Published in final edited form as:

Reprod Toxicol. 2007 ; 24(2): 240–252.

An Evaluation of Evidence for the Carcinogenic Activity of Bisphenol A

Ruth A. Keri¹, Shuk-Mei Ho², Patricia A. Hunt³, Karen E. Knudsen⁴, Ana M. Soto⁵, and Gail S. Prins⁶

¹ Department of Pharmacology and Division of General Medical Sciences—Oncology, Case Western Reserve University, Cleveland, OH 44160

² Department of Environmental Health, University of Cincinnati, Cincinnati, OH, 45267

³ School of Molecular Biosciences, Washington State University, Pullman, WA 99164

⁴ Department of Cell Biology, University of Cincinnati, Cincinnati, OH, 45267

⁵ Department of Anatomy and Cell Biology, Tufts University, Boston, MA 02111

⁶ Department of Urology, University of Illinois at Chicago, Chicago, IL, 60612

Abstract

The National Institutes of Health (NIEHS, NIDCR) and the United States Environmental Protection Agency convened an expert panel of scientists with experience in the field of environmental endocrine disruptors, particularly with knowledge and research on Bisphenol A (BPA). Five subpanels were charged to review the published literature and previous reports in five specific areas and to compile a consensus report with recommendations. These were presented and discussed at an open forum entitled “Bisphenol A: An Expert Panel Examination of the Relevance of Ecological, In Vitro and Laboratory Animal Studies for Assessing Risks to Human Health” in Chapel Hill, NC on November 28-30, 2006. The present review consists of the consensus report on the evidence for a role of BPA in carcinogenesis, examining the available evidence in humans and animal models with recommendations for future areas of research.

Keywords

bisphenol-A; cancer; prostate; breast; mammary; uterus; estrogen receptor

Introduction

The incidence rates for breast and prostate cancers in the United States have progressively risen since 1975 [1]. This trend has been attributed to multiple factors including increased exposure to endocrine disrupting agents. In response to this claim, studies evaluating the impact of exposure to agents such as bisphenol A (BPA) on reproductive health and carcinogenesis have been conducted both supporting and contesting the contributions of BPA to tumor formation in various organs. Herein, we will evaluate the assessment of the carcinogenic activity of BPA

To Whom Correspondence Should Be Addressed: Gail S. Prins, Ph.D., Department of Urology, University of Illinois at Chicago, M/C 955 820 S. Wood, Chicago, IL 60612, (312)413-9766, FAX: (312)996-1291, g.prins@uic.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

that was completed by the National Toxicology Program (NTP) in the 1980s. Conclusions that can be drawn from that study and areas requiring additional research will be discussed. We will then consider the potential modes of action by which BPA may induce cancer or increase cancer susceptibility and evaluate the available experimental data supporting those modes. Our evaluations primarily focus on cancer studies using in vivo models since in vitro results of BPA action are separately analyzed in a companion review paper. It is noteworthy that route of exposure differs between studies with some experiments using oral exposures and others administering BPA through non-oral routes. Nonetheless, “low dose” BPA exposures via non-oral routes in most of the studies evaluated were administered at doses that result in circulating, *non-conjugated* BPA serum levels that are within the range reported for human *non-conjugated* BPA serum levels (see companion review on Human Exposures). Thus although route of exposure may vary, the final serum levels of free BPA are within comparable ranges. We close our review with suggestions for standardization of assays and provide a list of areas that warrant further research.

Assessment of Bisphenol A-induced Carcinogenicity in Adult Rodents

The NTP evaluated the carcinogenic activity of BPA using a chronic feed study of Fischer 344 rats (0, 1,000 and 2,000 ppm in feed) and B6C3 F1 hybrid mice (0, 5,000, and 10,000 ppm in feed) [2]. BPA was administered in the diet of males and females for 103 weeks beginning peripubertally (5 weeks of age). A conclusion from this study was that there is equivocal evidence for carcinogenicity in male rats and mice and in female rats (Table I). No evidence for carcinogenicity was found in female mice. However, the NTP report also noted that there were slight increases in hematological malignancies in rats and male mice. In addition, a significant increase in testicular interstitial cell tumors was observed in male rats as well as a trend for an increase in fibroadenomas, a benign tumor of the mammary gland (8% in high dose vs. 0% in controls). While the increase in testicular tumors was significant, the frequency of tumors in controls was lower than in historical controls (88%). Thus, it is unclear whether the difference in testicular tumor incidence was a reflection of an atypical control population or to a specific effect of BPA.

The NTP analysis suggested that BPA was not a robust carcinogen in the context of adult exposure. The U.S. Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) have used these studies, with a 1,000 fold reduction in dose to conclude that a daily dose of 50 µg/kg/day was safe for humans (www.epa.gov/iris/subst/0356.htm). However, several limitations of the NTP study precluded concluding that BPA is not carcinogenic. The use of a scaling factor by the FDA to assign a safe exposure limit assumes that BPA follows a monotonic dose response curve and that the high doses of BPA used in the NTP study would have revealed any carcinogenic effect. This is not an accurate assumption for endocrine disrupting agents that often display an inverted-U dose-response curve [3;4]. For example, the morphometric changes in the mammary gland induced by treating prepubertal CD-1 females with estradiol from post-natal days 25-35 follow a non-monotonic curve, *i.e.*, the greatest changes in these parameters occurred at intermediate doses, with higher doses being less effective [5]. Similarly, post-natal exposure of Sprague-Dawley male rats to low doses of estradiol benzoate led to an increase in prostate weight at post-natal day 35 while high doses caused a reduction in prostate weight [6]. Thus, analyses using environmentally relevant, low doses are necessary for assessing the carcinogenic impact of an endocrine disruptor such as BPA.

The NTP study used single strains of rats and mice whose relative susceptibility to carcinogenic events induced by BPA or other chemical carcinogens is unclear. Of note, the F1 hybrid mice that were utilized involved the C57BL/6 genetic background, which has been shown to be resistant to transgene-induced mammary carcinogenesis [7;8] and the promotion of

chemically-induced skin tumors [9]. In contrast, the Fischer rats that were used in this study have varying susceptibility to diverse tumors that is dependent upon both the inducing agent and tumor site [10-13]. Given the variations in tumor susceptibility observed among different strains of rodents, use of multiple strains of mice and rats may be more reflective of the genetic variability observed in humans.

In addition to the use of single strains of rodents, only peri-pubertal animals were used by the NTP. This type of analysis is restricted to identifying agents that are carcinogenic in mature or nearly mature organs. However, numerous rodent studies have shown that programming during fetal development affects tissue function later in life [14-17]. Much of this programming involves epigenetic modifications that control gene expression and disturbing the establishment of these “marks” during fetal development induces high rates of various types of cancers in mice [15]. With regard to the assessment of BPA-induced carcinogenesis, it has been established that developing organs can have an increased susceptibility to endocrine disruption [18]. One of the clearest examples involves diethylstilbestrol (DES). While adult exposure to diethylstilbestrol (DES) modestly increases the risk of breast cancer and mortality from this disease later in life (RR = 1.27-1.35 for risk of disease and mortality with DES exposure) [19-21], fetal exposure to this agent apparently increases the incidence breast cancer in adult women much more dramatically (RR = 3.0 after the age of 50 yr) [22]. Similarly, clear cell adenocarcinomas of the vagina, a very rare cancer, has been observed in patients exposed prenatally to DES, but not after adult exposure [19;23;24]. Given the estrogenic properties of BPA, a thorough analysis of its impact should include test subjects exposed during different developmental windows, including fetal, peri-natal, and pubertal periods.

Lastly, it is important to note that the cages used in the NTP study were made of polycarbonate, a BPA polymer, which is known source of environmental BPA exposure in laboratory rodents [25;26]. Thus, it is likely that control animals were also exposed to some level of BPA during this analysis. No assessments of circulating BPA were made in this study, leaving the question of whether these animals were accidentally and chronically exposed to BPA unanswered.

In summary, the NTP analysis of BPA carcinogenicity indicates that adult exposure to this compound may increase the incidence of hematological and testicular malignancies. Additional *in vivo* studies that incorporate the broad range of genetic and developmental variables that may occur with relevant human exposure are essential prior to concluding that this agent poses little carcinogenic risk to humans. In addition, the NTP study focused solely on determining if BPA is directly carcinogenic. Since recent studies, discussed below, indicate that BPA increases susceptibility to carcinogenic events [17;27], the possibility that low-dose exposures of this endocrine disruptor may act as initiators which require subsequent promoting events to induce carcinogenesis must be considered.

A more recent *in vivo* analysis performed by RTI International and sponsored by GE Plastics, Aristech Chemical Corp., Shell Chemical Co., Bayer Corp., and The Dow Chemical Co. utilized a three generational assessment of BPA-induced toxicity in Sprague-Dawley rats (Table I) [28]. Bisphenol A-free caging was used, the phytoestrogen content in food was monitored, and the impact of BPA exposure extending throughout gestation and into adulthood was evaluated. Although significant toxicity was observed at 50 and 500 mg/kg/day (750 and 7500 ppm in feed), multiple organ systems were evaluated and no increase in cancer was observed. Reproductive organs that were examined included epididymis, preputial gland, coagulating gland, pituitary, prostate, seminal vesicles, and testis. In addition, in the low dose range of 0.001-5 mg/kg-day (0.015-75 ppm in feed), no effects of BPA were observed for any other parameters measured.

While the RTI International report indicates that multigenerational exposure to BPA does not induce tumors, further analysis is warranted for several reasons. Firstly, this study utilized the Sprague-Dawley rat model. Using comparisons among various published reports, it has been determined that this strain is much less sensitive to endocrine disruption than other rodent models [4;29]. In addition, this study did not examine the mammary glands of females, a proposed target of BPA. Moreover, histological evaluation of the ovaries was not reported. It is also important to note that qualitative histological examination may overlook the presence of preneoplastic lesions and subtle alterations of tissue organization. Secondly, this work also focused solely on development of cancers in the absence of additional carcinogenic insults. As indicated above, recent reports have suggested that developmental exposure to BPA may regulate susceptibility to carcinogenic processes, possibly by developmentally reprogramming carcinogenic risk [17;27] a subject to be discussed in more detail below. Lastly, as discussed above, endocrine disrupting chemicals often do not follow a monotonic dose-response curve [3]. Thus, the lack of a carcinogenic effect of prenatal exposure to BPA in this study could be due to a number of factors including the selection of tissues analyzed, the lack of a carcinogenic challenge, and the animal model used.

Potential Carcinogenic Modes of Action of Bisphenol A

An accurate assessment of the carcinogenicity of BPA requires an understanding of its potential modes of action. The current literature supports four modes of action that may be interrelated. These include estrogenic endocrine disruption, promotion of tumorigenic progression, genotoxicity, and developmental reprogramming that increases susceptibility to other carcinogenic events. When considering potential carcinogenic modes of action of BPA *in vivo*, it is necessary to keep in mind that the circulating levels of unconjugated BPA that have been reported in human serum range from 0.2-20 ng/ml [29].

Evidence for Estrogenic Endocrine Disruption by Bisphenol A

A key question in evaluating the carcinogenic potential of BPA is its potential function as an estrogenic endocrine disruptor. Estradiol-17 β has been classified as a carcinogen by the International Agency for Research on Cancer [37;107;108]. Thus natural levels of estrogens in every male and female have the ability to be carcinogenic. For example, early menarche and late menopause are risk factors for breast cancer as a result of longer estrogen exposures. [108]. It is also well established that chronic exposure to elevated estrogens contributes to carcinogenesis of multiple reproductive organs. Prophylactic treatment of women with tamoxifen, a selective estrogen receptor modulator (SERM) that acts as an estrogen receptor (ER) antagonist in the breast reduces the incidence of breast cancer in patients at high risk of developing this disease [30;31]. In contrast, tamoxifen acts as an ER agonist in the endometrium and hence increases susceptibility to endometrial cancer [30;31]. As indicated above, DES exposure during fetal development also increases the risk of breast and vaginal cancers in women [22;23] and may increase the risk of testicular cancer in men [32]; the carcinogenic effects of DES have been confirmed in multiple rodent models [33;34]. In men, chronically elevated estrogens have also been associated with increased risk of prostate cancer [35]. In rodents, estrogens in combination with androgens induce prostate cancer [36;37], as well as increase the incidence of testicular tumors [38;39].

The relative binding affinity of BPA for either estrogen receptor (ER α and ER β) is ~10,000 lower than estradiol or diethylstilbestrol [40]. This low affinity is mirrored by the ~10,000 fold lower capacity for BPA to activate ER-dependent transcription when compared to estradiol dipropionate *in vitro* [41]. These data suggest that BPA has only modest estrogenic activity. However, using a luciferase reporter assay, Kuiper et al found that BPA is 50% as efficacious as estradiol in activating an estrogen responsive luciferase reporter when both compounds are used at the same high concentration of 1 μ M [40]. These studies as well as others indicate that,

although BPA may have a significantly lower potency than endogenous estrogens *in vitro*, it is a full agonist for both ER α and ER β [42-44]. *In vivo* analyses using rat and mouse uterine wet weight and vaginal cornification endpoints have been equivocal particularly due to a lack of monotonic dose-response curve [45-49]. However, when dose-response covering a wide range of doses (several orders of magnitude) is assayed, BPA behaves as an ER agonist, albeit at high doses (100 mg/kg body weight/day, s.c. implants) [47]. While this has been interpreted as indicating low estrogenic potency of BPA *in vivo*, uterine wet weight may not faithfully report estrogen receptor activation within the uterus [47;50]. BPA (0.8 mg/kg body weight, single s.c. injection) induces estrogen receptor activity in the uterus of ovariectomized transgenic mice harboring an ER activated reporter gene [50]. BPA exposure also increases the height of the luminal epithelium in the immature mouse uterus (5 mg/kg body weight/day, s.c. implant) as well as expression of lactoferrin (75 mg/kg body weight/day), an established estrogen-responsive gene [47]. Transplacental estrogenic actions of BPA have been further demonstrated in a transgenic model that reports ER activity. In this case, 1-10 mg/kg body weight BPA, administered intraperitoneally to pregnant dams at 13.5 dpc, resulted in rapid accumulation of luciferase reporter activity in embryos that were isolated from their associated amniotic membranes [41]. While the specific tissues capable of activating the estrogen-responsive reporter gene were not determined, these data indicate that BPA stimulates the transcriptional activity of the estrogen receptor in fetuses. They also reveal that BPA crosses the placenta in rodents, resulting in activation of ER-dependent transcription within embryos. Transplacental movement of BPA also occurs in humans as evidenced by detectable BPA in amniotic fluid and fetal serum (0.2-9.2 ng/ml) [51-53]. Cancer susceptibility in humans and rodents has been linked to fetal exposure to pharmacologic doses of estrogens [22;23;34;54; 55]. Hence, assessments of the carcinogenic impact of BPA should include experimental paradigms involving fetal exposure. This is discussed in more detail below.

Bisphenol A has also been reported to induce non-genomic effects of ER *in vitro* with an EC₅₀ that is comparable to that of estradiol [56;57]. These data suggest that estrogenic activity of BPA *in vivo* may be due to non-genomic activation of ER. This possibility is more difficult to assess *in vivo*, but studies aimed at evaluating the potential non-genomic actions of BPA may yield important information regarding the mechanism of action of this xenoestrogen.

Evidence for Promotion of Tumorigenic Progression and Inhibition of Therapeutic Efficacy by Bisphenol A

Significant morbidity associated with prostate cancer has been attributed to a lack of therapies to control recurrent tumors. While locally-confined prostatic adenocarcinomas are effectively managed through prostatectomy or radiation therapy, non-organ confined disease is treated using therapies that block the action of the androgen receptor (AR). This modality is the first line of treatment, as prostate tumors require AR for survival and proliferation yet, due to their indolent nature are resistant to standard cytotoxic therapies. The goal of intervention is to block AR activity, either through prevention of ligand (androgen) synthesis or through the use of direct AR antagonists. This therapy is initially effective, and the majority of tumors undergo remission. However, recurrent tumors ultimately arise, wherein the AR has been inappropriately re-activated. Strikingly, there is no effective treatment for recurrent tumors, which lead to patient death. As such, there is a significant need to identify the factors that contribute to AR re-activation and recurrent tumor formation. Multiple mechanisms for AR re-activation have been identified, including: i) AR amplification; ii) overexpression of AR co-activators; iii) ligand-independent AR activation, or iv) gain-of-function AR mutations. The competence of these mechanisms to initiate tumor recurrence is strongly responsive to factors that enhance AR signaling, and data in *in vitro* and xenograft systems demonstrate that BPA promotes tumor progression in tumor cells harboring specific AR mutations.

Mutation of AR is believed to represent a significant mechanism by which tumor cells evade therapeutic intervention. Wetherill, *et al* observed that low levels (1 nM) of BPA activate the most common tumor-derived mutant of AR (AR-T877A) in transcriptional assays; however, in parallel experiments BPA had no impact on the wild type AR (wtAR) [58;59], thus indicating that the gain-of-function mutant had attained the ability to utilize BPA as an agonist. Comprehensive study revealed that BPA-mediated activation of AR-T877A led to unscheduled cell cycle progression and cellular proliferation in the absence of androgen [59]. These data had significant implications, as factors which activate AR-T877A are known to facilitate therapeutic relapse during tumor management. *In vivo* analyses of the impact of BPA on human prostate tumor growth and recurrence was performed utilizing a xenograft model (Table 1) [60]. Tumor size increased in response to BPA administration (12.5 mg, 21 day release, subcutaneous implants) as compared to placebo control and mice in the BPA cohort demonstrated an earlier rise in PSA (biochemical failure), indicating that BPA significantly shortened the time to therapeutic relapse [60]. Analyses of BPA levels within the sera of these mice (quantified by mass spectrometry) showed that BPA reached 27 ng/ml on day 7 post-implantation and were undetectable by day 35. Thus, the ability of BPA to promote AR-T877A activity and tumor growth *in vivo* occurred at low doses that fall within the reported ranges of human exposure. These outcomes underscore the need for further study of the effects of BPA on tumor progression and therapeutic efficacy.

Evidence for Genotoxicity induced by Bisphenol A

A widely accepted model of carcinogenesis states that progressive accumulation of genetic alterations ultimately conveys a growth advantage over normal cells (somatic mutation theory) [61;62]. An alternative model points to a fetal origin of disease where changes in the epigenome play a central role in carcinogenesis [15;63;64]. Both the genetic and epigenetic theories of carcinogenesis imply that cancer originates in a cell that has undergone genetic and/or epigenetic changes, which ultimately result in dysregulated growth. These two views of carcinogenesis are not mutually exclusive. The third model, the tissue organization field theory, postulates that cancers arise due to disruptions in tissue organization [65]. According to this theory, carcinogens as well as teratogens, would disrupt the normal dynamic interaction between neighboring cells and tissues during early development and throughout adulthood. From this perspective, mutations would not be necessary for neoplastic development. In this section, we consider the role of BPA in inducing genetic mutations.

Classical carcinogens induce a variety of genetic changes that can range from accumulation of point mutations by direct nucleotide alterations to chromosomal copy number changes due to defects in spindle assembly and/or non-disjunction of chromosomes during mitosis. Thus, consideration of BPA as a carcinogen warrants assessment of its genotoxic capabilities. Admittedly, most studies evaluating BPA-induced genetic alterations have involved *in vitro* assays, many of which have reported a lack of mutagenic activity [66-68]. However, some *in vitro* assays have reported genotoxic activity that correlates with morphological transformation [69] or aneuploidy in oocytes [70] and *in vivo* analyses have also indicated that BPA can induce aneuploidy in germ cells following embryonic exposure of C57BL/6 female mice to BPA via the maternal circulation beginning on 11.5 dpc [26;71].

Bisphenol A has been evaluated in standard screens for mutagenicity including the Ames test, the mouse lymphoma mutagenesis assay (MOLY), sister chromatid exchange (SCE) induction, chromosomal aberration (ABS) analysis, and mammalian gene mutation assay (V79/HPRT). Most of these analyses have indicated that BPA is not mutagenic [66;67]. For example, although BPA was highly toxic in both the Ames and V79 assays, no mutagenic activity was detected when assessed in the non-toxic range (0.1 mM for V79) for either assay [68]. Although

the majority of studies have suggested that BPA is not mutagenic, some reports have indicated that BPA can induce point mutations, double stranded DNA breaks, and aneuploidy.

Bisphenol A treatment has been shown to induce DNA adduct formation at all doses examined (50-200 μ M) in Syrian Hamster Embryo (SHE) cells [69]. Although treatment with 100-200 μ M BPA induced significant toxicity, 50 μ M BPA was generally well tolerated. Thus, DNA adduct formation could occur in the absence of overt toxicity to these cells. The formation of adducts was also correlated with the generation of aneuploidy and morphological transformation. The direct impact, however, of adduct formation on specific gene function and whether intrinsic DNA repair mechanisms can ultimately repair these adducts remains unknown. Two specific genes were assessed for alterations in nucleotide sequence and BPA did not induce changes in either, calling into question the significance of adduct formation [69]. It should also be noted that the doses utilized in these studies (11-44 μ g/ml) are ~ 1 - 10×10^3 fold higher than the reported levels in human serum (0.2-20 ng/ml) [53;72-74].

While specific mutations were not detected in the analysis of BPA effects on SHE cells, BPA has been shown to induce K-ras mutations in RSa transformed human embryo fibroblast cells and mutations that lead to ouabain resistance at concentrations of 10^{-7} - 10^{-5} M [75]. These changes were correlated with an increase in unscheduled DNA synthesis, reflecting DNA repair. As observed in other studies, BPA did not follow a linear dose-response curve. Taken together, these data indicate that BPA can induce mutations that may ultimately lead to cell transformation although use of RSa transformed cells precludes a direct analysis of transforming abilities of BPA. An unanswered question is the mechanism(s) underlying BPA-induced point mutations. One possibility is the formation of quinone derivatives of BPA [76], that form DNA adducts. As described above, DNA adducts have been observed in SHE cells treated with BPA. BPA also induces DNA adducts *in vivo* in CD1 male rat livers when given as a single dose of 200 mg/kg [77]. While DNA adduct formation has been observed in several studies, the ability of environmentally relevant doses of BPA to induce such adducts remains to be determined.

In addition to quinone derivatives, Cherry and colleagues have shown that BPA can react with sodium nitrite under acidic conditions to form nitrosylated BPA [67]. It is anticipated that such compounds could be formed following migration of BPA from packaging material into foods with high nitrite concentrations or following ingestion of nitrite containing foods that also contain BPA. Nitrosylated BPA is an electrophilic compound that produces a variety of mutations in the Ames II test at 100 μ g/plate), including both transition and transversion point mutations and frameshifts. Mutagenic activity was observed, *in vitro*, in the presence and absence of rat liver S9 fractions, indicating that metabolic activation is not required. Although these studies indicate that nitrosylated BPA has mutagenic activity, the degree of human exposure to this compound is unknown. In addition, the mutagenic activity of nitrosylated BPA is significantly less than that of well-established mutagens such as benzo[a]pyrene or 2-aminoanthracene [67].

Bisphenol A has also been shown to induce DNA double strand breaks in MCF-7 breast cancer cells [78]. Although the extent of DNA breaks induced by estradiol and BPA were similar, BPA was approximately 1,000 fold less efficient than estradiol. Doses of 10^{-6} - 10^{-4} M BPA were required to observe changes in DNA integrity that were assessed by Comet assays and accumulation of γ H2AX in foci. In contrast, double strand breaks were observed at a dose of 10^{-7} M estradiol, a pharmacological concentration that exceeds the dose required from maximal induction of proliferation by three orders of magnitude. This difference in efficacy may be due to the lower affinity of BPA than estradiol for binding to ER. Indeed, pretreatment of these cells with the pure anti-estrogen, ICI182780, prevents the induction of double stranded breaks, indicating that the accumulation of such damage requires activation of ER. Furthermore, the

extent of double strand breaks induced by BPA or estradiol was significantly reduced in ER negative breast cancer cells (MDA-MB-231), further suggesting a requirement for ER to convey this DNA damaging property of BPA and estradiol [78]. While this study suggests that BPA can induce double strand breaks in DNA, it is important to note that the doses required are much higher than the levels of human exposure. Thus, its relevance to potential carcinogenic mechanisms of BPA is currently unclear.

A common genetic alteration in cancer is aneuploidy. Several *in vitro* studies have shown that BPA treatment of Chinese hamster ovary cells (CHO) induces chromosomal aberrations. However, these reports indicate that clastogenic activity is correlated with cytotoxicity [79; 80]. It is currently unclear whether the chromosomal changes observed in these cells are a direct result of cytotoxicity or if cytotoxicity and aneuploidy are independent but coincidentally occur at similar doses. BPA has also been reported to induce aneuploidy in SHE cells *in vitro* [81]. Bisphenol A reduced the accumulation of SHE cells in a dose dependent manner from 50-200 μM ; 200 μM induced a complete blockade in the accumulation of cells that may reflect either an arrest in proliferation or cytotoxicity [69;81]. In contrast, cells treated with 50-100 μM BPA experienced chromosome losses and gains that correlated with morphological cellular transformation. Similar data were obtained for additional bisphenols (50-100 μM), including BP3, BP4, and BP5 which are components of pesticides, polycarbonate resins, and rubber bridging material, respectively [81]. These data indicate that BPA induces aneuploid changes in a subset of SHE cells (6-11%) in the absence of overt toxicity.

Although the ability of BPA to induce somatic cell aneuploidy has not been assessed in intact animals, BPA-induced meiotic aneuploidy has been shown in mice (Table I) [26;82]. These data stemmed from the accidental exposure of female C57BL/6 mice to BPA from damaged polycarbonate caging and an associated increase in aneuploidy in concurrent control animals compared to historical controls. Oral doses were estimated to be in the range of 14-72 $\mu\text{g/kg/day}$. Subsequent direct demonstration of the induction of meiotic aneuploidy involved daily oral dosing of juvenile females with 20-100 $\mu\text{g/kg}$ BPA for 1 week prior to collection of oocytes. All doses increased the incidence of meiotic defects, with the highest dose causing chromosome misalignment in ~11% of metaphase II-arrested oocytes, compared to ~2% in controls. Exposure of isolated oocytes to BPA (10 μM or 2.3 $\mu\text{g/ml}$) *in vitro* induced a delay in cell cycle progression and disruption of spindle function due to interference with microtubule and centrosome dynamics [70]. Similar disturbances in microtubule dynamics have been observed in somatic cells *in vitro*. Treatment of CHO V79 cells with 100 μM (23 $\mu\text{g/ml}$) BPA resulted in a ~12 fold increase in cells with tripolar and multipolar spindles within 6 hours [83]. In this latter report, the concentration of BPA used also inhibited metabolic activity by 30-40%, thus, the direct impact of BPA on microtubule activity vs. generalized toxicity is an area that requires further study.

More recent meiotic studies have examined the effect of BPA exposure *in utero*. Exposure of pregnant C57BL/6 mice (20 $\mu\text{g/kg/day}$, s.c. implant) beginning on embryonic day 11.5 was found to influence the earliest events of oocyte development in the female fetuses, disrupting the synapsis and recombination between homologous chromosomes. These defects during fetal development were translated into a dramatic increase in aneuploid oocytes and embryos in adult females that were previously exposed to BPA *in utero* [71]. Phenotypically identical defects in early oocyte development were observed in untreated ER β but not ER α deficient mice [71], suggesting that the actions of BPA in the fetal ovary are mediated by ER β . Together, these studies suggest an entirely new mechanism of aneuploidy induction and, although the relevance in somatic cells is currently unknown, these findings are important because they suggest that at least some of the effects of BPA on meiosis appear to involve direct actions of this agent on oocytes.

The studies described above have assessed the ability of BPA to act as an aneugen. Although carcinogens are classically considered to act in this manner, it is important to reinforce the concept that agents that induce epigenetic changes or alterations in tissue organization may also induce cancer without directly inducing changes in genomic sequence. The potential for BPA to induce cancers through these mechanisms is discussed below.

Evidence for Developmental Reprogramming by Bisphenol A that Alters Cancer Susceptibility

Components of numerous adult diseases, including cancer, may have their origins during fetal development when tissue architecture and homeostasis is established [63;84-86]. This is particularly evident for endocrine disruption which can lead to dysfunction of multiple target organs of sex steroids [87-90]. Of particular relevance for BPA are studies examining cancer risk following prenatal exposure to estrogens. As indicated above, women that were prenatally exposed to DES have an increased risk for breast and vaginal cancers [22;23]. Similarly, early postnatal DES treatment of CD-1 mice during days 1-5 of life resulted in a dose dependent increase in uterine cancers [91]. Thus, exposure to exogenous estrogens during fetal and/or neonatal life can increase the incidence of various cancers. Consequently, it is imperative that the potential carcinogenic activity of BPA, an estrogenic compound, be assessed using fetal and early postnatal exposure protocols. This is further underscored by the clear presence of BPA in the plasma of newborn humans (0.2-9.2 ng/ml) [53;74].

The ability of BPA to influence cancer risk has been evaluated in three target organs: prostate, mammary gland, and uterus. These tissues have been the focus of significant attention because earlier studies have indicated that prenatal exposure to BPA as well as other estrogenic compounds led to altered morphology of sex steroid target organs. In fetal CD-1 mice, estrogen responsiveness of the prostate followed an inverted-U shaped curve: fetal exposure to low doses of estradiol or DES resulted in enlarged prostates in adults while high doses reduced prostate size [88]. However, a dose-response study using neonatal estradiol exposures in Fischer and Sprague-Dawley rats did not find a permanent low-dose effect on prostate size [6], indicating that timing of perinatal exposure as well as species and strain may be confounding variables. Fetal BPA exposure in CD-1 mice (via 10 µg/kg/day oral dosing to pregnant dams on days 14-18 of gestation) resulted in an increase in the number, size, and proliferative index of dorsolateral prostate ducts, an increase in prostate volume, and malformation of the urethra were observed [92]. In contrast, other large studies aimed at examining the impact of BPA on the prostate were unable to identify a significant change following BPA exposure using Sprague-Dawley rats [28], Fischer 344 rats [93], or CF-1 mice [94], leading to significant controversy on this topic [4;95-98]. Further, although prostate size changed in some studies following BPA exposure, it is unclear whether such alterations are linked to an increase in cancer susceptibility. In an evaluation of published studies comparing changes in prostate weight with pathology, Milman and colleagues reported that enlargement of the prostate failed to correlate with subsequent development of cancer in rodents [96]. This might be due to the frequent increase in glandular activity that can occur in the prostate, generating an increase in size, without corresponding pathology. Hence, studies that are limited to assessing changes in prostate weight in rodents without evidence of preneoplastic histopathological changes are inadequate for inferring subsequent carcinogenic activity. Importantly, vom Saal and colleagues have shown that prenatal exposure of CD-1 male mice to BPA (10 µg/kg/day administered orally to pregnant dams from days 14-18 of gestation) resulted in an increase in the proliferative rate of basal epithelial cells in the primary dorsolateral prostate ducts on embryonic day 19 [92]. The basal epithelial cell layer of the prostate has been postulated to be a source of prostate cancer stem cells [99]. According to the somatic mutation theory of carcinogenesis, an increase in the proliferative rate of these cells would support the hypothesis that BPA may increase prostate cancer susceptibility.

Two studies have directly addressed the carcinogenic impact of developmental exposure to BPA on prostate carcinogenesis. Both have examined whether BPA induces cancer on its own, or if it increases susceptibility to tumorigenic stimuli. In F344 rats, a dose range of 0.05-120 mg/kg/day was given orally to pregnant dams beginning upon confirmation of pregnancy and throughout lactation (3 weeks) [100]. The male progeny were then treated with the carcinogen 3,2'-dimethyl-4-aminophenyl (DMAB) or vehicle beginning at 5 weeks of age and ending after 60 weeks. Anterior, ventral, and dorsolateral prostate lobes were assessed for intraepithelial neoplasias (PIN) and carcinomas. While DMAB induced tumors in all mice exposed to this agent, there was no statistically significant change in the incidence of PIN or carcinomas in these rats regardless of the dose of BPA utilized (Table I).

While the above study suggests that fetal BPA exposure does not change carcinogenic risk, another analysis utilizing a different rat tumor model indicated that neonatal exposure to BPA does increase tumor susceptibility. In this case, male Sprague-Dawley pups were given subcutaneous injections of BPA (10 µg/kg) or vehicle on post-natal days 1, 3, and 5. As a positive control for exposure to an estrogenic compound, 17β-estradiol 3-benzoate (EB, 0.1 and 2,500 µg/kg) was administered to additional sets of animals. At 90 days of age, a carcinogenesis regimen was initiated where half of the animals were chronically exposed to estradiol and testosterone using silastic implants while the other half received an empty implant for 16 weeks. These doses of estradiol and testosterone in adult Sprague-Dawley rats result in serum estradiol and testosterone levels of 75 pg/ml and 3 ng/ml, respectively, thus modeling the relative increase in serum estradiol levels in aging men. This hormonal treatment was selected because it is an established approach to induce PIN in 100% of Noble rats, but only 35% of Sprague-Dawley rats [37]. The investigators sought to determine if developmental BPA or estradiol exposure could augment PIN incidence in the Sprague-Dawley model. Although high dose EB resulted in high grade PIN, BPA did not induce PIN in aged rats that had not received supplemental sex steroids. However, both high dose EB and exposure to BPA significantly increased the incidence of PIN to 100% under chronic hormonal stimulation (Table I). Lesions frequently contained areas of severe nuclear atypia, cellular piling, and adenocarcinomas that were accompanied by increased proliferative and apoptotic rates. These data support the hypothesis that early exposure to estrogenic compounds can increase sensitivity to the carcinogenic activity of sex steroids, principally estradiol. As discussed below, estrogen sensitivity of the mammary gland also appears to be increased with perinatal exposure to BPA [90], suggesting that specific developmental windows of BPA exposure can reprogram the degree of estrogen responsiveness of an adult tissue. The mechanism underlying this reprogramming remains unclear; however, the BPA-induced change in prostate responsiveness is associated with a number of epigenetic changes including alterations of the phosphodiesterase type 4 variant 4 locus, which encodes an enzyme responsible for cAMP breakdown. While this gene normally becomes methylated and is silenced with age in the prostate, early life BPA exposure prevents this process and leads to inappropriate expression of this gene in aged animals [17]. Whether these changes directly contribute to regulating estrogen responsiveness requires further study.

The two *in vivo* analyses of BPA-regulated prostate carcinogenesis discussed above culminated in distinct results. The analysis reported by Ichihara, *et al.* found no increase in risk with BPA exposure [100], while the work of Ho, Prins, and colleagues reported a ~2 fold increase in PIN incidence [17]. Unfortunately, these are the only two studies that have specifically addressed the impact of developmental exposure to BPA on prostate cancer risk. The conflicting results of these two reports may be attributed to differences in the experimental protocols. These include developmental age at the time of exposure (fetal vs. neonatal), strain of rat used (F344 vs. Sprague-Dawley), carcinogenic insult (DMAB vs. estradiol + testosterone), housing conditions (unknown vs. environmental estrogen exposure clearly defined), and dosage (high dose vs. low dose). These differences underscore the need for further studies evaluating the

specific experimental parameters that may reveal an ability of BPA to dictate fundamental changes in tissue susceptibility to carcinogenic events. Such studies will be essential for future extrapolations to human risk.

Similar to the prostate, early exposure of the mammary gland to an altered estrogenic environment has been shown to induce fundamental changes in the homeostasis of the gland. Diethylstilbestrol treatment of neonatal mice caused accelerated ductal outgrowth during puberty and led to inappropriate alveolar development in adult virgin females [101]. Furthermore, prenatal exposure of rats to DES shortened the latency and increased the frequency of 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary cancers in adults [33;55]. These data indicated that developmental exposure to estrogenic compounds can program susceptibility of the mammary gland to subsequent tumorigenic events.

Perinatal exposure of female CD-1 mice to BPA (osmotic pump delivery of 25 or 250 ng/kg/day to pregnant dams from day 9 of gestation throughout post-natal day 4) induced a variety of changes in the mammary gland that were manifested during postnatal life [90]. Such changes included an increase in terminal end bud number and size and more rapid ductal growth during puberty. Factors that may have contributed to these morphological changes include a decrease in apoptotic rate and an increase in progesterone receptor expression. In addition, the mammary glands of BPA-exposed mice responded more robustly to exogenous estradiol by producing an even greater increase in terminal end buds (TEB) compared to their control counterparts. In adult animals, an increase in the number of side branches and inappropriate development of alveoli in virgin mice was also observed following perinatal BPA exposure when compared to glands from mice that had only received vehicle [89;90]. The mechanism underlying these sustained developmental changes that occur long after exposure to BPA is unknown but may involve structural and functional reorganization of the tissue during the stage of developmental plasticity, referred to as developmental reprogramming. Supporting this notion, changes in the mammary gland have been observed on embryonic day 18, where BPA (subcutaneous dosing of pregnant dams with 250 ng/kg/day during embryonic days 8-18) induced early maturation of the mammary fat pad and increased ductal area of the fetal mammary gland. However, the epithelial cells within a BPA exposed mammary gland rudiment were smaller and more tightly packed than their control counterparts. The mammary epithelium also displayed a significant reduction in apoptotic rates that led to a delay in lumen formation within the developing gland. In addition to changes in epithelial cells, the stroma immediately surrounding the gland contained less collagen, suggesting that stromal changes, may also be involved in developmentally reprogramming the mammary gland following BPA exposure [102]. Moreover, estrogen receptors at this age are predominantly expressed in stromal cells, suggesting that the stroma may be a primary and direct target of BPA. Testing this hypothesis will require mammary gland transplantation/tissue recombination studies.

As was discussed for changes in prostate architecture, BPA-induced changes in the mammary gland can not be directly translated to a change in carcinogenic susceptibility. Rather, a direct evaluation of the tumor-inducing capacity of BPA is required. Two very recent reports have shown that perinatal exposure to BPA does increase mammary tumor formation in rats. Wistar-Furth rats were exposed perinatally (subcutaneous delivery by a mini-osmotic pump implanted in pregnant dams beginning on embryonic day 9) to doses of BPA ranging from 2.5 to 1,000 µg/kg/day and the development of neoplastic lesions assessed histologically at postnatal day (PND) 50 and 95 [103] (Table I). The lowest dose utilized (2.5 µg/kg/day) resulted in a 3-4 fold increase in the number of hyperplastic ducts compared to vehicle treated animals. Most importantly, these lesions appeared to progress to carcinoma *in situ* as defined by an increase in duct size due to proliferation of luminal epithelium, formation of trabecular or secondary luminal structures, and nuclear atypia. Cells in these lesions expressed elevated levels of estrogen receptors suggesting that they may retain responsiveness to estrogen input. Whether

such preneoplastic lesions ultimately progress to invasive and metastatic cancers remains to be determined. In a companion analysis, Wistar rats were treated with 25 µg/kg/day using the same dosing protocol [27]. Again, this treatment resulted in the development of an increased number of hyperplastic ducts (~2 fold) compared to glands from vehicle treated rats. In addition, the glands had accumulated a dense stroma around the mammary epithelium that was reminiscent of a desmoplastic reaction. This study also evaluated the tumorigenic susceptibility of the mammary gland following developmental exposure to BPA. In this case, adolescent rats who had been prenatally exposed to BPA or vehicle were treated with subcarcinogenic doses of the known mammary carcinogen, N-nitroso-N-methylurea (NMU), and development of mammary lesions histologically assessed at PND 110 or 180. Tumors were observed in 20% of rats treated with BPA followed with NMU, whereas rats exposed only to NMU failed to form any tumors. Together, these reports indicate that perinatal exposure to BPA results in the formation of an adult mammary gland that has an increased susceptibility to tumorigenic insults that may either be spontaneous in nature or the result of a carcinogenic challenge.

Thus far, analysis of the potential of prenatal BPA exposure to increase uterine cancer susceptibility has been examined in only one study. Given the estrogenic activity of BPA, and the ability of early life exposure to DES to induce uterine cancers in mice [91], and vaginal cancers in women [24], it is expected that BPA would increase the risk of reproductive tract cancers. Thus, it is somewhat surprising that fetal and post-natal exposure of Donryu rats to BPA (0.006 and 6 mg/kg/day by oral gavage) did not increase the frequency of N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)-induced uterine cancers (Table I) [104]. These data suggest that BPA may not alter uterine cancer susceptibility. However, multiple methodological details of this study raise concerns about the conclusions drawn. Donryu rats were used in this analysis because they develop spontaneous uterine adenocarcinomas with a high incidence that corresponds with an age-dependent hormonal imbalance [105]. The high incidence of preneoplastic and neoplastic lesions in the uterus of these rats (~88%) [104], may limit the ability to detect significant increases induced by endocrine disruption. While previous studies indicated that treatment of this strain of rats with p-t-octylphenol accelerates development of uterine adenocarcinomas, this did not involve changes in female reproductive tract development or estrous cyclicity [106], two established outcomes of developmental estrogenic endocrine disruption. In the evaluation of the effects of BPA on uterine cancer susceptibility, BPA treatment also failed to impact the timing of vaginal opening or estrous cycling and a positive control for estrogenic endocrine disruption was not included [104]. Thus, it is unclear if the Donryu rat model is susceptible to uterine changes in response to early exposure to a known estrogen. This concern is further underscored by the detection of BPA in the sera of control, vehicle-treated rats. Further analysis of the drinking water and food used in this study revealed the presence of BPA in all samples indicating that all animals had been exposed to this compound [104]. As a result, this study can not be used to conclude that fetal and post-natal exposure to BPA does not impact uterine cancer susceptibility.

Conclusions

Based on existing evidence, we are **confident** of the following:

1. Natural estradiol-17β is a carcinogen as classified by the International Agency for Research on Cancer [37;107;108].
2. BPA acts as an endocrine disruptor with some estrogenic properties among other hormonal activities.

Based on existing evidence, we believe the following to be **likely** but requiring more evidence:

1. BPA may be associated with increased cancers of the hematopoietic system and significant increases in interstitial-cell tumors of the testes.

2. BPA alters microtubule function and can induce aneuploidy in some cells and tissues.
3. Early life exposure to BPA may induce or predispose to pre-neoplastic lesions of the mammary gland and prostate gland in adult life.
4. Prenatal exposure to diverse and environmentally relevant doses of BPA alters mammary gland development in mice, increasing endpoints that are considered markers of breast cancer risk in humans.

Based on existing evidence, the following are **possible**:

1. BPA may induce *in vitro* cellular transformation.
2. In advanced prostate cancers with androgen receptor mutations, BPA may promote tumor progression and reduce time to recurrence.

Summary and Recommendations for Future Studies

Due to the paucity of the current literature, it is premature to conclude that BPA is carcinogenic on its own. However, the weight of evidence suggests that BPA increases cancer susceptibility through developmental reprogramming, potentially involving changes in target organ morphogenesis as a result of epigenetic alterations (epigenetic changes to DNA and morphogenetic mechanisms involving tissue interactions). It is important to underscore that studies examining changes in carcinogenic susceptibility have only focused on the mammary and prostate glands, two obvious targets of potential endocrine disruption. In addition to identifying the mechanisms underlying BPA-induced risk in these tissues, further analysis of other estrogen targets is necessary including the vagina, uterus, ovary, and testes.

Another take home message from the evaluation of current data available on BPA and cancer risk is the lack of consistent practices among groups evaluating this compound. A degree of variability is necessary for translation of information gained from rodent studies to humans, however, there are some areas where consistent experimental design is necessary. These include the use of environmentally relevant doses of BPA. Ideally, these doses would be given orally, the main exposure route for humans. When analyzing the impact of fetal exposure to BPA, the most important factor is the levels of BPA within the maternal circulation. For example, a single s.c. injection of pregnant CD-1 mice on gestational day 17 with 20-23 µg/kg BPA results in a fetal level of unconjugated parent compound of ~4 ng/g tissue, 30 minutes after exposure and declines to ~0.1 ng/g by 24 hours [52]. This is comparable to the levels of unconjugated BPA observed in human fetal serum at the time of parturition (0.2-9 ng/ml) [53]. Further, studies examining human fetal exposure suggest chronic, long-term, steady state transmission of BPA from mother to fetus [29]. These data support the use of subcutaneous implants that generate continuous, low dose levels of BPA during fetal development. Thus, the availability of such exposure data correlating levels of parental BPA in mice with that observed in humans supports the use of subcutaneous routes of administration for assessments of BPA-induced fetal programming that ultimately increases cancer susceptibility. If alternative routes are utilized, measurement of exposure levels in the test animal is necessary to confirm that they are indeed comparable to human exposure. This is of particular concern because the route of administration will dictate whether BPA can undergo first pass metabolism by hepatic cytochrome P450 enzymes. In addition, the metabolic enzymes involved in BPA clearance may be distinct among species, further emphasizing the need for accurate assessment of BPA levels following administration. Doses should also be reported in a consistent manner. Specifically, the mass of BPA given to an animal should be expressed relative to its body weight and the dosing interval should be included. The serum levels of BPA in humans and the short half-life of this agent suggest that humans are chronically exposed rather than

ingesting a single daily bolus [29]. Thus, approaches that simulate this exposure pattern should be considered.

In addition to the route of administration, it is imperative that consistent environmental conditions be used to assess BPA impact on carcinogenesis in laboratory animals [98]. Specifically, care should be taken to minimize exposure to polycarbonate caging and plastic water bottles, an established source of BPA [25]. Phytoestrogen content of food should be made negligible and the estrogenicity of bedding should also be examined [98].

Choice of model organism is a key variable in studies examining BPA. Multiple strains of rats and mice have been utilized. While use of a single strain of mouse and/or rats would likely generate more consistent data, a central question is which strains are the most appropriate? In some cases a strain examined may be particularly resistant to a variety of carcinogenic stimuli or insensitive to endocrine disruption. Thus, a negative result in such strains may not be particularly informative or necessarily translatable to human susceptibility. It is important to keep in mind that each mouse within an inbred strain is essentially a clone of every other mouse. The use of inbred strains would reduce variability; however, it would also reduce the ability to translate the work to the vast genetic variability in the human population. While not identical, outbred strains are highly similar, generating the same problem. To circumvent this issue, it is recommended that multiple strains of mice and rats continue to be utilized. Of utmost importance, species and strain choice should be dictated by susceptibility to endocrine disruption by positive controls such as DES rather than convenience or familiarity [109]. If BPA contributes to a select type of cancer in only one strain of mouse, this would suggest that the genetic background of this strain includes modifiers that may contribute to tumor susceptibility. While still an unproven concept, identification of such modifiers may lend insight into the genetic basis of human susceptibility. A more effective approach for identifying candidate pathways involved in BPA-induced carcinogenesis may utilize comparisons of BPA-induced changes with phenotypes observed in genetically engineered mice that overexpress a candidate protein or which have a targeted disruption of a specific allele.

Lastly, the requirement for appropriate controls should be emphasized. Studies that conclude that BPA has no impact on tumor susceptibility require controls where an estrogenic compound does increase susceptibility. If such controls are not included, it would be impossible to conclude that the study was adequately designed and/or executed to reveal a tumorigenic action, if present.

The following issues require more studies:

1. Does BPA exposure induce or promote cancers in mammary and prostate? What is its mode of action?
2. Does BPA increase cancer susceptibility in estrogen-target organs (prostate, mammary gland, uterus, vagina, testis, ovary, etc.)?
3. Does BPA reprogram target tissues during development through epigenetic mechanisms, including epigenetic marking of genes and morphogenetic processes involving tissue interactions?
4. What are the most appropriate life stages for examining BPA-induced cancer susceptibility?
5. Under what conditions might BPA promote DNA and/or microtubule aberrations?
6. Identify biological consequence of long term, low dose exposure on genomic integrity, cooperation with oncogenic insult and tumor management.

7. Development of carcinogenesis paradigms with relevance to humans for assessing the ability of BPA to alter cancer risk.
8. What species/strains are the most appropriate for assessing BPA-induced cancer susceptibility?
9. Developing three dimensional culture models to assess the mechanisms involved in altered morphogenesis of the target organs that may lead to neoplastic development.
10. Epidemiology studies and development of new methodologies to evaluate BPA-cancer risks in humans.
11. Development of markers for total xenoestrogen insult in humans.

Acknowledgements

This work was supported by the following grants from the NIH: ES015768 (to RAK), DK40890 (to GSP), ES12282 (to GSP and SMH), ES013071 (to SMH), ES013527 (to PH), CA93404 (to KEK), ES012301, ES08314 and ES015182 (to AMS).

Reference List

1. Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ, Thun MJ. Cancer Statistics, 2004. *CA Cancer J Clin* 2006;54:8–29. [PubMed: 14974761]
2. NTP. Carcinogenesis Bioassay of Bisphenol A (CAS No. 80-05-7) in F344 rats and B6C3F1 mice (feed study). National Toxicology Program Technical Report 1982;215:1–116.
3. Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ Health Perspect* 2003;111:994–1006. [PubMed: 12826473]
4. vom Saal FS, Hughes C. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. *Environ Health Perspect* 2005;113:926–933. [PubMed: 16079060]
5. Vandenberg LN, Wadia PR, Schaeberle CM, Rubin BS, Sonnenschein C, Soto AM. The mammary gland response to estradiol: monotonic at the cellular level, non-monotonic at the tissue-level of organization. *J Steroid Biochem Mol Biol* 2006;101:263–274. [PubMed: 17010603]
6. Putz O, Schwartz CB, Kim S, LeBlanc GA, Cooper RL, Prins GS. Neonatal low- and high-dose exposure to estradiol benzoate in the male rat: I. effects on the prostate gland. *Biol Reprod* 2001;65:1496–1505. [PubMed: 11673267]
7. Lifsted T, Le Voyer T, Williams M, Muller W, Klein-Szanto A, Buetow KH, Hunter KW. Identification of inbred mouse strains harboring genetic modifiers of mammary tumor age of onset and metastatic progression. *Int J Cancer* 1998;77:640–644. [PubMed: 9679770]
8. Rowse GJ, Ritland SR, Gendler SJ. Genetic modulation of neu proto-oncogene-induced mammary tumorigenesis. *Cancer Res* 1998;58:2675–2679. [PubMed: 9635596]
9. DiGiovanni J, Imamoto A, Naito M, Walker SE, Beltran L, Chenicek KJ, Skow L. Further genetic analyses of skin tumor promoter susceptibility using inbred and recombinant inbred mice. *Carcinogenesis* 1992;13:525–531. [PubMed: 1576703]
10. Chan PC, Dao TL. Enhancement of mammary carcinogenesis by a high-fat diet in Fischer, Long-Evans, and Sprague-Dawley rats. *Cancer Res* 1981;41:164–167. [PubMed: 7448756]
11. Shisa H, Hiai H. Genetically determined susceptibility of Fischer 344 rats to propyl nitrosourea-induced thymic lymphomas. *Cancer Res* 1985;45:1483–1487. [PubMed: 3978615]
12. Moore CJ, Tricomi WA, Gould MN. Comparison of 7,12-dimethylbenz[a]anthracene metabolism and DNA binding in mammary epithelial cells from three rat strains with differing susceptibilities to mammary carcinogenesis. *Carcinogenesis* 1988;9:2099–2102. [PubMed: 3141078]
13. Stanley LA, Carthew P, Davies R, Higginson F, Martin E, Styles JA. Delayed effects of tamoxifen in hepatocarcinogenesis-resistant Fischer 344 rats as compared with susceptible strains. *Cancer Lett* 2001;171:27–35. [PubMed: 11485825]

14. Hilakivi-Clarke L, Cho E, Cabanes A, DeAssis S, Olivo S, Helferich W, Lippman M, Clarke R. Dietary modulation of pregnancy estrogen levels and breast cancer risk among female rat offspring. *Clin Cancer Res* 2002;8:3601–3610. [PubMed: 12429652]
15. Holm TM, Jackson-Grusby L, Brambrink T, Yamada Y, Rideout WM 3, Jaenisch R. Global loss of imprinting leads to widespread tumorigenesis in adult mice. *Cancer Cell* 2005;8:275–285. [PubMed: 16226703]
16. Savabieasfahani M, Kannan K, Astapova O, Evans NP, Padmanabhan V. Developmental programming: differential effects of prenatal exposure to bisphenol-A or methoxychlor on reproductive function. *Endocrinol*. 2006epub
17. Ho SM, Tang WY, Belmonte de Frausto J, Prins G. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase Type 4 Variant 4. *Cancer Res* 2006;66:5624–5632. [PubMed: 16740699]
18. Newbold RR, Padilla-Banks E, Jefferson WN. Adverse effects of the model environmental estrogen diethylstilbestrol are transmitted to subsequent generations. *Endocrinol* 2006;147:S11–S17.
19. Titus-Ernstoff L, Troisi R, Hatch EE, Palmer JR, Wise LA, Ricker W, Hyer M, Kaufman R, Noller K, Strohshitter W, Herbst AL, Hartge P, Hoover RN. Mortality in women given diethylstilbestrol in pregnancy. *Br J Cancer* 2006;95:107–111. [PubMed: 16786044]
20. Titus-Ernstoff L, Hatch EE, Hoover RN, Palmer J, Greenberg ER, Ricker W, Kaufman R, Noller K, Herbst AL, Colton T, Hartge P. Long term risk in women given diethylstilbestrol (DES) during pregnancy. *Br J Cancer* 2001;84:126–133. [PubMed: 11139327]
21. Colton T, Greenberg ER, Noller K, Resseguie L, Van Bennekom C, Heeren T, Zhang Y. Breast cancer in mothers prescribed diethylstilbestrol in pregnancy. Further follow-up. *JAMA* 1993;269:2096–2100. [PubMed: 8468763]
22. Palmer JR, Wise LA, Hatch EE, Troisi R, Titus-Ernstoff L, Strohshitter W, Kaufman R, Herbst AL, Noller KL, Hyer M, Hoover RN. Prenatal diethylstilbestrol exposure and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 2006;15:1509–1514. [PubMed: 16896041]
23. Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *N Engl J Med* 1971;284:878–881. [PubMed: 5549830]
24. Herbst AL, Poskanzer DC, Robboy SJ, Friedlander L, Scully RE. Prenatal exposure to stilbestrol. A prospective comparison of exposed female offspring with unexposed controls. *N Engl J Med* 1976;292:334–339. [PubMed: 1117962]
25. Howdeshell KL, Peterman PH, Judy BM, Taylor JA, Orazio CE, Ruhlen RL, vom Saal FS, Welshons WV. Bisphenol A is released from used polycarbonate animal cages into water at room temperature. *Environ Health Perspect* 2003;111:1180–1187. [PubMed: 12842771]
26. Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, Thomas S, Thomas BF, Hassold TJ. Bisphenol A exposure causes meiotic aneuploidy in the female mouse. *Curr Biol* 2003;13:546–553. [PubMed: 12676084]
27. Durando M, Kass L, Piva J, Sonnenschein C, Soto AM, Munoz de Toro M. Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland of Wistar rats. *Environ Health Perspect*. 2006in press
28. Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, Seely JC, Joiner RL, Butala JH, Dimond SS, Cagen SZ, Shiotsuka RN, Stropp GD, Waechter JM. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. *Toxicol Sci* 2002;68:121–146. [PubMed: 12075117]
29. Welshons WV, Nagel SC, vom Saal FS. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. *Endocrinol* 2006;147:S56–S69.
30. Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, Vogel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L, Wolmark N. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst* 1998;90:1371–1388. [PubMed: 9747868]
31. Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, Vogel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L, Wolmark N. Tamoxifen for the

- prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst* 2005;90:1371–1388. [PubMed: 9747868]
32. Strohsnitter WC, Noller KL, Hoover RN, Robboy SJ, Palmer JR, Titus-Ernstoff L, Kaufman RH, Adam E, Herbst AL, Hatch EE. Cancer risk in men exposed *in utero* to diethylstilbestrol. *J Natl Cancer Inst* 2001;93:545–551. [PubMed: 11287449]
 33. Boylan ES, Calhoon RE. Transplacental action of diethylstilbestrol on mammary carcinogenesis in female rats given one or two doses of 7,12- dimethylbenz(a)anthracene. *Cancer Res* 1983;43:4879–4884. [PubMed: 6411335]
 34. Rothschild TC, Boylan ES, Calhoon RE, Vonderhaar BK. Transplacental effects of diethylstilbestrol on mammary development and tumorigenesis in female ACI rats. *Cancer Res* 1987;47:4508–4516. [PubMed: 3607779]
 35. Modugno F, Weissfeld JL, Trump DL, Zmuda JM, Shea P, Cauley JA, Ferrell RE. Allelic variants of aromatase and the androgen and estrogen receptors: toward a multigenic model of prostate cancer risk. *Clin Cancer Res* 2001;7:3092–3096. [PubMed: 11595700]
 36. Leav I, Ho SM, Ofner P, Merk FB, Kwan PW, Damassa D. Biochemical alterations in sex hormone-induced hyperplasia and dysplasia of the dorsolateral prostates of Nobel rats. *J Natl Cancer Inst* 1988;80:1045–1053. [PubMed: 2457709]
 37. Bosland MC, Ford H, Horton L. Induction of a high incidence of ductal prostate adenocarcinomas in NBL and Sprague Dawley rats treated with estradiol-17- β or diethylstilbestrol in combination with testosterone. *Carcinogenesis* 1995;16:1311–1317. [PubMed: 7788848]
 38. Fowler KA, Gill K, Kirma N, Dillehay DL, Tekmal RR. Overexpression of aromatase leads to development of testicular Leydig cell tumors: an *in vivo* model for hormone-mediated testicular cancer. *Am J Pathol* 2000;156:347–353. [PubMed: 10623684]
 39. Bosland MC. Hormonal factors in carcinogenesis of the prostate and testis in humans and in animal models. *Prog Clin Biol Res* 1996;394:309–352. [PubMed: 8778804]
 40. Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β . *Endocrinol* 1998;139:4252–4263.
 41. Lemmen JG, Arends RJ, van der Saag PT, van der Burg B. *In vivo* imaging of activated estrogen receptors *in utero* by estrogens and bisphenol A. *Environ Health Perspect* 2004;112:1544–1549. [PubMed: 15531440]
 42. Recchia AG, Vivacqua A, Gabriele S, Carpino A, Fasanella G, Rago C, Bonofiglio D, Maggiolini M. Xenoestrogens and the induction of proliferative effects in breast cancer cells via direct activation of oestrogen receptor alpha. *Food Addit Contam* 2004;21:134–144. [PubMed: 14754635]
 43. Kurosawa T, Hiroi H, Tsutsumi O, Ishikawa T, Osuga Y, Fujiwara T, Inoue S, Muramatsu M, Momoeda M, Taketani Y. The activity of bisphenol A depends on both the estrogen receptor subtype and the cell type. *Endocr J* 2002;49:465–471. [PubMed: 12402979]
 44. Leung YK, Mak P, Hassan S, Ho SM. Estrogen receptor (ER)- β isoforms: a key to understanding ER- β signaling. *Proc Natl Acad Sci USA* 2006;103:13162–13167. [PubMed: 16938840]
 45. Ashby J, Tinwell H. Uterotrophic activity of bisphenol A in the immature rat. *Environ Health Perspect* 1998;106:719–720. [PubMed: 9799186]
 46. Tinwell H, Joiner R, Pate I, Soames A, Foster J, Ashby J. Uterotrophic activity of bisphenol A in the immature mouse. *Regul Toxicol Pharmacol* 2000;32:118–126. [PubMed: 11029274]
 47. Markey CM, Michaelson CL, Veson EC, Sonnenschein C, Soto AM. The mouse uterotrophic assay: a reevaluation of its validity in assessing the estrogenicity of bisphenol A. *Environ Health Perspect* 2001;109:55–60. [PubMed: 11171525]
 48. Steinmetz R, Mitchner NA, Grant A, Allen DL, Bigsby RM, Ben-Jonathan N. The xenoestrogen bisphenol A induces growth, differentiation, and c-fos gene expression in the female reproductive tract. *Endocrinol* 1998;139:2741–2747.
 49. Coldham NG, Dave M, Sivapathasundaram S, McDonnell DP, Connor C, Sauer MJ. Evaluation of a recombinant yeast cell estrogen screening assay. *Environ Health Perspect* 1997;105:734–742. [PubMed: 9294720]
 50. Nagel SC, Hagelbarger JL, McDonnell DP. Development of an ER action mouse for the study of estrogens, selective ER modulators (SERMs), and xenobiotics. *Endocrinol* 2001;142:4721–4728.

51. Yamada H, Furuta I, Kato EH, Kataoka S, Usuki Y, Kabashi G, Sata F, Kishi R, Fujimoto S. Maternal serum and amniotic fluid bisphenol A concentrations in the early second trimester. *Reprod Toxicol* 2002;16:735–739. [PubMed: 12401500]
52. Zalko D, Soto AM, Dolo L, Dorio C, Rathahao E, Debrauwer L, Faure R, Cravedi JP. Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. *Environ Health Perspect* 2003;111:309–319. [PubMed: 12611660]
53. Schonfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I. Parent bisphenol A accumulation in the human maternal-fetal-placental unit. *Environ Health Perspect* 2002;110:A703–A707. [PubMed: 12417499]
54. Boylan ES, Calhoon RE. Prenatal exposure to diethylstilbestrol: ovarian-independent growth of mammary tumors induced by 7,12-dimethylbenz[a]anthracene. *J Natl Cancer Inst* 1981;66:649–652. [PubMed: 6785506]
55. Boylan ES, Calhoon RE. Mammary tumorigenesis in the rat following prenatal exposure to diethylstilbestrol and postnatal treatment with 7,12-dimethylbenz[a]anthracene. *J Toxicol Environ Health* 1979;5:1059–1071. [PubMed: 119055]
56. Song KH, Lee K, Choi HS. Endocrine disruptor bisphenol a induces orphan nuclear receptor Nur77 gene expression and steroidogenesis in mouse testicular Leydig cells. *Endocrinol* 2002;143:2208–2215.
57. Walsh DE, Dockery P, Doolan CM. Estrogen receptor independent rapid non-genomic effects of environmental estrogens on $[Ca^{2+}]$ in human breast cancer cells. *Mol Cell Endocrinol* 2005;230:23–30. [PubMed: 15664448]
58. Wetherill YB, Fisher NL, Staubach M, Danielson RW, de Vere White RW, Knudsen KE. Xenoestrogen action in prostate cancer: pleiotropic effects dependent on androgen receptor status. *Cancer Res* 2005;65:54–65. [PubMed: 15665279]
59. Wetherill YB, Petre CE, Monk KR, Puga A, Knudsen KE. The xenoestrogen bisphenol A induces inappropriate androgen receptor activation and mitogenesis in prostatic adenocarcinoma cells. *Mol Cancer Ther* 2002;1:515–524. [PubMed: 12479269]
60. Wetherill YB, Hess-Wilson JK, Comstock CES, Shah SA, Buncher CR, Sallans L, Limbach PA, Schwemberger S, Babcock GF, Knudsen KE. Bisphenol A facilitates bypass of androgen ablation therapy in prostate cancer. *Mol Cancer Ther* 2006;5:3181–3190. [PubMed: 17172422]
61. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70. [PubMed: 10647931]
62. Cairns J, Logan J. Step by step into carcinogenesis. *Nature* 1983;304:582–583. [PubMed: 6877381]
63. Barker DJ. The developmental origins of adult disease. *J Am Coll Nutr* 2004;23:588S–595S. [PubMed: 15640511]
64. Joyce JA, Schofield PN. Genomic imprinting and cancer. *Mol Pathol* 1998;51:185–190. [PubMed: 9893743]
65. Soto AM, Sonnenschein C. Emergentism as a default: cancer as a problem of tissue organization. *J Biosci* 2005;30:103–118. [PubMed: 15824446]
66. Tennant RW, Margolin BH, Shelby MD, Zeiger E, Haseman JK, Spalding J, Caspary W, Resnick M, Stasiewicz S, Anderson B, Minor R. Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. *Science* 1987;236:933–941. [PubMed: 3554512]
67. Schrader TJ, Langlois I, Soper K, Cherry W. Mutagenicity of bisphenol A (4,4'-isopropylidenediphenol) *in vitro*: effects of nitrosylation. *Teratog Carcinog Mutagen* 2002;22:425–441. [PubMed: 12395404]
68. Schweikl H, Schmalz G, Rackebrandt K. The mutagenic activity of unpolymerized resin monomers in *Salmonella typhimurium* and V79 cells. *Mutat Res* 1998;415:119–130. [PubMed: 9711268]
69. Tsutsui T, Tamura Y, Yagi E, Hasegawa K, Takahashi M, Maizumi N, Yamaguchi F, Barrett JC. Bisphenol-A induces cellular transformation, aneuploidy and DNA adduct formation in cultured Syrian Hamster Embryo cells. *Int J Cancer* 1998;75:290–294. [PubMed: 9462721]
70. Can A, Semiz O, Cinar O. Bisphenol-A induces cell cycle delay and alters centrosome and spindle microtubular organization in oocytes during meiosis. *Mol Hum Reprod* 2005;11:389–396. [PubMed: 15879462]

71. Susiarjo M, Hassold TJ, Freeman E, Hunt PA. Bisphenol A exposure *in utero* disrupts early oogenesis in the mouse. *PLoS Genet*. 2006;in press
72. Sajiki J, Takahashi K, Yonekubo J. Sensitive method for the determination of bisphenol-A in serum using two systems of high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1999;736:255–261. [PubMed: 10677006]
73. Inoue K, Kato K, Yoshimura Y, Makino T, Nakazawa H. Determination of bisphenol A in human serum by high-performance liquid chromatography with multi-electrode electrochemical detection. *J Chromatogr B Biomed Sci Appl* 2000;749:17–23. [PubMed: 11129074]
74. Dash C, Marcus M, Terry PD. Bisphenol A: Do recent studies of health effects among humans inform the long-standing debate? *Mutat Res* 2006;613:68–75. [PubMed: 16757209]
75. Takahashi S, Chi XJ, Yamaguchi Y, Suzuki H, Sugaya S, Kita K, Hiroshima K, Yamamori H, Ichinose M, Suzuki N. Mutagenicity of bisphenol A and its suppression by interferon- α in human RSa cells. *Mutat Res* 2001;490:199–207. [PubMed: 11342245]
76. Atkinson A, Roy D. In vitro conversion of environmental estrogenic chemical bisphenol A to DNA binding metabolite(s). *Biochem Biophys Res Comm* 1995;210:424–433. [PubMed: 7755618]
77. Atkinson A, Roy D. *In vivo* DNA adduct formation by bisphenol A. *Environ Mol Mutagen* 1995;26:60–66. [PubMed: 7641708]
78. Iso T, Watanabe T, Iwamoto T, Shimamoto A, Furuichi Y. DNA damage caused by bisphenol A and estradiol through estrogenic activity. *Biol Pharm Bull* 2006;29:206–210. [PubMed: 16462019]
79. Hilliard CA, Armstrong MJ, Bradt CI, Hill RB, Greenwood SK, Galloway SM. Chromosomal aberrations *in vitro* related to cytotoxicity of nonmutagenic chemicals and metaboloid poisons. *Environ Mol Mutagen* 1998;31:316–326. [PubMed: 9654240]
80. Galloway SM, Miller JE, Armstrong MJ, Bean CL, Skopek TR, Nichols WW. DNA synthesis inhibition as an indirect mechanism of chromosome aberrations: comparison of DNA-reactive and non-DNA-reactive clastogens. *Mutat Res* 1998;400:169–186. [PubMed: 9685628]
81. Tsutsui T, Tamura Y, Suzuki A, Hirose Y, Kobayashi M, Nishimura H, Metzler M, Barrett JC. Mammalian cell transformation and aneuploidy induced by five bisphenols. *Int J Cancer* 2000;86:151–154. [PubMed: 10738239]
82. Susiarjo M, Hassold TJ, Freeman E, Hunt PA. Bisphenol A exposure *in utero* disrupts early oogenesis in the mouse. *PLoS Genet* 2007;3:e5. [PubMed: 17222059]
83. Ochi T. Induction of multiple microtubule-organizing centers, multipolar spindles and multipolar division in cultured V79 cells exposed to diethylstilbestrol, estradiol-17 β and bisphenol A. *Mutat Res* 1999;431:105–121. [PubMed: 10656490]
84. Fowden AL, Forhead AJ. Endocrine mechanisms of intrauterine programming. *Reproduction* 2004;127:515–526. [PubMed: 15129007]
85. Zandi-Nejad K, Luyckx VA, Brenner BM. Adult hypertension and kidney disease: the role of fetal programming. *Hypertension* 2006;47:502–508. [PubMed: 16415374]
86. Ismail-Beigi F, Catalano PM, Hanson RW. Metabolic programming: fetal origins of obesity and metabolic syndrome in the adult. *Am J Physiol Endocrinol Metab* 2006;291:E439–E440. [PubMed: 16638823]
87. Xita N, Tsatsoulis A. Review: fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. *JCEM* 2006;91:1660–1665. [PubMed: 16522691]
88. vom Saal FS, Timms BG, Montano MM, Palanza KA, Thayer KA, Nagel SC, Dhar MG, Gangam S, Parmigiani S, Welshons WV. Prostate enlargement in mice due to fetal exposure to low doses of estradiol of diethylstilbestrol and opposite effects at high doses. *Proc Natl Acad Sci USA* 1997;94:2056–2061. [PubMed: 9050904]
89. Markey CM, Luque EH, Munoz de Toro M, Sonnenschein C, Soto AM. In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol Reprod* 2001;65:1215–1223. [PubMed: 11566746]
90. Munoz de Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonnenschein C, Soto AM. Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. *Endocrinol* 2005;146:4138–4147.

91. Newbold RR, Bullock BC, McLachlan JA. Uterine adenocarcinoma in mice following developmental treatment with estrogens: a model for hormonal carcinogenesis. *Cancer Res* 1990;50:7677–7681. [PubMed: 2174729]
92. Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS. Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. *Proc Natl Acad Sci USA* 2005;102:7014–7019. [PubMed: 15867144]
93. Yoshino H, Ichihara T, Kawabe M, Imai H, Hagiwara A, Asamoto M, Shirai T. Lack of significant alteration in the prostate or testis of F344 rat offspring after transplacental and lactational exposure to bisphenol A. *J Toxicol Sci* 2002;27:433–439. [PubMed: 12533913]
94. Ashby J, Tinwell H, Haseman J. Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed *in utero*. *Regul Toxicol Pharmacol* 1999;30:156–166. [PubMed: 10536110]
95. Ashby J. Toward resolution of the divergent effects of estrogens on the prostate gland of CF-1 mice. *Environ Health Perspect* 2001;109:A109. [PubMed: 11333197]
96. Milman HA, Bosland MC, Walden PD, Heinze JE. Evaluation of the adequacy of published studies of low-dose effects of bisphenol A on the rodent prostate for use in human risk assessment. *Regul Toxicol Pharmacol* 2002;35:338–346. [PubMed: 12202049]
97. Maffini MV, Rubin BS, Sonnenschein C, Soto AM. Endocrine disruptors and reproductive health: the case for bisphenol-A. *Mol Cell Endocrinol* 2006;254–255. 179–186.
98. vom Saal FS, Richter CA, Ruhlen RR, Nagel SC, Timms BG, Welshons WV. The importance of appropriate controls, animal feed, and animal models in interpreting results from low-dose studies of bisphenol A. *Birth Defects Res A* 2005;73:140–145.
99. Rizzo S, Attard G, Hudson DL. Prostate epithelial stem cells. *Cell Prolif* 2005;38:363–374. [PubMed: 16300650]
100. Ichihara T, Yoshino H, Imai N, Tsutsumi T, Kawabe M, Tamano S, Inaguma S, Suzuki S, Shirai T. Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol A in rats. *J Toxicol Sci* 2003;28:165–171. [PubMed: 12974608]
101. Hovey RC, Asai-Sato M, Warri A, Terry-Koroma B, Colyn N, Ginsburg E, Vonderhaar BK. Effects of neonatal exposure to diethylstilbestrol, tamoxifen, and toremifene on the BALB/c mouse mammary gland. *Biol Reprod* 2005;72:423–435. [PubMed: 15470002]
102. Vandenberg LN, Maffini MV, Wadia PR, Sonnenschein C, Rubin BS, Soto AM. Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland. *Endocrinol*. 2006in press
103. Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM. Induction of mammary gland ductal hyperplasias and carcinoma *in situ* following fetal bisphenol A exposure. *Reproductive Tox*. 2006in press
104. Yoshida M, Shimomoto T, Katashima S, Watanabe G, Taya K, Maekawa A. Maternal exposure to low doses of bisphenol A has no effects on development of female reproductive tract and uterine carcinogenesis in Donryu rats. *J Reprod Dev* 2004;50:349–360. [PubMed: 15226600]
105. Nagaoka T, Takeuchi M, Onodera H, Matsushima Y, Ando-Lu J, Maekawa A. Sequential observation of spontaneous endometrial adenocarcinoma development in Donryu rats. *Toxicol Pathol* 1994;22:261–269. [PubMed: 7817117]
106. Yoshida M, Katsuda S, Tanimoto T, Asai S, Nakae D, Kurokawa Y, Taya K, Maekawa A. Induction of different types of uterine adenocarcinomas in Donryu rats due to neonatal exposure to high-dose p-t-octyl. *Carcinogenesis* 2002;23:1745–1750. [PubMed: 12376485]
107. Post-menopausal oestrogen therapy. *IARC Mongr Eval Carcinog Risks Hum* 1992;72:399–530.
108. Liehr JG. Is estradiol a genotoxic mutagenic carcinogen? *Endocrine Rev* 2000;21:40–54. [PubMed: 10696569]
109. Melnick R, Lucier G, Wolfe M, Hall R, Stancel G, Prins G, Gallo M, Reuhl K, Ho SM, Brown T, Moore J, Leakey J, Haseman J, Kohn M. Summary of the National Toxicology Program's report of the endocrine disruptors low-dose peer review. *Environ Health Perspect* 2002;110:427–431. [PubMed: 11940462]

Table I
In Vivo Assessment of Carcinogenic Properties of Bisphenol A

Author (Year)	Species/Strain/Sex	Dose(s)	Route	Timing	Additional Carcinogenic Treatment	End Point
NTP (1982)	Rats/Fischer 344/ Both	1,000-2,000 ppm	Feed	Chronic, peripubertal to 2 years	-	Equivocal risk of leukemia, testicular, and mammary (male) malignancy
NTP (1982)	Mice/ C3B6 F1 Hybrids/ Both	5,000-10,000 ppm	Feed	Chronic, peripubertal to 2 years	-	Equivocal risk of hematological malignancy
Tyl, R. W. (2002)	Rats/Sprague- Dawley/Both	0.001-5 mg/kg/day	Feed (Transplacental for fetal exposure)	Fetal-adulthood over three generations	-	No increased risk of cancers in multiple organ systems
Wetherill, Y.B. (2006)	Mice with Human Tumor Xenografts, NCR/nr/nr (athymic)/Male	12.5 mg/21 days (highest mean serum concentration = 27 ng/ml, 7 days after implant)	s.c. implant	Adults with xenografts of 50-100 mm ³ , over a three week period	-	Accelerated tumor growth rate following castration
Hunt, P.A. (2003)	Mice/C57BL/6/ Female	20-100 µg/kg/day	Oral gavage	Juvenile (28 day old)	-	Meiotic Aneuploidy
Susiarjo, M. (2006)	Mice/C57BL/6/ Female	20 µg/kg/day	s.c. implants	Fetal, e11.5- parturition	-	Meiotic Aneuploidy
Timms, B.G. (2005)	Mice/CD-1/Male	10 µg/kg/day	Oral gavage	Fetal, e14-18	-	Increased proliferation of basal cells of the prostate
Ichihara, T. (2003)	Rats/F344/Male	0.05-120 mg/kg/day	Oral gavage and s.c. injections	Fetal/infant, early pregnancy (~ e0.5) → weaning	DMAB	No increase in prostate cancer
Ho, S.-M. (2006)	Rats/Sprague-Dawley/ Male	10 µg/kg	s.c. injections	Postnatal days 1, 3, and 5	Estradiol + testosterone	Prostate intraepithelial neoplasia and adenocarcinomas
Murray, T.J. (2006)	Rats/Wistar-Furth/ Female	2.5-1,000 µg/kg/day	s.c. implants	Fetal, e9 → post- natal day 1	-	Mammary ductal hyperplasia and carcinoma <i>in situ</i>
Durando, M. (2006)	Rats/Wistar/Female	25 µg/kg/day	s.c. implants	Fetal, e9 → post- natal day 1	NMU	Mammary ductal hyperplasia and solid carcinomas
Yoshida, M. (2004)	Rats/Crj:Donryu/ Female	0.006 and 6 mg/kg/ day + environmental exposure	Oral gavage	Fetal/infant, e2 → weaning (post-natal day 21)	ENNG	No increase in uterine cancer