Article

The bisphenol A toxicological paradox: The more we learn the less we know for sure

Francisco J.R. Paumgartten, **Ana C.A.X. De-Oliveira**

Laboratory of Environmental Toxicology, Department of Biological Sciences, National School of Public Health – FIOCRUZ, Brazil

E-mail: paum@ensp.fiocruz.br

Received 11 July 2014; *Accepted 15 August 2014*; *Published online 1 December 2014*

Abstract

Despite the overwhelming need for toxicological data on unstudied substances, a search in Pubmed reveals >7300 entries for a single chemical (bisphenol A, BPA), most of which published in the last 25 years. BPA, a component of plastics and resins and a putative xenoestrogen, is certainly the molecule for which there are more studies in the toxicological literature. It was reported that fetal / perinatal exposures of mice to BPA (in the ppb range) alter prostate weight in adult males, and cause persistent changes of mammary gland morphogenesis in females. Several studies, however, failed to replicate these findings. More recently, debate on BPA health risks was boosted by a few cross-sectional epidemiology studies that reported associations between total-BPA (in urine) and cardiovascular diseases, diabetes and other health problems. The urine levels, however, reflect recent BPA exposures (within hours), and aforementioned disorders start much earlier in individuals' life.

Keywords endocrine disrupting chemicals; prostate hyperplasia; mammary gland; cancer; developmental toxicity; xenoestrogens.

Environmental Skeptics and Critics
ISSN 2224-4263
URL: http://www.iaees.org/publications/journals/environsc/online-version.asp
RSS: http://www.iaees.org/publications/journals/environsc/rss.xml
E-mail: environsc@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Bisphenol A (CAS No. 80-05-7; BPA) is found in a variety of consumer products such as polycarbonate plastics, resins, dental sealants, adhesives, and others. Owing to its multiple uses and large volume production, BPA is a ubiquitous environmental contaminant as well (Vanderberg et al., 2009). As far as health hazards are concerned, BPA is certainly the most studied environmental chemical. A search in the PubMed data basis, using "bisphenol A" as searching term, resulted in 7549 records as to May 30th, 2013. Similar searches for other compounds topping lists of chemicals of concern such as "phthalates", "dichloro-diphenyl-dichloroethilene" (DDE), polybrominated diphenyl ethers (PBDEs), "tetrachloro-dibenzo-*p*-dioxin" (*2,3,7,8*-TCDD), and "polychlorinated biphenyls" (PCBs), undertaken on the same day, resulted in 1321, 1859, 2684, 4664 and 14454 records, respectively. Polychlorinated biphenyls, the searching term with the greatest number

of records, however, refers to an entire class of chemicals the individual members of which (209 PCB congeners, 12 of which are “dioxin-like” compounds) exhibit distinct toxicological features. From a toxicological standpoint, BPA is not only the most studied substance but also the most controversial one.

The extraordinary interest of researchers on the health risks posed by BPA stands mainly on its putative endocrine (estrogenic) disrupting activity, on apparent low-dose effects and non-monotonic dose response relationships and on its developmental toxicity.

The focus of this critical review of the literature was placed on two of the most instigating and at the same time most controversial findings from toxicological studies on BPA, i.e., the effects of pre- and/or early post-natal exposure to low doses of BPA on prostate size and mammary gland morphology in adult animals.

2 Methods

Electronic data sources including PubMed and ToxLine were used to conduct the literature search. Searching terms were “BPA”, “bisphenol A”, “toxicity”, “endocrine disruptors”, “estradiol”, “DES”, “endocrine disrupters”, “prostate”, “mammary gland”, “breast”, “kinetics” “cancer”, “tumors”, “epidemiology” and several combinations of these terms. References cited in retrieved articles were also reviewed to identify papers not captured by the search in electronic data basis. The search and selection of studies was conducted independently by both authors who consensually agreed on the inclusion of the study in the review. In all cases of articles listed in the Box 1, a publication (full text) containing details of study methods and results was reviewed.

Box 1 Time line of landmark studies about health hazards posed by BPA.

Year	Authors	Report
1891	-	First synthesis of BPA
1938	Dodds & Lawson	First report of BPA estrogenic activity / DES more potent
1986	Berthois et al.	Phenol red stimulates proliferation of MCF-7 breast cancer cells
1991	Soto et al.	xenoestrogen nonylphenol released from plastics
1993	Krishnan et al.	BPA weak estrogen released from plastics stimulates MCF-7 cells proliferation
1995	Brotons et al.	estrogenicity of BPA released from cans in food products
1996	Olea et al.	estrogenicity of BPA released from dental sealants
1997	vom Saal et al.	Inverted U dose response of prenatal DES and EST on prostate size in adult mice
1997	Nagel et al.	Prenatal BPA mimics DES and EST effects on mouse prostate
2001_a	Markey et al.	Prenatal BPA (low doses) affected mammary gland architecture and cell proliferation in adult female mice
2008	Lang et al.	Urinary BPA levels in adults associated with cardio vascular disease and diabetes/cross-sectional study.
2012	Trasande et al.	Urinary BPA levels in children and adolescents associated with obesity / cross-sectional study
2012	LaKind et al.	Urinary BPA levels not significantly associated with adverse health outcomes for any of the NHANES surveys

BPA: bisphenol A; DES: diethylstilbestrol; EST: estradiol; NHANES: US National Health and Nutrition Examination Survey

3 Results and Discussion

3.1 The initial studies

The oldest studies found in the PubMed-indexed literature, published in early 1960s, focused on BPA sensitizing potential and its role in the hypersensitivity to epoxy resins (Fregert and Rorsman, 1960; Gaul, 1960; Fregert and Rorsman, 1962). In 1966, a study by Knaak and Sullivan (1966) described the metabolic fate of ^{14}C-labelled BPA given to rats by the oral route. The authors (Knaak and Sullivan, 1966) found that 28% of administered ^{14}C was excreted in the urine and 56% in the feces. They also noted that rats eliminate BPA primarily as the glucuronide and that less than 1% of the BPA-related material in the urine was unconjugated (free) BPA.

From 1960 to 1990, most citations to BPA in biomedical publications referred to its use in dentistry as a constituent of resins (employed as dental sealants, cements and so on), and related safety issues such as BPA-resins biocompatibility, and also BPA sensitizing effects and the compound's role in allergic reactions to BPA-containing resins and plastic materials. In 1982, the National Toxicology Program (NTP) published a long-term carcinogenesis study of BPA (feed study) in F344 rats and B6C3F1 mice (NTP, 1982). Results of NTP study led to the overall conclusion that, under the conditions of the assay, there was no convincing evidence that BPA was carcinogenic for F344 rats or B6C3F1 mice of either sex (NTP, 1982). A subsequent investigation of skin carcinogenicity of technical-grade epoxy resin (Araldite®), the main component of which was a diglycidyl ether of BPA, found that it caused no effect on survival and no excess incidence of skin tumours in CF1 mice (Zakova et al., 1985).

An investigation of the developmental toxicity of BPA in rodents was published in 1987. Morrissey et al. (1987) reported that BPA, given by gavage to CD rats (0, 160, 320 or 640 mg/kg/d) and CD-1 mice (0, 500, 750, 1000 or 1250 mg/kg/d) on gestation days (GD) 6-15, enhanced the resorption rate and decreased the fetal body weight in mice (only at 1250 mg/kg/d, a maternally toxic dose level) but not in rats and did not alter morphologic development in either species. In 1984/5, a two-generation study of the effects of BPA on reproduction and fertility in Swiss CD-1 mice was conducted by NTP (Anonymous, 1997). Mice were exposed to concentrations at 0, 0.25, 0.5 and 1% in feed with estimate daily intakes of approximately 437, 875 and 1750 mg/kg body wt/d. Results indicated that exposure of the first generation to the two highest levels of BPA resulted in reductions in number of litters per pair, live pups per litter, and pup body weight. At the highest dose, liver and kidneys weights were increased as well. The second generation was not more sensitive than the first to BPA reproductive toxicity (Anonymous, 1997).

3.2 Landmarks on the road leading to an unprecedented controversy

In early 1990s, while investigating whether yeast (*Saccharomyces cerevisae*) culture would produce estrogens, David Feldman and his group made a serendipitous discovery thanks to which concerns on BPA safety took center stage for years to come (Krishnan et al., 1993). The authors noted that *S. cerevisae* culture medium contained a substance that competed with [^{3}H] estradiol for binding to estrogen receptors from rat uterus. Subsequent experiments showed that this estrogenic substance was not a product of the yeast grown in culture but it was leached out of the polycarbonate flasks during the autoclaving procedure (Krishnan et al., 1993). The product released from plastic flasks was identified by nuclear magnetic resonance spectroscopy and mass spectrometry as BPA. The authors subsequently found that BPA (25 nM) increased proliferation rate (^{3}H-thymidine incorporation) of human breast cancer cells (MCF-7), and induced (10-25 nM) progesterone receptors in these cells at a potency of approximately 1:5000 compared to estradiol, being the latter effect blocked by anti-estrogen drug tamoxifen. Feldman et al.'s (1993) results showed that BPA was active albeit being much less potent than ß-estradiol at the human estrogen receptor (Krishnan et al., 1993; Feldman and Krishnan, 1995). The MCF-7 cell line is highly sensitive to estrogenic compounds and a few years earlier a

similar study by Berthois et al. (1986) had found that phenol red – a pH indicator used in tissue culture media – stimulated proliferation of estrogen receptor positive MCF-7 cells. The authors noted that phenol red exhibited significant estrogenic activity at concentrations (15-45 μM) at which it is commonly found in tissue culture media (Berthois et al., 1986). Along the same line, in 1991, a study by Ana Soto and coworkers had shown that an alkylphenol (nonylphenol) released from plastic centrifuge tubes induced both cell proliferation and progesterone receptors in estrogen-sensitive MCF-7 cells, and mitotic activity in rat endometrium (Soto et al., 1991). Based on the foregoing findings, Feldman and Krishnan (1995) highlighted that estrogenic compounds can be found in unexpected places and thus might be inadvertently added to sensitive experimental systems thereby confounding study results and conclusions. The authors also expressed their concerns regarding a possible addition of BPA to food products because polycarbonate plastic is used in a myriad of food and beverages packing materials (Feldman and Krishnan, 1995).

A study by Brotons et al. published in the same year (1995) lent support to Feldman and Krishnan's concerns on the presence of estrogenic activity mediated by BPA in food products. Brotons et al.'s data (1995) showed that BPA leached out from the lacquer coating of cans and could be found as a contaminant not only in the liquid of preserved vegetables but also in water autoclaved in the cans. Additionally, the authors also demonstrated that BPA-containing fractions (obtained after chromatographic elution) of the liquid phase of extracts from food packed in lacquer-coated cans, stimulated proliferation of estrogen sensitive MCF-7 cells (Brotons et al., 1995). Although the authors did not rule out the presence of xenoestrogens other than BPA in the canned food extracts, the magnitude of MCF-7 cell proliferative effect seemed to correlate with the concentration of BPA in the assayed fraction.

In the following year (1996), another study by Nicolás Olea and colleagues raised concerns about the presence of BPA and estrogenic activity in resin-based composites and sealants widely employed in dentistry (Olea et al., 1996; Editorial, 1996; Habib ang Kugel., 1996). Olea et al. (1996) identified BPA (range 90-931 μg) in the saliva collected from 18 patients within one hour after treatment with 50 mg of a BPA diglycidylether methacrylate based dental sealant. The authors did not detect BPA or any other composite component in samples of saliva collected prior to treatment (Olea et al., 1996). The estrogen sensitive MCF-7 cell assay showed that addition of samples of saliva containing the highest amounts of BPA and BPA-dimethylmethcrylate to culture medium stimulated cell proliferation (Olea et al., 1996). Olea et al. (1996) also noted that, although being at least 4 orders of magnitude less potent than 17β-estradiol, both BPA and its methacrylate derivative displaced [^{3}H]-estradiol binding and enhanced estrogen responsive MCF-7 cells proliferation.

3.3 Effects of BPA on prostate development: an endless scientific dispute?

The origins of what is possibly the most controversial topic of endocrine disruption research can be traced back to an instigating study published in 1997. Frederick vom Saal and colleagues (1997) treated pregnant CF-1 mice with 17-β-estradiol (administered through subcutaneously implanted Silastic capsules) from GD13 to C-section (GD19) or with diethylstilbestrol (DES, 0.02; 0.2, 2.0, 20.0 and 200.0 ng/g body wt/day) given orally on GD11-17. Pups prenatally exposed to supra-physiological levels of estradiol were delivered by C-section (so that their intrauterine positions were determined) and further reared by foster mothers. Offspring exposed to DES during prenatal development were born through vaginal delivery and nursed by their biological mothers. When 8 months old, male offspring were killed, and their prostates were removed and weighed. Results showed that a 50% increase in free-serum estradiol resulted in a prostate enlargement (by 30% due to hyperplasia) and also in an enhanced number of prostatic androgen receptors relative to control males. Additional increases in free-serum estradiol levels (from 2- to 8-fold), however, resulted in a reduction of adult prostate weight relative to males exposed to 50% increase in estradiol concentrations. DES effect on

prostate weight exhibited a similar non-monotonic (inverted-U) dose-response relationship: DES doses of 0.02, 0.2 and 2.0 ng/g body wt/d increased, whereas 200.0 ng/g body wt/d decreased adult prostate weight relative to that of control males (vom Saal et al., 1997). The report that maternal supra-physiological levels of estradiol (50% increase) and low doses of a xenoestrogen (DES) during early development gave rise to a long-term effect on prostate size and androgen receptor expression (hyperplasia, possibly relevant to male ageing medical conditions), and their inverted-U dose response relationship aroused a great deal of interest.

A second study by vom Saal's group (Nagel et al., 1997) using essentially the same procedures employed for testing DES, revealed that prenatal exposure of CD-1 mice to low doses of BPA (2.0 and 20.2 ng/g body wt/d) led to heavier prostates in adult (6-month old) males as well. In their conclusions, the authors commented that these oral doses of BPA lie near the reported ranges of human exposure to this phenolic compound. A further paper by the same group reported that similar prenatal exposures of CF-1 mice to methoxychlor (MCZ), another putative xenoestrogen, produced a prostate enlargement in adults even greater that those caused by BPA and DES in previous studies (Judy et al., 1999).

Table 1 Effects of prenatal exposure to BPA, methoxychlor (MXC), DES and 17β-estradiol (EST) on the mouse prostate weight.

Strain (N/group)	Exposure				Results	Study (year)
	Chemical	Period	Doses	Route		
CF-1 (7)	BPA	GD 11-17	2, 20 μg/kg/d	po	Increase (both doses) in prostate wt relative to controls (6 month old)	Nagel et al., 1997
CF-1 (8)	BPA	GD 11-17	2, 20 μg/kg/d	po	No effect on prostate wt (6 month old)	Ashby et al., 1999
CF-1 (18-24)	BPA	GD 11-17	0.2, 2, 20, 200 μg/kg/d	po	No effect on prostate weight (3 month old)	Cagen et al., 1999b
CD-1 (15)	BPA	GD 16-18	50 μg/kg/d	po	Increase in prostate wt relative to controls (3, 21 and 60 days old)	Gupta, 2000
CD-1 (28)	BPA	2-generation study	0.003, 0.3, 5, 50, 600 mg/kg/d	diet	No effect on prostate wt	Tyl et al., 2008
CF-1	MXC	GD 11-17	20, 2000 μg/kg/d	po	Increase (both doses) in prostate wt relative to controls (9.5 months old)	Judy et al., 1999
CF-1 (6-8)	DES	GD 11-17	0.002, 0.02, 0.2, 2, 20, 200 μg/kg/d	po	Increase (0.02, 0.2, 2 μg/kg/d), no effect (0.002, 20 μg/kg/d) and decrease (200 μg/kg/d) in prostate wt (8 month old)	Vom Saal et al., 1997
CF-1 (8)	DES	GD 11-17	0.2 μg/kg/d	po	No effect on prostate wt (6 month old)	Ashby et al., 1999
CF-1 (23)	DES	GD 11-17	0.2 μg/kg/d	po	No effect on prostate weight (3 month old)	Cagen et al., 1999b
CD-1 (15)	DES	GD 16-18	0.1, 200 μg/kg/d	po	Increase (0.1 μg/kg/d) and decrease (200 μg/kg/d) in prostate wt relative to controls (3, 21 and 60 days old)	Gupta, 2000
CF-1 (6-8)	EST	GD 13-19	0.21, 0.32, 0.56, 0.78, 1.7 pg/ml	sc (implant)	Increase (0.32 pg/ml) relative to physiological (0.21 pg/ml); decrease (1.7 pg/ml) relative to 0.32 and 0.56 pg/ml in prostate wt (8 month old)	Vom Saal et al., 1997
CD-1 (28)	EST	2-generation study	80 μg/kg/d (0.5 ppm in food)	diet	No effect on prostate wt	Tyl et al., 2008

BPA: bisphenol A; DES: diethylstilbestrol; EST: estradiol; MXC: methoxychlor; GD: gestation day; po: per os; sc: subcutaneous; wt: weight.

Table 2 Effects of prenatal exposure by the oral route to BPA, DES or EE on the prostate weight in adult rats.

Strain (N)	Exposure				Results	Study (year)
	Chemical	Period	Doses	Route		
Han-Wistar (28)	BPA	PM +G +L	0.01, 0.1, 1, 10 ppm	dwt	No effect on prostate wt (3 month old)	Cagen et al., 1999a
SD	BPA	GD11-PD20	3.2, 32, 320 mg/kg/d	po	No effect on prostate wt (6 month old)	Kwon et al., 2000
SD	BPA	GD2-PD21	0.005, 0.05, 0.5, 5, 50 ppm (≈ 0.01–10 mg/kg/d)	dwt	Increase in prostate (V) wt (PND 177) [+]	Elswick et al., 2000
SD-IGS	BPA	2-generation	0.2,2,20,200 µg/kg/d	po	No effect on prostate wt	Ema et al., 2001
SD	BPA	GD6-21	20, 100, 50 000 µg/kg/d	po	No effect on prostate wt (3 month old)	Tinwell et al., 2002
AP-Wistar	BPA	GD6-21	20, 100, 50 000 µg/kg/d	po	No effect on prostate wt (3 month old)	Tinwell et al., 2002
F344	BPA	GD0-PD21	7.5, 120 mg/kg/d	po	No effect on prostate (V,A,DLl) wt (PND 23, 28, 91)	Yoshino et al., 2002
SD	BPA	3-generation	0.001,0.02,0.3,5,50 ,500 mg/kg/d	diet	No effect on prostatate wt (in the low dose range 0.001-5 mg/kg/d)	Tyl et al., 2002
F344	BPA	GD0-PD21	0.05, 7.5, 30, 120 mg/kg/d	po	No effect on prostate (V,A,DLl) wt (65 week old)	Ichihara et al., 2003
LE	BPA	GD7-PD18	2, 20, 200 µg/kg/d	po	No effect on prostate wt	Howdeshell et al., 2008
Wistar	DES	PM+G+L	0.1 ppm	dwt	Decrease in prostate (V) wt (3 month old)	Sharpe et al., 1995
Han-Wistar (28)	DES	PM+G+L	0.1 ppm	dwt	No effect on prostate wt (3 month old)	Cagen et al., 1999
SD	DES	GD11-PD20	15 µg/kg/d	po	No effect on prostate wt (6 month old)	Kwon et al., 2000
AP-Wistar	EE	GD6-21	200 µg/kg/d	po	Decrease in prostate wt (3 month old)	Tinwell et al., 2002
LE	EE	GD7-PD18	0.05, 50 µg/kg/d	po	No effect on prostate wt (5 month old)	Howdeshell et al., 2008

SD: Sprague-Dawley rats; dwt: drinking water; [+] According to authors' interpretation the effect was probably due to the sampling design and thus was considered of no toxicological significance. V: ventral; A: anterior; DL: dorso lateral prostate; PM: premating, G: gestation; L: lactation period exposures; AP-Wistar: Alderly Park-Wistar, BPA: bisphenol A; DES: diethylstilbestrol; EE: ethinyl estradiol.

As shown in Table 1, in the following two years, two different groups, replicating essentially the same design and methods employed by vom Saal and cowokers, failed to reproduce their findings on the effects of BPA and DES on the prostate (Ashby et al., 1999; Cagen et al., 1999a). Gupta (2000), however, using a somewhat different study design (CD-1 strain, a shorter and later exposure period, GD 18-19, and a higher BPA dose, 50 g/kg b wt/d), examined prostate weights at younger ages (3, 21 and 60 days old) and confirmed prior findings by vom Saal et al. (vom Saal et al., 1997; Nagel et al., 1997) suggesting that prenatal exposure to low doses of BPA or DES caused prostate enlargement in adult mice. More recently, a large two-generation reproductive toxicity study in CD-1 mice by Tyl et al. (2008) found no effect of BPA on prostate size and morphology either in F_1 or in F_2 generation.

A number of rat studies found no effect of prenatal and prenatal plus lactation exposures to a broad range of doses of BPA or DES (including low doses), given by the oral route, on the prostate size in adulthood (Table 2).

The lack of consistency among studies regarding the effects of low doses of BPA during early development on prostate size in adulthood is intriguing. In principle, failure of rat studies to reproduce initial findings in mice could reflect species differences in responsiveness to BPA and DES. Discrepancies among the mouse studies, however, are difficult to explain. Failures to replicate vom Saal et al's findings by three subsequent studies cannot be attributed to differences regarding strain, BPA purity, dose, route or mode of administration, developmental period of exposure or age of animals at necropsy (Table 1). Lack of replication of effects on prostate wt, on the other side, is unlikely to have arisen from random variations in prostate size, from genetic drift of CF-1 breeding stock, or from a reduced statistical power of further studies either. An analysis of statistical power of Nagel et al. (1997), Cagen et al. (1999) and Ashby et al. (1999) studies revealed that the two subsequent replicating studies were in fact more sensitive to detect changes in prostate wt than the original study (Owens and Chaney, 2005). According to Owens and Chaney's (2005) analysis it is highly unlikely for the replicating studies to have missed a true effect.

3.4 BPA and prostate: The lack of an "appropriate" positive control (PC) issue

A recent and extensive review (Vanderberg et al., 2012) on low-dose effects and nonmonotonic dose responses of endocrine-disrupting chemicals identified nine rodent studies that showed no effect of low doses of BPA on the prostate weight, some of which were designed specifically to replicate the original positive study. The authors highlighted that all nine negative rodent studies had one or another problem regarding the use of a positive control (Vanderberg et al., 2012). Three rat studies (Ema et al., 2001; Tyl et al., 2002; Ichihara et al., 2003; Tyl, 2003) failed to include a positive control (PC) of any kind, one mouse study (Tyl et al., 2008) used 17β-estradiol (EST) as a PC, administered by the oral route, and two rat studies used ethinyl estradiol (EE) as a PC but only at inappropriately high doses (Tinwell et al., 2002; Howdeshell et al., 2008). The remaining two mouse studies (Ashby et al., 1999; Cagen et al., 1999a) and one rat study (Cagen et al., 1999b), although using an "appropriate" dose of DES as PC, found no effect on prostate weight (Tables 1 and 2).

The lack of appropriate PC has been repeatedly presented by vom Saal et al. (2005, 2010) and vom Saal and Welshons (2006) as one of the main reasons why different rodent studies have failed to replicate previously described low-dose effects of BPA, including those on the adult mouse prostate size. This view is emphatically expressed in one of their articles where the authors stated that *"...due to lack of attention to the importance of appropriate positive controls, a small number of studies reporting negative effects of bisphenol A have created a false sense of controversy regarding low-dose effects of bisphenol A*" (vom Saal et al., 2005). According to them "*.. if the study fails to detect low-dose effects of a test chemical, no convincing conclusion can be made; in this case, a positive control is required to demonstrate that the experimental system was capable of detecting such effects* ".

Actually, PCs are used to assess test validity. A PC substance has a well-established effect on the test system and is used to demonstrate that, under the conditions prevailing when that particular assay was conducted, it was sensitive to detect similar effects of other chemicals. Use of an appropriate PC therefore ensures that, if there is indeed an effect, it is likely to be detected by the test system under the conditions the assay was carried out. For instance, if an *in vivo* assay is performed to evaluate the ability of a test chemical to elicit activities consistent with agonists of estrogen receptor (e.g., rodent Uterotrophic Assay), it is required to include a PC group treated with a reference estrogen agonist (EPA guidance recommends 17α-ethynyl estradiol, EE, 0.3 µg/kg/d by *sc* or 1.0 µg/kg/d by gavage) to show that the test system is working (i.e. appropriate uterine weight increase is observed) (US EPA, 2011). The same holds true for an *in vitro*

estrogenicity assay. There are a number of factors that can alter responsiveness of *in vitro* test systems to estrogen agonists, and a concurrent PC can demonstrate that the test system is operative under the assay conditions and to provide a base for experiment-to-experiment comparisons (Zacharewsky, 1997). In both cases (*in vitro* and *in vivo* assays), appropriate PCs are particularly important to strengthen the predictive value of a negative result, *i.e*., a conclusion that the test chemical has no estrogenic activity.

The weakness of vom Saal et al.'s argument on the lack of appropriate PCs is that prostate enlargement due to prenatal exposure to low doses of xenoestrogens, or even to supra-physiological levels of natural estrogens, is far from being a well established effect. Except for Gupta's (2000) findings with DES, vom Saal et al.'s results with EST or xenoestrogens other than BPA (DES, MCX) were not replicated by any further study. As shown in Tables 1 and 2, a vast majority of subsequent rodent studies failed to reproduce not only BPA low dose effects but also effects of DES or any other putative xenoestrogen. Therefore, their statement that "…*the study fails to detect low-dose effects of a test chemical.."* because a PC (positive response) "…*is required to demonstrate that the experimental system was capable of detecting such effects"* (and thus test validity) is based on a false premise, i.e., that prostate enlargement is a well-established low dose effect of xenoestrogens. As far as PCs are concerned, the criticism of studies that did not replicate vom Saal et al.'s findings looks like a circular reasoning, *i.e.*, ".the negative studies did not demonstrate a low dose response of prostate to a xenoestrogen (BPA) because they did not show a prostate wt response to low doses of another xenoestrogen (PC) either". The question is not whether BPA is a xenoestrogen, it is whether prenatal exposure to xenoestrogens induces prostate enlargement.

3.5 Pre- and/or perinatal exposure to BPA and mammary gland development

A series of studies by Soto and coworkers published between 2001 and 2008 found that pre- and early postnatal exposure of CD-1 mice and Wistar rats to low doses of BPA (in the μg/kg or even ng/kg body wt/d range) affected further mammary gland histogenesis and architecture (increased duct branching, ductal hyperplasia, cell proliferation rates) (Table 3). Soto et al. administered BPA continuously through an osmotic pump implanted under the rodent skin. A study by the same group (Tharp et al., 2012) in which a higher dose of BPA (400 μg/kg/day) was given orally to Rhesus monkeys yielded equivocal results.

The foregoing findings by Soto et al. were to some extent contradicted by other researchers' results (Table 4). Nikaido et al. treated CD-1 mice with higher doses of BPA (in the mg dose range, *sc* injections) during gestation (Nikaido et al., 2004) or after birth before puberty (Nikaido et al., 2005). The authors found an acceleration of puberty onset and mammary gland differentiation after prenatal exposure (Nikaido et al., 2004) and no alteration of mammary gland growth in mice treated postnatally (Nikaido et al., 2005). Yin et al. (2006) injected (*sc*) BPA into rats (0.1 to 10 mg/rat) before puberty and observed an inhibition of terminal ducts (TD) and alveolar buds (AB) and a decrease of mammary gland tumors induced by MNU. Vandenberg, Soto and others (Vanderberg et al., 2012) argued that discrepancies between their own data and those provided by Nikaido et al. (2004, 2005) and Yin et al. (2006) could possibly arise from the fact that these authors exposed rodents to higher doses of BPA for shorter developmental periods. In fact, so far no independent investigation has replicated exactly the design and experimental conditions of studies by Soto et al. and thus their primary findings were not either directly contradicted or confirmed. A study by Moral et al. (2008), for instance, treated orally pregnant rats with BPA and noted that female offspring exposed to 250 μg/kg/d (but not those treated with 25 μg/kg/d) exhibited mammary gland architectural changes such as increased numbers of terminal end buds (TEB), terminal ducts (TD) and lobules type 1. These alterations of undifferentiated epithelial structures, however, were transient as a higher number of TEB was noted on PND 21 but not on PND 35 and 50, TD was increased on PND 21 and 100 but not on PND 35 and 50, and lobule 1 was augmented on PND35 but not thereafter. The authors, on the other hand, found no effect of BPA on the

mammary tissue proliferative index (Moral et al., 2008). Colerangle and Roy (1997) reported that peripubertal exposure to BPA by the *sc* route, in the mg/kg/d dose range, caused a dose-related increase in the proliferative activity of mammary epithelial cells. The authors also noted that BPA had a profound effect on epithelial cell proliferation compared to that of an equivalent dose of DES, a by far more potent xenoestrogen (Colerangle and Roy, 1995, 1997). Lamartiniere et al. (2011) reported that the offspring of rats treated orally with BPA (25 and 250 μg/kg bwt/d) during lactation (i.e., transfer through maternal milk) were more susceptible to mammary tumors induced by DMBA [*7,12*-dimethylbenz(*a*)anthracene]. The authors also described that offspring of dams treated during pregnancy (transplacental transfer) did not differ from controls regarding to susceptibility to DMBA-induced mammary tumors (Lamartiniere et al., 2011). It is of note that Lamartinere et al.'s results (2011) are not consistent with those reported by Durando et al. (2007) who found an increased susceptibility to MNU (neoplastic lesions in mammary gland) in rats exposed in utero to BPA (Table 3). Along the same line, Yoshida et al. (2004) treated rats with BPA (0.006 and 6 mg/kg/d po) during pregnancy and lactation and found no effect on age-related changes in reproductive organs and carcinogenesis (uteri) of the exposed offspring at 15 months of age.

Some of the foregoing results are to some extent consistent with Soto et al.'s findings. Taken together, however, these studies have a number of inconsistencies among them regarding BPA doses, routes of administration, developmental periods of exposure and the biological response.

Table 3 Effects of developmental exposure to BPA on the long-term growth and organization of mammary glands in rodents and non-human primates: Studies by Soto and co-workers.

Strain/species	Exposure				Results	Study (year)
	Chemical	Period	Doses	Route		
CD-1 mice	BPA	GD9-delivery (GD20)	25, 250 μg/kg/d	sc (op)	(PD 10, 30; 6 month old) 6 mo: increase % of ducts, TD, TEB, AB. %cells BrdU decreased PD10, increased 6 mo	Markey et al., 2001a
CD-1 mice	BPA	GD-9-PD4	25, 250 ng/kg/d	sc (op)	(PD 20/30, 4 month old) 30 day old: Increased area and numbers of terminal end buds relative to the gland ductal area; apoptotic activity decreased. Adult (ovariectomized) enhanced sensitivity to EST	Muñoz-de-Toro et al., 2005
CD-1 mice	BPA	GD-8-18	250 ng/kg/d	sc (op)	(term fetuses): Increased ductal extension and area; Stroma, promoted maturation of the fat pad and altered the localization of collagen; Epithelium, decrease in cell size and delayed lumen formation	Vandenberg et al., 2007
CD-1 mice	BPA	G+L (GD8-PD16)	0.25, 2.5, 25 μg/kg/d	sc (op)	(3, 9, 12-15 mo.) AB, intraductal hyperplasias, increased proliferation indexes	Vandenberg et al., 2008
Wistar rats	BPA	GD 8-23	25 μg/kg/d	sc (op)	(PD 30, 50, 110,180) increased proliferation/apoptosis ratio, hyperplastic ducts. MNU (PD 50), increased hyperplastic ducts, neoplastic lesions	Durando et al., 2007
Wistar-Furth rats	BPA	GD9-PD1	2.5, 25, 250, 1000 μg/kg/d	sc (op)	(PD 50, 95) increased ductal hyperplasias and neoplastic lesions	Murray et al., 2007
Rhesus monkeys	BPA	GD100 - 165	400 μg/kg/d[#]	po	At birth, MG: no difference ER expression, increase in the number of buds per ductal units (bud density). Although ductal area, number of buds, TEB, branching points were increased, differences were not statistically different (N, control=5; BPA=4)	Tharp et al., 2012

op: osmotic pump; SD: Sprague-Dawley rats; dwt: drinking water; G: gestation; L: lactation period exposures; BPA: bisphenol A; [#] 0.68± 0.312 ng of unconjugated BPA per ml maternal serum; EST: 17β-estradiol; MNU: methylnitrosourea; TD: terminal duct; TED: terminal end bud; AB: alveolar bud; ER: estrogen receptor; PD: postnatal day.

Table 4 Effects of developmental exposure to BPA on the long-term growth and organization of mammary glands in rodents: Studies by research groups other than that of Soto and coworkers.

Species/ strain	Exposure				Results	Study (year)
	Chemical	Period	Doses	Route		
Noble rats	BPA	Peripubertal 4-5 week old (11 days)	0.1, 54 mg/kg/d	sc (op)	(4-5 week old) Increased proliferative activity (LD 143%, HD 220%)	Colerangle and Roy, 1997
Noble rats	DES	Peripubertal (11 days)	0.1 mg/kg/d	sc (op)	(4-5 week old) Increased proliferative activity	Colerangle and Roy, 1995
SD-CD rats	BPA	GD10-delivery	25, 250 μg/kg/d	po	(PD 25, 35, 50, 100) HD: changes in the number of undifferentiated epithelial structures. No change in proliferative index, both doses changed gene expression signature of MG.	Moral et al., 2008
CD-1 mice	BPA	GD15-18	0.5, 10 mg/kg/d	sc	(PD 4, 8, 12, 16 weeks) both doses: transient effects on mammary gland differentiation. Earlier puberty onset. Differentiation accelerated at 4 weeks of age	Nikaido et al., 2004
CD-1 mice	BPA	Prepubertal PD15, 4 d	10 mg/kg/d	sc	No alteration of mammary gland growth	Nikaido et al., 2005
SD-CD rats	BPA	Prepubertal PD 2,4,6	0.1, 10 mg/pup/d (3 injections)	sc	A: PD 35: decreased Terminal end bud, terminal duct, alveolar bud, BPA decreased TD and AB B: 33 weeks: did not increase incidence and multiplicity of MNU (7 wk sc) induced MG tumors	Yin et al., 2006
C57BL6/ J mice	BPA	M+G+L	≈ 0.12 μg to 1.2 mg/kg/d	dwt	(PD 30) altered response to progesterone	Ayyanan et al., 2011
SD rats	BPA	L (PD2-21)	25, 250 μg/kg/d (dam)	po	Increased (multiplicity) DMBA (PD 50) induced MG tumors (6 mo later)	Lamartiniere et al., 2011
SD rats		GD 2-20	25, 250 μg/kg/d (dam)	po	No enhancement of DMBA (PD 50) induced MG tumors (6 mo later)	Lamartiniere et al., 2011

op: osmotic pump; SD: Sprague-Dawley rats; dwt: drinking water; [+] According to authors' interpretation the effect was probably due to sampling design and thus was considered of no toxicological significance. V: ventral; A: anterior; DL: dorso lateral prostate; M: mating period; PM: premating; G: gestation; L: lactation period exposures; AP-Wistar: Alderly Park-Wistar; BPA: bisphenol A; DES: diethylstilbestrol; EE: ethinyl estradiol; PD: postnatal day; DMBA: dimethylbenzantrhracene; LD: lower dose; HD: higher dose.

3.6 The kinetic enigma

In vitro assays have consistently shown that BPA is a weak estrogenic compound, some orders of magnitude less potent than EST the endogenous ligand of estrogen receptor-α. A study by Gaido et al. (1997) with the Yeast based estrogen receptor assay (*S. cerevisae* expressing human estrogen receptor) found EC_{50}s of 2.25 $x10^{-10}$ for EST, 3.53 $x10^{-10}$ for DES and 3.40 $x10^{-6}$ for BPA, that gave rise to calculated potency ratios (chemical-EC_{50}/EST-EC_{50}) of 1.00, 1.57 and 15,000 for EST, DES and BPA, respectively. Another study by Harris et al. (1997) using the yeast assay for testing estrogenicity of phthalates and BPA found that BPA is active only at molar concentrations >10,000 times higher than those of the positive control substance (EST).

An inter-laboratory study of YES assay by Dhooge et al. (2006) reported EC_{50}s for BPA (5.5-7.0 $x10^{-5}$; molar concentrations) >10,000 higher than those found for EST (3.5-8.1 $x10^{-11}$). Recently, Lee et al. (2012) also demonstrated that, in the STTA assay (i.e., stably transfected transcriptional activation assay), estrogenic activity of BPA (PC_{50} = 1.26 x 10^{-7} M) was 5,000 fold less than that of EST (PC_{50} = 2.43 x 10^{-11} M). Furthermore, the relative potencies (ratio of EC_{50}s) of EST, EE, DES and BPA determined by Andersen et al.'s (1999) inter-laboratory study in the E-screen (E-screen, MCF-7cell proliferation assay) and yeast assay (YES), were as follows: E-screen, EST =1, EE= 0.9 and 0.03, DES=0.05, BPA = $1x10^{-5}$, $3x10^{-6}$, $5x10^{-7}$; YES, EST=1, EE=1.6 and 3.3, DES= 0.2 and 0.4, BPA= $8x10^{-5}$, $4x10^{-5}$).

Not only in functional assays of estrogenicity (YES, E-screen, STTA), but also in the relative binding assays, BPA has showed a much lower affinity for ERα compared to that of the natural ligand EST (i.e., $2x10^2$, 10^5 or 10^4, $8x10^3$ fold less) (Krishnan et al., 1993; Olea et al., 1996; Nagel et al., 1997; Andersen et al., 1999).

In the *in vivo* uterotrophic assay, BPA (sc injection) exhibited an estrogen agonistic effect in ovariectomized mice at 100 mg/kg/d (7 d) and above (Ohta et al., 2012). A BPA dose of 300 mg/kg/d induced a 2-fold increase in uterine wt which was 2/3 of that produced by 0.2 μg/kg/d of EE (Ohta et al., 2012). Markey et al. (2001b) noted, in immature female CD-1 mice treated (sc implanted osmotic pumps) with doses of BPA ranging from 0.1 to 100 mg/kg/d (3 days), that only the highest dose tested increased uterine wt. A dose of EST as low as 5 μg/kg/d (osmotic pump, 3 d) caused an increase in uterine wt that was 2-fold that produced by 100 mg/kg/day of BPA (Markey et al., 2001b). A comparable sensitivity to detect estrogenic effects of BPA was reported for rat uterotrophic assays using different test protocols (Kanno et al., 2003).

In summary, data from different rat and mouse uterotrophic assays suggested that *in vivo* BPA is at least 10,000-fold less potent than EE (Markey et al., 2001b; Kanno et al., 2003; Tinwell and Ashby, 2004; Ohta et al., 2012).

Studies of BPA kinetics showed that this phenolic compound is converted by liver enzymes (UGT and sulfotransferases, SULT isoforms) into BPA monoglucuronide and (to a lesser extent) sulfate conjugates and rapidly cleared from the blood (Pottenger et al., 2000; Vanderberg et al., 2010; Taylor et al., 2011). It has also been demonstrated in rodents and humans that BPA conjugates are the dominant species in the blood (serum/plasma) (Pottenger et al., 2000; Taylor et al., 2011), urine (Vanderberg et al., 2010; Liao and Kannan, 2012) and maternal milk (Mendonça et al., 2014). Both metabolites (BPA-glucuronide and BPA-sulfate) proved to be devoid of any estrogenic activity (Matthews et al., 2001; Shimizu et al., 2002). The rapid clearance of BPA and the fact that its major metabolites are inactive at the estrogen receptor suggest that BPA health risk from BPA is negligible. Some authors (Ginsberg and Rice, 2009; Vanderberg et al., 2010), however, argued that BPA metabolites could be deconjugated at the site of action by local tissue β-glucuronidases. Although being a plausible hypothesis (glucuronidases are in fact present in placenta and other key tissues), it remains undemonstrated whether local deconjugation of BPA metabolites account for estrogenic effects of low doses of BPA reported in the literature. Owing to the marked pre-systemic clearance, low doses of orally administered BPA (e.g., vom Saal et al.'s and Gupta et al.'s studies) give rise to very low blood levels of unconjugated BPA. It remains an enigma how very low blood levels of a weak xenoestrogen, orders of magnitude less potent than the natural receptor ligand, disrupt endocrine function in rodents.

3.7 Epidemiology studies

In 2008, an observational (cross-sectional) study by Lang et al. (2008) found an association between urinary levels of BPA (conjugated plus unconjugated BPA) and cardiovascular disease and diabetes in American adults. Lang et al.´s study fueled the debate on the health risks associated with exposure to low (environmental) levels of BPA. Urine levels, however, reflect recent BPA exposures (within a few hours), and cardiovascular disorders and diabetes start much earlier in individuals' life. Along the same line, a cross-sectional study by

Trasande et al. (2012) described an association of urinary levels of BPA in children and adolescents and obesity. Causality, however, is an unlikely explanation for this association. Obese children and adolescents' higher urinary levels of BPA (recent exposure) could be a consequence of their current food habits rather than a cause of obesity.

A further study by LaKind et al. (2012) found no associations between urinary BPA and heart disease or diabetes and authors highlighted that using cross-sectional data bases (like the US National Health and Nutrition Examination Survey used by Lang et al. (2008) and Trasande et al. (2012) to draw conclusions about associations between exposure to short-lived chemicals and chronic diseases is inappropriate. A systematic review of both consistency and the quality of epidemiological evidence concluded that assertions about a causal link between BPA and cardiovascular diseases, diabetes and obesity are unsubstantiated (LaKind et al., 2014).

4 Concluding Remarks

The scientific value and applicability of conclusions from any experimental study stands on its reliability – or the extent to which their findings are consistent over time – and also on its validity, or the extent to which the experimental study truly measured what it was intended to measure. The reliability is therefore a cornerstone principle of experimental sciences. Reliability is demonstrated by repeatability (i.e., internal consistency) and reproducibility or replication of original findings by independent researchers. In toxicological sciences, therefore, researchers other than those who conducted the initial study must be able to replicate the experiment and, by doing so under exactly the same experimental conditions, to achieve the same findings.

The reported effects of BPA on the prostate and mammary gland have hallmarks of endocrine disruption: a putative endocrine (estrogenic) mode of action, a developmental window (gestation and/or pre-pubertal) of sensitivity, low-dose effects, non-monotonic dose response curves, and possibly related adverse health effects that manifest later in life (prostate hyperplasia and/or cancer, mammary gland tumors). As aforementioned, both findings were consistently repeated by those who originally reported them but were not reproduced by independent researchers.

Reports that were not reproduced by independent researchers and even those that proved to be irreproducible are not uncommon in the scientific literature. In fundamental sciences, experimental findings are not fully accepted until they proved to be reproducible. In toxicology, however, results suggesting that a chemical poses health hazards may raise high concerns before they are confirmed by independent replication. If uncertainties persist due to a lack of sufficient toxicity data to conduct a reliable risk assessment, then regulatory decisions to protect public health can be taken with basis on the so-called "precautionary principle". In 2011 European Union, Brazil and some other countries banned baby bottles containing BPA. Paradoxically for one of, if not the most toxicologically studied chemical, EU (2011) and Brazilian (2011) regulatory decision on BPA was based on the precautionary principle due to uncertainties concerning its effects on infants (EU Commission Directive, 2011).

Recently, Silbergeld and Scherer (2013) proposed an evidence-based toxicology approach for decision-making. They highlighted that systematic reviews are seldom undertaken in toxicology by regulatory agencies, international organizations or academic scientists. According to them (Silbergeld and Scherer, 2013), this approach, albeit not obviating the role of judgment and values in decision-making, ensures that regulatory decisions stand on all available information analyzed in a transparent and unbiased manner. This critical review on the most controversial topics of BPA toxicity is to be regarded as an academic contribution to evidence-based approaches in toxicology and regulatory decision-making.

5 Highlights

- In vitro and in vivo assays demonstrated consistently that BPA is a weak estrogenic compound, orders of magnitude less potent than 17β-estradiol.
- In the liver, BPA is promptly converted into conjugated metabolites BPA-glucuronides and BPA-sulfate that are rapidly cleared from the blood. After oral exposure, BPA undergoes a marked pre-systemic clearance.
- In vitro assays showed that BPA-conjugates (BPA-monoglucuronid and BPA-sulfate) are devoid of any estrogenic activity.
- Except for a study by Gupta et al., no independent research has reproduced vom Saal et al.'s results showing that prenatal exposure of rodents to low doses of BPA increased prostate size in adulthood. Three replication studies in CF-1 and CD-1 mice directly contradicted vom Saal et al.´s reports.
- Owing to a lack of replication studies, results by Soto et al. suggesting that pre- and/or postnatal (prebubertal) exposure of rodents to low doses of BPA alter tissue architecture and epithelial cells proliferative activity in mammary tissue were not either contradicted or confirmed by independent investigations.
- Epidemiological evidence for associations between exposure to BPA and chronic diseases remains elusive.

Acknowledgments

The sentence in the title is from a poem by Ted Sheridan. FJRP is the recipient of a research fellowship from CNPq (Brazilian National Research Council). The authors certify that there is no conflict of interest with any financial organization regarding the topic discussed in the article. Authors' research projects are supported by grants from CNPq, Rio de Janeiro State Agency for Supporting Research (FAPERJ) and the National School of Public Health – FIOCRUZ Program for Innovation in Health Sciences (INOVA-ENSP).

References

Andersen HR, Andersson AM, Arnold SF, et al. 1999. Comparison of short-term estrogenicity tests for identification of hormone-disrupting chemicals. Environmental Health Perspectives, 107(Suppl 1): 89-108

Anonymous. 1997. Reproductive toxicology. Bisphenol A. Environmental Health Perspectives, 105(Suppl 1): 273-274

Ashby J, Tinwell H, Haseman J. 1999. Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed in utero. Regulatory Toxicology and Pharmacology, 30(2 Pt 1): 156-166

Ayyanan A, Laribi O, Schuepbach-Mallepell S, et al. 2011. Perinatal exposure to bisphenol A increases adult mammary gland progesterone response and cell number. Molecular Endocrinology, 25(11): 1915-1923

Berthois Y, Katzenellenbogen JA, Katzenellenbogen BS. 1986. Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. Proceedings of the National Academy of Sciences of the United States, 83(8): 2496-2500

BRASIL-ANVISA RDC 41/2011. Dispõe sobre a proibição de uso de bisfenol A em mamadeiras destinadas a alimentação de lactentes e dá outras providências. Brasília, DF, 16.09.2011

Brotons JA, Olea-Serrano MF, Villalobos M, et al. 1995. Xenoestrogens released from lacquer coatings in food cans. Environmental Health Perspectives, 103(6): 608-612

Cagen SZ, Waechter JM Jr, Dimond SS, Breslin WJ, et al. 1999a. Normal reproductive organ development in Wistar rats exposed to bisphenol A in the drinking water. Regulatory Toxicology and Pharmacology, 30(2 Pt 1): 130-139

Cagen SZ, Waechter JM Jr, Dimond SS, Breslin WJ, et al. 1999b. Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A. Toxicological Sciences, 50(1): 36-44

Colerangle JB, Roy D. 1995. Perturbation of cell cycle kinetics in the mammary gland by stilbene estrogen, diethylstilbestrol (DES). Cancer Letters, 94(1): 55-63

Colerangle JB, Roy D. 1997. Profound effects of the weak environmental estrogen-like chemical bisphenol A on the growth of the mammary gland of Noble rats. The Journal of Steroid Biochemistry and Molecular Biology, 60(1-2): 153-160

Dhooge W, Arijs K, D'Haese I, Stuyvaert S, et al. 2006. Experimental parameters affecting sensitivity and specificity of a yeast assay for estrogenic compounds: results of an interlaboratory validation exercise. Analytical and Bioanalytical Chemistry, 386(5): 1419-1428

Dodds EC, Lawson W. 1938. Molecular structure in relation to oestrogenic activity. Compounds without a phenanthrene group. Proceedings of the Royal Society of London, 125(839): 222-232

Durando M, Kass L, Piva J, Sonnenschein C, et al. 2007. Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. Environmental Health Perspectives, 115(1): 80-86

Editorial. 1996. Dangerous dental sealants? Environmental Health Perspectives, 104(4): 373-374

Ema M, Fujii S, Furukawa M, Kiguchi M, et al. 2001. Rat two-generation reproductive toxicity study of bisphenol A. Reproductive Toxicology, 15(5): 505-523

Elswick BA, Janszen DB, Gould JC, Stedman DB, Welsch F. 2000. Effects of perinatal exposure to low doses of bisphenol A in male offspring of Sprague-Dawley rats. Toxicologist, 54: 256

EU Commission Directive 2011/8/EU. Restriction of use of bisphenol A in plastic infant feeding bottles. Official Journal of the European Union, 29.01.2011. L26/11

Feldman D, Krishnan A. 1995. Estrogens in unexpected places: possible implications for researchers and consumers. Environmental Health Perspectives, 103(Suppl 7): 129-133

Fregert S, Rorsman H. 1960. Hypersensitivity to diethylstilbestrol. Cross-sensitization to dienestrol, hexestrol, bisphenol A, p-benzyl-phenol, hydroquinone-monobenzyle ther, and p-hydroxybenzoic-benzyl ester. Acta Dermato-venereologica, 40: 206-219

Fregert S, Rorsman H. 1962. Hypersensitivity to epoxy resins with reference to the role played by bisphenol A. The Journal of Investigative Dermatology, 39: 471-472

Gaido KW, Leonard LS, Lovell S, Gould JC, et al. 1997. Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicology and Applied Pharmacology, 143(1): 205-212

Gaul LE. Sensitivity to bisphenol A. 1960. Archives of Dermatology, 82: 1003

Ginsberg G, Rice DC. 2009. Does rapid metabolism ensure negligible risk from bisphenol A? Environmental Health Perspectives, 117(11): 1639-1643

Gupta C. 2000. Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. Proceedings of the Society for Experimental Biology and Medicine, 224(2): 61-68

Habib CM, Kugel G. 1996. Estrogenicity of resin-based composites and sealants in dentistry. Environmental Health Perspectives, 104(8): 808

Harris CA, Henttu P, Parker MG, Sumpter JP. 1997. The estrogenic activity of phthalate esters in vitro. Environmental Health Perspectives,105(8): 802-811

Howdeshell KL, Furr J, Lambright CR, Wilson VS, et al. 2008. Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male long Evans hooded rat. Toxicological Sciences, 102(2): 371-382

Ichihara T, Yoshino H, Imai N, Tsutsumi T, et al. 2003. Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol A in rats. The Journal of Toxicological Sciences, 28(3): 165-171

Judy BM, Nagel SC, Thayer KA, Vom Saal FS, Welshons WV. 1999. Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicology and Industrial Health, 15(1-2): 12-25

Kanno J, Onyon L, Peddada S, Ashby J, et al. 2003. The OECD program to validate the rat uterotrophic bioassay. Phase 2: dose-response studies. Environmental Health Perspectives, 111(12): 1530-1549

Knaak JB, Sullivan LJ. Metabolism of bisphenol A in the rat. 1966. Toxicology and Applied Pharmacololy, 8(2): 175-184

Krishnan AV, Stathis P, Permuth SF, Tokes L, Feldman D. 1993. Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology, 132(6): 2279-2286

Kwon S, Stedman DB, Elswick BA, Cattley RC, Welsch F. 2000. Pubertal development and reproductive functions of Crl:CD BR Sprague-Dawley rats exposed to bisphenol A during prenatal and postnatal development. Toxicological Sciences, 55(2): 399-406

LaKind JS, Goodman M, Naiman DQ. 2012. Use of NHANES data to link chemical exposures to chronic diseases: a cautionary tale. PLoS One, 7(12): e51086

LaKind JS, Goodman M, Mattison DR. 2014. Bisphenol A and indicators of obesity, glucose metabolism/type 2 diabetes and cardiovascular disease: A systematic review of epidemiologic research. Critical Reviews in Toxicology (in print)

Lamartiniere CA, Jenkins S, Betancourt AM, Wang J, Russo J. 2011. Exposure to the endocrine disruptor Bisphenol A alters susceptibility for mammary cancer. Hormone Molecular Biology and Clinical Investigation, 5(2): 45-52

Lang IA, Galloway TS, Scarlett A, Henley WE, et al. 2008. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA: The Journal of the American Medical Association, 300(11): 1303-1310

Lee HK, Kim TS, Kim CY, Kang IH, et al. 2012. Evaluation of in vitro screening system for estrogenicity: comparison of stably transfected human estrogen receptor-α transcriptional activation (OECD TG455) assay and estrogen receptor (ER) binding assay. The Journal of Toxicological Sciences, 37(2): 431-437

Liao C, Kannan K. 2012. Determination of free and conjugated forms of bisphenol A in human urine and serum by liquid chromatography-tandem mass spectrometry. Environmental Science and Technology, 46(9): 5003-5009

Markey CM, Luque EH, Munoz De Toro M, Sonnenschein C, Soto AM. 2001a. In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. Biology of Reproduction, 65(4): 1215-1223

Markey CM, Michaelson CL, Veson EC, Sonnenschein C, Soto AM. 2001b. The mouse uterotrophic assay: a reevaluation of its validity in assessing the estrogenicity of bisphenol A. Environmental Health Perspectives, 109(1): 55-60

Matthews JB, Twomey K, Zacharewski TR. 2001. In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. Chemical Research in Toxicology, 14(2): 149-157

Mendonça K, Hauser R, Calafat AM, Arbuckle TE, Duty SM. 2014. Bisphenol A concentrations in maternal breast milk and infant urine. International Archives of Occupational Environmental Health, 87(1): 13-20

Moral R, Wang R, Russo IH, Lamartiniere CA, et al. 2008. Effect of prenatal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. The Journal of Endocrinology, 196(1): 101-112

Morrissey RE, George JD, Price CJ, Tyl RW, et al. 1987. The developmental toxicity of bisphenol A in rats and mice. Fundamental and Applied Toxicology, 8(4): 571-582

Muñoz-de-Toro M, Markey CM, Wadia PR, Luque EH, et al. 2005. Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. Endocrinology, 146(9): 4138-4147

Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM. 2007. Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. Reproductive Toxicology, 23(3): 383-390

Nagel SC, vom Saal FS, Thayer KA, Dhar MG, et al. 1997. Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. Environmental Health Perspectives, 105(1): 70-76

National Toxicology Program. Carcinogenesis Bioassay of Bisphenol A (CAS No. 80-05-7) in F344 Rats and B6C3F1 Mice (Feed Study). 1982. National Toxicology Program Technical Report Series, 215: 1-116

Nikaido Y, Yoshizawa K, Danbara N, Tsujita-Kyutoku M, et al. 2004. Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. Reproductive Toxicology, 18(6): 803-811

Nikaido Y, Danbara N, Tsujita-Kyutoku M, Yuri T, et al. 2005. Effects of prepubertal exposure to xenoestrogen on development of estrogen target organs in female CD-1 mice. In Vivo, 19(3): 487-494

Ohta R, Takagi A, Ohmukai H, Marumo H, et al. 2012. Ovariectomized mouse uterotrophic assay of 36 chemicals. The Journal of Toxicological Sciences, 37(5): 879-889

Olea N, Pulgar R, Pérez P, Olea-Serrano F, et al. 1996. Estrogenicity of resin-based composites and sealants used in dentistry. Environmental Health Perspectives, 104(3): 298-305

Owens JW, Chaney JG. 2005. Weighing the results of differing 'low dose' studies of the mouse prostate by Nagel, Cagen, and Ashby: quantification of experimental power and statistical results. Regulatory Toxicology and Pharmacology, 43(2): 194-202

Pottenger LH, Domoradzki JY, Markham DA, Hansen SC, et al. 2000. The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. Toxicological Sciences, 54(1): 3-18

Sharpe RM, Fisher JS, Millar MM, Jobling S, Sumpter JP. 1995. Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production. Environmental Health Perspectives, 103(12): 1136-1143

Shimizu M, Ohta K, Matsumoto Y, Fukuoka M, et al. 2002. Sulfation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells. Toxicology in Vitro, 16(5): 549-556

Silbergeld E, Scherer RW. 2013. Evidence-based toxicology: strait is the gate, but the road is worth taking. ALTEX, 30(1): 67-73

Soto AM, Justicia H, Wray JW, Sonnenschein C. 1991. p-Nonyl-phenol: an estrogenic xenobiotic released from "modified" polystyrene. Environmental Health Perspectives, 92: 167-173

Taylor JA, Vom Saal FS, Welshons WV, Drury B, et al. 2011. Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. Environmental Health Perspectives, 119(4): 422-430

Tharp AP, Maffini MV, Hunt PA, VandeVoort CA, et al. 2012. Bisphenol A alters the development of the rhesus monkey mammary gland. Proceedings of the National Academy of Sciences of the United States, 109(21): 8190-8195

Tinwell H, Haseman J, Lefevre PA, Wallis N, Ashby J. 2002. Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. Toxicological Sciences, 68(2): 339-348

Tinwell H, Ashby J. 2004. Sensitivity of the immature rat uterotrophic assay to mixtures of estrogens. Environmental Health Perspectives, 112(5): 575-582

Trasande L, Attina TM, Blustein J. 2012. Association between urinary bisphenol A concentration and obesity prevalence in children and adolescents. JAMA: The Journal of the American Medical Association, 308(11): 1113-1121

Tyl RW, Myers CB, Marr MC, Thomas BF, et al. 2002. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicological Sciences, 68(1): 121-146

Tyl RW. 2003. Bisphenol A: findings of a multigenerational rat study. Environmental Health Perspectives, 111(12): A632

Tyl RW, Myers CB, Marr MC, Sloan CS, et al. 2008. Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. Toxicological Sciences,104(2): 362-384

U.S.EPA. Uterotrophic Assay. OCSPP Guideline 890.1600 *Standard Evaluation Procedure (SEP)* Endocrine Disruptor Screening Program 2011; U.S. Environmental Protection Agency, Washington DC, USA

Vandenberg LN, Maffini MV, Wadia PR, Sonnenschein C, et al. 2007. Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland. Endocrinology, 148(1): 116-127

Vandenberg LN, Maffini MV, Schaeberle CM, Ucci AA, et al. 2008. Perinatal exposure to the xenoestrogen bisphenol-A induces mammary intraductal hyperplasias in adult CD-1 mice. Reproductive Toxicology, 26(3-4): 210-219

Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM. 2009. Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocrine Reviews, 30(1): 75-95

Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, et al. 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environmental Health Perspectives, 118(8): 1055-1070

Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, et al. 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocrine Reviews, 33(3): 378-455

vom Saal FS, Timms BG, Montano MM, Palanza P, et al. 1997. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proceedings of the National Academy of Sciences of the United States, 94(5): 2056-2061

vom Saal FS, Richter CA, Ruhlen RR, Nagel SC, et al. 2005. The importance of appropriate controls, animal feed, and animal models in interpreting results from low-dose studies of bisphenol A. Birth Defects Research. Part A, Clinical and Molecular Teratology, 73(3):140-145

vom Saal FS, Welshons WV. 2006. Large effects from small exposures. II. The importance of positive controls in low-dose research on bisphenol A. Environmental Research, 100(1): 50-76

vom Saal FS, Akingbemi BT, Belcher SM, Crain DA, et al. 2010. Flawed experimental design reveals the need for guidelines requiring appropriate positive controls in endocrine disruption research. Toxicological Sciences, 115(2): 612-613

Yoshida M, Shimomoto T, Katashima S, Watanabe G, et al. 2004. Maternal exposure to low doses of bisphenol a has no effects on development of female reproductive tract and uterine carcinogenesis in Donryu rats. The Journal of Reproduction and Development, 50(3): 349-360

Yoshino H, Ichihara T, Kawabe M, Imai N, et al. 2002. Lack of significant alteration in the prostate or testis of F344 rat offspring after transplacental and lactational exposure to bisphenol A. The Journal of Toxicological Sciences, 27(5): 433-439

Yin H, Ito A, Bhattacharjee D, Hoshi M. 2006. A comparative study on the protective effects of 17beta-estradiol, biochanin A and bisphenol A on mammary gland differentiation and tumorigenesis in rats. Indian Journal of Experimental Biology, 44(7): 540-546

Zacharewski T. 1997. *In Vitro* Bioassays for Assessing Estrogenic Substances. Environmental Science & Technology, 31(3): 613-623

Zakova N, Zak F, Froehlich E, Hess R. 1985. Evaluation of skin carcinogenicity of technical 2,2-bis-(p-glycidyloxyphenyl)-propane in CF1 mice. Food and Chemical Toxicology, 23(12): 1081-1089