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Endocrine Disruption Potentials of Bisphenol A Alternatives - Are Bisphenol A Alternatives Safe from Endocrine Disruption?

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ABSTRACT

Objectives: Although a great body of knowledge is available on the toxicity of bisphenol A (BPA), little is known about that of BPA alternatives, such as bisphenol analogues (BPs) or Tritan™ copolyesters. This review provides a summary of the available information on the toxicity of BPs and three components of Tritan™, with a special focus on endocrine disruption.

Methods: We collected from the literature a battery of *in vitro* and *in vivo* assay data developed to assess endocrine disruption of four BPs (bisphenol AF, B, F, and S) and three major components of Tritan™ ((di-methylterephthalate (DMT), 1,4-cyclohexanedimethanol (CHDM), and 2,2,4,4-tetramethyl-1,3-cyclobutanediol (TMCD)).

Results: Several alternative compounds were identified as possessing comparable or even greater endocrine-disrupting effects than BPA in *in vitro* and *in vivo* studies.

Conclusions: Potential endocrine disruption of BPA alternatives requires further studies on health consequences in experimental animals and in humans following longer term exposure.

Keywords: bisphenols, endocrine disruption, Tritan

I. Introduction

Endocrine disruptions due to exposure to chemicals in various consumer products, e.g., plastics have received great attention.[1] Among them, bisphenol A (BPA; 2,2-bis(4-hydroxydiphenyl)propane, which has been produced over eight billion pounds each year worldwide, is frequently used as a monomer in the manufacture of polycarbonates and epoxy resins.[2] As BPA can disrupt steroidogenesis and act as a weak estrogen receptor agonist, concerns on adverse health outcomes, especially on reproduction and development, are increasing.[3,4] A large number of biomonitoring studies indicate widespread exposure to BPA in adults, adolescents, and children from several different countries,[5] while the results from toxicokinetic studies that determined the disposition of BPA in humans after oral administration of BPA are at odds with them.[6,7] In 2011, the European Commission has applied the precautionary principle on BPA and restricted its use in plastic infant feeding bottles.[8] In response to this restriction, a number of alternative compounds, such as bisphenol AF (BPAF; 2,2-bis(4-hydroxyphenyl)hexafluoropropane), bisphenol B (BPB; 2,2-bis(4-hydroxyphenyl)butane), bisphenol F (BPF; bis(4-hydroxydiphenyl)methane), and bisphenol S (BPS; bis(4-hydroxyphenyl)sulfone), began to be often used increasingly as component of plastic substitutes.[2] In addition, a novel plastic is also manufactured by Eastman Chemical Company (Kingsport, TN, USA) utilizing three monomers, di-methylterephthalate (DMT), 1,4-cyclohexanedimethanol (CHDM), and 2,2,4,4-tetramethyl-1,3-cyclobutanediol (TMCD) in various ratios, marketed under a trade name of Tritan™.[9]

The production and consumption of bisphenol analogues (BPs; Table 1) that are structurally similar to BPA with two hydroxyphenyl functionalities have

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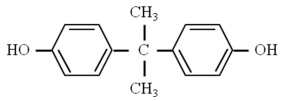
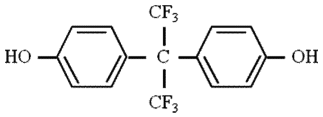
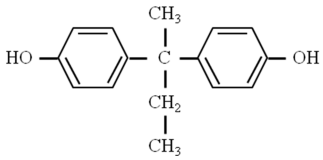
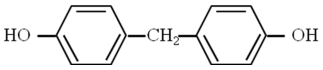
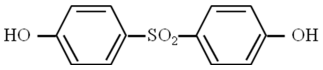
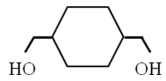
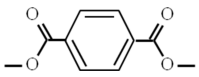
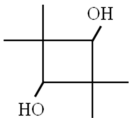
Table 1. Chemical structures of bisphenol A and its alternatives which are commonly used in consumer products

Abbreviation	Systematic name	CAS Number	Structure
BPA	2,2-bis(4-hydroxydiphenyl)propane	80-05-7	
BPAF	2,2-bis(4-hydroxyphenyl)hexafluoropropane	1478-61-1	
BPB	2,2-bis(4-hydroxyphenyl)butane	77-40-7	
BPF	Bis(4-hydroxydiphenyl)methane	87139-40-0	
BPS	Bis(4-hydroxyphenyl)sulfone	80-09-1	
CHDM	1,4-cyclohexanedimethanol	105-08-8 (mixture of cis and trans)	
DMT	Dimethyl terephthalate	120-61-6	
TMCD	2,2,4,4-tetramethyl-1,3-cyclobutanediol	3010-96-6 (mixture)	

increased recently.[10] BPAF, a fluorinated derivative of BPA, is widely used in polycarbonate copolymers in high-temperature composites, electronic materials, gas-permeable membranes, and specialty polymer applications.[11-14] Approximately 10,000-500,000 pounds of BPAF are produced annually in the United States.[15] BPB, a BPA analogue having a butyl chain instead of a propyl chain between the two phenol moieties, is utilized in the manufacture of resins and plastics.[16] BPF, which differs from BPA only by the lack of two methyl groups on the central carbons, has a broad range of industrial applications such as lacquers, varnishes, liners, adhesives plastics, food packaging, dental sealants, and water pipes.[17] BPS, whose two phenolic rings are joined together with sulfur, has excellent stability against high temperature and resistance to sunlight.[18] BPS has been introduced to the market as a component of plastic substitutes for the production of babybottles[19] or used as a developer in dyes for thermal paper.[20]

Tritan copolyester is used in packaging of beverages, edible oil, and foods, as well as for food contact films and foils including microwave packaging.[21] Three important co-monomers of Tritan, namely CHDM, DMT, and TMCD, were used for production of polyethylene terephthalate (PET) bottle in Lock & Lock® company. DMT is nominated as a high production volume chemical, both in the United States[22] and Organization for Economic Co-operation and Development.[23]

Recent studies have reported the occurrence of BPA alternatives in environmental samples, consumer products, food, and human specimens (Table 2). BPAF has been found in 76% of the 41

Table 2. Concentrations of bisphenol A alternatives in environment, consumer products, and biota

Compounds	Location	Sample	N	Median	Range	LOQ	References
BPAF	USA	Sediment	82	ND	ND	0.25 ng/g dw	Liao et al., 2012c
	Japan	Sediment	56	ND	ND	0.25 ng/g dw	Liao et al., 2012c
	Korea	Sediment	34	ND	<LOQ ~ 4.23 ng/g dw	0.25 ng/g dw	Liao et al., 2012c
	USA	Indoor dust	38	ND	ND	0.5 ng/g	Liao et al., 2012d
	China	Indoor dust	55	ND	ND	0.5 ng/g	Liao et al., 2012d
	Japan	Indoor dust	22	ND	ND	0.5 ng/g	Liao et al., 2012d
	Korea	Indoor dust	41	4.8 ng/g	<LOQ ~ 91 ng/g	0.5 ng/g	Liao et al., 2012d
BPB	Italy	Peeled canned tomatoes in canes with epoxyphenolic lining	6	33.4 μg/kg[a]	27.1 ~ 85.7 μg/kg	2.3 μg/kg	Grumetto et al., 2008
	Italy	Peeled canned tomatoes in canes with low BADGE coating	3	37.7 μg/kg[a]	31.3 ~ 45.5 μg/kg	2.3 μg/kg	Grumetto et al., 2008
	Italy	Serum	69	5.15 ng/mL[a]	0.88 ~ 11.94 ng/mL	0.18 ng/mL	Cobellis et al., 2009
	Portugal	Serum[b]	20	0.68 ng/mL	<LOQ ~ 1.15 ng/mL	0.05 ng/mL	Cunha and Fernandes, 2010
	Spain	Glass beverage soft-drink cola	1	ND	ND	167 ng/L	Gallart-Ayala et al., 2011
	Spain	Glass beverage soft-drink soda	5	ND	ND	167 ng/L	Gallart-Ayala et al., 2011
	Spain	Glass beverage soft-drink tonic	1	ND	ND	167 ng/L	Gallart-Ayala et al., 2011
	USA	Sediment	82	ND	ND	0.5 ng/g dw	Liao et al., 2012c
	Japan	Sediment	56	ND	ND	0.5 ng/g dw	Liao et al., 2012c
	Korea	Sediment	34	ND	<LOQ ~ 10.6 ng/g dw	0.5 ng/g dw	Liao et al., 2012c
	USA	Indoor dust	38	ND	ND	1.0 ng/g	Liao et al., 2012d
	China	Indoor dust	55	ND	ND	1.0 ng/g	Liao et al., 2012d
	Japan	Indoor dust	22	ND	ND	1.0 ng/g	Liao et al., 2012d
	Korea	Indoor dust	41	ND	ND	1.0 ng/g	Liao et al., 2012d
BPF	Germany	Surface-water	30	-	<LOQ ~ 180 ng/L	2 ng/L	Fromme et al., 2002
	Germany	Sewage water	25	-	22 ~ 123 ng/L	2 ng/L	Fromme et al., 2002
	Germany	Sediment	7	-	1,200 ~ 7,300 ng/kg dw	5 ng/kg	Fromme et al., 2002
	Spain	Glass beverage soft-drink cola	1	ND	ND	132 ng/L	Gallart-Ayala et al., 2011
	Spain	Glass beverage soft-drink orange soda	1	218 ng/L	218 ng/L	132 ng/L	Gallart-Ayala et al., 2011
	Spain	Glass beverage soft-drink lemon soda	1	141 ng/L	141 ng/L	132 ng/L	Gallart-Ayala et al., 2011

Table 2. Continued

Compounds	Location	Sample	N	Median	Range	LOQ	References
BPF	Spain	Glass beverage soft-drink tonic	1	ND	ND	132 ng/L	Gallart-Ayala et al., 2011
	USA	Sediment	82	1.44 ng/g dw	<LOQ ~ 27.5 ng/g dw	1.0 ng/g dw	Liao et al., 2012c
	Japan	Sediment	56	3.57 ng/g dw	<LOQ ~ 9.11 ng/g dw	1.0 ng/g dw	Liao et al., 2012c
	Korea	Sediment	34	ND	<LOQ ~ 9,650 ng/g dw	1.0 ng/g dw	Liao et al., 2012c
	USA	Indoor dust	38	49 ng/g	<LOQ ~ 240 ng/g	2.0 ng/g	Liao et al., 2012d
	China	Indoor dust	55	38 ng/g	<LOQ ~ 1,890 ng/g	2.0 ng/g	Liao et al., 2012d
	Japan	Indoor dust	22	57 ng/g	<LOQ ~ 2,780 ng/g	2.0 ng/g	Liao et al., 2012d
	Korea	Indoor dust	41	450 ng/g	<LOQ ~ 1,070 ng/g	2.0 ng/g	Liao et al., 2012d
BPS	Spain[c]	Peas and carrots (supernatant)	3	175 ng/mL[a]	-	0.0083 ng/mL	Viñas et al., 2010
	Spain[c]	Peas and carrots (food)	3	36.1 ng/g[a]	-	0.073 ng/g	Viñas et al., 2010
	Spain[c]	Peas (supernatant)	3	16.7 ng/mL[a]	-	0.0083 ng/mL	Viñas et al., 2010
	Spain[c]	Natural peas (supernatant)	3	30.9 ng/mL[a]	-	0.0083 ng/mL	Viñas et al., 2010
	Spain[c]	Artichoke (supernatant)	3	34.3 ng/mL[a]	-	0.0083 ng/mL	Viñas et al., 2010
	Spain[c]	Mushroom (supernatant)	3	11.5 ng/mL[a]	-	0.0083 ng/mL	Viñas et al., 2010
	Spain[c]	Bean shoot (supernatant)	3	14.0 ng/mL[a]	-	0.0083 ng/mL	Viñas et al., 2010
	Spain[c]	Mixed vegetables (supernatant)	3	70.1 ng/mL[a]	-	0.0083 ng/mL	Viñas et al., 2010
	Spain[c]	Natural peas, sweet corn, artichoke, mushroom, bean shoot, and mixed vegetables (food)	3	ND	ND	0.073 ng/g	Viñas et al., 2010
	Spain	Glass beverage soft-drink cola	1	ND	ND	167 ng/L	Gallart-Ayala et al., 2011
	Spain	Glass beverage soft-drink soda	5	ND	ND	167 ng/L	Gallart-Ayala et al., 2011
	Spain	Glass beverage soft-drink tonic	1	ND	ND	167 ng/L	Gallart-Ayala et al., 2011
	USA(Albany)	Thermal receipt paper	81	7,440 μg/g	0.0138 ~ 22,000 μg/g	0.0001 μg/g	Liao et al., 2012a
	Japan	Thermal receipt paper	6	5,500 μg/g	0.546 ~ 6,130 μg/g	0.0001 μg/g	Liao et al., 2012a
	Korea	Thermal receipt paper	11	0.8 μg/g	0.0896 ~ 11 μg/g	0.0001 μg/g	Liao et al., 2012a
	Vietnam	Thermal receipt paper	3	0.3 μg/g	0.105 ~ 0.554 μg/g	0.0001 μg/g	Liao et al., 2012a
	USA(Albany)	Several paper products	157	8.5 μg/g	<LOQ ~ 8,380 μg/g	0.0001 μg/g	Liao et al., 2012a
	USA	Urine[b]	31	0.263 ng/mL	<LOQ ~ 21.0 ng/mL	0.02 ng/mL	Liao et al., 2012b
	China	Urine[b]	89	0.297 ng/mL	<LOQ ~ 3.16 ng/mL	0.02 ng/mL	Liao et al., 2012b
	India	Urine[b]	38	0.055 ng/mL	<LOQ ~ 0.881 ng/mL	0.02 ng/mL	Liao et al., 2012b

Table 2. Continued

Compounds	Location	Sample	N	Median	Range	LOQ	References
BPS	Japan	Urine[b]	36	1.040 ng/mL	0.147 ~ 9.57 ng/mL	0.02 ng/mL	Liao et al., 2012b
	Korea	Urine[b]	33	0.014 ng/mL	<LOQ ~ 1.98 ng/mL	0.02 ng/mL	Liao et al., 2012b
	Kuwait	Urine[b]	30	0.371 ng/mL	<LOQ ~ 12.1 ng/mL	0.02 ng/mL	Liao et al., 2012b
	Malaysia	Urine[b]	29	0.084 ng/mL	<LOQ ~ 0.922 ng/mL	0.02 ng/mL	Liao et al., 2012b
	Vietnam	Urine[b]	29	0.157 ng/mL	0.037 ~ 0.932 ng/mL	0.02 ng/mL	Liao et al., 2012b
	USA	Sediment	82	ND	<LOQ ~ 4.65 ng/g dw	0.25 ng/g dw	Liao et al., 2012c
	Japan	Sediment	56	ND	<LOQ ~ 4.46 ng/g dw	0.25 ng/g dw	Liao et al., 2012c
	Korea	Sediment	34	ND	<LOQ ~ 1,970 ng/g dw	0.25 ng/g dw	Liao et al., 2012c
	USA	Indoor dust	38	630 ng/g	5.6 ~ 25,500 ng/g	0.5 ng/g	Liao et al., 2012d
	China	Indoor dust	55	170 ng/g	0.83 ~ 12,600 ng/g	0.5 ng/g	Liao et al., 2012d
	Japan	Indoor dust	22	810 ng/g	250 ~ 2,550 ng/g	0.5 ng/g	Liao et al., 2012d
	Korea	Indoor dust	41	360 ng/g	90 ~ 26,600 ng/g	0.5 ng/g	Liao et al., 2012d

[a] Mean value.
[b] Values indicate creatinine-unadjusted concentration.
[c] Values are analyte concentrations with derivatization procedures using bis-(trimethylsilyl)trifluoroacetamide (BSTFA).
LOQ: limit of quantification, ND: non-detected, -: not available.

indoor dust samples collected in South Korea (median 4.8 ng/g).[24] BPB has been found in human serum from Italy (mean 5.15 ng/mL)[25] and Portugal (mean 0.68 ng/mL),[26] and in canned foods (mean 42.3 ng/g).[27] BPF has been reported in surface water, sewage, and sediments at concentrations ranging from below the limit of quantification (LOQ) to 0.180 μg/L, 0.022 to 0.123 μg/L, and 1.2 to 7.3 μg/kg, respectively.[17] BPF was reported to occur in soft drinks at concentrations ranging from below LOQ to 0.22 μg/L.[28] The highest median concentration of BPF (450 ng/g) was found in dust from South Korea, which was ten folds higher than that detected in samples of USA, China, and Japan.[24] BPS has been found in thermal receipt papers at concentrations comparable to those of BPA (several tens of milligrams per gram)[29,30] and in sediment samples collected from various countries.[31] Widespread exposure of the general population in various countries to BPS has been demonstrated through biomonitoring studies.[2] BPS has been found in canned foodstuffs at concentrations on the order of several tens of nanograms per gram.[27,32]

Since the discharges into the environment of BPs and Tritan are estimated to increase rapidly,[9,33] environmental and health risk potentials of BPA alternatives are of growing concern. Unlike BPA of which endocrine toxicity and various health consequences have received thorough investigations, very limited attention has been paid to the toxicity of BPA alternatives until now. This review focuses on endocrine disruption and presents what we know about the endocrine disruption potentials of BPs and the three monomers of Tritan to understand the current status of knowledge and to identify areas of future research.

II. Methods

In this review, we provides a summary of the available information on the estrogenicity and androgenicity of four BPs (BPAF, BPB, BPF, and BPS) and three monomers of Tritan copolyesters (CHDM, TMCD, and DMT) which have been frequently used as BPA alternatives. Only toxicity data that measured estrogenicity/anti-estrogenicity and androgenicity/anti-androgenicity in *in vitro* cell-based and in *in vivo* assay in rat were summarized. Specifically, the following studies were summarized:

- *In vitro* estrogen receptor binding assays (alpha and beta isoforms)
- *In vitro* androgen receptor binding assays
- *In vitro* estrogen and androgen receptor transactivation assays (mammalian cells and yeast)
- *In vivo* estrogenicity assays (uterotrophic assay and steroidogenic assay)
- *In vivo* androgenicity assays (Hershberger assay)

III. Results and Discussion

A. Estrogenic activities of BPs

Several studies have been published confirming the estrogenic and anti-androgenic activity of BPA alternatives in diverse *in vitro* and *in vivo* assay which are summarized in Tables 3-4. Analysis of the structure-activity relationship of BPA and its related compounds implied that key structural requirement for estrogenic and anti-androgenic activity of BPs is the phenolic hydroxyl group (Fig. 1).[34] In addition, 4-hydroxyl group on the A-phenyl ring and a hydrophobic group of the propane moiety are suggested to regulate estrogenic and anti-androgenic activities (Fig. 1).[34] For example, the increase of E2 activity by BPAF and BPB could be explained by hydrophobic substituents in place of the 1-methyl group of the propane moiety. Unhindered hydroxyl group on an aryl ring and a hydrophobic group attached *para* to the hydroxyl group are also important factors for estrogen receptor (ER) ligand activity.[35] It was predicted that BPF and BPS, which have a *para* hydroxyl group on each of the phenol rings, may have modulating effects toward ER binding potency.[18,36]

1. Estrogenic activities of BPAF

BPAF may possess greater toxicological implication than BPA because trifluoromethyl (CF3) group which is substituted for methyl (CH3) group of BPA is much more electronegative and therefore potentially more reactive. This type of substitution has been reported to increase estrogenic activity *in vivo* and *in vitro*.[12,13,34,37] An ER-luciferase reporter assay using MCF-7 cell line demonstrated that the estrogen activity of BPAF was about one order of magnitude greater than that of BPA.[34] Daily subcutaneous injections of 100 mg/kg BPAF to

Table 3. Estrogenicity/anti-estrogenicity and androgenicity/anti-androgenicity of bisphenol A alternatives in *in vitro* studies

Compounds	Test type	Endpoint	Toxicity data	Reference
BPAF	Recombinant gene assay in yeast	EC_{50}, Estrogenic activity	$7.44E^{-7}$ M	Zhang et al., 2009
	ER transactivation assay in human T47D-KBluc cell	EC_{50}, Estrogenic activity	$2.248E^{-8}$ M	Bermudez et al., 2010
	ER transactivation assay in human Ishikawa cell	LOEC, ERα luciferase activity	$1E^{-9}$ M	Li et al., 2012
	ER transactivation assay in human Ishikawa cell	NOEC, ERα luciferase activity	$<1E^{-9}$ M	Li et al., 2012
	ER transactivation assay in human Ishikawa cell	LOEC, ERβ luciferase activity	$1E^{-6}$ M	Li et al., 2012
	ER transactivation assay in human Ishikawa cell	NOEC, ERβ luciferase activity	$1E^{-7}$ M	Li et al., 2012
	ER transactivation assay in human HeLa cell	LOEC, ERα luciferase activity	$1E^{-7}$ M	Li et al., 2012
	ER transactivation assay in human HeLa cell	NOEC, ERα luciferase activity	$1E^{-8}$ M	Li et al., 2012
	ER transactivation assay in human HeLa cell	EC_{50}, ERα luciferase activity	$5.87E^{-8}$ M	Matsushima et al., 2010
	ER transactivation assay in human HeLa cell	LOEC, ERβ luciferase activity	$1E^{-7}$ M	Li et al., 2012
	ER transactivation assay in human HeLa cell	LOEC, ERβ luciferase activity	$1E^{-8}$ M	Li et al., 2012
	ER transactivation assay in human HepG2 cell	LOEC, ERα luciferase activity	$1E^{-8}$ M	Li et al., 2012
	ER transactivation assay in human HepG2 cell	NOEC, ERα luciferase activity	$1E^{-9}$ M	Li et al., 2012
	ER transactivation assay in human HepG2 cell	LOEC, ERβ luciferase activity	$1E^{-8}$ M	Li et al., 2012
	ER transactivation assay in human HepG2 cell	LOEC, ERβ luciferase activity	$1E^{-9}$ M	Li et al., 2012
	ER binding assay to human ERá	Log relative ER binding affinity	-0.11	Akahori et al., 2008
	E-screen (cell proliferation) assay in human MCF-7 cell	Proliferative effect over control [a]	5.5 (E2: 6.7, BPA:6.0)	Perez et al., 1998
	E-screen (cell proliferation) assay in human MCF-7 cell	Proliferative effect over control [a]	5.5	Rivas et al., 2002
	E-screen (cell proliferation) assay in human MCF-7 cell	Relative proliferative effect [b]	78.94	Rivas et al., 2002
	E-screen (cell proliferation) assay in human MCF-7 cell	LOEC, Estrogenic activity	$1E^{-7}$ M	Rivas et al., 2002
	E-screen (cell proliferation) assay in human MCF-7 cell	Relative proliferative potency [c]	0.01	Rivas et al., 2002
	ERE-luciferase reporter assay in human MCF-7 cell	EC_{50}, Estrogenic activity	$5E^{-8}$ M	Kitamura et al., 2005
	ERE-luciferase reporter assay in human MCF-7 cell	LOEC, Estrogenic activity	$1E^{-7}$ M	Kitamura et al., 2005
	ERE-luciferase reporter assay in human MCF-7 cell	NOEC, Anti-estrogenic activity at $1E^{-11}$ M E2	$1E^{-5}$ M	Kitamura et al., 2005
	Radioligand binding assay for saturation binding of ER	IC_{50}, inhibit ability to [^{3}H]17β-estradiol binding in ERα ligand	$5.34E^{-8}$ M	Matsushima et al., 2010
	Radioligand binding assay for saturation binding of ER	IC_{50}, inhibit ability to [^{3}H]17β-estradiol binding in ERβ ligand	$1.89E^{-8}$ M	Matsushima et al., 2010

Table 3. Continued

Compounds	Test type	Endpoint	Toxicity data	Reference
BPAF	Radioligand binding assay for saturation binding of ER	IC_{50}, inhibit ability to [^{3}H]17β-estradiol binding in ERRγ ligand	3.58E^{-7} M	Matsushima et al., 2010
	ARE-luciferase reporter assay in mouse NIH3T3 cell	NOEC, Androgenic activity	1E^{-4} M	Kitamura et al., 2005
	ARE-luciferase reporter assay in mouse NIH3T3 cell	IC_{50}, Anti-androgenic activity at 1E^{-10} M dihydrotestosterone	1.3E^{-6} M	Kitamura et al., 2005
BPB	Two-hybrid system in yeast	10% REC, β-galactosidase activity	Greater than bisphenol A	Chen et al., 2002
	Two-hybrid system in yeast without S9 mix	LOEC, Estrogenic activity based on relative β-galactosidase activity	1E^{-5} M	Hashimoto et al., 2001
	Two-hybrid system in yeast without S9 mix	NOEC, Estrogenic activity based on relative β-galactosidase activity	1E^{-6} M	Hashimoto et al., 2001
	Two-hybrid system in yeast with S9 mix	LOEC, Estrogenic activity based on relative β-galactosidase activity	1E^{-5} M	Hashimoto et al., 2001
	Two-hybrid system in yeast with S9 mix	NOEC, Estrogenic activity based on relative β-galactosidase activity	1E^{-6} M	Hashimoto et al., 2001
	Fluorescence polarization system	LOEC, Estrogenic activity	1E^{-7} M	Hashimoto et al., 2001
	Fluorescence polarization system	NOEC, Estrogenic activity	<1E^{-7} M	Hashimoto et al., 2001
	ER transactivation assay in human HeLa cell	PC_{10}, ERα luciferase activity	4.09E^{-8} M	Yamasaki et al., 2002
	ER transactivation assay in human HeLa cell	PC_{50}, ERα luciferase activity	6.63E^{-7} M	Yamasaki et al., 2002
	ER transactivation assay in human HeLa cell	EC_{50}, ERα luciferase activity	1.67E^{-7} M	Yamasaki et al., 2002
	E-screen (cell proliferation) assay in human MCF-7 cell	LOEC, Estrogenic activity	1E^{-9} M	Hashimoto et al., 2001
	E-screen (cell proliferation) assay in human MCF-7 cell	NOEC, Estrogenic activity	<1E^{-9} M	Hashimoto et al., 2001
	E-screen (cell proliferation) assay in human MCF-7 cell	Proliferative effect over control [a]	5.9	Rivas et al., 2002
	E-screen (cell proliferation) assay in human MCF-7 cell	Relative proliferative effect [b]	85.96	Rivas et al., 2002
	E-screen (cell proliferation) assay in human MCF-7 cell	LOEC, Estrogenic activity	1E^{-7} M	Rivas et al., 2002
	E-screen (cell proliferation) assay in human MCF-7 cell	Relative proliferative potency [c]	0.01	Rivas et al., 2002
	ERE-luciferase reporter assay in human MCF-7 cell	EC_{50}, Estrogenic activity	7E^{-8} M	Kitamura et al., 2005
	ERE-luciferase reporter assay in human MCF-7 cell	LOEC, Estrogenic activity	1E^{-7} M	Kitamura et al., 2005
	ERE-luciferase reporter assay in human MCF-7 cell	NOEC, Estrogenic activity	1E^{-8} M	Kitamura et al., 2005
	ERE-luciferase reporter assay in human MCF-7 cell	LOEC, Anti-estrogenic activity at 1E^{-11} M E2	1E^{-6} M	Kitamura et al., 2005
	ERE-luciferase reporter assay in human MCF-7 cell	NOEC, Anti-estrogenic activity at 1E^{-11} M E2	1E^{-7} M	Kitamura et al., 2005

Table 3. Continued

Compounds	Test type	Endpoint	Toxicity data	Reference
BPB	ER competitive-binding assay in Sprague-Dawley rat uterine cytosol	IC_{50}, Inhibition of [^{3}H]-E2 binding	$1.05E^{-6}$ M	Blair et al., 2000
	ER competitive-binding assay in Sprague-Dawley rat uterine cytosol	Log relative ER binding affinity	-1.07	Blair et al., 2000
	ARE-luciferase reporter assay in mouse NIH3T3 cell	NOEC, Androgenic activity	$1E^{-4}$ M	Kitamura et al., 2005
	ARE-luciferase reporter assay in mouse NIH3T3 cell	LOEC, Anti-androgenic activity at $1E^{-10}$ M dihydrotestosterone	$1E^{-5}$ M	Kitamura et al., 2005
	ARE-luciferase reporter assay in mouse NIH3T3 cell	NOEC, Anti-androgenic activity at $1E^{-10}$ M dihydrotestosterone	$1E^{-6}$ M	Kitamura et al., 2005
	ARE-luciferase reporter assay in mouse NIH3T3 cell	IC_{50}, Anti-androgenic activity at $1E^{-10}$ M dihydrotestosterone	$1.7E^{-6}$ M	Kitamura et al., 2005
BPF	Two-hybrid system in yeast	10% REC, β-galactosidase activity	Similar to bisphenol A	Chen et al., 2002
	Two-hybrid system in yeast without S9 mix	LOEC, Estrogenic activity based on relative β-galactosidase activity	$1E^{-4}$ M	Hashimoto and Nakamura, 2000; Hashimoto et al., 2001
	Two-hybrid system in yeast without S9 mix	NOEC, Estrogenic activity based on relative β-galactosidase activity	$1E^{-5}$ M	Hashimoto and Nakamura, 2000; Hashimoto et al., 2001
	Two-hybrid system in yeast with S9 mix	LOEC, Estrogenic activity based on relative β-galactosidase activity	$1E^{-5}$ M	Hashimoto et al., 2001
	Two-hybrid system in yeast with S9 mix	NOEC, Estrogenic activity based on relative β-galactosidase activity	$1E^{-6}$ M	Hashimoto et al., 2001
	Recombinant gene assay in yeast	EC_{50}, Estrogenic activity	$7.52E^{-6}$ M	Zhang et al., 2009
	Fluorescence polarization system	LOEC, Estrogenic activity	$1E^{-4}$ M	Hashimoto and Nakamura, 2000; Hashimoto et al., 2001
	Fluorescence polarization system	NOEC, Estrogenic activity	$1E^{-5}$ M	Hashimoto and Nakamura, 2000; Hashimoto et al., 2001
	E-screen (cell proliferation) assay in human MCF-7 cell	Proliferative effect over control [a]	7.1 (E2: 6.7, BPA:6.0)	Perez et al., 1998
	E-screen (cell proliferation) assay in human MCF-7 cell	LOEC, Estrogenic activity	$<1E^{-7}$ M	Hashimoto and Nakamura, 2000
	E-screen (cell proliferation) assay in human MCF-7 cell	LOEC, Estrogenic activity	$1E^{-8}$ M	Hashimoto et al., 2001
	E-screen (cell proliferation) assay in human MCF-7 cell	NOEC, Estrogenic activity	$1E^{-9}$ M	Hashimoto et al., 2001
	E-screen (cell proliferation) assay in human MCF-7 cell	EC_{50}, Estrogenic activity	$8.48E^{-8}$ M	Stroheker et al., 2004
	ERE-luciferase reporter assay in human MCF-7 cell	EC_{50}, Estrogenic activity	$1E^{-6}$ M	Kitamura et al., 2005
	ERE-luciferase reporter assay in human MCF-7 cell	NOEC, Anti-estrogenic activity at $1E^{-11}$ M E2	$1E^{-5}$ M	Kitamura et al., 2005

Table 3. Continued

Compounds	Test type	Endpoint	Toxicity data	Reference
BPF	ER transactivation assay in human HeLa cell	PC_{10}, ERα luciferase activity	$2.84E^{-6}$ M	Yamasaki et al., 2002
	ER transactivation assay in human HepG2 cell	LOEC, ERα transcriptional activity	$1E^{-7}$ M	Cabaton et al., 2009
	ER transactivation assay in human HepG2 cell	EC_{50}, ERα transcriptional activity	$2.39E^{-6}$ M	Cabaton et al., 2009
	ER transactivation assay in human HepG2 cell	LOEC, ERβ transcriptional activity	$1E^{-6}$ M	Cabaton et al., 2009
	ER transactivation assay in human HepG2 cell	EC_{50}, ERβ transcriptional activity	$6.04E^{-6}$ M	Cabaton et al., 2009
	ER competitive-binding assay in Sprague-Dawley rat uterine cytosol	IC_{50}, Inhibition of [^{3}H]-E2 binding	$9.50E^{-5}$ M	Blair et al., 2000
	ER competitive-binding assay in Sprague-Dawley rat uterine cytosol	Log relative ER binding affinity	-3.02	Blair et al., 2000
	AR-binding assay in hamster CHO-K1 cell	IC_{50}, AR binding activity	$9.0E^{-6}$ M	Satoh et al., 2004
	ARE-luciferase reporter assay in hamster CHO-K1 cell	NOEC, Androgenic activity	$1E^{-4}$ M	Satoh et al., 2004
	ARE-luciferase reporter assay in hamster CHO-K1 cell	NOEC, Anti-androgenic activity	$4.8E^{-6}$ M	Satoh et al., 2004
	ARE-luciferase reporter assay in mouse NIH3T3 cell	NOEC, Androgenic activity	$1E^{-4}$ M	Kitamura et al., 2005
	ARE-luciferase reporter assay in mouse NIH3T3 cell	LOEC, Anti-androgenic activity at $1E^{-10}$ M dihydrotestosterone	$1E^{-5}$ M	Kitamura et al., 2005
	ARE-luciferase reporter assay in mouse NIH3T3 cell	NOEC, Anti-androgenic activity at $1E^{-10}$ M dihydrotestosterone	$1E^{-6}$ M	Kitamura et al., 2005
	ARE-luciferase reporter assay in mouse NIH3T3 cell	IC_{50}, Anti-androgenic activity at $1E^{-10}$ M dihydrotestosterone	$1.2E^{-5}$ M	Kitamura et al., 2005
	ARE-luciferase reporter assay in human MDA0MB453 cell	LOEC, Anti-androgenic activity at $4E^{-10}$ M dihydrotestosterone	$1E^{-10}$ M	Stroheker et al., 2004
	AR transactivation assay in human MDA-kb2 cell	LOEC, AR transcriptional activity	$1E^{-5}$ M	Cabaton et al., 2009
BPS	Two-hybrid system in yeast	10% REC, β-galactosidase activity	Lesser than BPA	Chen et al., 2002
	Yeast two-hybrid system without S9 mix	LOEC, Estrogenic activity based on relative β-galactosidase activity	$>1E^{-3}$ M	Hashimoto and Nakamura, 2000; Hashimoto et al., 2001
	Yeast two-hybrid system without S9 mix	NOEC, Estrogenic activity based on relative β-galactosidase activity	$1E^{-3}$ M	Hashimoto and Nakamura, 2000; Hashimoto et al., 2001
	Yeast two-hybrid system with S9 mix	LOEC, Estrogenic activity based on relative β-galactosidase activity	$1E^{-3}$ M	Hashimoto et al., 2001
	Yeast two-hybrid system with S9 mix	NOEC, Estrogenic activity based on relative β-galactosidase activity	$1E^{-4}$ M	Hashimoto et al., 2001
	Fluorescence polarization system	LOEC, Estrogenic activity	$1E^{-3}$ M	Hashimoto and Nakamura, 2000; Hashimoto et al., 2001

Table 3. Continued

Compounds	Test type	Endpoint	Toxicity data	Reference
BPS	Fluorescence polarization system	NOEC, Estrogenic activity	$1E^{-4}$ M	Hashimoto and Nakamura, 2000; Hashimoto et al., 2001
	ERE-luciferase reporter assay in human BG1Luc4E2 cell	EC_{50}, Estrogenic activity	$4.93E^{-6}$ M	Grignard et al., 2012
	E-screen (cell proliferation) assay in human MCF-7 cell	LOEC, Estrogenic activity	$<1E^{-7}$ M	Hashimoto and Nakamura, 2000
	E-screen (cell proliferation) assay in human MCF-7 cell	LOEC, Estrogenic activity	$1E^{-7}$ M	Hashimoto et al., 2001
	E-screen (cell proliferation) assay in human MCF-7 cell	NOEC, Estrogenic activity	$1E^{-8}$ M	Hashimoto et al., 2001
	ERE-luciferase reporter assay in human MELN cell	EC_{50}, Estrogenic activity	$4.24E^{-6}$ M	Grignard et al., 2012
	ERE-luciferase reporter assay in human MCF-7 cell	EC_{50}, Estrogenic activity	$1.75E^{-6}$ M	Kuruto-Niwa et al., 2005
	ERE-luciferase reporter assay in human MCF-7 cell	EC_{50}, Estrogenic activity	$1.1E^{-6}$ M	Kitamura et al., 2005
	ERE-luciferase reporter assay in human MCF-7 cell	NOEC, Anti-estrogenic activity at $1E^{-11}$ M E2	$1E^{-5}$ M	Kitamura et al., 2005
	ER competitive-binding assay in Sprague-Dawley rat uterine cytosol	IC_{50}, Inhibition of [^{3}H]-E2 binding	$1.05E^{-4}$ M	Blair et al., 2000
	ER competitive-binding assay in Sprague-Dawley rat uterine cytosol	Log relative ER binding affinity	-3.07	Blair et al., 2000
	ARE-luciferase reporter assay in mouse NIH3T3 cell	NOEC, Androgenic activity	$1E^{-4}$ M	Kitamura et al., 2005
	ARE-luciferase reporter assay in mouse NIH3T3 cell	IC_{50}, Anti-androgenic activity at $1E^{-10}$ M dihydrotestosterone	$1.7E^{-5}$ M	Kitamura et al., 2005
CHDM	E-screen (cell proliferation) assay in human MCF-7 cell	Estrogenic activity	Yes	Yang et al., 2011
	ER binding assay to human ERá and ERâ	NOEC, binding to ERα and ERβ	$1E^{-3}$ M	Osimitz et al., 2012
	ER transactivation assay in yeast	NOEC, Estrogenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	ER transactivation assay in human T47D-KBluc cell	NOEC, Estrogenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	ER transactivation assay in human T47D-KBluc cell	NOEC, Anti-estrogenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	ER transactivation assay in yeast	NOEC, Estrogenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	AR binding assay	NOEC, binding to AR	$1E^{-3}$ M	Osimitz et al., 2012
	AR transactivation assay in human MDA-kb2 cell	NOEC, Androgenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	AR transactivation assay in human MDA-kb2 cell	NOEC, Anti-androgenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	AR transactivation assay in yeast	NOEC, Androgenic activity	$1E^{-3}$ M	Osimitz et al., 2012
DMT	E-screen (cell proliferation) assay in human MCF-7 cell	Estrogenic activity	Yes	Yang et al., 2011
	ER binding assay to human ERá and ERâ	NOEC, binding to ERα and ERβ	$1E^{-3}$ M	Osimitz et al., 2012
	ER transactivation assay in yeast	NOEC, Estrogenic activity	$1E^{-3}$ M	Osimitz et al., 2012

Table 3. Continued

Compounds	Test type	Endpoint	Toxicity data	Reference
DMT	ER transactivation assay in human T47D-KBluc cell	NOEC, Estrogenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	ER transactivation assay in human T47D-KBluc cell	NOEC, Anti-estrogenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	ER transactivation assay in yeast	NOEC, Estrogenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	AR binding assay	NOEC, binding to AR	$1E^{-3}$ M	Osimitz et al., 2012
	AR transactivation assay in human MDA-kb2 cell	NOEC, Androgenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	AR transactivation assay in human MDA-kb2 cell	NOEC, Anti-androgenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	AR transactivation assay in yeast	NOEC, Androgenic activity	$1E^{-4}$ M	Osimitz et al., 2012
TMCD	ER binding assay to human ERá and ERâ	NOEC, binding to ERα and ERβ	$1E^{-3}$ M	Osimitz et al., 2012
	ER transactivation assay in yeast	NOEC, Estrogenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	ER transactivation assay in human T47D-KBluc cell	NOEC, Estrogenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	ER transactivation assay in human T47D-KBluc cell	NOEC, Anti-estrogenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	ER transactivation assay in yeast	NOEC, Estrogenic activity	$1E^{-4}$ M	Osimitz et al., 2012
	AR binding assay	NOEC, binding to AR	$1E^{-3}$ M	Osimitz et al., 2012
	AR transactivation assay in human MDA-kb2 cell	NOEC, Androgenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	AR transactivation assay in human MDA-kb2 cell	NOEC, Anti-androgenic activity	$1E^{-3}$ M	Osimitz et al., 2012
	AR transactivation assay in yeast	NOEC, Androgenic activity	$1E^{-3}$ M	Osimitz et al., 2012

AR: Androgen receptor, EC_{50}: Median effective concentration, ER: Estrogen receptor, IC_{50}: Median inhibition concentration, LOEC: lowest observed effective concentration, NOEC: no observed effective concentration, PC_{10}: concentrations estimated to show 10% of the transcriptional activity of 1 nM E2, PC_{50}: concentrations estimated to show 50% of the transcriptional activity of 1 nM E2, REC: Relative effective concentration.

[a] Proliferative effect over control = maximal cell count of test compounds/cell count of control.

[b] Relative proliferative effect = (proliferative effect of test compounds-1/proliferative effect of E2-1)×100.

[c] Relative proliferative potency = ratio between E2 and test compounds doses to produce maximal yield×100.

Table 4. Summary of *in vivo* studies published on the estrogenic and androgenic activity of bisphenol A alternatives

Compounds	Test type	Test organisms	Exposure duration	Endpoint	Toxicity data	Reference
BPAF	Steroidogenic assay	Adult male rat	14 d	NOED, T levels in serum	50 mg/kg/d	Feng et al., 2012
	Steroidogenic assay	Adult male rat	14 d	LOED, T levels in serum	200 mg/kg/d	Feng et al., 2012
	Steroidogenic assay	Adult male rat	14 d	NOED, LH levels in serum	10 mg/kg/d	Feng et al., 2012
	Steroidogenic assay	Adult male rat	14 d	LOED, LH levels in serum	50 mg/kg/d	Feng et al., 2012
	Steroidogenic assay	Adult male rat	14 d	NOED, FSH levels in serum	2 mg/kg/d	Feng et al., 2012
	Steroidogenic assay	Adult male rat	14 d	LOED, FSH levels in serum	10 mg/kg/d	Feng et al., 2012
	Steroidogenic assay	Adult male rat	14 d	NOED, transcription in genes in steroidogenesis	50 mg/kg/d	Feng et al., 2012
	Steroidogenic assay	Adult male rat	14 d	LOED, transcription in genes in steroidogenesis	200 mg/kg/d	Feng et al., 2012
	Uterotrophic assay	Immature female rat	1 d	Log LOED, estrogenic effects	1.08 ìmol/kg/d	Akahori et al., 2008
	Uterotrophic assay	Immature female rat	3 d	LOED, estrogenic effects	8 mg/kg/d	Yamasaki et al., 2003
	Hershberger assay	Adult male rat	10 d	LOED, anti-androgenic effects	200 mg/kg/d	Yamasaki et al., 2003
BPB	Uterotrophic assay	Immature female rat	3 d	LOED, estrogenic effects	200 mg/kg/d	Yamasaki et al., 2002
	Hershberger assay	Adult male rat	10 d	LOED, anti-androgenic effects	600 mg/kg/d	Yamasaki et al., 2003
BPF	Uterotrophic assay	Immature female rat	3 d	LOED, estrogenic effects	200 mg/kg/d	Yamasaki et al., 2002
	Hershberger assay	Adult male rat	10 d	LOED, anti-androgenic effects	1,000 mg/kg/d	Yamasaki et al., 2003
CHDM	Uterotrophic assay	Female rat	3 d	NOED, estrogenic effects	10 mg/kg/d	Osimitz et al., 2012
	Hershberger assay	Male rat	10 d	NOED, androgenic effects	10 mg/kg/d	Osimitz et al., 2012
DMT	Uterotrophic assay	Female rat	3 d	NOED, estrogenic effects	10 mg/kg/d	Osimitz et al., 2012
	Hershberger assay	Male rat	10 d	NOED, androgenic effects	10 mg/kg/d	Osimitz et al., 2012
TMCD	Uterotrophic assay	Female rat	3 d	NOED, estrogenic effects	10 mg/kg/d	Osimitz et al., 2012
	Hershberger assay	Male rat	10 d	NOED, androgenic effects	10 mg/kg/d	Osimitz et al., 2012

NOED: no observed effective dose, LOED: lowest observed effective dose, T: testosterone, LH: luteinizing hormone, FSH: follicle-stimulating hormone.

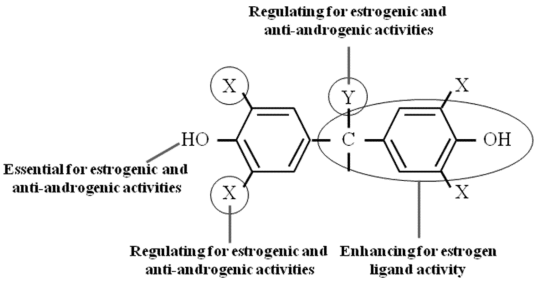


Fig. 1. Structural characteristics of bisphenol-related compounds which might possess endocrine-disrupting activity (modified from Kitamura et al. (2005)[34])

immature female rats for three days led to a 337% increase in uterus size, compared to only a 197% increase when exposed to 200 mg/kg BPA.[15)]

BPAF has been shown to induce estrogen-dependent responses via binding to ERα and ERβ.[11,12,13,37)] The binding affinity of BPAF was approximately 20 times stronger and 48 times stronger than that of BPA as a ligand for ERα and ERβ, respectively.[13)] High binding activity of BPAF for ERβ suggests that the binding pocket of ERβ possesses specific structural elements that interact much more favorably with the CF3 groups of BPAF than with the CH3 groups of BPA. In Ishikawa and HepG2 cells, the agonistic effects of BPAF for ERα were stronger than that of BPA (10 nM BPAF vs. 100 nM BPA).[12)] However binding to estrogen-related receptor gamma (ERRγ) was weaker than BPA, suggesting less favorable interaction of ERRγ-ligand binding domain (LBD) with the CF3 groups.[13)]

In uterotrophic assay employing immature rat, uterine weight increased significantly in rats given 8, 40, and 100 mg/kg BPAF, suggesting estrogen agonist.[38)] The results of Hershberger assay showed that BPAF decreased body weight-gain and spontaneous locomotion in the rats treated with 200 and 600 mg/kg BPAF, suggesting anti-androgen activity.[38)]

BPAF may impair pituitary-gonadal function at different levels by increasing LH and FSH concentrations and decreasing testosterone levels in serum.[39)] It appears that the inhibition of androgen biosynthesis was not a result of altered regulatory function of the LH-dependent signaling pathway but was more likely a direct result of BPAF's ability to reduce the expression of steroidogenic genes such as *SR-B1*, *StAR*, *P450scc*, and *17βHSD*. Sharp decrease in testosterone concentration and reduction in gene transcriptions and protein levels involved in steroidogenesis suggested that the testes may be a primary target organ of BPAF exposure.

2. Estrogenic activities of BPB

Estrogenicity of BPB is suspected in part because of the substitution of the propane bridge of BPA to butane, which is related to its estrogenic activity. It was reported that higher estrogenic responses were obtained from longer alkyl substituent at the bridging carbon in MCF-7 cells.[14)] Recently Chen et al. ranked diphenylalkanes without any modifications by their estrogenic potency, and reported the order of BPB (C4) > BPA (C3) bisphenol E (1,1-bis(4-hydroxyphenyl)ethane; BPE) (C2) BPF (C1).[33)]

BPB has been shown to possess estrogenic properties in various *in vitro* and *in vivo* experiments. BPB showed considerably higher estrogenic activity than BPA in the yeast two-hybrid assay.[33)] This compound also exhibited significant increase of MCF-7 cell growth in the E-screen test[40,41)] as well as greater luciferase activity in MCF-7 cells.[34)] Moderate binding affinity to ER was also reported in Sprague-Dawley rat uterine cytosol.[35)] Yamasaki et al. found that BPB has estrogenic activity both in ER transactivation assay in HeLa cell and uterotrophic assay in female rat, however, the estrogenic potencies obtained in *in vitro* assay do not completely correspond to the uterotrophic potency in *in vivo* test.[42)]

3. Estrogenic activities of BPF

BPF has been shown to induce estrogenic activity *in vivo* and *in vitro*. In *in vivo*, BPF exhibited estrogen agonistic properties in the uterotrophic assay.[42)] In *in vitro* assay using a yeast two-hybrid system BPF was identified as the most estrogenic compound among the tested chemicals that were present in food packaging material or used in dentistry.[40,43)] BPF has also exhibited estrogenic activity in yeast recombinant gene assay.[44)] In human cells, the proliferative response of MCF-7 cells (E-Screen assay) increased in a concentration dependent manner.[14,40,43,45)] The latter authors showed that, according to the respective median effective concentration (EC_{50}) values for proliferation of MCF-7 cell, BPF was more pronounced than BPA.

Moderate binding affinities of BPF to ER in MCF-7 cell,[34] HeLa cell,[42] HepG2 cell,[46] and Sprague-Dawley rat uterine cytosol[35] were reported.

Some of effects of BPF exposure are mediated by binding to nuclear steroid receptors (ERα and ERβ) and inducing estrogenic signals, which may subsequently modify estrogen-responsive gene expression in HepG2 cells.[46] These data are in agreement with Kitamura et al. who used an ERE-luciferase reporter assay in MCF-7 cells.[34]

Anti-androgenic activities of BPF were reported in *in vitro* and *in vivo* systems. BPF can compete with 5-α dihydro-testosteron (5-α DHT) for binding with AR and exhibits a significant anti-androgenic activity in MDA-kb2 cells.[46] These results are in agreement with Satoh et al. (2004) who observed a decrease of 5-α DHT-induced luciferase at 10^{-6} M in CHO-K1 cells using the AR-EcoScreen assay.[47] BPF decreased luciferase induction by DHT in mouse NIH3T3 cells[30] as well as human MDA0MB453 cells.[45]

4. Estrogenic activities of BPS

Estrogenic activity of BPS is rather controversial. It was first reported that BPS had no estrogenic activity using the yeast two-hybrid system.[33] Several authors, however, reported that BPS possessed estrogenic activity in MCF-7 cell[40,43] as well as weak estrogenic transcriptional activities in human MELN cells derived from MCF-7 cells.[36] Since the basic structural features which have been linked to ER binding,[48] in particular the presence of a *para* hydroxyl group on each of the phenol rings, are shared by both BPA and BPS, BPS also may have modulatory effects toward ER binding potency.[18,34,35,36] BPS is also reported to have anti-androgenic activity in mouse NIH3T3 cells.[34]

B. Estrogenic activities of Tritan copolyesters

Three monomers of Tritan exhibited no evidence of interaction with either the AR or the *alpha* or *beta* ER receptors.[9] Similarly, the AR and ER transactivation assays, conducted with human cells and yeast reporters were negative as well. The lack of an estrogenic effect in *in vitro* assays was in good agreement with the *in vivo* uterotrophic assay in which none of the monomers demonstrated biological activities consistent with agonism of natural estrogens when administrated orally to ovariectomized female rats using a very wide range of dose levels. Similarly, the *in vivo* Hershberger assay shows no evidence of androgenic or anti-androgenic effects.

These results, however, are contrary to those reported by Yang et al.[49] They reported estrogenic effects of CHDM and DMT using the MCF-7 cell. Although details of the test results are not given, the authors report both compounds to be “estrogenic active”. Authorities in the US and Europe have reviewed Tritan copolyesters for safety for food contact use,[50,51] however, further study of polymer as well as monomer on endocrine disruption appear to be warranted.

IV. Conclusion

According to the investigations that employed several estrogenicity and androgenic assays, most BPs used as alternatives of BPA appear to have estrogenic and anti-androgenic activity as a common property. The modification of phenolic rings and bridging carbon, or the longer length of the alkyl substituents seems to influence the estrogen and anti-androgen activity, although the apparent relationship between their structure and estrogenic activity was not clarified.[33] For components of Tritan, no evidence of estrogen- or androgen-related effects was reported in one study, but a report suggesting otherwise is available. More studies with thorough study design, e.g., with long-term exposure period are warranted.

If current trends continue, production and subsequent environmental release of BPA alternatives are expected to increase. As some BPA substitutes such as BPF and BPS could be more persistent in environments compared to BPA,[52] toxicological consequences in ecosystem should receive more attention. Further toxicological information of BPA alternatives is required to understand the environmental health implications of these alternatives and to develop proper management plans.

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