous l’effet de la chaleur et considérée comme présentant un effet de perturbateur endocrinien estrogén-mimétique. Il est essentiellement absorbé dans l’espèce humaine par voie alimentaire et est retrouvé sous forme libre active dans le sang et sous forme conjuguée dans les urines en vue de son élimination, chez la majorité des individus. La mise en évidence de troubles du développement, de la reproduction, du métabolisme chez les rongeurs exposés à des taux équivalents, pendant des périodes critiques d’exposition foetale et/ou périnatale, a conduit à s’interroger sur sa responsabilité dans les pathologies humaines correspondantes. D’autant qu’il est capable d’induire in vitro sur des cellules humaines, via des récepteurs membranaires des estrogènes, la prolifération de cellules malignes mammaires ou testiculaires ou d’adipocytes et de réguler la sécrétion insulinaire par les îlots β pancréatiques. Bien que des études épidémiologiques transversales, rétrospectives mettent en évidence une corrélation entre obésité, insulinorésistance, diabète, hypofertilité et les taux de BPA, elles ne permettent pas à elles seules d’attester d’une relation causale et d’évaluer exactement la part de ce potentiel facteur de risque. Seules des études prospectives longitudinales pourraient conforter cette hypothèse ainsi que la caractérisation de biomarqueurs permettant à la fois d’évaluer l’exposition prolongée et continue à de faibles doses de BPA et en même temps de mettre en évidence des modifications moléculaires conduisant à faire la part de l’exposition chronique et de la susceptibilité individuelle.

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1. Introduction

The concept of endocrine disruptor was proposed 20 years ago [1] when various observations of deleterious effects on wildlife or humans were put together, in association with the intensive use of pesticides in agriculture. It refers to natural or synthetic chemical products, which are able to mimic and/or interfere with hormone receptors generating as unexpected ligands deleterious effects on animal or human health [2]. Many of these products, such as dichlorodiphenyltrichloroethane (DDT) or other organochlorides, demonstrated estrogenic or anti-androgenic effects and could interact during development and/or

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reproduction [3]. These observations have been compared with the human unwilling experiment of fetal exposure to diethylstilbestrol (DES) used in prevention of miscarriages from 1950 to 1975. An increased risk of different pathologies, including genital malformations, infertility, or hormone dependent cancers, have been reported [4] for these exposed children, sometimes several decades later [5], underlining the critical window of fetal period for exposure to environmental exposure disruptors (EED). This concept is very similar to the one proposed by David Barker, based on the fetal nutritional environment influencing the developmental origin of chronic adult diseases [6].

However, an objective causative role for EED exposure in human chronic disease remains very difficult to assess, except for this unique “experimental” story of distilbene, because of methodological limitations. These difficulties concern epidemiological designs as well as toxicological and mechanistic aspects. These concerns represent a real challenge for the scientists and the physicians who have to assess the possible toxicity or the harmlessness of a given molecule, and for the politicians who will have to establish regulation for allowed thresholds or decide the authorization for common use of a new molecule (REACH program).

The case of bisphenol A represents a perfect example for this emerging concept and should be of particular interest for French endocrinologists and diabetologists, considering that its links with this compound has been pointed out for reproduction, diabetes, obesity and endocrine related cancers in the very recent ANSES document (Agence nationale de sécurité sanitaire de l’alimentation, de l’environnement et du travail) published in December 2011 by the French Ministry of Environment [7].

2. Historical record

BPA is a chemical product, first synthesized at the beginning of the 20th century from two phenol molecules (bisphenol) linked by one acetone molecule (A) (Fig. 1). BPA was first designed as a synthetic estrogen because of its action on rodent’s uterus [8]. In fact, diethylstilbestrol, a structural analog, was found to be much more potent. Later, BPA has been widely used for its cross-linking properties in the manufacture of polycarbonate plastics and epoxy resins. Due to real advantages in terms of heat resistance and elasticity, the use of BPA has progressively increased all over the world with an annual production, higher than 10 millions of tons. It has become ubiquitous in a lot of domestic products such as plastic bags, bottles, coated tins, dental sealants, paintings, CD-Rom, etc. Because of incomplete polymerization and degradation of the polymers by exposure to higher than usual temperatures such as microwave’s use with plastic film or containers [9], BPA has been found to leach out from food and beverage, as well as from dental sealants. Exposure to environmental nanomolar concentrations of BPA is ubiquitous and continuous, via different routes: oral absorption, air, skin. In humans, free active unconjugated BPA is metabolized by rapid glucuronos- or sulfon-conjugation and eliminated via renal clearance. It is present in the urine of 90% of cases in an American reference population (0.2–1.6 ng/mL) [10] and in most bloods, maternal milk or amniotic fluid [11].

Its structural analogy with DES (Fig. 1), with its supposed xenoestrogenic effect, as well as its increasing underspread background exposure have led to numerous toxicological tests in rodents. After fetal or perinatal exposure, these tests have shown, at low, environmentally relevant concentrations, to affect the brain, liver, gut, adipose tissue, endocrine pancreas, mammary gland and reproductive tract and function. Similar concentrations are also able in vitro to impact human malignant breast, prostate, male germ or adipocyte cell lines with a promoting effect [12,13], or by interfering with chemotherapy drugs, or to stimulate pancreatic β cell insulin secretion. High levels of free active BPA have recently been correlated with obesity, diabetes, cardiovascular diseases, polycystic ovarian disease or low sperm count. All these experimental and epidemiological data have led to increasing concerns about the real impact of chronic BPA exposure on human health and what should be an objective and realistic regulatory policy concerning the determination of maximum thresholds or even its prohibition, as was recently decided [14] in France concerning baby bottles in 2011 leading to complete prohibition after 2014.

3. Obesity and metabolic syndrome

A recent working document of ANSES proposed the recommendation of a total BPA prohibition because of indirect evidence supporting its involvement in obesity, metabolic syndrome and diabetes [7]. In mice, fetal or perinatal exposure to low, environmentally relevant doses of BPA induces in the offspring an increase of adult weight, depending on sex, dose and window of exposure [15–19], associated to metabolic syndrome [20]. Similar effects have been described after neonatal exposure to DES [21]. BPA stimulates in vitro differentiation of murine fibroblasts into adipocytes and increases lipid storage in a dose-dependent manner [22–24]. Low doses of BPA inhibit adiponectin secretion by human adipocytes cultures in vitro and stimulate the secretion of interleuvin 6 and TNFα, two inflammatory adipokines, suggesting its possible involvement in obesity, metabolic syndrome and insulin resistance [25–27]. It also results in deleterious effects observed both on the energetic imbalance and glucose homeostasis via its effects on liver, adipose tissue and pancreatic islets [27]. In humans, although rapidly metabolized in the liver into inactivated glucurono- or sulfo-conjugates and theoretically immediately eliminated by renal excretion, BPA was shown to accumulate into the adipose tissue. This could explain its blood persistence during fasting [28]. A recent French epidemiological study reported a non-monotonous, inversed U shape dose-response relation between maternal urinary BPA and newborn weight [29] quite concordant with what was observed in rodents [15]. Several recent epidemiological studies in adults have tried to correlate urinary BPA and obesity. First an American study concerning 2700 adults reported a positive correlation with general obesity, central obesity, and insulin resistance [30]. A Chinese study involving 3400 middle age adults found quite similar results [31]. However, both studies were retrospective, cross-sectional with a single urinary sample, traducing only the recent exposure. Both wondered with honesty whether this correlation was causal or linked.
Fig. 1. Xenoestrogens: notice the bisphenol A structural analogy with various xenoestrogens like diethylstilbestrol, un estrogène de synthèse, DDT, an organochloride pesticide, PCB an industrial chemical and resveratrol, a phytoestrogen.

4. Diabetes

Animal experimental models, cell cultures using murine or human pancreatic β cells and epidemioligical studies, suggest also that exposure to BPA might participate to the increasing incidence of type 2 diabetes. Adult male mice exposed to BPA develop both hyperinsulinemia and mild insulin resistance [20]. This impairment occurs through a direct effect on pancreatic cells, as described later, but also by impairment of insulin signal transduction at the level of peripheral tissues (muscle, liver, adipose tissue). BPA induces an oxidative stress in rodent liver by decreasing expression of anti-oxidative enzymes [34]. Saku-rai et al. [35] have shown that in adipocytes, BPA increases, basal and insulin-activated glucose transport, by stimulating GLUT4 expression. At the level of pancreatic islets, BPA is able to suppress in vitro, glucose-induced calcium oscillations, which control glucagon secretion [36] in α cells. On β cells, very low doses of BPA (0.1 nM) increase glucose-induced calcium oscillations frequency leading to a higher insulin secretion [37]. These rapid effects observed in vitro are confirmed on entire murine pancreatic islets [37]. These effects mimic the rapid effects induced by estrogens, which are mediated by non-classical membrane estrogen receptors as described later [20]. In vivo BPA is able as estrogens to decrease very rapidly (30 minutes) glucose blood level by stimulating insulin secretion [20] and by stimulating AMP dependent transcription factor CREB which regulates both insulin gene expression and β cells survival [38]. A longer exposure to BPA in rats, induces after 4 days, chronic hyperinsulinemia and insulin resistance [20]. Two kinds of arguments allow a possible extrapolation to humans. First, identical rapid effects of BPA have been reported on human pancreatic β cells [39]. Secondly, several epidemiological, retrospective cross-sectional studies found a correlation. All these studies belong to the American study of the National Health and Nutrition Examination Survey (NHANES). The first one was performed during the period of 2003–2004 with adults aged 18 to 74 years from the general. After normalization for age, sex, ethnic background, education level, smoking, BMI, waist circumference, and urinary creatinine level, it found a positive correlation between urinary BPA and the presence of
a self-reported diabetes [13]. The highest levels of BPA were associated with the highest BMI values and one may wonder one more time whether it represents the recent exposure to BPA leached out from soda cans, which are more frequently used by the obese people or if it could be a marker of exposure to multiple EED including BPA [13]. Several similar studies performed from 2005 to 2008 found discordant results [40,41]. However, Shankar et al. [42], selecting from these different studies of NHNAES the patients for whom biological results were provided, could find after similar normalization, a positive correlation with diabetes diagnosed either by fasting glycaemia greater than 1.26 g/L; fasting or not fasting glycaemia greater than 2.00 g/L or HBA1c greater than 6.5%. This possible risk factor independent from classical risk factors of type 2 diabetes has to be confirmed by prospective longitudinal studies.

5. Reproductive studies

5.1. Polycystic ovaries and BPA exposure

Polycystic ovaries (PCO) is considered to be the result of both genetic and environmental factors. Neonatal exposure to BPA in rats induces an adult syndrome very similar to human PCO [43]. In vitro BPA stimulates testosterone secretion by ovarian theca cells in rats [44]. In adult women with PCO, blood BPA level is higher than in control women with normal ovulation and without hyperandrogenia, independently of the presence or not of obesity [33], and BPA correlates with androgens.

5.2. Cryptorchidism

Cryptorchidism or undescended testis (UDT) is the most frequent male congenital malformation and its incidence at birth (>2%) may have increased over the last decades. UDT is a risk factor for hypofertility and testicular cancer. It remains idiopathic in most cases but genetic and environmental factors have been suggested [45,46]. We and others have found in mother milk higher levels of some EED in UDT [46,47]. While cord blood BPA was not found significantly increased in UDT [48] (Fig. 2), we could identify in the whole population (UDT and controls) a negative correlation between cord blood BPA and insulin-like peptide 3 (iNSL3) a Leydigian hormone involved in testicular descent, with INSL3 significantly lower in the UDT group versus control [49].

5.3. Male hypofertility

Estrogenic and anti-androgenic effects of BPA have been demonstrated in vivo and in vitro in rodents [50]. For this reason, testicular function has been particularly studied. In rodents, BPA reduces Leydigian secretion of testosterone [51]. In Sertoli cells, it induces an impairment of junctional proteins [52] and stimulates in vitro human seminoma cell proliferation [53,54]. In utero exposure in rats, leads in adult male to impaired spermatogenesis [55]. Few human epidemiological studies have been able to correlate urinary BPA and sperm quality. Recently, Ly et al. [56] could find a correlation between urinary BPA, number, motility and sperm vitality.

6. Hormone dependent cancer studies

6.1. Breast cancer

Breast cancer is the most frequent cancer in women with one million of new cases every year all over the world including 40,000 in France corresponding to 32% of feminine cancers [57]. It represents 2% of all causes of women deaths and the first cause by cancer. Its incidence is still increasing (two-fold increase within the last 20 years), 2 to 5% more every year depending on the part of the world, with a great disparity between the one hand North America, Europe and Australia which are
high-incidence areas, versus on the other hand, Asia or Africa with a lower incidence [58]. These geographical differences suggest environmental factors supported by the data obtained from migrant populations from Asia to America and the fact that less than 10% of breast cancers are associated with germinal mutations [59,60]. For all these reasons, nutritional and/or toxic factors including EED with estrogenic effects have been suggested. Indeed, the carcinogenic effect of fetal programming by exposure to low doses of some of them, including BPA, has been clearly established in rodent models. Fetal exposure to BPA induces in mice at the peripubertal period, an impaired development of the histological architecture of the mammary glands with an enhanced sensitivity to estrogens, an increase in area and number of terminal buds when normalized to the total canalar structures, a decrease of apoptotic activity, an increase of the number of epithelial cells expressing the progesterone receptor and an increase of lateral canalar ramifications [61,62]. These observed abnormalities suggest that development and morphogenesis have been disturbed during the fetal exposed period. This was identified and confirmed during this period in fetal mice [63]. Moreover, in Wister rats, prenatal exposure to BPA was associated in adults with changes in epithelial and stromal tissues, an increased ratio proliferation/apoptosis and a clear decrease of the doses of carcinogens necessary to induce neoplastic lesions, doses which remain without any effect in control animals non exposed in utero to BPA. All these elements support an increased susceptibility to develop a mammary cancer. BPA is able to stimulate in vitro human breast cancer cell proliferation [64]. Strikingly, BPA is also able at very low doses (nM) similar to the blood level found in the general population, to induce in vitro a chemo-resistance on several human malignant breast cell lines whether these cells are ERα positive or negative, while it increases expression of several anti-apoptotic proteins [65]. Nevertheless, to up to now, no epidemiological study has been reported correlating chronic exposure to BPA with an increased risk of breast cancer as it has been shown for some EED [59], although one has to consider the methodological difficulties associated to such a demonstration [66].

6.2. Prostate cancer

Prostate cancer is the most frequent cancer of the elderly man more than 50 years and the second cause of male death by cancer. Its incidence is still increasing independently of age [67]. Despite a better screening and treatment, its mortality remains high when the androgen dependent phase is over. Among the risk factors, only nutritional (high fat diet) and genetic (familial and ethnic) have been well identified but here too the role of EED has been suggested. The prostate gland is classically androgen dependent, develops during puberty under androgen control. Androgen dependence of prostate cancer allows anti-androgenic treatment. But additive hormonal control through estrogens in normal and malignant prostate development is supported by many experimental data [68]. Aromatase and ER (both ERα and ERβ) are expressed in human prostate [68]. Maternal estrogens allow at the third trimester of pregnancy the growth of the prostate gland in men. When exposed to DES given during the pregnancy to their mothers the newborn presented prostate hyperplasia [69]. A recent study performed in the French Antilles, has clearly illustrated the likely risk factor of chronic exposure to chlordene, an estrogenic organochloride pesticide in the occurring of prostate cancer [70]. Chlordene or kepone has been widely used in the French Antilles during 1973 and 1993 in the culture of banana. Its degradation is very slow and it will persist in groundwater during several decades. In rodents, it acts as a carcinogen able to induce liver cancer. In men this case/control study has been performed in a limited geographical area with a controlled exposure, which has allowed to support the relation between chlordene exposure and prostate cancer risk independently of ethnical origin or other statistical bias or confounders. The risk was positively correlated to chlordene blood levels and enhanced in men greater than 60 years, with familial antecedent and/or in presence of a specific polymorphism for the chlordene reductase gene coding for an enzyme involved in hepatic detoxification [70]. Moreover, tumoral aggressiveness was correlated with chlordene blood levels [70]. These epidemiological data are supported by experimental ones. Fetal or perinatal BPA or DES exposure in rodents, are associated in adults with prostate hyperplasia and pre-malignant lesions [68]. This perinatal exposure to BPA or to estradiol increases the susceptibility to develop a prostate cancer either spontaneously or after a second estrogen shot [71]. In order to explain the therapeutic escape to anti-androgens in prostate cancer becoming androgen independent, several mechanisms have been proposed including the occurring of secondary mutation of the androgen receptor gene [72]. In some of these cases, it has been shown in vitro that cancer cells became sensitive to BPA, activating proliferation through this mutated receptor [73].

6.3. Testicular cancer

Testicular germ cell cancers (TGCC) represent only 2% of all cancers but they are the most frequent cancer in young man with an increasing incidence all over the world estimated as 2 to 3% every year [74]. This increase remains unexplained but the possible role of in utero exposure to EEP interfering in the malignant transformation of fetal stem germ cells, the gonocytes, into TGCC [75], are supported by several epidemiological and experimental data. In the USA, among the agriculture workers exposed to pesticides, an odd ratio of 6 has been found for testicular cancer when compared to control [76,77] especially for atrazine, an organochloride estrogenic pesticide which is still widely used in the West of France. Hardell et al. [78] have studied in Sweden the correlation between TGCC and the blood levels of several EED in patients and their mothers. Strikingly, no correlation was observed with the patient’s levels but the mother’s levels when elevated were associated with an increasing risk with an odd ratio of 4 for PCB (bisphenols polychlorés), HCB (hexachlorobenzene), nonachlordane, all estrogenic EED, but not with ppDDE a stable anti-androgenic derivative of DDT supporting the role of fetal exposure to xenoestrogens [78]. However is TTGC really estrogen dependent as other classical estrogen dependent cancers? In fact we and others have shown that
Fig. 3. Bisphenol A (BPA) regulates human seminoma cell proliferation. Very low doses of BPA (pM) stimulates human seminoma cell proliferation in vitro. Notice that the dose/response curve is not monotonic but an inverted U shape, which can be explained by two different effects mediated by two different receptors. FIRST BPA stimulates at low doses cell proliferation through a non-classical membrane estrogen receptor, GPR30 for whom it presents a very high affinity; secondly BPA triggers a suppressive effect through ERβ at high doses because of a relative low affinity.

From Bouskine et al. [53].

seminoma, the most frequent testicular germ cell cancer, expresses both aromatase and ERβ but not ERα [79]. At the opposite of prostate or breast cancer, it is not possible to induce experimentally a TGCC in rodents by fetal exposure to BPA or other estrogenic EED and seminoma rodent models do not exist at all. For this reason we have used a human seminoma cell line to demonstrate that estrogens and xenoestrogens were able to stimulate cell proliferation in vitro [79,80]. Estradiol 17β, the natural estrogen induced a suppressive effect on seminoma cell proliferation through the classical estrogen receptor ERβ [79]. Estradiol when coupled to bovine serum albumin which cannot cross the cell membrane, stimulate at the opposite of the free molecule, cell proliferation through a non-classical G protein coupled estrogen receptor (GPR30/GPER) [54,80]. Human seminoma are therefore estrogen dependent and xenoestrogens may act through two different receptors depending to their respective affinity for them. We have screened several EED such as atrazine, DES and BPA and observed different effects depending on the resultant of the two receptors. Concerning BPA (Fig. 3), we were able to demonstrate that very low doses of BPA (pM) under the levels found in male cord blood [48] stimulate seminoma cell proliferation through GPR30, a membrane associated estrogen receptor, different from classical ERs [48]. The dose-response curve was not monotonic but showed a inverted U shape (Fig. 3), which could be explained by the resultant of the double opposite effect on ERβ and GPR30 [53,81]. At low doses (nM or pM), BPA acted only through GPR30 by a promotive effect. At higher doses (nM), BPA acted also through ERβ, which counteracted the promotive GPR30 mediated effect [79]. Low doses of BPA (nM) are similar to the one able in rodents when exposed in utero, to induce mammary or prostatic malignant lesions in adult [82,83] as described before. We have recently shown that seminoma over express GPR30 [81] likely through a specific polymorphism abnormally present in men with TGCC conferring likely a genetic susceptibility to such cancers.

7. Exposure to BPA and thyroid function

BPA may interfere with thyroid hormone action [84]. A recent human epidemiological study reported an inverse relation between BPA concentration in maternal urine and maternal serum total T4, during pregnancy [85]. The association was stronger when maternal urinary BPA and serum total T4 were measured closer together, suggesting a transient effect of BPA, and when measured in the third trimester of pregnancy [85]. They also reported an inverse correlation between maternal BPA and neonatal TSH but only in male, which could be explained by a sexual differentiation of UDP-glucuronosyl transferase expression as shown in rat [86]. A negative correlation between cord blood fT4 and maternal milk PCB118, PCB180 and DDE has also been reported [87].

8. Metabolism, storage, waste, dosage

Free BPA, the active form, is theoretically in humans rapidly glucurono – or – sulfo conjugated by the liver and evacuated by the kidney as demonstrated by the pharmacokinetic study after absorption of an unique oral dose [88]. This kinetics is different from rodents and this difference has been used to contest any chronic potential deleterious effect [89,90]. However, lots of studies have nevertheless identified relatively high levels of free BPA in cord blood, amniotic fluid, plasma or urine of adults from general populations [11]. Its blood persistence during fasting [28] suggests that BPA is stored in adipose tissue and unless a relative lipophilicity [28]. Moreover daily oral absorption contributes to maintain constant high blood levels. Moreover sulfatases and/or glucuronidases are present in several adults organs and highly expressed in the placenta which could explain that relative high free BPA levels are present in the blood flow, which is the only form able to induce deleterious effects as EED. The fetus and the newborn seem to be particularly exposed [91,92] in part due hepatic immaturity of detoxification enzymes. Chromatography and mass spectrometry represent the reference in term of free or conjugated BPA in blood or urine. Moreover a RIA (rotter interaction analysis) has been proposed and validated by H. Déchaud at Lyon [48,93]. It allowed us to confirm the presence of free BPA in cord blood and pregnant women between 1 and 10 ng/mL [48]. However, one must be very careful to avoid plastic contamination from transport, storage and manipulation of the samples at the
laboratory and the simple perfusion of the patient before increases the BPA concentrations due to release from the tubules and perfusion bottles.

8.1. BPA, an endocrine disruptor: which receptors?

One important question concerning the endocrine disruption mediated by BPA is whether it acts really as a weak estrogen since its affinity for the classical nuclear ERα is 10,000 times weaker as for estradiol 17β and 1000 weaker than affinity for ERβ [94]. For this reason BPA at the nanomol concentration found in humans should not be considered as an estrogen-mimetic at all [95]. Different explanations have been proposed for this apparent paradoxical criteria, especially the involvement of non-classical membrane estrogen receptors. One of this receptor G protein coupled, GPR30 have been identified. This GPCR is present in breast cancer cells, β Langerhans islets, adipocytes with a very high affinity for BPA and mediates activation of signaling pathways classically involved in the control of cell proliferation, apoptosis and survey. In our laboratory, we have shown its promoter role on human seminoma cells [53,54]. Another receptor, ERKγ (estrogen-related receptor-gamma), a nuclear receptor, has also a good affinity for BPA [96]. It is present on adipocytes [97]. GPR30 and ERKγ are both expressed by ERα and ERβ negative breast cancer cells which respond to estrogens and BPA [98]. An anti-androgen effect has also been identified at the AR level for BPA [99]. It can also interact with PPARγ as an antagonist [100] able to inhibit adipocyte proliferation and also as an antagonist for thyroid hormone receptors TR (Table 1) [101].

8.2. Molecular mechanisms programming in utero the developmental origin of adult diseases induced by EED

Fetal or perinatal developmental origin of adult diseases has been nicely analyzed for bisphenol A concerning mammary carcinogenesis consecutive to very low doses administrated during this critical period in rats and mice which have later developed pre- or malignant lesions. Bromer et al. in 2009 have characterized the imprinted molecular mechanisms, which have led after in utero exposure to DES, to uterus cancer in mice. They identified one gene HOXA10 coding for a transcription factor involved in uterus growth and which promoter region was hypomethylated through BPA exposure [102]. This epigenetic modification is traduced into adult overexpression of HOXA10, influencing carcinogenesis. Other DES induced epigenetic changes have been reported for the oncogene c-fos which showed a similar gene hypomethylation leading to its overexpression in adult uterus stimulating cell proliferation [103]. Similar modifications concerning HOXA10 gene have been reported with BPA [104] and methoxychlor an estrogenic organochloride pesticide [105]. DNA methylation is an epigenetic hallmark involved in the initiation and/or promoting phase of carcinogenesis [106]. Ho and Prins [71,83] have studied the epigenetic changes induced in prostate rat by BPA or estrogens fetal or perinatal exposure. Out of 50 gene sequence changes they identified a gene coding for a particularly interesting enzyme phosphodiesterase 4D4, which degrades cAMP. Hypomethylation of this gene is correlated with the overexpression of the protein. Hypomethylation could be identify before adult age before any histological modification and could therefore serve as an early a biomarker [83]. Hypomethylation of this, gene is also present in human prostate cancer. These modifications observed after EED exposure are likely induced through the action of DNA methyltransferases (DNMT), enzymes which control DNA methylation on the CpG islets [102]. There are some data suggesting that these enzymes are regulated by classical estrogen receptors [103] and/or nuclear or not nuclear receptors [37,107,108]. Fig. 4 summarizes the proposed mechanism through which EED will imprint epigenetic changes after fetal in utero exposure which

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**Table 1**

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From Chiam et al. [109].

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![Diagram](image-url)
will contribute with others deleterious factors to malignant transformation by enhancing chromosomal instability [109].

9. Conclusion

In conclusion there are solid arguments to support a deleterious role for bisphenol A after chronic exposure but its real participation in human health remain to be demonstrated. BPA is ubiquitously present in the current environment. Low doses of BPA environmentally relevant might interfere in the development of chronic metabolic diseases, reproductive pathologies, hormone dependent cancers (prostate, breast, testes). However, prospective longitudinal studies are needed to confirm such a responsibility. It is also necessary to identify biomarkers of duration and intensity of exposure and to recognize early induced molecular imprints (gene methylation, histone modifications, microRNAs). ANSES works has led first to the prohibition of baby bottles in 2011 in France and for 2014 to the complete prohibition of BPA use.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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