



Review

In vitro molecular mechanisms of bisphenol A action[☆]

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Abstract

Bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl)propane; CAS# 80-05-7) is a chemical used primarily in the manufacture of polycarbonate plastic, epoxy resins and as a non-polymer additive to other plastics. Recent evidence has demonstrated that human and wildlife populations are exposed to levels of BPA which cause adverse reproductive and developmental effects in a number of different wildlife species and laboratory animal models. However, there are major uncertainties surrounding the spectrum of BPA's mechanisms of action, the tissue-specific impacts of exposures, and the critical windows of susceptibility during which target tissues are sensitive to BPA exposures. As a foundation to address some of those uncertainties, this review was prepared by the "In vitro" expert sub-panel assembled during the "Bisphenol A: An Examination of the Relevance of Ecological, In vitro and Laboratory Animal Studies for Assessing Risks to Human Health" workshop held in Chapel Hill, NC, Nov 28-29, 2006. The specific charge of this expert panel was to review and assess the strength of the published literature pertaining to the mechanisms of BPA action.

Abbreviations: 4-*tert*-OP, 4-tertiary octylphenol; 17 α -EE, 17alpha-ethinyl estradiol; AR, androgen receptor; ARE, androgen response element; BADGE, bisphenol A diglycidyl ether; BFDGE, bisphenol F diglycidyl ether; BP-3, *p,p'*-dihydroxybenzophenone; BP-4, 2,2-bis(*m*-methyl-*p*-hydroxyphenyl)propane; BP-5, 2,2-bis(*p*-hydroxyphenyl)perfluoropropane; BPA, 2,2-bis(4-hydroxyphenyl)propane; BPA-OMe, 2,2-bis(4-methoxyphenyl)propane; BPE, 1,1-bis(4-hydroxyphenyl)ethane; BPF, 4,4'-methylenebisphenol; BPM, 4,4'-(1,3-phenylenedisopropylidene)bisphenol; BPP, 4,4'-(1,4-phenylenedisopropylidene)phenol; BPS, 4,4'-sulfonyldiphenol; BPZ, 4,4'-cyclohexylidenebisphenol; DDE, *o,p'*-dichlorodiphenylethylene; *o,p'*-DDT, 1,1,1-trichloro-2-[*p*-chlorophenyl]-2-[*p*-chlorophenyl]ethane; DEHP, bis(2-ethylhexyl)phthalate; DES, diethylstilbesterol; DHT, dihydrotestosterone; DMSO, dimethyl sulfoxide; E2, 17beta-estradiol; EDC, endocrine disrupting chemical; ELISA, enzyme-linked immunosorbent assay; ER, estrogen receptor; ERE, estrogen response element; ETOH, ethanol; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; GH, growth hormone; HBSS, Hank's buffered saline solutions; h, hour; HRP, horseradish peroxidase; inos, inducible nitric oxide synthase; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; min, minute; NO, nitric oxide; NP, nonylphenol; OP, octylphenol; PCB, polychlorinated biphenyl; PMA, phorbol 12-myristate 13-acetate; PR, progesterone receptor; PSA, prostate specific antigen; RIA, radio-immuno assay; RT-PCR, reverse transcription-polymerase chain reaction; RXR, retinoid X receptor; s, second; TCDD, 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin; T3, L-3,3',5-triiodothyronine; T4, 3,3',5,5'-tetraiodo-L-thyronine; TR, thyroid hormone receptor.

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The resulting document is a detailed review of published studies that have focused on the mechanistic basis of BPA action in diverse experimental models and an assessment of the strength of the evidence regarding the published BPA research.

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Keywords: Bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl) propane; Endocrine disruption; Endocrine disrupting chemical (EDC); Rapid signaling; Estrogen receptor; Androgen receptor; Thyroid receptor; Thyroid hormone; GPR30; Non-classical membrane estrogen receptor; Immune system; Allergic response; *In vitro* mechanisms; Cell specificity; Expert panel review

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1. Introduction

Bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl) propane; CAS# 80-05-7) is a chemical used primarily in the manufacture of polycarbonate plastic, epoxy resins and as a non-polymer additive to other plastics. Because of BPA's extensive use in the manufacture of consumer goods and products, including polycarbonate food containers and utensils, dental sealants, protective coatings, some flame retardants, and water supply pipes, there is a widespread and well-documented human exposure to BPA [1,2]. Recent discoveries regarding the environmental distribution and presence of BPA in humans and wildlife have generated persistent scientific, regulatory, and public interest in assessing the potential health risks associated with BPA exposure. The detection of adverse health effects in a number of laboratory animal models upon exposure to environmentally relevant doses of BPA, as well as potential effects on human reproduction and development, have fueled additional concern. While the exposure of wildlife species and humans to BPA has been increasingly reported in the literature, there are research gaps regarding its molecular mechanisms of action, the tissue-specific impacts of exposure, and knowledge of the critical windows of susceptibility, during which target tissues are especially sensitive to BPA.

The lack of an integrated and systemic understanding of BPA's endocrine disruptive actions has considerably complicated risk assessment efforts and safety recommendations by regulatory agencies. Current limitations in understanding the global consequences of BPA exposures include incomplete understanding of the cell/tissue specific actions and effects, a limited understanding of the receptor systems and signaling cascades through which BPA acts, and understanding when these receptors and signaling systems are present and active in target cells at different critical times of sensitivity during the entire life-span.

2. Overview

This white paper was prepared for the *in vitro* expert sub-panel assembled during the “Bisphenol A: An Examination of the Relevance of Ecological, *In vitro* and Laboratory Animal Studies for Assessing Risks to Human Health” workshop at the NIEHS. The specific charge of this expert panel was to review and assess the strength of the published literature pertaining to the mechanisms of BPA action. Particular attention was paid to studies employing *in vitro* models and the results, findings and conclusions from those studies were integrated into the broader biological/physiologic context of BPA action. Thus, the goal of

the white paper was to review in detail peer-reviewed published studies that have focused on the mechanistic basis of BPA action in diverse experimental models.

3. Definition of endocrine disruptor

The U.S.-Environmental Protection Agency (EPA) has defined an environmental endocrine disruptor or endocrine disrupting chemical (EDC) as “an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes” [3]. This definition is not limited to endocrine disrupting effects exclusive of the estrogen system. Rather, endocrine disruption encompasses effects on other endocrine systems including effects mediated by androgens, thyroid hormone, prolactin, and insulin, among others.

4. Definition of “low dose”

The primary focus of this review is on *in vitro* studies that investigate the molecular mechanisms of BPA action. However, full consideration of BPA’s mode of action cannot rely solely on *in vitro* studies performed at a range of concentrations. It is critically important to consider experimental findings from numerous *in vivo* studies investigating BPA actions in animals at a physiologically relevant exposure dose, also known as a “low-dose”. Thus, a low-dose effect was determined based on the U.S.-EPA criterion for low-dose effects of EDCs as effects observed at concentrations below those used for traditional toxicological studies. Levels below the current lowest observed effect level (LOAEL) of 50 µg/kg/day were considered as low dose for *in vivo* studies. For *in vitro* cell or organ culture studies, estimates of circulating levels of BPA at the LOAEL cut-off have defined an equivalent low dose concentration as <50 ng/mL or $<2.19 \times 10^{-7}$ M [4]. Based on experimental designs of most studies, we elected to consider an alternative and more conservative definition of low-dose for *in vitro* BPA effects as $\leq 1 \times 10^{-7}$ M.

5. Definition of estrogen

When considering the mechanisms of BPA action, it was considered critical to determine whether the molecular and physiological EDC effects of BPA could be fully explained by its ability to act as an agonist or antagonist of endogenous estrogens in target cells, or whether the molecular and physiological EDC effects of BPA extend beyond the molecular, subcellular and/or cellular mechanism of estrogens’ actions. According to the Merriam-Webster Medical Dictionary of the U.S. National Library of Medicine, (<http://www.nlm.nih.gov/medlineplus/medlineplusdictionary.html>) estrogen is defined as: any of various natural steroids (as estradiol) that are formed from androgen precursors, that are secreted chiefly by the ovaries, placenta, adipose tissue, and testes, and that stimulate the development of female secondary sex characteristics and promote the growth and maintenance of the female reproductive system; also: any of various synthetic

or semisynthetic steroids (as ethinyl estradiol) that mimic the physiological effect of natural estrogens.

In this review, “estrogen” is used as a class name for endogenous estrogenic compounds, and “estradiol” or “17β-estradiol” will be used more specifically in reference to 1,3,5(10)-estratrien-3,17β-diol.

6. Mechanisms of BPA action

Diverse biological effects have been attributed to low-dose environmental BPA exposure in a variety of tissues. Molecular studies employing an array of *in vitro* models have revealed a variety of molecular pathways through which BPA may elicit cellular responses. As a prototypical non-steroidal estrogen, BPA interferes with the activity of endogenous estrogens (e.g. 17β-estradiol) by disrupting the proper activity of the estrogen nuclear hormone receptors in a diverse set of target tissues [5–21]. However, the primary endocrine disrupting activities of BPA extend beyond its ability to mimic, enhance or inhibit the activity of endogenous estrogens and/or disrupt estrogen nuclear hormone receptor action, and include the following: effects upon the androgen systems [22–29]; disruption of thyroid hormone function [30–40]; diverse influences on development, differentiation and function of the central nervous system [40–46]; and influences on the immune system [21,47–65]. There is additional clear evidence for BPA to directly impact intracellular signal transduction pathways through mechanisms independent of the transactivational activity of nuclear hormone receptors [43,46,66–79]. Secondary metabolic and pharmacokinetic actions of BPA that impact its bioavailability and bioavailability of endogenous steroid hormones have also been described. Those secondary effects include modification of cytochrome P450 enzyme expression and activity [80–83] and alterations of serum hormone binding protein expression and interactions [84–87]. Recent studies have also begun to highlight the potential for transmission of early exposures to physiological expression later in life and across generations via epigenetic mechanisms such as methylation-mediated promoter silencing [88–90]. Table 1 summarizes the results from published studies that were used in the preparation of this review. Clearly, BPA is an endocrine disrupting compound or EDC in the broad sense of the definition. However, because of the pleiotropic mechanisms of BPA action, narrowly defining BPA as a selective estrogen receptor modulator [SERM; a class of pharmacological compounds (such as raloxifene or tamoxifen) that bind nuclear estrogen receptors and act as estrogen agonists in some tissues and estrogen antagonists in other tissues], or exclusively as an environmental estrogen, is inaccurate. The use of these self-limiting terms only partially describes the extent of BPA’s endocrine disrupting and other biological activities.

7. Nuclear estrogen receptors, coregulators, and the estrogen-response element (ERE)

It is well established that BPA can exert some of its effects by binding at the nuclear steroid receptors ERα and ERβ to induce estrogenic signals that modify estrogen-responsive gene expression. Mechanisms for ER-mediated gene regulation are

Table 1
Published papers reporting physiological effects of BPA *in vitro*

Reference	Species	Model	Endpoints	Exposure: vehicle	Exposure time	LOEC (M) (<i>*P</i> < 0.05)	Doses tested (M) (<i>*P</i> < 0.05)	Inhibitors	Other chemicals tested
Adipose models									
Masuno et al. 2005 [167]	Mice	3T3-L1 (preadipocyte)	Adipocyte differentiation	Culture medium. Not indicated	6 days	2×10^{-5}	4×10^{-6} , 2×10^{-5} , 4×10^{-5} , 8×10^{-5}	LY294002	BPA-derivatives; NP; OP
Masuno et al. 2002 [168]	Mice	3T3-L1 (preadipocyte)	Adipocyte differentiation	Culture medium. Not indicated	11 days	2×10^{-6}	2×10^{-6} , 10^{-5} , 2×10^{-5}		BPA-derivatives
Bone models									
Kanno et al. 2004 [169]	Mice	MC3T3-E1 (C57BL/6) osteoblast-like	Cell proliferation. Differentiation (ALP activity). Ca^{2+} content	Culture medium. ETOH	3–4 days	10^{-6} , $10^{-6.5}$, 10^{-7}	10^{-8} , $10^{-8.5}$, 10^{-9} , $10^{-9.5}$, 10^{-10}		E2; cumestrol; genistein; daidzein; NP; DEHP
Breast cancer models									
Buettner et al. 2006 [101]	Human	T47D/Luc, MCF7	ERE-promoter activation. Cell proliferation	Culture medium. DMSO	3–24 h, 24 h	10^{-7} (24 h)	10^{-10} to 10^{-5} , 10^{-5}		E2; genistein; PCB54; PCB126
Cappelletti et al. 2003 [170]	Human	T47D, BT20	Cell growth, ER, PR mRNA (RT-PCR)	Culture medium. Not indicated	6 days, 10, 24, 48 h	$10^{-6.5}$, $10^{-5.5}$	10^{-8} , 10^{-7} , $10^{-6.5}$, $10^{-5.5}$		E2; 4- <i>tert</i> -OP; 2-OH-biphenol
Imadera et al. 2000 [60]	Human	MCF7	IL-1 induced MCP-1 expression	Culture media. <0.1% ETOH	24 h	5×10^{-7}	$10^{-7.5}$, $5 \times 10^{-7.5}$, $10^{-6.5}$	ICI 182,780	E2; NP
Iso et al. 2006 [171]	Human	MCF7, MDA-MB-231	DNA damage (comet tail length assay)	Culture medium. <0.1% ETOH	1, 3, 24 h	10^{-6}	10^{-5} , 10^{-6} , $10^{-6.5}$	ICI 182,780	E2
Olsen et al. 2003 [115]	Human	MCF7	Cell proliferation. Estrogen-responsive gene expression	Culture medium. $\leq 0.2\%$ ETOH	6 days, 3 days	5×10^{-7}	10^{-8} , 10^{-7} , 5×10^{-7} , 10^{-6} , 10^{-5}	4-OHTamoxifen; ICI 182,780	E2; tetra-Cl-BPA; tetra-Br-BPA; T3; T4; 4-OH-biphenol; 4-OH-biphenol
Recchia et al. 2004 [9]	Human	MCF7, T47D	Cell proliferation. ER α -expression & nuclear localization, ERE-luciferase reporter	Culture medium. Not indicated	6 days, 2 h	10^{-6}	10^{-6}	4-OH Tamoxifen	4-NP
Embryonic/development models									
Samuelsen et al. 2001 [142]	Human	MCF7, T47D	Cell proliferation. PR, pS2	Culture medium. $\leq 0.1\%$ ETOH	6 days	10^{-7}	10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , 10^{-4}		E2; Br-BPA derivatives
Singleton et al. 2006 [98]	Human, <i>S. cerevisiae</i>	MCF7-C4-12 (HA-ER α) Ishikawa (endometrial)	Differential mRNA expression, ERE-LacZ	Culture media. ETOH	3 h, 24 h	10^{-6} , 10^{-7}	10^{-7} , 3×10^{-7} , 10^{-6} , 3×10^{-6}		E2
Vivacqua et al. 2003 [10]	Human	MCF7, MCF7SH	ERE-luciferase, ER α , pS2 expression, Cell growth	Culture medium. Not indicated	30–24, 6 days	10^{-9}	10^{-10} , 10^{-9} , 10^{-8} , $10^{-7.5}$, 10^{-6} , 10^{-5}	4-OH Tamoxifen	E2; 4-NP
Walsh et al. 2005 [74]	Human	MCF7, MDA-MB-231, SKBR-3	Intracellular Ca^{2+}	Krebs' solution. $\leq 0.001\%$ methanol	0–15 min	10^{-10}	10^{-10} , 10^{-9} , 10^{-8} , $10^{-7.5}$, 10^{-7}	ICI 182,780	<i>o,p'</i> -DDT; DES; 4- <i>tert</i> -OP
Embryonic/development models									
Iwamura et al. 2003 [146]	<i>Xenopus laevis</i>	Tadpole tail organ culture	Tail length regression (also see <i>in vivo</i>)	Culture medium. ETOH	1–4 days	10^{-6}	10^{-6} , 10^{-5} , ($\pm 10^{-7}$ T3)		E2
Iwamura et al. 2006 [32]	<i>Xenopus laevis</i>	Tadpole tail organ culture	Tail length regression. TR α , TR β , RXR γ , mRNA expression (RT-PCR)	Culture medium. ETOH	1–5 days	10^{-7}	10^{-7} , 10^{-6} , 10^{-5} , ($\pm 10^{-7}$ T3)		E2
Tsutsui et al. 1998 [172]	Hamster (Syrian)	Primary embryo cells	Cell growth, morphological chromosomal aneuploidy, DNA adduct formation	Culture medium. 0.6% DMSO	24, 48, 72 h	5×10^{-5}	5×10^{-5} , 10^{-4} , 2×10^{-4}		BPH; BP-3; BP-4; BPS
Tsutsui et al. 2000 [173]	Hamster (Syrian)	Primary embryo cells	Cell growth, morphological, DNA adduct formation, aneuploidy	Culture medium. 0.6% DMSO	24, 48, 72 h	10^{-4}	5×10^{-5} , 10^{-4} , 2×10^{-4}		BPH; BP-3; BP-4; BPS
Endothelium models									
Lee et al. 2003 [25]	Human	Female primary endothelial cells	Cell proliferation	Culture medium. Not indicated	24, 48, 72 h	10^{-6}	10^{-8} , 10^{-6} , 10^{-4}		Aroclor 1254
Female reproductive tissue models									
Jin et al. 2005 [174]	Human	BeWo (h30) placental trophoblast	Calcium AM efflux	HBSS 2.5 mM glucose. ETOH	48 h	10^{-7}	10^{-7} , 10^{-6} , 5×10^{-5}		E2
Xu et al. 2002 [112]	Mice B6C3F1	Primary granulosa cells	Cell viability/apoptosis	Culture medium. 0.1% ETOH	72 h	10^{-10}	10^{-13} , 10^{-10} , 10^{-7} , 10^{-4}		
Immune system models									
Alfradeh et al. 2006 [47]	Mice BALB/c	Primary splenocytes; Sex not specified	Cytokine production, (IL-4, IL-12; IFN- γ ELISA)	<i>In vivo</i> IP-injection, corn oil	8 days	100 mg/kg every other days	100 mg/kg for IL-4; IFN- γ		DES; NP
Canesi et al. 2004 [75]	Mussels	Primary hemocytes	Lysosomal membrane stability, ERK2, p38, STAT3, STAT3 phosphorylation	Artificial sea water/serum. ETOH	15–110 min, 5–30 min	2.5×10^{-5} , 2.5×10^{-3}	2.5×10^{-5} , 2.5×10^{-3} , 2.5×10^{-5}	4-OHTamoxifen; SB203580; PD98059; wortmannin; GF109203X	DES; NP

Table 1 (Continued)

Reference	Species	Model	Endpoints	Exposure: vehicle	Exposure time	LOEC (M) (*P < 0.05)	Doses tested (M) (**P < 0.05)	Inhibitors	Other chemicals tested
Goto et al., 2004 [64]	Mice BALB/c	Female primary splenocytes	Cell proliferation	Culture medium, 0.156% DMSO	72 h	2×10^{-5}	2.5×10^{-6} , 5×10^{-6} , 1×10^{-5} , 2×10^{-5}		
Han et al., 2002 [63]	Mice BALB/c	Male primary splenocytes	Immunoglobulin production (ELISA)	Culture medium, <0.1% ETOH	7 days	10^{-8} (lgE, IgM)	10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , 10^{-3}		E2, tamoxifen; DES; diadzin genistein quercetin; luteolin Various EDCs
Hong et al., 2004 [62]	Mice BALB/c	RAW 264 (macrophage), Female Abdominal macrophages	LPS-induced TNF- α production, NO-production	Culture medium, DMSO.	4 h	nd	4.4×10^{-5} (NO only), NS		
Kim et al., 2003 [48]	Mice	RAW 264.7 (macrophage), Female Abdominal macrophages	TNF- α production (total nitrite), iNOS expression (RT-PCR)	DMSO/corn oil Culture medium (\pm LPS), <0.1% DMSO	6, 24 h	10^{-5}	10^{-6} , 10^{-5} , 5×10^{-5} , * vs. LPS control	ICI 182,780	E2
Lee et al., 2003 [61]	Mice BALB/c	Female primary lymph node cells, hybridoma cells (3.155, GK1.5, EL4)	PMA/ILK1H-primed IL-4 production	Culture medium, Not indicated	2, 4 days	10^{-5}	10^{-7} , 10^{-6} , 10^{-5} , 5×10^{-5} , 10^{-4}	Nitrendipine; BATA-AM; thapsigargin; FK506	NP
Nödebele et al., 2004 [49]	Human	CD4+ Jurkat T cell line	IL-2 production (ELISA; RT-PCR)	Culture medium	48 h	ns	10^{-9} , 10^{-8} , 10^{-7} , 10^{-6}		Coumestrol; TCDD; DDT
Reisand et al., 2005 [160]	Human	Primary neutrophil granulocytes	Reactive oxygen species, Intracellular Ca^{2+} (secondary endpoints)	HEPES-buffered salt solution, $\leq 0.1\%$ ETOH	60 min, 250 s	2×10^{-6}	2×10^{-6} , 6×10^{-6} , 1.2×10^{-5} , 2×10^{-5}		4-Br-BPA
Sakabe et al., 1999 [50]	Mice	Primary thymus epithelial cells	Thymosin- α 1 suppression (ELISA; HPLC)	Culture medium, Not indicated	7 days	3×10^{-6}	3×10^{-5} , 3×10^{-7} , 3×10^{-6} , 3×10^{-5} , $3 \times 10^{-3} \times 10^{10}$		E2; genistein; coumestrol; α -Zeranol; progesterone; cholesterol
Sawar et al., 2003 [51]	Mice C57BL/6j	Primary splenic mononuclear cells HL-60	ConA induced IFN- γ secretion (ELISA)	Culture medium, Not indicated	24 h	5×10^{-8}	5×10^{-10} , 5×10^{-8} , 5×10^{-6}		
Watanabe et al., 2003 [53]	Human	HL-60	Neutrophilic differentiation, O_2^- production, CD18 expression	Culture medium, DMSO	6 days	10^{-10}	10^{-12} , 10^{-10} , 10^{-8} , 10^{-6} , 10^{-5}	4-OH Tamoxifen	
Liver models									
Jevng et al., 2000 [83]	Mice	Hepa-1c1e7 (hepatoma)	Cyp1a-1 activity, Cyp1a-1 mRNA expression (RT-PCR)	Culture medium, DMSO <0.2%	18 h, 6 h	10^{-5}	10^{-6} , 10^{-5} , 5×10^{-5}	4-OH Tamoxifen	TCDD
Kubo et al., 2004 [78]	Human	Hep1B (hepatoma)	Inhibition of hypoxic response, Erythropoietin, HIF-1 α expression	Culture medium, Not indicated	6 h	10^{-4}	5×10^{-5} , 10^{-4} , 5×10^{-4}	CoCl ₂ , Mg132	BPA OMe; BPE; BPF
Male reproductive tissue models									
Fiorini et al., 2004 [136]	Rat	SerW3 (Sertoli cells testis)	Qualitative junctional protein expression	Culture medium, DMSO <0.1%	24 h	4.5×10^{-5}	4.5×10^{-5}		BPA-methacrylate; <i>o,p'</i> -DDT
Ida et al., 2003 [175]	Rat Wistar	Primary Sertoli cells	Viability/apoptosis	Culture media	<12–48 h	1.5×10^{-4}	5×10^{-5} , 10^{-4} , 1.5×10^{-4} , 2×10^{-4} , 3×10^{-4}		
Lee et al., 2003 [29]	<i>S. cerevisiae</i> , Human, Mice	Hela, HepG2, 1Sp-1 (Sertoli cell line)	2-hybrid AR/ASCI, GFP-AR translocation, Inhibition of ARE transcription (luciferase)	Culture medium, ETOH	3 h, 24 h, 24 h	10^{-6}	10^{-7} , 10^{-6} , 10^{-5} , 10^{-4}		E2, NP, cyproterone
Nikula et al., 1999 [135]	Mice	mLTC-1 (Leydig tumor cell line)	cAMP, progesterone production	Culture medium, ETOH	48 h	10^{-7}	10^{-7} , 10^{-6} , 10^{-5} , 10^{-4}		E2, <i>tert</i> -OP, OP, DES
Song et al., 2002 [137]	Mice	K28 (Leydig tumor cell line)	Nur77 expression S.	Culture media, 0.1% ETOH	30 min – 24 h	10^{-8}	10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5}	HR9; PD98039; GFX; wortmannin	BPE; BPF; BFM; BPP; BPS; BPZ
Tabuchi et al., 2006 [176]	Mice	TTE3 Sertoli cells (testis)	Steroidogenic function mRNA expression	Culture media, 0.1% DMSO	3, 6, 12 h	2×10^{-4}	2×10^{-4}		
Wetherill et al., 2002 [26]	Human	LNCaP (prostate)	Cell proliferation, AR-translocation	Culture medium, 0.1% ETOH	72 h, 2, 7 h	10^{-9} , 10^{-10}	10^{-10} , 10^{-9} , 10^{-8} , 10^{-7}	Bicalutamide	DHT
Wetherill et al., 2005 [28]	Human	LNCaP (prostate)	PSA, AR expression, AR transactivation	Culture medium, 0.1% ETOH	48 h	10^{-9}	10^{-9} , 10^{-5}	Bicalutamide	DHT
Sarob et al., 2004 [112]	Hamster	CHO-K1 (ARE—luciferase)	AR/ARE—luc expression (\pm DHT), Cell proliferation	Culture medium, DMSO, $\leq 0.1\%$ ETOH	16–24 h	10^{-6} , 10^{-7}	10^{-11} , 10^{-10} , 10^{-9} , 10^{-7} , 10^{-7} , 10^{-5}		BADGE, BADGE-2H; O; BADGE-2HCl; BPF; BFDGE; BFDGE-2H; O; BFDGE-2HCl; Cyproterone; Nifedipine
Nervous system models									
Miyatake et al., 2006 [44]	Mice ICR	Primary culture midbrain astrocytes & neuroglia	GFAP/Neu-N immunoreactivity, $[Ca^{2+}]_i$	Culture medium, Not indicated	24 h	10^{-13}	10^{-14} , 10^{-13} , 10^{-12} , 10^{-11} , 10^{-10} , 10^{-9} , 10^{-8} , 10^{-7} , 10^{-6}	ICI 182,780; mifepristone; flutamide	E2

Table 1 (Continued)

Reference	Species	Model	Endpoints	Exposure: vehicle	Exposure time	LOEC (M) (*P < 0.05)	Doses tested (M) (*P < 0.05)	Inhibitors	Other chemicals tested
Selwa et al., 2004 [177]	Mice ICR	Primary oligodendrocyte precursor cells	T3-induced differentiation	Culture media, Not indicated	2 days	10 ⁻⁵	10 ⁻⁹ , 10 ⁻⁷ , 10 ⁻⁶ , 10 ⁻⁵	ICI 182,780	E2; 17 α -EE; DES; p-NP
Sato et al., 2002 [178]	Rat Wistar	Hippocampus organotypic primary culture	Glutamate-induced cell death (CA3)	Culture medium	24 h	10 ^{-12.6}	10 ^{-12.6}	ICI 182,780	E2; 17 α -EE; DES; p-NP
Yamaguchi et al., 2006 [40]	Mice	SFME cells	NMDA-receptor expression, Spine & mossy fiber density LIF/BMP-2 induced astrocyte differentiation	$\leq 0.1\%$ ETOH Culture medium, Not indicated	48 h	4.4 \times 10 ^{-12.6}	4.4 \times 10 ^{-11.6} , 4.4 \times 10 ^{-12.6} , 4.4 \times 10 ^{-13.6} , 4.4 \times 10 ^{-14.6} , 4.4 \times 10 ^{-15.6} , 4.4 \times 10 ^{-16.6} , 4.4 \times 10 ^{-17.6} , 4.4 \times 10 ^{-18.6}	4-OH Tamoxifen	
Yoneda et al., 2003 [76]	Rat	PC12 (adrenal)	Dopamine secretion	Culture medium, $\leq 0.1\%$ DMSO	10 min	2.5 \times 10 ^{-6.6}	2.5 \times 10 ^{-6.6} , 5 \times 10 ^{-6.6} , 1 \times 10 ^{-5.6} , 1.5 \times 10 ^{-5.6}	Diltiazem; clorgylin; pargyline; ω -agatoxin; ω -conotoxin; H7; H-89; ryanodine; ruthenium red; Rp-cAMPS; GDP β s; U0128	E2
Zsarnovszky et al., 2005 [46]	Rat Sprague-Dawley	Developing cerebellar neurons (male & female)	ERK1/2 phosphorylation	Culture medium, artificial cerebrospinal fluid, DMSO	5 min	10 ^{-12.6}	10 ^{-12.6} , 10 ^{-11.6} , 10 ^{-10.6} , 10 ^{-9.6} , 10 ^{-8.6} , 10 ^{-7.6} , 10 ^{-6.6} , 10 ^{-5.6}	Actinomycin-D; ICI 182,780	E2; NP
Pancreatic models Adachi et al., 2005 [21]	Rat Wistar	Male; primary pancreatic islets	Insulin release-RIA	Krebs-Ringer bicarbonate buffer	60 min, 24 h	nd, 4.4 \times 10 ^{-9.6}	4.4 \times 10 ^{-10.6} , 4.4 \times 10 ^{-9.6} , 4.4 \times 10 ^{-8.6} , 4.4 \times 10 ^{-7.6}		
Alonso-Magdalena et al., 2005 [66]	Mice Swiss albino OF1	Male; primary pancreatic islets and isolated islet cells	Intracellular Ca ²⁺	Modified Ringers—5 or 0.5 mM glucose, DMSO	5 min	10 ^{-9.6}	10 ^{-9.6}	ICI 182,780; KT-5823;	E2; E2-HRP; DES; DDT
Alonso-Magdalena et al., 2006 [67]	Mice Swiss albino OF1	Male; primary pancreatic islets and isolated islet cells	Insulin release-RIA; insulin content	<i>In vivo</i> sc injection; tocopherol-stripped corn oil			10; 100 μ g/kg/day	perussis toxin; ODD; L-NAME	E2
Nadal et al., 2000 [70]	Mice Swiss albino OF1	Male; primary pancreatic islets and isolated islet cells	Fluo-3 AM [Ca ²⁺] _i	Perfusion buffer	4–8 min	10 ⁻¹⁰	10 ⁻¹⁰ , 10 ⁻¹¹ , 10 ⁻⁹ , 10 ⁻⁷		E2; E2-HRP; DES; α , β -DDT
Quesada et al., 2002 [73]	Mice Swiss albino OF1	Male; primary pancreatic islets and isolated islet cells	Intracellular Ca ²⁺ , Phospho-CREB-IR	Perfusion buffer	0–15 min	10 ⁻⁹	10 ⁻⁹	ICI 182,780	E2; E2-HRP
Primary models Bulayeva et al., 2004 [69]	Rat	GH3/B6/F10 (ER α -positive)	ERK1/2 phosphorylation immunoreactivity	Culture medium; ETOH	3–30 min	nd	10 ⁻⁹		E2; NP; endosulfan; DDE; dieldrin; comestrol
Ghisanzi et al., 2005 [30]	Rat	GH3	Cell proliferation (T-screen)	Culture medium	6 days	10 ^{-7.6}	10 ^{-8.6} , 10 ^{-7.6} , 10 ^{-6.6} , 10 ^{-5.6}	ICI 182,780	E2; T3; BPA; methacrylate; 4-OP; 4-n-NP; iprodione; prochloraz; chlorpyrifos; 4-OH-PCB 106; 4-OH-PCB 69; 4-OH-PCB 121
Kitamura et al., 2002 [33]	Rat	GH3	Cell proliferation	Culture medium; ETOH	7 days	10 ^{-6.6}	10 ^{-7.6} , 10 ^{-8.6} , 10 ^{-5.6}	4-OH Tamoxifen	T3; E2; TBBPA; TC8PA
Kitamura et al., 2005 [35]	Human, Mice, Rat	MCF7, NIH3T3, GH3	ERE α -luciferase, ARE-luciferase, GH-induction	Culture medium; ETOH	24 h, 24 h, 2 days	10 ^{-7.6} , 10 ^{-6.6} , NS	10 ^{-8.6} , 10 ^{-7.6} , 10 ^{-6.6} , 10 ^{-5.6} , 10 ^{-4.6}	4-OHTamoxifen	E2; various BPA-related compounds
Stemmetz et al., 1997 [157]	Rat	GH3	Prolactin release, Cell proliferation, Prolactin-luciferase	Culture media, $\leq 0.001\%$ ETOH	1–8 days, 8, 24 h	10 ⁻⁶ , 10 ⁻⁹	10 ⁻⁶ , 10 ⁻⁹		E2
Watson et al., 2005 [72]	Rat	GH3/B6/F10 GH3/B6/D9	Intracellular Ca ²⁺ , Prolactin release	Ringer's solution, Culture medium	1, 3 min	10 ⁻¹²	10 ⁻¹² , 10 ^{-10.6} , 10 ^{-9.6} , 10 ^{-8.6} , 10 ^{-7.6}		Comestrol; DDE; DES; dieldrin; endosulfan; p-NP
Wozniak et al., 2005 [77]	Rat	GH3/B6/F10 GH3/B6/D9	Intracellular Ca ²⁺ , Prolactin release	Ringer's solution, Culture medium	0–20 min, 1–15 min	10 ^{-12.6}	10 ^{-12.6} , 10 ^{-11.6} , 10 ^{-10.6} , 10 ^{-9.6} , 10 ^{-8.6} , 10 ^{-7.6} , 10 ^{-6.6} , 10 ^{-5.6} , 10 ^{-4.6}	Nifedipine	Comestrol; DDE; DES; dieldrin; endosulfan; p-NP

complex, and depend on the recruitment of tissue-specific co-regulatory factors that differentially affect the interaction of ERs with EREs of different target genes [91,92]. BPA selectively binds to ER α and ER β , and has a higher affinity for ER β in target cells [6–8]. Kuiper et al. reported a K_i for BPA of 195 and 35 nM at ER α and ER β , respectively [6]. It was also found that the binding affinity relative to 17 β -estradiol for BPA at ER β was 6.6-fold higher than at ER α ; 0.33 and 0.05, respectively [6]. Satoh et al. also showed that BPA could bind to human ER α and reported an IC_{50} (the concentration of chemical required to reduce specific 17 β -estradiol binding by 50%) of 7.8 μ M [12]. Gould et al. demonstrated that as an endocrine disruptor BPA is not merely a weak estrogen mimic, but it exhibits characteristics of a distinct molecular mechanism of action at ER α , with BPA interacting differently within the ligand-binding domain [5]. These findings reveal that the interactions of BPA and 17 β -estradiol with an estrogen receptor can induce differential molecular effects, thus ultimately impacting the physiological response of sensitive cells.

In addition to the differential ER binding properties, there are major differences in the effects of BPA on co-activator recruitment by ER α and ER β [8]. Specifically, the BPA/ER β receptor/ligand complex showed over 500-fold greater potency than the BPA/ER α complex in recruiting the co-activator TIF2. Moreover, the enhanced (relative to ER α) ability of ER β to recruit co-activators in the presence of BPA was consistent with a greater capacity of ER β to potentiate reporter gene activity in transiently transfected HeLa cells expressing ER β and TIF2 over a concentration range of 1–100 μ M [8]. A higher relative binding affinity (RBA) for BPA at ER β was also observed (estradiol RBA for BPA at ER β was 0.75 versus 0.073 for ER α). Based on those data it was concluded that ligand-dependent differences in the ability of ER α and ER β to recruit co-activator proteins would contribute to the complex tissue-dependent agonistic or antagonistic responses observed with exposure to endocrine disruptors such as BPA.

Numerous isoforms of ER α and ER β have been identified and they likely play unique roles as mediators of the actions of endogenous estrogens and BPA. For example, Ramsey et al. used different ERE-sequence containing reporter-constructs to demonstrate that expression of different isoforms of ER β differentially impacted ERE-regulated gene expression in a ligand dependent manner [93]. These results suggest that the cell-type specific expression of different ER isoforms together with availability of a specific ligand, contributes to the cellular complexity of ERE-regulated estrogen responsive target gene expression. Moreover, a study by Masuyama et al. showed that compared to 17 β -estradiol, BPA blocks the ubiquitination and degradation of ER β , suggesting that BPA may affect ER β -mediated transcription of target genes by differential degradation of nuclear receptors [94].

8. Differential responsiveness of sensitive cells and tissues

Regarding cell-specific differences in response to BPA, it was proposed that the nature of the estrogen-responsive promoter

would also influence the activity of endocrine disrupting chemicals through ERs, similar to the way in which SERMs manifest their gene-specific activities. The idea that ligands play an active role in ER function was proposed by McDonnell and others who demonstrated that different ligands induce distinct conformational changes in the ER, correlating with the observed diversity in transcriptional and biological responses [95–97].

Pennie et al. showed that the nature of the ERE, along with the ratio of ER α and ER β in a particular cell or tissue could influence ER-responsive genes in the presence of estrogenic compounds such as BPA [14]. In those studies, the effects of BPA at doses from 1 to 100 μ M and 17 β -estradiol (1 nM to 1 μ M) in COS-1 cells transiently transfected with either vitellogenin ERE or LH β ERE reporters were characterized. A follow-up study by Hall et al. confirmed these findings by demonstrating that the transcriptional activities of EDCs are mediated in a promoter-specific manner, and that estrogen-responsive promoters communicate differentially with ER α and ER β by influencing the receptor structures, thus resulting in a diversity of transcriptional responses [13]. Specifically, HepG2 (human hepatoma), Ishikawa (human endometrial carcinoma) or UCI107 (human ovarian carcinoma) cells were co-transfected with ER α or ER β expression plasmids together with different firefly luciferase reporter constructs. The reporter constructs contained either three copies of the vitellogenin ERE, the estrogen-responsive promoters from pS2, lactoferrin, or complement 3 (C3). Notably, BPA action through ER β was significantly influenced by the nature of the promoter utilized. In particular, BPA displayed remarkably high potency on pS2 promoter through ER β , with higher ER α -mediated activity at vitellogenin and lactoferrin promoters [13]. The authors concluded that different estrogen-responsive promoters may influence the ability of the ER to form a transcriptionally active conformation, providing a mechanism for the promoter-specific activities observed. These studies are in agreement with Kurosawa et al. who demonstrated that BPA exhibits estradiol-like agonist activity via ER β , and has mixed agonist and antagonist activity in some types of cells via ER α [16]. Thus, for a given cell type, the activity of BPA likely depends in part on the levels of different ER isoforms and variants expressed in the sensitive cell or tissue.

Although altered ER-regulated gene expression through the ERE is a major mechanism of BPA action, recently it was shown by Singleton et al., that high concentrations of BPA could regulate expression of a distinct set of genes involved in growth and development, such as HOXC1 and C6, Wnt5A, Frizzled, TGF β -2, and STAT inhibitor 2 in MCF-7 breast cancer cells stably expressing HA-tagged ER α [98]. When HOXC6 mRNA expression was examined by quantitative RT-PCR, three independent ER α -HA cell cultures showed down-regulation (2.2–2.8-fold) following 3 h treatment with 1 μ M BPA. HOX genes encode transcription factors containing the highly conserved 183-base-pair homeodomain sequence and are critically important for embryonic development [99]. Although biological functions of the HOXC6 gene product are not well defined, targeted disruption of this gene in mice has indicated a role in mammary ductal development [100]. In contrast to Singleton's study, Buterin et al. demonstrated that exposure of MCF-7 and T47D breast

cancer cells to pharmacological concentrations of BPA (10 μ M) resulted in identical transcriptional effects as induced by physiological concentrations of estradiol [101]. These authors suggest that endocrine disrupting compounds may act in conjunction with endogenous steroid hormones to induce additive effects in target tissues. Another study demonstrated that exposure to low BPA doses (ranging from 0.1 nM to 25 μ M) increased HOXA10 protein expression in the uterus [102]. Thus, the results from these studies reveal novel non-ERE-regulated effects on gene expression by BPA, suggesting a role for additional transcriptional mechanisms of BPA action in reproductive and mammary gland development.

In order to identify tissues and cells that contain functionally active ERs, and to define their ability to respond to different ligands, Nagel et al. developed ER action indicator (ERIN) transgenic mice (strain C57BL/6-SJL). This model system integrates the upstream requirements of ER action, including the receptor, ligand, and accessory comodulators with activation of the ER, resulting in expression of the enzyme β -galactosidase (β -gal), which allows for enzymatic amplification of the estrogenic signal and histological localization of its activity [103]. In these studies, BPA elicited a distinct cellular profile of gene expression, similar to SERMs. BPA stimulated ER transcriptional activity at a dose of 800 μ g/kg (40% efficacy as compared to DES-maximum) and exhibited 20% of DES-induced maximum response at a low dose of 25 μ g/kg; however, BPA had little uterotrophic activity in this system [103]. In contrast, in the same model, the well-established SERM tamoxifen significantly increased uterine weight at 25 mg/kg, while minimally inducing ER reporter activity in this tissue [103]. The authors suggest that these differential actions of BPA may be due to this ligand's selective ER modulatory activities, resulting in tissue-selective ER activity.

In agreement with Nagel and colleagues, a recent study by Hong et al., also demonstrated distinct expression patterns of responsive genes following exposure to either estradiol or endocrine disrupting compounds, including BPA, thus further implicating differential effects of estradiol versus BPA in reproductive tissues [104]. Based on the studies described above, it is likely that a BPA-induced ER conformation results in recruitment of cell- and tissue-specific co-modulatory factors responsible for the differential responses to this ligand.

9. BPA effects on cells and tissues of the reproductive system

The findings described above raise further questions about the differential roles of endocrine disruptors in human development and reproduction. Based on recent molecular studies, it was proposed by Welshons et al., that BPA is a SERM [4]. An investigation into SERMs/endocrine disruptors confirmed that BPA effects in uterus, vagina and the bone are not purely estrogenic. Utilizing low doses of BPA (37 and also 370 μ g/kg/body weight) in Sprague–Dawley rats, Seidlova-Wuttke et al. found no significant effect on uterine weight, but a clear increase in epithelial thickness, which was contrasted by a reduction in the myometrial thickness [11]. Following 3 months treatment

with 37 μ g/kg body weight/day BPA, a modest reduction in endometrial thickness was observed. These effects were at much lower doses than used in previous experiments [105]. Hence, the uterotrophic assay in this study revealed subtle, low-dose effects of BPA that cannot be defined as “classical” estrogenic effects. Seidlova-Wuttke et al. also used quantitative RT-PCR analysis to characterize the effects of BPA on ER α , ER β and C3 transcripts in the uterus. It was found that estradiol had no effect on ER α mRNA, decreased ER β mRNA, and greatly increased C3 mRNA expression. Interestingly, these effects were not mimicked by BPA. In fact, BPA exposure increased ER α , did not effect ER β , and decreased C3 mRNA expression [11]. Treatment with both 37 and 370 μ g/kg BPA also resulted in significant reductions of bone mineral density, which was in stark contrast to estradiol, whose action is essential for maintenance of normal bone structure. The authors suggest that BPA-induced effects on bone metabolism were through mechanisms other than those regulated by ER α or ER β . It is known that the bone expresses the aryl hydrocarbon receptor (AhR) [106,107], but whether BPA serves as a ligand for the AhR still needs to be established. While the rapid signaling effects of BPA on bone cells have not been investigated, sex-specific rapid effects of estradiol on intracellular signal transduction in osteoblasts are well documented [108], and the signaling network for the rapid actions of the phytoestrogen diadzein has also been described [109]. Thus, it is likely that BPA acts via membrane ER isoforms, contributing to regulation of bone physiology. Additionally, BPA may modulate the available co-regulatory factors (enhancers or repressors of ER) in the uterus and bone. Clearly, further investigations are needed to decipher BPA's molecular mode of action in these tissues.

Based on studies described in this section, it is evident that some actions of BPA are compatible with its characterization as a SERM. However, mounting research has demonstrated that BPA cannot be defined exclusively as a SERM. The actions of BPA extend beyond its ability to mimic, enhance, or inhibit the activity of endogenous estrogens at the estrogen receptor: as described below, BPA is not ER “selective,” because it also binds and affects other nuclear receptors, including the androgen and thyroid receptors.

10. BPA effects related to fertility

Numerous studies have implicated BPA and other EDCs in reduced female fertility and the role of estrogens in oocyte development is a topic of considerable research interest [110]. It is known that theca cells of the late-stage follicle express both the ER α and ER β , whereas the granulosa cells express predominantly ER β . Accordingly, BPA has the potential to affect both theca and granulosa cells, however the degree to which the differential expression of ER α and ER β , and the differences in receptor affinity for BPA might impact these ovarian cells is unknown [6–8,11].

In studies using a primary culture system of human endometrial cells, inhibition of cell growth was observed following exposure to 1 μ M BPA [25]. Granulosa cells express ERs and are essential for ovarian folliculogenesis and steroidogenesis (reviewed in [111]). The impact of BPA, at low 0.1 nM dose,

on murine ovarian granulosa cells was investigated by Xu et al., who found that this low and physiologically relevant dose of BPA decreased granulosa cell viability by inducing apoptosis and G2-to-M cell cycle arrest, suggesting that granulosa cells are highly sensitive to BPA [112].

In contrast, others have reported that pharmacological concentrations of 17 β -estradiol (1 μ g/mL) exert antiapoptotic effect on ovarian granulosa cells [113,114]. Based on previous findings demonstrating that BPA could partially antagonize the effects of 17 β -estradiol at ER α , but not ER β [115], Xu et al. (2002) suggested that their results can be explained by BPA's ability to antagonize antiapoptotic effect of intrinsic estrogens produced by granulosa cells. While these researchers showed that BPA increased the expression of Bax and concomitantly decreased the expression of Bcl2 at both protein and mRNA levels, the precise mechanism of BPA action to induce apoptosis in granulosa cells still remains to be determined.

An *in vivo* study by Hunt et al., found that chronic low-dose exposure to BPA causes meiotic aneuploidy in mouse oocytes, thus implicating BPA in disruption of female meiosis. Specifically, this study demonstrated that low oral BPA exposure to BPA at 20, 40, or 100 μ g/kg body weight/day for 6–8 days preceding the analysis, adversely affects oocyte chromosome segregation [116]. The molecular mechanism(s) through which BPA may exert effects on meiosis is an important research topic that is in need of further investigation.

11. BPA actions at the androgen receptor

Several studies using *in vitro* yeast-based assays revealed that BPA exhibits strong anti-androgenic activity [23,29]. Using ligand and competition assays, it was demonstrated that BPA could compete with 5 α -dihydrotestosterone (DHT) for binding at the androgen receptor (AR) with an IC₅₀ (the concentration of chemical required to reduce the specific DHT binding by 50%) value of 2.14 μ M [24,29]. By utilizing an elegant yeast two-hybrid system, Lee et al. demonstrated that BPA affects AR interaction with its co-regulator ASC1, to subsequently modulate AR-transactivation. In addition, analysis of GFP-AR fusion protein in the presence of BPA showed inhibition of AR nuclear localization [29]. These findings suggest that BPA can affect multiple steps of the activation and function of AR, including modulation of AR interaction with its co-regulator ASC1 and inhibition of the binding of native androgens to the AR, resulting in antagonistic action of BPA on the AR.

A recent study by Xu et al. has confirmed that BPA can act as an anti-androgen in a mammalian system [27]. In this study, a human androgen receptor (hAR) reporter gene assay using African monkey kidney cell line CV-1 transiently transfected with the reporter gene plasmid pMMTV-CAT was utilized. BPA showed significant inhibitory effects on the transcriptional activity induced by DHT with an IC₅₀ value of \sim 0.8 μ M; the highest anti-androgenic activity of any endocrine-disrupting compounds tested [27].

Using two stably transfected Chinese hamster ovarian cell lines (CHO-K1 cells) as an AR-EcoScreen for androgenic activity and c-luc for cell toxicity evaluation, Satoh et al. investigated

in vitro hormonal activity of BPA and its derivatives bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE) [12]. These BPA derivatives are used as interior coating for food cans and components of some dental sealants. One of the CHO-K1 derived cell lines stably expressed androgen-inducible luciferase, while the other stably expressed luciferase independent of androgen induction. It was found that BPA, BADGE and BFDGE exhibited anti-androgenic activity, suggesting that chlorohydroxy compounds such as BADGE and BFDGE act as androgen antagonist through binding to the AR. BPA had a potent binding affinity for AR, and inhibited specific testosterone binding to the receptor in a concentration-dependent manner. The IC₅₀ value for AR was 7.9 μ M, and complete inhibition appeared at 19 μ M [12]. A follow-up study by Roy et al., using CHO-K1 stably expressing hAR and a mouse mammary tumor virus-neomycin-luciferase reporter gene, confirmed that BPA is an anti-androgenic compound with an IC₅₀ = 19.6 μ M [22]. However, a study by Gaido et al., failed to demonstrate antiandrogenic activities of BPA in HepG2 cells [117].

Thus, research results show that BPA can act as AR antagonist in some cell systems. Once bound to AR, the AR/BPA complex may prevent endogenous androgens from regulating androgen-dependent transcription. The specificity of AR–ligand interaction may be critical in eliciting adverse effects on male reproductive system. However, discrepancies between different research groups on antiandrogenic activities of BPA exist – this area of research requires, further investigation.

Similar to endogenous estrogens, several endocrine disrupting compounds have been hypothesized to alter androgen action or prostate growth and development [118]. In fact, developmental (*in utero* or neonatal) exposure to low dose BPA and other compounds, including polychlorinated bisphenols (PCBs), hexachlorobenzene (HCB) and diethylstilbestrol (DES), has been reported to increase prostate size and weight, suggesting that these agents influence early prostate growth patterns [119–125]. Although the mechanism of BPA action in the prostate is yet to be determined, recent studies have shown its ability to influence AR function and activity.

Given the importance of the AR for prostate development and tumorigenesis, several studies monitored effects of BPA exposure on AR expression and function *in vivo*. While two studies demonstrated that both BPA (25 or 250 μ g/kg/day) and estradiol (0.02–2 μ g/kg) increased AR expression in dorsolateral and ventral prostate lobes [123,126], a report by Ramos et al. observed no change in the ventral prostate upon BPA exposure [122]. However, the differentiation pattern of periductal stromal cells of the ventral prostate was found altered by prenatal exposure to BPA at 25 or 250 μ g/kg/day [122]. Together, the mitogenic effect of BPA on prostate cells often correlates with changes in the AR status, although future studies are needed to elucidate the relationship between BPA and AR in the developing prostate.

The impact of BPA on AR regulation in prostate cancer cells and the response to androgen-deprivation therapy (ADT) has also been examined. Because of the androgen dependence of prostatic adenocarcinomas, the mainline therapeutic intervention in advanced prostate cancer is ADT. Although ADT

is initially highly effective, within 2–3 years of therapy most patients develop ADT-resistant tumors, for which no effective treatment is available [127,128].

It was shown by Wetherill and colleagues that low concentrations of BPA (1–10 nM) are capable of activating the mutant AR (AR-T877A), that is selected for during ADT therapy in prostate cancer cells. Under conditions of androgen ablation, BPA activation of the mutant AR induced cellular proliferation [26]. In contrast, BPA has no agonistic activities on the wild-type AR [23,24,29]. Wetherill et al. also demonstrated that BPA competes with DHT for AR-T877A binding by a non-competitive mechanism, and enhances mutant AR activity in both androgen-dependent (mutant AR-T877A) and androgen-independent (mutant AR-H874Y) prostate cancer cell lines in the presence of DHT [28]. In contrast, in an androgen-dependent prostate cancer cell line LAPC-4 that expresses a wild-type AR, low doses of BPA only marginally activate the receptor and fail to show additive effects with DHT on AR transactivation [28]. In addition, this group showed that the ability of BPA to activate tumor-derived AR is conserved across multiple mutant ARs (AR-V715M, AR-T877S, and AR-H874Y), as demonstrated by both yeast-based and mammalian cell assays. These mutations in the AR were identified in patients with metastatic androgen-independent prostate tumors following ADT [129,130]. At higher doses of 1–10 μ M BPA, growth inhibition of androgen-dependent prostate cancer cell lines (LNCaP and LAPC-4) was observed. Thus, the differential effects of lower and higher doses of BPA were demonstrated, as the growth inhibitory effect at higher doses is seen only in androgen-dependent prostate cancer cells [28]. These results are in agreement with previous studies that showed inhibition of AR activity at high dose exposure to other endocrine disruptors [131,132] and BPA [22–24,27,29,35]. Collectively, the above *in vitro* studies, offer a mechanism by which environmentally relevant BPA exposures in prostate cancer might lead to tumor cell proliferation and suggest that BPA can interfere with therapy in prostate cancer patients by activating mutant ARs that are frequently selected for during ADT; these effects of BPA could contribute to the disease relapse. In fact, a recent study by Wetherill and Hess-Wilson et al. suggests that exposure of advanced prostate tumors expressing specific somatic mutations (AR-T877A) in the androgen receptor gene to environmentally relevant doses of BPA may facilitate ADT bypass and advance tumor recurrence [133].

12. Effects of BPA on cells and tissues of the male reproductive system

A study in adult Leydig cells by Akingbemi et al. demonstrated that low-dose BPA (0.01 nM) decreases testosterone biosynthesis by 25% as a result of decreased expression of the steroidogenic enzyme 17 α -monooxygenase (17 α -hydroxylase/17-20 lyase) [134]. Further, post-natal exposure to BPA decreased serum 17 β -estradiol levels in Long-Evans rats exposed to 2.4, 10, or 100 μ g/kg/day of BPA by inhibiting Leydig cell aromatase activity and exposure to lowest and environmentally relevant BPA dose (2.4 μ g/kg/day) decreased the mRNA levels of luteinizing hormone (LH)- β and increased

ER β expression in the pituitary [134]. These findings demonstrate that the perinatal period is a sensitive window of exposure to BPA in this animal system. The effects of BPA on steroidogenesis in Leydig cells was also analyzed by measuring the LH receptor-mediated cAMP and progesterone production in cultured mouse Leydig tumor cells [135]. This study found that BPA inhibited cAMP production and progesterone synthesis by preventing coupling between LH receptor and adenylate cyclase. However, estradiol did not inhibit cAMP production, thus further emphasizing the complexity of endocrine disruption.

In the testis, Sertoli cells establish intercellular junctions that are essential for spermatogenesis. The SerW3 Sertoli cell line displays some features of native Sertoli cells. Fiorini et al. studied the mechanism of BPA action on Sertoli cells [136]. Using Western blot and immunofluorescence analyses it was found that SerW3 Sertoli cells express typical components of tight (occludin and zonula occludens-1), anchoring (N-cadherin) and gap (connexin 43) junctions. Similar to other endocrine disruptors, BPA affected intercellular junctions by either reducing their amount, or by inducing aberrant intracellular localization of these membrane proteins [136].

Song et al. investigated the action of BPA on orphan nuclear receptor Nur77 gene expression and steroidogenesis, as it plays an important role in the regulation of LH-mediated steroidogenesis in testicular mouse K28 cells [137]. The K28 mouse Leydig tumor cell line has been characterized as a suitable cell culture model for steroidogenesis [138,139]. It was found that exposure to 10 nM BPA induces Nur77 gene expression in K28 cells after 30 min treatment and BPA-mediated induction of Nur77 gene expression is ERK mitogen-activated protein kinase (MAPK) and PKA dependent. At high BPA concentrations (1 μ M), progesterone biosynthesis was increased in K28 cells; this effect was suppressed by overexpression of dominant negative Nur77. Moreover, high dose BPA effects on steroidogenesis were confirmed *in vivo* where BPA injection into prepubertal mice (25 mg/kg; 18-day old) resulted in induction of Nur77 mRNA and increased concentrations of testicular testosterone *in vivo* [137]. Taken together, these results demonstrate that BPA can induce Nur77 gene expression, which subsequently alters the steroidogenesis in testicular Leydig cells. Post-natal exposure to a low dose of BPA (2.4 μ g/kg/day) was also found to reduce expression of cortactin, an actin-binding protein, in mouse testes [140]. Thus, the studies described above implicate adverse effects of low dose environmental BPA exposure on male fertility.

13. Thyroid hormone action, thyroid receptor and BPA

Thyroid hormone (TH) is essential for normal brain development, and recent studies indicate that the developing brain is very sensitive to small perturbations in thyroid hormone status. As a result, understanding the action of environmental chemicals that can interfere with TH signaling mechanisms is highly important to human health. The first environmental chemical shown to bind to the thyroid hormone receptor (TR) was BPA and its brominated and chlorinated derivatives. It was demonstrated by Meerts et al. in 2001 that polybrominated BPA compounds have estro-

genic activities and are able to bind to the ER α [141]. A study in 2002 by Kitamura et al. showed that BPA, tetrabromo- and tetrachloro-BPA (TBBPA and TCBPA, respectively) could displace thyroid hormone $^{125}\text{I-T}_3$ from cell nuclei isolated from the estrogen-responsive pituitary cell line MTt/E-2 [33]. Although these data indicate TR binding, it is not clear whether this binding is specific to the TR β , TR α or both. In the thyroid hormone-responsive pituitary cell line GH3 (in which the growth hormone secretion depends markedly on thyroid hormones, but little on estrogen), TCBPA and TBBPA increase cell proliferation at the dose of 100 μM and 1–100 μM , respectively [33]. These results indicate that brominated BPA derivatives act as TR agonists in pituitary GH3 cells. Further support for this interpretation was the observation that these two halogenated BPA compounds increase GH3 cell proliferation, and produce growth hormone (GH) production in the presence of thyroid hormone T3 in an additive way. In contrast, Moriyama et al., who utilized rat liver nuclei that express both the TR α and TR β , showed that BPA binds to the TR and acts as an antagonist on this receptor [36]. This conclusion was based on two types of experiments. In the first approach, they fused the ligand binding domain (LBD) of either TR α or TR β to the Gal4 DNA binding domain and performed *in vitro* expression assays using a Gal4 response element. It was found that BPA at 1.0 μM inhibited both the TR α - and TR β -stimulated response induced by thyroid hormone T₃. In the second approach, investigators co-transfected full length TR α and TR β into TSA-201 cell (a clone of human embryonic kidney 293 cells) with one of three types of thyroid hormone response elements (TRE): a palindrome, the malic enzyme TRE, and the TSH α TRE. They found that in the presence of both TR α and TR β , BPA produced a dose-dependent inhibition in the T₃ response (10 nM T₃) on both the palindromic and the malic enzyme TRE, but produced a dose-dependent induction in the T₃ response on the TSH α TRE. Because the TSH α TRE is a negative response element, the authors speculated that BPA stabilized the interaction between TR α 1 or TR β 1 and a nuclear repressor N-CoR. To test this hypothesis, Moriyama et al. performed a mammalian two-hybrid experiment using GAL4-NCoR and TR-VP16 with their GAL4 reporter construct in TSA-201 cells. These experiments fully supported the concept that BPA binding to TR stabilized the interaction between the TR and N-CoR. This hypothesis that is consistent with the observations that BPA suppresses T₃ action on positive TREs (malic enzyme and a DR4), and enhances T₃ action on a negative TSH α TRE.

The above studies indicate that BPA is an antagonist on the TR, but that halogenated BPA derivatives (commonly used as flame retardants) are TR agonists. Moreover, the affinity of the TR for brominated TBBPA or TCBPA appears to be higher than for BPA. Finally, these data indicate that BPA and its halogenated derivatives can bind two classes of TRs equally well. However, the latter concept is inconsistent with the work of Ghisari and Bonefeld-Jorgensen where BPA and TB-BPA were found to exert thyroid hormone-like activity in GH3 cells by increasing cell proliferation [30]. Interestingly, BPA, but not TBBPA, increased GH3 cell proliferation in the presence of a pure estrogen receptor antagonist ICI 182,780, suggesting that ER-mediated transactivation is not involved and that BPA may

exert effects directly on the TR [30]. Other studies have found that TBBPA has a lower affinity for the ER than does BPA [142]. It is unclear why BPA appeared to have a stronger action on the TR than TB-BPA in this study. Moreover, BPA appeared to act as an agonist, which is not consistent with previous *in vitro* studies.

The contribution of rapid intracellular signaling effects are well described for T₃, estradiol, and BPA and cannot be ruled out as contributing to the actions of BPA in this cell model system (reviewed in [143,144]). A potential contribution of rapid signaling effects in TH/TR-mediated effects of BPA and/or TB-BPA are supported by the work of Canesi et al. who showed that BPA (25 μM) and TBBPA (5 μM) stimulate rapid and transient activation of ERK-MAPK and PKC in hemocytes isolated from the marine mussel *Mytilus galloprovincialis* [75]. While these studies reinforce the potential contribution of rapid signaling to BPA-mediated actions at the TRs, the high concentrations of BPA required to activate signaling effects limit their significance.

Other halogenated BPA derivatives were shown to act as TR antagonists in the study by Kitamura et al. [34]. The investigators used CHO cells in a transient transfection assay in which human TR α or TR β were co-transfected with a palindromic TRE. In these experiments, TBBPA and TCBPA were potent antagonists of T₃, producing significant inhibition of T₃ action at concentrations as low as 3 μM , but this action depended upon the compound and the TR isoform. In general, TBBPA was more potent than TCBPA, and TR α was more sensitive to each of these compounds. These findings are not consistent with the initial studies reviewed above in which the halogenated BPA derivatives acted as agonists and parent BPA acted as an antagonist. Differences in the cellular context (i.e., use of different cell lines), and/or differences in the reporter construct (i.e., the use of a DR4 versus a palindrome) may account for the reported experimental differences.

Kitamura et al. followed these observations with a comparison of effects of BPA, TBBPA and TC-BPA on ER, AR and TR signaling [35]. This study supported their previous observations that halogenated derivatives of BPA act as TR agonists in pituitary GH3 cells [35]. Interestingly, the biologically active dose in these experiments was one or two orders of magnitude higher than doses producing estrogenic or androgenic effects, concentrations ranged from 1 to 100 μM . In contrast to previous studies by Moriyama et al. [36], an agonistic or antagonistic action of BPA on the TR in this system was not observed.

Hamers et al. have profiled a number of polyhalogenated aryl hydrocarbons for their ability to signal through the AhR receptor, ER, AR, and TR [31]. In addition, they characterized the ability of these compounds to displace T₄ from transthyretin, or to affect estrogen sulfotransferases. In these studies, TB-BPA was a very potent T₄ competitor in the TTR-binding assay ($\text{IC}_{50} < 0.1 \mu\text{M}$) with a 1.6 times higher TTR-binding potency than the natural ligand T₄. In their GH3 cell-based assays, BPA, TB-BPA and TC-BPA were nearly inactive, yet TB-BPA was found to potentiate the effects of T₃ in the T-screen assay.

Finally, Schricks et al. modified the T-screen to produce a more quantifiable assay [145]. This assay utilized a GH3 rat pituitary cell line that specifically proliferates when exposed to thyroid hormone T₃. The growth stimulatory effect is mediated

via T_3 -receptors. In this proliferation assay, resazurine is used to measure cell proliferation. It was found that neither BPA nor the halogenated derivatives were active as agonists or antagonists in this system. However, it was shown that BPA significantly potentiated the effect of T_3 .

Taken together, *in vitro* studies demonstrate that BPA binds to both the $TR\alpha$ and $TR\beta$ with relatively low affinity. In addition, the halogenated flame retardants, TB-BPA and TC-BPA bind with higher affinity. However, some studies indicate that BPA acts as an antagonist on the TR [32,34–36], whereas other studies conclude that BPA acts as an agonist [30,33], and some studies indicated that BPA and its halogenated derivatives are not active on thyroid hormone signaling. It is unclear why such differences in experimental results were obtained. Several investigators have employed GH3 cells in their studies, and have still obtained different results. This could be partially due to experimental design differences: initial reports utilized the MTT assay to evaluate relative growth, as well as growth hormone production (GH is controlled by thyroid hormone) [33], whereas in later studies the authors used a modified version of a cell proliferation assay (the use of resazurine), which may differ in its sensitivity [145]. It is also important to consider the possibility that differences in cell lines may have developed and that the differing results might reflect differences in transcriptional machinery that impact the ability of the TR to drive gene expression. If these compounds bind to the TR but produce a slightly different conformational response in the protein, it is theoretically possible that the TR will interact differentially with various cofactors. The initial observation of Moriyama et al. indicating that BPA can alter the strength of the interaction between TR and NCoR supports this interpretation [36].

If BPA and its halogenated derivatives interfere with thyroid hormone signaling, *in vivo* effects that are consistent with this concept might be observed. In this regard, Iwamuro et al. found that BPA reduces the rate of metamorphosis in *Xenopus* and inhibits T_4 -accelerated metamorphosis [146]. These investigators also showed *in vitro* that T_4 -induced tail resorption inhibited by BPA in a dose-dependent manner. These findings are fully consistent with the hypothesis that BPA acts as an antagonist of the *Xenopus* thyroid receptor/thyroid receptor. This finding is consistent with those of Kitamura et al. where T_3 -induced tail shortening is inhibited by TB-BPA in a Ranid [34]. Further, Kudo et al. found that 3,3',5-tribromobisphenol A and TB-BPA both exhibited T_3 agonist and antagonist activities at submicromolar concentrations in a *Xenopus laevis* cell line and in tadpoles [147]. These compounds competed with T_3 binding to α TTR and α TR *in vitro*, suggesting these proteins are possible target sites for BPA action *in vivo*. In addition, the dose at which TR signaling was influenced was lower than the IC_{50} values for the receptor. Therefore, the authors suggest that novel molecular mechanisms of interaction with the TR may be at play. Interestingly, Sugiyama et al. reported that TC-BPA could inhibit the effect of T_3 on a luciferase construct driven by a *Xenopus* TRE (the TH/bZIP TRE), but that TB-BPA acts as a TR agonist in the absence of T_3 [148].

Iwamuro et al. investigated the effects of BPA on the expression of $TR-\alpha$ and $TR-\beta$, and retinoid X receptor (RXR) gamma

mRNA in tails of stage 52–54 *Xenopus* tadpoles in organ culture in the presence or absence of different concentrations of T_3 [32]. In the absence of T_3 , BPA at all concentrations examined did not show remarkable effects on tail length, but blocked T_3 -induced tail resorption in a concentration-dependent manner. Changes in expression of $TR\alpha$ and $TR\beta$ mRNAs from the tail specimens (measured by RT-PCR) indicated that low BPA dose (0.1 μ M) showed an apparent antagonistic effect towards the receptors and reduced mRNA levels. When administered together with T_3 , the antagonistic effects of BPA were more pronounced. Auto-induction of both $TR\alpha$ and $TR\beta$ genes by T_3 was inhibited by BPA, but the effect was less marked on $TR\alpha$ than on $TR\beta$. Gene expression of RXR γ , a partner for heterodimer formation of TRs, was moderately suppressed either by T_3 or by BPA, but no additive effects were observed.

Those studies show that somewhat different findings are reported using amphibian models as well as those reviewed above for other *in vitro* models. Although some of these data appear conflicting, it is clear that BPA and its halogenated derivatives can interfere with TH signaling in the amphibian model. The differences among studies characterizing these compounds as agonists or antagonists likely reflect the complexity of mechanisms underlying BPA actions on the TR.

Findings from studies in mammals are also complex. For example, Zoeller et al. [37] reported that BPA could increase serum total T_4 in rat pups, but also increased RC3 expression in the dentate gyrus. Their interpretation was that BPA acted as a selective $TR\beta$ antagonist, causing an increase in serum T_4 . However, because RC3 expression is driven by the $TR\alpha$ in the dentate gyrus, these authors speculated that BPA was not exerting the same action on the $TR\alpha$. In contrast, Nakamura et al., who studied whether prenatal exposure to low-doses of BPA by injection of pregnant mice with 20 μ g/kg/day BPA affected the brain morphology and expression of genes related to brain development, reported that BPA exposure causes changes in the histogenesis of the cerebral cortex and increases the expression of a number of genes, including the $TR\alpha$ gene. These results were interpreted as reflecting a thyroid hormone-like effect of BPA in the cortex [149]. A similar observation was also made in *Xenopus* where TB-BPA increased $TR\alpha$, but not $TR\beta$, expression [150].

These studies suggest that BPA and a variety of its derivatives interfere with thyroid hormone signaling during development. The mechanism(s) underlying these effects are likely to be complex given that the dose of BPA required to produce effects on molecular events may be lower than the IC_{50} for binding to the thyroid receptor. It is also likely that BPA and its derivatives alter the relationship of the TR to various co-modulatory molecules. Moreover, BPA and its derivatives may also influence the abundance of the receptor. Thus, the mechanisms associated with BPA endocrine disrupting effects on the TH system are currently ill-defined and warrant further detailed investigation.

14. BPA and rapid signaling systems

BPA also exerts cell- and tissue-specific effects that act through mechanisms which modulate a variety of cell signaling pathways. The impact of BPA exposure is observed within

seconds to minutes, and is initially independent of the nuclear hormone receptor mediated transactivation activity that induces later effects on gene expression. However, these rapid signaling pathways interact with the traditional nuclear hormone receptor pathways [144,151].

For specific cell types, the features of the mechanisms associated with rapid signaling for estradiol, thyroid hormone and some endocrine disruptors, including BPA, are becoming more understood with recent research findings showing the presence of extracellularly accessible binding sites that act to modulate intracellular signaling [12,143,144,152]. Although the properties of the receptors involved in the initiation of rapid signaling effects in different tissues/cells are often related to those of a nuclear hormone receptor-like protein [20], some receptor/signaling pathways can be explained by an alternative transmembrane heptahelical G-protein coupled receptor [153]. A candidate G-protein coupled membrane estrogen receptor (GPR30) that binds estradiol and the endocrine disruptor *ortho,para*-dichlorodiphenyldichloroethylene (*o,p'*-DDE) with relatively low affinities has been described [71]. At GPR30, an IC_{50} for BPA was found to be 630 nM and its RBA (when compared to 17β -estradiol) equals 2.83 nM [79]. It is notable that the RBA for BPA at GPR30 is higher than that observed for ER α or ER β . In addition, this study demonstrated that following a 30 min treatment, both estradiol and 200 nM BPA increase cAMP activity, a GPR30-dependent signaling pathway activated by estrogens, in stably transfected ER-negative HEK 293 cells [79]. Thus, it is likely that BPA exerts some effects through this novel seven-transmembrane estrogen receptor GPR30, although the consequences of inappropriate activation of this signaling pathway are currently unknown. It is important to caution that even in controlled recombinant over-expression systems, the role of GPR30 receptor in rapid estradiol-mediated signaling is still controversial [154,155].

14.1. Rapid signaling effects in pituitary cells

Investigations of potential impact of BPA on pituitary physiology have been largely focused on examining pituitary tumor cell growth and disruption of prolactin release. Established GH3 rat pituitary tumor cell line has been a valuable model in characterizing the mechanisms involved in the estrogenic release of prolactin [156]. Steinmetz et al. demonstrated that 1 μ M BPA stimulates prolactin release in GH3 cells and induces hyperprolactinemia in F344 rats [157]. Watson et al. identified an extracellularly accessible plasma membrane ER on GH3/B6 rat pituitary tumor cell line [158,159], and have concluded that concentrations of BPA as low as 1 fM can cause an influx of calcium from extracellular sources via nifedapine-sensitive L-type calcium channels within 1 min of exposure [20,72,77]. In addition, these low concentrations of BPA could induce rapid release of prolactin from pituitary cells; effects that are mediated by the elevation of intracellular calcium and similar to those observed for estradiol [77]. Because immunoselected or limiting dilution selected GH3/B6 cells with very low expression levels of a cell surface ER α epitope were non-responsive, these rapid signaling effects of BPA were associated with expression of an

extracellular accessible ER α -like protein. In this GH3/B6 cell model, several other potential endocrine disrupting compounds (DDE, coumestrol, endosulfan, and nonylphenol) induced small increases in ERK1/2 phosphorylation; however, rapid increases in ERK-phosphorylation were not observable in response to 1 nM BPA [68,69].

In contrast to the results from the GH3 cell model, rapid modulation of ERK-signaling in response to BPA has been observed in neurons and immune cells; observations lending further support for the cell/tissue specific effects of BPA. For example, Canesi et al. showed that concentrations of BPA on *Mytilus* hemocytes induced small, but significant, and transient increases in ERK1/2 and PKC pathways with later increases in ERK2 and STAT3 phosphorylation [75]. These results are in agreement with a study of tetrabromobisphenol A (TB-BPA) toxicity, where Reistad et al. found that TBBPA can rapidly (2–4 min) activate the ERK1/2 pathway in human neutrophil granulocytes and that in high concentrations TBBPA or BPA (2–12 μ M) stimulate production of reactive oxygen species. Both TBBPA and high concentrations of BPA (20 μ M) were shown to induce a concentration dependent increase in intracellular free calcium levels [160].

14.2. Rapid and low dose actions of BPA related to the developing CNS

Rapid low dose BPA-mediated effects on ERK signaling were characterized *in vivo* and *in vitro* in isolated primary cultures of cerebellar granule cell neurons [46,153,161]. An analysis of BPA concentration dose response by Zsarnovszky et al. revealed that BPA stimulates an inverted U-shaped curve with efficacy and potency equal to estradiol in the low dose range (10 fM to 10 nM) [46]. In the presence of pharmacological concentrations (micromolar range) of BPA and estradiol, ERK-phosphorylation was also observed, demonstrating a clear non-monotonic and biphasic dose response. Developmental and pharmacological studies of the rapid actions of estrogen and BPA on cerebellar signaling by Belcher et al. revealed a mechanism that induces cell specific activation of ERK1/2-phosphorylation that involves G-proteins, PKA and the Src-family tyrosine kinase, but not EGF receptor or the PI3kinase/AKT pathway [161]. Further, the results of experiments assessing the effects of binary mixtures of estradiol and BPA highlighted the complexity of the rapid signaling mechanism in these developing neurons [46]. These results demonstrate the ability of BPA to act as a highly potent EDC with a potential to disrupt the rapid signaling of estradiol at very low concentrations during brain development.

Numerous *in vivo* studies have shown that prenatal and neonatal exposure to low doses of BPA can disrupt normal sexual differentiation in the rodent brain [162,163]. BPA at 40 μ g/kg dose can antagonize the action of estradiol in the adult rat hippocampus by blocking the stimulatory effect of estradiol on synaptogenesis [43]. Additional studies have also implicated BPA in modifying the activity of the mesolimbic dopamine system through upregulation of dopaminergic neurotransmission, an effect that in part involves increased dopamine D1 receptor expression. These endocrine disrupting activities of BPA could

potentially influence reward-seeking behaviors associated with drugs of abuse. In the pheochromocytoma PC12 cell model, high concentrations of BPA (25–150 μM) stimulate rapid release of dopamine through guanine nucleotide-binding protein and N-type calcium channels, illustrating that exposure to BPA may influence the function of dopaminergic neurons [76]. While high concentrations of BPA were used in this study, it is possible that rapid signaling mechanisms involving mobilization of intra- and extracellular calcium contribute to the impact of BPA through presynaptic influences on dopaminergic neurotransmission.

In an effort to discriminate rapid signaling actions of BPA from BPA-mediated gene expression, Yamaguchi et al. examined the effects of low dose BPA (1 pg/mL to 1 $\mu\text{g/mL}$) on the differentiation of serum-free mouse embryo (SFME) cells and astrocyte progenitor cells [40]. These investigators monitored glial fibrillary acidic protein (GFAP) expression as a marker of differentiation and found that GFAP expression was significantly increased in SFME cells in the presence of 1–100 pg/mL BPA. The BPA-induced increases in GFAP were due to activation of signal transducer and activator of transcription 3 (STAT3) and Smad1. In isolated murine midbrain astrocytes or in astrocyte/neurons co-culture Miyatake et al. demonstrated increased GFAP immunoreactivity and increased intracellular calcium in response to dopamine at BPA concentrations as low as 100 fM and 1 pM, respectively [44]. An inverted U-shaped low dose response was observed between 100 fM and 10 pM, with a second phase of effects observed at concentrations of 10 nM to 1 μM . Similar effects were not observed in response to estradiol. The investigators suggest that these results provide evidence that BPA alters dopamine responsiveness in neurons and astrocytes, which may contribute to potentiate the development of psychological dependence on drugs of abuse.

Together, the results from these CNS-related models highlight the complexity of BPA endocrine disruption effects at low physiological concentration, and the extreme importance of appropriate experimental design, such as including positive and negative controls for each experiment and doing complete dose response analysis for each end-point.

14.3. Rapid BPA effects on the endocrine pancreas

A “non-classical membrane ER” (ncmER) has been described in the endocrine pancreas that mediates the actions of BPA at concentrations as low as 0.1 nM [70]. This action is also involved in the activation of the transcription factor calcium-dependent cAMP-responsive element binding protein (CREB) where 1 nM BPA induces activation [73]. This study showed that BPA and estradiol activate CREB with the same potency as estradiol. In another study, 1 nM BPA potentiated glucose-induced calcium ion oscillations in freshly isolated islets of Langerhans [70], also implicating ncmER.

Involvement of the ncmER in modulation of glucose induced calcium signals in glucagon releasing alpha-cells of the pancreas has also been demonstrated [66]. It was shown that 1 nM BPA can suppress low-glucose induced calcium ion oscillations in alpha cells, the signal that triggers glucagon secretion, through the action of ncmER, G-proteins, and PKG. Moreover, it

was demonstrated that BPA exposure rapidly increases insulin release in adult mice following a single dose of 10 $\mu\text{g/kg/day}$ [67]. This effect was insensitive to an antiestrogen ICI 182,780, indicating that classic ERs may not be involved. In contrast, long-term exposure alters pancreatic insulin content and induces insulin resistance in a classic ER-dependent manner [67].

In agreement with previous findings, Adachi et al. reported that long-term exposure to 10 $\mu\text{g/L}$ BPA induces insulin secretion in rat pancreatic islets following stimulation with 16.7 mM glucose. This effect is significantly suppressed by 1 μM ICI 182,780, suggesting that long-term exposure to BPA potentiates glucose-induced insulin secretion via a classic ER-mediated pathway [21]. In the same study, the maximal effective dose of estradiol for insulin secretion was also 10 $\mu\text{g/L}$. However, in contrast to estradiol, BPA exhibited only long-term effects and did not induce acute insulin secretion from islets via plasma membrane ERs.

The rapid effects of BPA on intracellular calcium ion concentration have also been measured and compared to that of estradiol in breast cancer cell models (MCF-7 and MDA-MB-231). A rapid (within 1.5 min) influx of calcium was observed in response to BPA that was significant at the lowest dose tested (0.1 nM) [74]. This response was not blocked by anti-estrogens, demonstrating ER-transactivation independence of these rapid signaling effects of BPA at nanomolar concentrations. In sum, it has been shown that BPA can activate classic ERs in organelles other than the nucleus in target cells. In addition, BPA can bind and activate other estrogen binding proteins, for example ncmER and GPR30. BPA activates these receptor-mediated pathways at concentrations similar to those described for estradiol. However, further studies are needed to delineate BPA's mode of action in the pancreas, CNS and other tissues. Clearly, the studies described above are just starting to unveil the possible link between environmental estrogens, insulin resistance and glucose metabolism.

15. Immune system, allergic response and BPA exposure

It is well established that estrogens play a role in the immune system and recent research shows that BPA is capable of influencing the immune system functions [164,165]. It was demonstrated that at concentrations as low as 10 nM, BPA decreases the adherence index of rat peritoneal macrophages *in vitro* [166]. Based on the understanding that adhesion is the first step in the phagocytic process of macrophages and in antigen presentation, the authors of this study suggested that BPA may modulate immune and inflammatory responses.

To examine the effects of estradiol and BPA on chemokine production, expression of monocyte chemoattractant protein-1 (MCP-1, a member of the chemokine family) in human breast cancer cell line MCF-7 was studied [60]. MCF-7 cells produce a large quantity of MCP-1 in response to interleukin-1 alpha (IL-1 α). Addition of 0.1 μM BPA to MCF-7 cells inhibited MCP-1 mRNA and protein expression and electrophoretic mobility shift assay and supershift analysis revealed that treatment with 0.1–1 μM BPA diminished the IL-1 α -induced complex formation, although to a lesser degree than estradiol. These results

suggest that BPA represses MCP-1 expression in a different manner than estradiol, possibly depending on the presence of cell-type specific cooperating factors and/or signaling pathways. Further studies are needed to decipher the molecular mechanism of cell type- and stimulus-specific responses.

Regulation of immunoglobulin (Ig) production by BPA and estradiol was examined by Han et al. [63]. Mouse splenocytes (BALB/c mice) were treated with 10 nM to 1 μ M BPA or estradiol and the levels of IgM, IgE, IgA and IgG were measured. While IgA and IgG levels were not affected by either treatment, estradiol induced decreases in IgM and increased IgE levels. In contrast, 1 μ M BPA treatment resulted in enhanced IgM production and decreased IgE levels [63].

Youn et al. examined the immune response following BPA exposure in the mouse spleen cells [58]. BPA was administered to mice in drinking water for 4 weeks at 0.015, 1.5 and 30 mg/mL. It was found that BPA induced prolactin production in the spleen, and exposure of BPA increased the activity of splenocyte proliferation. Interestingly, the production of a strong Th-1 type cytokine (IFN- γ) was induced, while Th-2 type (IL-4) was suppressed by BPA treatment. Based on those findings, the authors speculated that stimulation of prolactin production by estrogenic effects of BPA can affect cytokine production, thus leading to imbalanced cellular immune response. A follow-up study by Yoshino et al. demonstrated that prenatal exposure to BPA results in up-regulation of immune responses [56]. This group measured proliferative responses of spleen cells to antigen, anti-HEL IgG2a and interferon- γ (IFN- γ) secreted from splenic lymphocytes were also measured as indicators of T helper 1 (Th1) immune responses, while anti-HEL IgG1 and interleukin-4 (IL-4) were measured as indicators of Th2 responses. The results showed that fetal exposure to BPA was followed by significant increases in anti-HEL IgG as well as antigen-specific cell proliferation, suggesting that prenatal exposure to BPA may result in the up-regulation of immune responses in adulthood.

Goto et al. examined the effects of BPA on mouse splenocyte (BALB/c female mice) proliferation and found that at high concentrations of BPA (20 μ M) enhanced Mac1+ splenocyte proliferation [64]. However, the dose used in this study is clearly outside of the physiologic range. To evaluate the effects of estradiol and BPA on the immune function, Sakazaki et al. investigated whether ER α is expressed in splenic T and B cells, and what effects these compounds have on mouse lymphocyte mitogenesis [65]. This group showed that ER α is expressed in both male and female mouse splenic lymphocytes. Next, lymphocytes from mouse spleen were exposed to estradiol or BPA under growth stimulation by lipopolysaccharide or concanavalin A as a mitogen specific for either B or T cells, respectively. It was found that exposure to estradiol (10 nM to 10 μ M) or BPA at 1 μ M inhibits lymphocytes mitogenesis, especially B cells. However, this group did not determine whether BPA-mediated suppression of lymphocyte proliferation was mediated by the ER- α in these cells.

Another study examined the effects of BPA at low concentrations (0.1–10 nM) on leukocyte differentiation by measuring superoxide production by differentiated HL-670 cells, a human

promyelocytic cell line, in the presence of absence of tamoxifen [53]. It was found that that BPA enhances leukocyte differentiation through an ER-independent pathway. Thus, it is possible that long-term exposure to low dose BPA might significantly affect the innate immunity in humans.

The effects of BPA on the production of interleukin-4 (IL-4), a pro-inflammatory cytokine closely associated with allergic immune responses, were examined by several investigators [52,61]. Using different experimental systems, these investigations are in agreement: treatment with BPA results in enhanced production of various cytokines, including IL-4. Yamashita et al. demonstrated BPA effects on murine thymocytes *in vitro* [55]. They showed that precultured thymocytes with 10 μ M BPA exhibited enhanced production of IL-3, IL-4 and interferon- γ . Tian et al. studied the effects of BPA on the *in vitro* production of Th1 and Th2 cytokines in mesenteric lymph node cells from *Trichinella spiralis* (Ts)-infected mice inoculated orally with BPA [52]. They found that IL-4 production by Th2-dominant mesenteric lymph node cells from Ts-infected mice increased significantly by addition of 3 μ M BPA. However, IL-5 production was not affected. This group demonstrated that the IL-4 production was increased both *in vitro* and *in vivo* by treatment with BPA, and this study suggests that BPA might cause allergic diseases by stimulating the IL-4 production by Th2 cells. It was also shown that BPA significantly enhanced IL-4 production in keyhole limpet haemocyanin (KLH)-primed CD4+ T cells in a concentration-dependent manner [61]. The lowest dose of BPA that had an effect on IL-4 production in this study was 10 μ M. Furthermore, BPA enhanced the activation of IL-4 gene promoter in hybridoma EL4 T cells transiently transfected with IL-4 promoter/reporter constructs. The authors also assessed the contribution of intracellular calcium-mediated signaling to BPA-enhanced IL-4 production by pre-treating lymph node cells from the immunized mice with various inhibitors known to interfere with calcium homeostasis. BPA increased intracellular calcium ion levels by inhibiting intracellular calcium pumps. The enhancement of IL-4 production by BPA was significantly reduced by nitrendipine, which blocks calcium ion influx and by FK506, a calcineurin inhibitor, demonstrating that the enhancement of IL-4 by is mediated by a calcium/calcineurin/NF-AT signaling pathway [61].

A study by Kim et al. demonstrated that BPA may affect the regulation of the immune system by reducing nitric oxide (NO) and tumor-necrosis factor- α (TNF- α) via the inhibition of nuclear factor (NF)- κ B transactivation. These effects of BPA are mediated through the ER, as shown in this study [48]. Peritoneal macrophages isolated from specific pathogen free-BALB/C mice and RAW 264.7 cells, a mouse macrophage cell line, were used in the study. Ten to 50 μ M BPA was used. In agreement with this study, a follow-up study by Hong et al. found that BPA treatment enhanced NO production in mouse macrophages *in vitro* [62].

The involvement of endocrine disruptors in autoimmune disease was examined in the study by Yurino and colleagues. In those studies it was found that demonstrated that endocrine disruptors including DES and BPA enhance autoantibody production by B1 cells both *in vitro* and *in vivo* [59].

The production of IL-2, a cytokine that plays an important role in adaptive immune response, was examined in CD4+ Jurkat T cells in response to BPA treatment [49]. Interestingly, other endocrine-disrupting compounds (coumestrol, DDT and TCDD), but not BPA, significantly suppressed IL-2 production at the transcriptional and translational levels. These results demonstrate that BPA elicits differential responses on cytokine production and may be cell-type and stimulus specific. Recently, Yamashita et al. examined BPA effects on murine spleen cells and thymus cells *in vitro* [54]. They reported that at 0.01–1 μ M BPA concentrations BPA stimulates production of IL-1, IL-6, IL-12, TNF and MCP-1. The maximum response was observed at 0.1 μ M BPA. Alizadeh et al. demonstrated that low dose BPA exposure (0.1 mg/g/body weight) results in augmentation of Th1 immune response [47], thus further implicating BPA involvement in the immune system functions.

16. Conclusions and levels of confidence from the results of mechanistic *in vitro* studies

16.1. Based on existing evidence, we are confident of the following

The criterion for achieving this confidence level is that multiple independent studies had been conducted that showed the same or similar outcome.

- BPA can act as an estrogen. Its effects are, however, cell type specific.
- Timing (developmental stage) of exposure and exposure dose/concentration are critical.
- When BPA binds to classic nuclear estrogen receptors and induce specific ERE binding, BPA is usually less potent than estradiol.
- When BPA action is mediated by estrogen receptors outside the nucleus, its potency is as high as that of estradiol, ranging within the pico- and nano-molar concentrations.
- Because of cell-type specific expression patterns and the role of varied specific co-regulatory factors, the effects of BPA might be different in individual cell types and these effects can vary depending on intrinsic and extrinsic influences.
- BPA is not simply a SERM (selective estrogen receptor modulator).
- BPA exerts pleiotropic cellular and tissue-type specific effects and non-monotonic dose–response at the cellular and intracellular levels at low physiologically relevant concentrations.

16.2. We consider the following to be likely but requiring confirmation

The criterion for achieving this confidence level is that significant effects have been reported, but the number of independent replications is limited. However, confidence in the findings is increased by the plausibility of the results, based on mechanistic information available from other related studies.

- BPA exerts some of its effects by binding to the nuclear steroid receptors for estrogen (ER α and ER β) to subsequently impact expression of estrogen-responsive gene products via the EREs. Thus, cell types that express specific ERs and co-modulatory elements can be especially sensitive to the effects of BPA.
- BPA exerts some of its effects by binding to nuclear steroid receptors for androgens—the (AR). BPA can act as an antagonist for the wild type AR in some tissues.
- Ligand-binding domain somatic mutants of ARs are important in prostate cancer: they can be activated by low dose (chronic exposure) of BPA.
- In cells from the male reproductive system BPA impacts the biosynthesis of steroids which results in altered steroid hormone concentrations.
- Compared to the impact of brominated BPA derivatives, these studies point toward BPA at physiological concentrations ($\leq 1 \times 10^{-7}$ M) has a relatively minor influence on the TH system.

16.3. Research on BPA suggests several broad themes that should be pursued in the future

- Because of the apparently complex nature of BPA action on the TH-signaling systems studied to date, additional studies are required in order to develop a mechanistic understanding of BPA's influence of TH-signaling, which has an important impact on the development of wildlife and humans.
- Increase our knowledge of the physiological/biological relevance of BPA binding to the ER, AR and TR.
- Investigate the mechanisms responsible for inverted U dose response.
- We need to develop sensitive cell model systems that detect altered patterns of differentiation and physiologically relevant responses, avoiding systems in which regulators are over-expressed.
- Identify other BPA-binding proteins and investigate GPR-30 actions in specific cell types upon exposure to endocrine disrupting compounds.
- Define additive and synergistic effects of BPA and endogenous hormones and other endocrine disruptors.
- While there are numerous *in vivo* studies demonstrating impact on the nervous system upon early exposures to BPA, much additional research is necessary in order to assign specific molecular mechanism through which BPA acts to impact specific neuronal and glial populations in the developing and mature CNS.
- Explore low dose BPA exposure on the immune system.
- Molecular mechanism(s) of acute versus chronic exposure to BPA need to be distinguished and deciphered.
- Define cell and tissue-type sensitivity and windows of susceptibility to BPA across the life span of experimental animals and humans.

References

- Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Ekong J, Needham LL. Urinary concentrations of bisphenol A and 4-nonylphenol in

- a human reference population. *Environ Health Perspect* 2005;113(4):391–5.
- [2] Kang JH, Kondo F, Katayama Y. Human exposure to bisphenol A. *Toxicology* 2006;226(2/3):79–89.
 - [3] Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S, et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. *Environ Health Perspect* 1996;104(Suppl 4):715–40.
 - [4] Welshons WV, Nagel SC, vom Saal FS. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. *Endocrinology* 2006;147(6 Suppl):S56–69.
 - [5] Gould JC, Leonard LS, Maness SC, Wagner BL, Conner K, Zacharewski T, et al. Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol. *Mol Cell Endocrinol* 1998;142(1/2):203–14.
 - [6] Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* 1997;138(3):863–70.
 - [7] Matthews JB, Twomey K, Zacharewski TR. In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. *Chem Res Toxicol* 2001;14(2):149–57.
 - [8] Routledge EJ, White R, Parker MG, Sumpter JP. Differential effects of xenoestrogens on coactivator recruitment by estrogen receptor (ER) alpha and ERbeta. *J Biol Chem* 2000;275(46):35986–93.
 - [9] Recchia AG, Vivacqua A, Gabriele S, Carpino A, Fasanella G, Rago V, et al. Xenoestrogens and the induction of proliferative effects in breast cancer cells via direct activation of oestrogen receptor alpha. *Food Addit Contam* 2004;21(2):134–44.
 - [10] Vivacqua A, Recchia AG, Fasanella G, Gabriele S, Carpino A, Rago V, et al. The food contaminants bisphenol A and 4-nonylphenol act as agonists for estrogen receptor alpha in MCF7 breast cancer cells. *Endocrine* 2003;22(3):275–84.
 - [11] Seidlova-Wuttke D, Jarry H, Wuttke W. Pure estrogenic effect of benzophenone-2 (BP2) but not of bisphenol A (BPA) and dibutylphthalate (DBP) in uterus, vagina and bone. *Toxicology* 2004;205(1/2):103–12.
 - [12] Satoh K, Ohyama K, Aoki N, Iida M, Nagai F. Study on anti-androgenic effects of bisphenol A diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE) and their derivatives using cells stably transfected with human androgen receptor, AR-EcoScreen. *Food Chem Toxicol* 2004;42(6):983–93.
 - [13] Hall JM, Korach KS. Analysis of the molecular mechanisms of human estrogen receptors alpha and beta reveals differential specificity in target promoter regulation by xenoestrogens. *J Biol Chem* 2002;277(46):44455–61.
 - [14] Pennie WD, Aldridge TC, Brooks AN. Differential activation by xenoestrogens of ER alpha and ER beta when linked to different response elements. *J Endocrinol* 1998;158(3):R11–4.
 - [15] Olsen CM, Meussen-Elholm ET, Samuelsen M, Holme JA, Hongslo JK. Effects of the environmental oestrogens bisphenol A, tetrachlorobisphenol A, tetrabromobisphenol A, 4-hydroxybiphenyl and 4,4'-dihydroxybiphenyl on oestrogen receptor binding, cell proliferation and regulation of oestrogen sensitive proteins in the human breast cancer cell line MCF-7. *Pharmacol Toxicol* 2003;92(4):180–8.
 - [16] Kurosawa T, Hiroi H, Tsutsumi O, Ishikawa T, Osuga Y, Fujiwara T, et al. The activity of bisphenol A depends on both the estrogen receptor subtype and the cell type. *Endocr J* 2002;49(4):465–71.
 - [17] Ackermann GE, Brombacher E, Fent K. Development of a fish reporter gene system for the assessment of estrogenic compounds and sewage treatment plant effluents. *Environ Toxicol Chem* 2002;21(9):1864–75.
 - [18] Mueller SO, Kling M, Arifin Firzani P, Mecky A, Duranti E, Shields-Botella J, et al. Activation of estrogen receptor alpha and ERbeta by 4-methylbenzylidene-camphor in human and rat cells: comparison with phyto- and xenoestrogens. *Toxicol Lett* 2003;142(1/2):89–101.
 - [19] Nagel SC, vom Saal FS, Thayer KA, Dhar MG, Boechler M, Welshons WV. Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. *Environ Health Perspect* 1997;105(1):70–6.
 - [20] Watson CS, Campbell CH, Gametchu B. Membrane oestrogen receptors on rat pituitary tumour cells: immuno-identification and responses to oestradiol and xenoestrogens. *Exp Physiol* 1999;84(6):1013–22.
 - [21] Adachi T, Yasuda K, Mori C, Yoshinaga M, Aoki N, Tsujimoto G, et al. Promoting insulin secretion in pancreatic islets by means of bisphenol A and nonylphenol via intracellular estrogen receptors. *Food Chem Toxicol* 2005;43(5):713–9.
 - [22] Roy P, Salminen H, Koskimies P, Simola J, Smeds A, Saukko P, et al. Screening of some anti-androgenic endocrine disruptors using a recombinant cell-based in vitro bioassay. *J Steroid Biochem Mol Biol* 2004;88(2):157–66.
 - [23] Sohoni P, Sumpter JP. Several environmental oestrogens are also anti-androgens. *J Endocrinol* 1998;158(3):327–39.
 - [24] Sun H, Xu LC, Chen JF, Song L, Wang XR. Effect of bisphenol A, tetrachlorobisphenol A and pentachlorophenol on the transcriptional activities of androgen receptor-mediated reporter gene. *Food Chem Toxicol* 2006;44(11):1916–21.
 - [25] Lee MS, Hyun SH, Lee CK, Im KS, Hwang IT, Lee HJ. Impact of xenoestrogens on the growth of human endometrial epithelial cells in a primary culture system. *Fertil Steril* 2003;79(6):1464–5.
 - [26] Wetherill YB, Petre CE, Monk KR, Puga A, Knudsen KE. The xenoestrogen bisphenol A induces inappropriate androgen receptor activation and mitogenesis in prostatic adenocarcinoma cells. *Mol Cancer Ther* 2002;1(7):515–24.
 - [27] Xu LC, Sun H, Chen JF, Bian Q, Qian J, Song L, et al. Evaluation of androgen receptor transcriptional activities of bisphenol A, octylphenol and nonylphenol in vitro. *Toxicology* 2005;216(2/3):197–203.
 - [28] Wetherill YB, Fisher NL, Staubach A, Danielsen M, de Vere White RW, Knudsen KE. Xenoestrogen action in prostate cancer: pleiotropic effects dependent on androgen receptor status. *Cancer Res* 2005;65(1):54–65.
 - [29] Lee HJ, Chattopadhyay S, Gong EY, Ahn RS, Lee K. Antiandrogenic effects of bisphenol A and nonylphenol on the function of androgen receptor. *Toxicol Sci* 2003;75(1):40–6.
 - [30] Ghisari M, Bonfeld-Jorgensen EC. Impact of environmental chemicals on the thyroid hormone function in pituitary rat GH3 cells. *Mol Cell Endocrinol* 2005;244(1/2):31–41.
 - [31] Hamers T, Kamstra JH, Sonneveld E, Murk AJ, Kester MH, Anderson PL, et al. In vitro profiling of the endocrine-disrupting potency of brominated flame retardants. *Toxicol Sci* 2006;92(1):157–73.
 - [32] Iwamuro S, Yamada M, Kato M, Kikuyama S. Effects of bisphenol A on thyroid hormone-dependent up-regulation of thyroid hormone receptor alpha and beta and down-regulation of retinoid X receptor gamma in *Xenopus* tail culture. *Life Sci* 2006.
 - [33] Kitamura S, Jinno N, Ohta S, Kuroki H, Fujimoto N. Thyroid hormonal activity of the flame retardants tetrabromobisphenol A and tetrachlorobisphenol A. *Biochem Biophys Res Commun* 2002;293(1):554–9.
 - [34] Kitamura S, Kato T, Iida M, Jinno N, Suzuki T, Ohta S, et al. Anti-thyroid hormonal activity of tetrabromobisphenol A, a flame retardant, and related compounds: affinity to the mammalian thyroid hormone receptor, and effect on tadpole metamorphosis. *Life Sci* 2005;76(14):1589–601.
 - [35] Kitamura S, Suzuki T, Sanoh S, Kohta R, Jinno N, Sugihara K, et al. Comparative study of the endocrine-disrupting activity of bisphenol A and 19 related compounds. *Toxicol Sci* 2005;84(2):249–59.
 - [36] Moriyama K, Tagami T, Akamizu T, Usui T, Saijo M, Kanamoto N, et al. Thyroid hormone action is disrupted by bisphenol A as an antagonist. *J Clin Endocrinol Metab* 2002;87(11):5185–90.
 - [37] Zoeller RT, Bansal R, Parris C. Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. *Endocrinology* 2005;146(2):607–12.
 - [38] Zoeller RT, Crofton KM. Thyroid hormone action in fetal brain development and potential for disruption by environmental chemicals. *Neurotoxicology* 2000;21(6):935–45.
 - [39] Zoeller RT. Environmental chemicals as thyroid hormone analogues: new studies indicate that thyroid hormone receptors are targets of industrial chemicals? *Mol Cell Endocrinol* 2005;242(1/2):10–5.
 - [40] Yamaguchi H, Zhu J, Yu T, Sasaki K, Umetsu H, Kidachi Y, et al. Low-level bisphenol A increases production of glial fibrillary acidic protein

- in differentiating astrocyte progenitor cells through excessive STAT3 and Smad1 activation. *Toxicology* 2006;226(2/3):131–42.
- [41] Ishido M, Masuo Y, Kunimoto M, Oka S, Morita M. Bisphenol A causes hyperactivity in the rat concomitantly with impairment of tyrosine hydroxylase immunoreactivity. *J Neurosci Res* 2004;76(3):423–33.
- [42] Kabuto H, Amakawa M, Shishibori T. Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. *Life Sci* 2004;74(24):2931–40.
- [43] MacLusky NJ, Hajsan T, Leran C. The environmental estrogen bisphenol A inhibits estradiol-induced hippocampal synaptogenesis. *Environ Health Perspect* 2005;113(6):675–9.
- [44] Miyatake M, Miyagawa K, Mizuo K, Narita M, Suzuki T. Dynamic changes in dopaminergic neurotransmission induced by a low concentration of bisphenol-A in neurones and astrocytes. *J Neuroendocrinol* 2006;18(6):434–44.
- [45] Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM. Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. *Endocrinology* 2006;147(8):3681–91.
- [46] Zsarnovszky A, Le HH, Wang HS, Belcher SM. Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex: potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A. *Endocrinology* 2005;146(12):5388–96.
- [47] Alizadeh M, Ota F, Hosoi K, Kato M, Sakai T, Satter MA. Altered allergic cytokine and antibody response in mice treated with Bisphenol A. *J Med Invest* 2006;53(1/2):70–80.
- [48] Kim JY, Jeong HG. Down-regulation of inducible nitric oxide synthase and tumor necrosis factor- α expression by bisphenol A via nuclear factor- κ B inactivation in macrophages. *Cancer Lett* 2003;196(1):69–76.
- [49] Ndebele K, Tchounwou PB, McMurray RW. Coumestrol, bisphenol-A, DDT, and TCDD modulation of interleukin-2 expression in activated CD4+ Jurkat T cells. *Int J Environ Res Public Health* 2004;1(1):3–11.
- [50] Sakabe K, Okuma M, Karaki S, Matsuura S, Yoshida T, Aikawa H, et al. Inhibitory effect of natural and environmental estrogens on thymic hormone production in thymus epithelial cell culture. *Int J Immunopharmacol* 1999;21(12):861–8.
- [51] Sawai C, Anderson K, Walser-Kuntz D. Effect of bisphenol A on murine immune function: modulation of interferon- γ , IgG2a, and disease symptoms in NZB X NZW F1 mice. *Environ Health Perspect* 2003;111(16):1883–7.
- [52] Tian X, Takamoto M, Sugane K. Bisphenol A promotes IL-4 production by Th2 cells. *Int Arch Allergy Immunol* 2003;132(3):240–7.
- [53] Watanabe H, Adachi R, Kusui K, Hirayama A, Kasahara T, Suzuki K. Bisphenol A significantly enhances the neutrophilic differentiation of promyelocytic HL-60 cells. *Int Immunopharmacol* 2003;3(12):1601–8.
- [54] Yamashita U, Sugiura T, Yoshida Y, Kuroda E. Effect of endocrine disruptors on macrophage functions in vitro. *J Uoeh* 2005;27(1):1–10.
- [55] Yamashita U, Sugiura T, Yoshida Y, Kuroda E. Effect of endocrine disruptors on thymocytes in vitro. *J Uoeh* 2003;25(2):161–70.
- [56] Yoshino S, Yamaki K, Li X, Sai T, Yanagisawa R, Takano H, et al. Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses, in mice. *Immunology* 2004;112(3):489–95.
- [57] Yoshino S, Yamaki K, Yanagisawa R, Takano H, Hayashi H, Mori Y. Effects of bisphenol A on antigen-specific antibody production, proliferative responses of lymphoid cells, and TH1 and TH2 immune responses in mice. *Br J Pharmacol* 2003;138(7):1271–6.
- [58] Youn JY, Park HY, Lee JW, Jung IO, Choi KH, Kim K, et al. Evaluation of the immune response following exposure of mice to bisphenol A: induction of Th1 cytokine and prolactin by BPA exposure in the mouse spleen cells. *Arch Pharm Res* 2002;25(6):946–53.
- [59] Yurino H, Ishikawa S, Sato T, Akadegawa K, Ito T, Ueha S, et al. Endocrine disruptors (environmental estrogens) enhance autoantibody production by B1 cells. *Toxicol Sci* 2004;81(1):139–47.
- [60] Inadera H, Sekiya T, Yoshimura T, Matsushima K. Molecular analysis of the inhibition of monocyte chemoattractant protein-1 gene expression by estrogens and xenoestrogens in MCF-7 cells. *Endocrinology* 2000;141(1):50–9.
- [61] Lee MH, Chung SW, Kang BY, Park J, Lee CH, Hwang SY, et al. Enhanced interleukin-4 production in CD4+ T cells and elevated immunoglobulin E levels in antigen-primed mice by bisphenol A and nonylphenol, endocrine disruptors: involvement of nuclear factor-AT and Ca2+. *Immunology* 2003;109(1):76–86.
- [62] Hong CC, Shimomura-Shimizu M, Muroi M, Tanamoto K. Effect of endocrine disrupting chemicals on lipopolysaccharide-induced tumor necrosis factor- α and nitric oxide production by mouse macrophages. *Biol Pharm Bull* 2004;27(7):1136–9.
- [63] Han D, Denison MS, Tachibana H, Yamada K. Effects of estrogenic compounds on immunoglobulin production by mouse splenocytes. *Biol Pharm Bull* 2002;25(10):1263–7.
- [64] Goto M, Ono H, Takano-Ishikawa Y, Shinmoto H, Yoshida M. Mac1 positive cells are required for enhancement of splenocytes proliferation caused by bisphenol A. *Biosci Biotechnol Biochem* 2004;68(1):263–5.
- [65] Sakazaki H, Ueno H, Nakamuro K. Estrogen receptor alpha in mouse splenic lymphocytes: possible involvement in immunity. *Toxicol Lett* 2002;133(2/3):221–9.
- [66] Alonso-Magdalena P, Laribi O, Ropero AB, Fuentes E, Ripoll C, Soria B, et al. Low doses of bisphenol A and diethylstilbestrol impair Ca2+ signals in pancreatic alpha-cells through a nonclassical membrane estrogen receptor within intact islets of Langerhans. *Environ Health Perspect* 2005;113(8):969–77.
- [67] Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance. *Environ Health Perspect* 2006;114(1):106–12.
- [68] Bulayeva NN, Gametchu B, Watson CS. Quantitative measurement of estrogen-induced ERK 1 and 2 activation via multiple membrane-initiated signaling pathways. *Steroids* 2004;69(3):181–92.
- [69] Bulayeva NN, Watson CS. Xenoestrogen-induced ERK-1 and ERK-2 activation via multiple membrane-initiated signaling pathways. *Environ Health Perspect* 2004;112(15):1481–7.
- [70] Nadal A, Ropero AB, Laribi O, Maillet M, Fuentes E, Soria B. Nongenomic actions of estrogens and xenoestrogens by binding at a plasma membrane receptor unrelated to estrogen receptor alpha and estrogen receptor beta. *Proc Natl Acad Sci USA* 2000;97(21):11603–8.
- [71] Thomas P, Pang Y, Filardo EJ, Dong J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology* 2005;146(2):624–32.
- [72] Watson CS, Bulayeva NN, Wozniak AL, Finnerty CC. Signaling from the membrane via membrane estrogen receptor- α : estrogens, xenoestrogens, and phytoestrogens. *Steroids* 2005;70(5–7):364–71.
- [73] Quesada I, Fuentes E, Viso-Leon MC, Soria B, Ripoll C, Nadal A. Low doses of the endocrine disruptor bisphenol-A and the native hormone 17 β -estradiol rapidly activate transcription factor CREB. *Faseb J* 2002;16(12):1671–3.
- [74] Walsh DE, Dockery P, Doolan CM. Estrogen receptor independent rapid non-genomic effects of environmental estrogens on [Ca2+]i in human breast cancer cells. *Mol Cell Endocrinol* 2005;230(1/2):23–30.
- [75] Canesi L, Lorusso LC, Ciacci C, Betti M, Zampini M, Gallo G. Environmental estrogens can affect the function of mussel hemocytes through rapid modulation of kinase pathways. *Gen Comp Endocrinol* 2004;138(1):58–69.
- [76] Yoneda T, Hiroi T, Osada M, Asada A, Funae Y. Non-genomic modulation of dopamine release by bisphenol-A in PC12 cells. *J Neurochem* 2003;87(6):1499–508.
- [77] Wozniak AL, Bulayeva NN, Watson CS. Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor- α -mediated Ca2+ fluxes and prolactin release in GH3/B6 pituitary tumor cells. *Environ Health Perspect* 2005;113(4):431–9.
- [78] Kubo T, Maezawa N, Osada M, Katsumura S, Funae Y, Imaoka S. Bisphenol A, an environmental endocrine-disrupting chemical, inhibits hypoxic response via degradation of hypoxia-inducible factor 1 α

- (HIF-1 α): structural requirement of bisphenol A for degradation of HIF-1 α . *Biochem Biophys Res Commun* 2004;318(4):1006–11.
- [79] Thomas P, Dong J. Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. *J Steroid Biochem Mol Biol* 2006.
- [80] Cannon JM, Kostoryz E, Russo KA, Smith RE, Yourtee DM. Bisphenol A and its biomaterial monomer derivatives alteration of in vitro cytochrome P450 metabolism in rat, minipig, and human. *Biomacromolecules* 2000;1(4):656–64.
- [81] Hanioka N, Jinno H, Nishimura T, Ando M. Suppression of male-specific cytochrome P450 isoforms by bisphenol A in rat liver. *Arch Toxicol* 1998;72(7):387–94.
- [82] Hanioka N, Jinno H, Tanaka-Kagawa T, Nishimura T, Ando M. Interaction of bisphenol A with rat hepatic cytochrome P450 enzymes. *Chemosphere* 2000;41(7):973–8.
- [83] Jeong HG, Kimand JY, Choi CY. Down-regulation of murine Cyp1a-1 in mouse hepatoma Hepa-1c1c7 cells by bisphenol A. *Biochem Biophys Res Commun* 2000;277(3):594–8.
- [84] Bendridi N, Mappus E, Grenot C, Lejeune H, Yves Cuilleron C, Pugeat M. Intravenous injection of human sex steroid hormone-binding globulin in mouse decreases blood clearance rate and testicular accumulation of orally administered [2–125I]iodobisphenol A. *Steroids* 2002;67(7):637–45.
- [85] Csanady GA, Oberste-Frielinghaus HR, Semder B, Baur C, Schneider KT, Filser JG. Distribution and unspecific protein binding of the xenoestrogens bisphenol A and daidzein. *Arch Toxicol* 2002;76(5/6):299–305.
- [86] Dechaud H, Ravard C, Claustrat F, de la Perriere AB, Pugeat M. Xenoestrogen interaction with human sex hormone-binding globulin (hSHBG). *Steroids* 1999;64(5):328–34.
- [87] Teeguarden JG, Barton HA. Computational modeling of serum-binding proteins and clearance in extrapolations across life stages and species for endocrine active compounds. *Risk Anal* 2004;24(3):751–70.
- [88] Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 2005;308(5727):1466–9.
- [89] Anway MD, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors. *Endocrinology* 2006;147(6 Suppl):S43–9.
- [90] Ho SM, Tang WY, Belmonte de Frausto J, Prins GS. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer Res* 2006;66(11):5624–32.
- [91] Klinge CM. Estrogen receptor interaction with co-activators and co-repressors. *Steroids* 2000;65(5):227–51.
- [92] Klinge CM. Estrogen receptor interaction with estrogen response elements. *Nucl Acids Res* 2001;29(14):2905–19.
- [93] Ramsey TL, Risinger KE, Jernigan SC, Mattingly KA, Klinge CM. Estrogen receptor beta isoforms exhibit differences in ligand-activated transcriptional activity in an estrogen response element sequence-dependent manner. *Endocrinology* 2004;145(1):149–60.
- [94] Masuyama H, Hiramatsu Y. Involvement of suppressor for Gal 1 in the ubiquitin/proteasome-mediated degradation of estrogen receptors. *J Biol Chem* 2004;279(13):12020–6.
- [95] McDonnell DP, Clemm DL, Hermann T, Goldman ME, Pike JW. Analysis of estrogen receptor function in vitro reveals three distinct classes of antiestrogens. *Mol Endocrinol* 1995;9(6):659–69.
- [96] Paige LA, Christensen DJ, Gron H, Norris JD, Gottlin EB, Padilla KM, et al. Estrogen receptor (ER) modulators each induce distinct conformational changes in ER alpha and ER beta. *Proc Natl Acad Sci USA* 1999;96(7):3999–4004.
- [97] Wijayarathne AL, Nagel SC, Paige LA, Christensen DJ, Norris JD, Fowlkes DM, et al. Comparative analyses of mechanistic differences among antiestrogens. *Endocrinology* 1999;140(12):5828–40.
- [98] Singleton DW, Feng Y, Yang J, Puga A, Lee AV, Khan SA. Gene expression profiling reveals novel regulation by bisphenol-A in estrogen receptor-alpha-positive human cells. *Environ Res* 2006;100(1):86–92.
- [99] Chariot A, Gielen J. The HOXC6 homeodomain-containing proteins. *Int J Biochem Cell Biol* 1998;30(6):651–5.
- [100] Garcia-Gasca A, Spyropoulos DD. Differential mammary morphogenesis along the anteroposterior axis in Hoxc6 gene targeted mice. *Dev Dyn* 2000;219(2):261–76.
- [101] Buterin T, Koch C, Naegeli H. Convergent transcriptional profiles induced by endogenous estrogen and distinct xenoestrogens in breast cancer cells. *Carcinogenesis* 2006;27(8):1567–78.
- [102] Smith C, Taylor HS. Exposure to the endocrine disruptor bisphenol A alters uterine developmental gene expression. In uterine biology and endometriosis. *Endometrium* 2005.
- [103] Nagel SC, Hagelbarger JL, McDonnell DP. Development of an ER action indicator mouse for the study of estrogens, selective ER modulators (SERMs), and Xenobiotics. *Endocrinology* 2001;142(11):4721–8.
- [104] Hong EJ, Park SH, Choi KC, Leung PC, Jeung EB. Identification of estrogen-regulated genes by microarray analysis of the uterus of immature rats exposed to endocrine disrupting chemicals. *Reprod Biol Endocrinol* 2006;4:49.
- [105] Diel P, Schmidt S, Vollmer G, Janning P, Upmeyer A, Michna H, et al. Comparative responses of three rat strains (DA/Han, Sprague–Dawley and Wistar) to treatment with environmental estrogens. *Arch Toxicol* 2004;78(4):183–93.
- [106] Naruse M, Ishihara Y, Miyagawa-Tomita S, Koyama A, Hagiwara H. 3-Methylcholanthrene, which binds to the arylhydrocarbon receptor, inhibits proliferation and differentiation of osteoblasts in vitro and ossification in vivo. *Endocrinology* 2002;143(9):3575–81.
- [107] Singh SU, Casper RF, Fritz PC, Sukhu B, Ganss B, Girard Jr B, et al. Inhibition of dioxin effects on bone formation in vitro by a newly described aryl hydrocarbon receptor antagonist, resveratrol. *J Endocrinol* 2000;167(1):183–95.
- [108] Lieberherr M, Grosse B, Kachkache M, Balsan S. Cell signaling and estrogens in female rat osteoblasts: a possible involvement of unconventional nonnuclear receptors. *J Bone Miner Res* 1993;8(11):1365–76.
- [109] De Wilde A, Heberden C, Chaumaz G, Bordat C, Lieberherr M. Signaling networks from Gbeta1 subunit to transcription factors and actin remodeling via a membrane-located ERbeta-related protein in the rapid action of daidzein in osteoblasts. *J Cell Physiol* 2006;209(3):786–801.
- [110] Britt KL, Findlay JK. Estrogen actions in the ovary revisited. *J Endocrinol* 2002;175(2):269–76.
- [111] Palter SF, Tavares AB, Hourvitz A, Veldhuis JD, Adashi EY. Are estrogens of import to primate/human ovarian folliculogenesis? *Endocr Rev* 2001;22(3):389–424.
- [112] Xu J, Osuga Y, Yano T, Morita Y, Tang X, Fujiwara T, et al. Bisphenol A induces apoptosis and G2-to-M arrest of ovarian granulosa cells. *Biochem Biophys Res Commun* 2002;292(2):456–62.
- [113] Quirk SM, Cowan RG, Harman RM. The susceptibility of granulosa cells to apoptosis is influenced by oestradiol and the cell cycle. *J Endocrinol* 2006;189(3):441–53.
- [114] Wang Y, Asselin E, Tsang BK. Involvement of transforming growth factor alpha in the regulation of rat ovarian X-linked inhibitor of apoptosis protein expression and follicular growth by follicle-stimulating hormone. *Biol Reprod* 2002;66(6):1672–80.
- [115] Hiroi H, Tsutsumi O, Momoeda M, Takai Y, Osuga Y, Taketani Y. Differential interactions of bisphenol A and 17beta-estradiol with estrogen receptor alpha (ERalpha) and ERbeta. *Endocr J* 1999;46(6):773–8.
- [116] Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, et al. Bisphenol a exposure causes meiotic aneuploidy in the female mouse. *Curr Biol* 2003;13(7):546–53.
- [117] Gaido KW, Maness SC, McDonnell DP, Dehal SS, Kupfer D, Safe S. Interaction of methoxychlor and related compounds with estrogen receptor alpha and beta, and androgen receptor: structure-activity studies. *Mol Pharmacol* 2000;58(4):852–8.
- [118] Jarred RA, Cancilla B, Prins GS, Thayer KA, Cunha GR, Risbridger GP. Evidence that estrogens directly alter androgen-regulated prostate development. *Endocrinology* 2000;141(9):3471–7.
- [119] Gupta C. Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. *Proc Soc Exp Biol Med* 2000;224(2):61–8.

- [120] Gupta C. The role of estrogen receptor, androgen receptor and growth factors in diethylstilbestrol-induced programming of prostate differentiation. *Urol Res* 2000;28(4):223–9.
- [121] Ralph JL, Orgebin-Crist MC, Lareyre JJ, Nelson CC. Disruption of androgen regulation in the prostate by the environmental contaminant hexachlorobenzene. *Environ Health Perspect* 2003;111(4):461–6.
- [122] Ramos JG, Varayoud J, Sonnenschein C, Soto AM, Munoz De Toro M, Luque EH. Prenatal exposure to low doses of bisphenol A alters the periductal stroma and glandular cell function in the rat ventral prostate. *Biol Reprod* 2001;65(4):1271–7.
- [123] Thayer KA, Ruhlen RL, Howdeshell KL, Buchanan DL, Cooke PS, Preziosi D, et al. Altered prostate growth and daily sperm production in male mice exposed prenatally to subclinical doses of 17alpha-ethinyl oestradiol. *Hum Reprod* 2001;16(5):988–96.
- [124] Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS. Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. *Proc Natl Acad Sci USA* 2005;102(19):7014–9.
- [125] vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, et al. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. *Proc Natl Acad Sci USA* 1997;94(5):2056–61.
- [126] Ramos JG, Varayoud J, Kass L, Rodriguez H, Costabel L, Munoz-De-Toro M, et al. Bisphenol A induces both transient and permanent histofunctional alterations of the hypothalamic-pituitary-gonadal axis in prenatally exposed male rats. *Endocrinology* 2003;144(7):3206–15.
- [127] Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. *Nat Rev Cancer* 2001;1(1):34–45.
- [128] Leewansangtong S, Soontrapa S. Hormonal ablation therapy for metastatic prostatic carcinoma: a review. *J Med Assoc Thai* 1999;82(2):192–205.
- [129] Culig Z, Hobisch A, Cronauer MV, Cato AC, Hittmair A, Radmayr C, et al. Mutant androgen receptor detected in an advanced-stage prostatic carcinoma is activated by adrenal androgens and progesterone. *Mol Endocrinol* 1993;7(12):1541–50.
- [130] Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, et al. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. *N Engl J Med* 1995;332(21):1393–8.
- [131] Mitchell SH, Zhu W, Young CY. Resveratrol inhibits the expression and function of the androgen receptor in LNCaP prostate cancer cells. *Cancer Res* 1999;59(23):5892–5.
- [132] Davis JN, Kucuk O, Sarkar FH. Expression of prostate-specific antigen is transcriptionally regulated by genistein in prostate cancer cells. *Mol Carcinog* 2002;34(2):91–101.
- [133] Wetherill YB, Hess-Wilson JK, Comstock CE, Shah SA, Buncher CR, Sallans L, et al. Bisphenol A facilitates bypass of androgen ablation therapy in prostate cancer. *Mol Cancer Ther* 2006;5(12):3181–90.
- [134] Akingbemi BT, Sottas CM, Koulouva AI, Klinefelter GR, Hardy MP. Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. *Endocrinology* 2004;145(2):592–603.
- [135] Nikula H, Talonpoika T, Kaleva M, Toppari J. Inhibition of hCG-stimulated steroidogenesis in cultured mouse Leydig tumor cells by bisphenol A and octylphenols. *Toxicol Appl Pharmacol* 1999;157(3):166–73.
- [136] Fiorini C, Tilloy-Ellul A, Chevalier S, Charuel C, Pointis G. Sertoli cell junctional proteins as early targets for different classes of reproductive toxicants. *Reprod Toxicol* 2004;18(3):413–21.
- [137] Song KH, Lee K, Choi HS. Endocrine disrupter bisphenol A induces orphan nuclear receptor Nur77 gene expression and steroidogenesis in mouse testicular Leydig cells. *Endocrinology* 2002;143(6):2208–15.
- [138] Lefevre A, Rogier E, Astraud C, Duquenne C, Finaz C. Regulation by retinoids of luteinizing hormone/chorionic gonadotropin receptor, cholesterol side-chain cleavage cytochrome P-450, 3 beta-hydroxysteroid dehydrogenase/delta (5–4)-isomerase and 17 alpha-hydroxylase/C17-20 lyase cytochrome P-450 messenger ribonucleic acid levels in the K9 mouse Leydig cell line. *Mol Cell Endocrinol* 1994;106(1/2):31–9.
- [139] Lee HK, Yoo MS, Choi HS, Kwon HB, Soh J. Retinoic acids up-regulate steroidogenic acute regulatory protein gene. *Mol Cell Endocrinol* 1999;148(1/2):1–10.
- [140] Anahara R, Yoshida M, Toyama Y, Maekawa M, Kai M, Ishino F, et al. 17beta-estradiol, bisphenol A, and diethylstilbestrol, decrease cortactin expression in the mouse testis. *Arch Histol Cytol* 2006;69(2):101–7.
- [141] Meerts IA, Letcher RJ, Hoving S, Marsh G, Bergman A, Lemmen JG, et al. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PDBEs, and polybrominated bisphenol A compounds. *Environ Health Perspect* 2001;109(4):399–407.
- [142] Samuelsen M, Olsen C, Holme JA, Meussen-Elholm E, Bergmann A, Hongslo JK. Estrogen-like properties of brominated analogs of bisphenol A in the MCF-7 human breast cancer cell line. *Cell Biol Toxicol* 2001;17(3):139–51.
- [143] Belcher SM, Zsarnovszky A. Estrogenic actions in the brain: estrogen, phytoestrogens, and rapid intracellular signaling mechanisms. *J Pharmacol Exp Ther* 2001;299(2):408–14.
- [144] Farach-Carson MC, Davis PJ. Steroid hormone interactions with target cells: cross talk between membrane and nuclear pathways. *J Pharmacol Exp Ther* 2003;307(3):839–45.
- [145] Schriks M, Vrabie CM, Gutleb AC, Faassen EJ, Rietjens IM, Murk AJ. T-screen to quantify functional potentiating, antagonistic and thyroid hormone-like activities of poly halogenated aromatic hydrocarbons (PHAHs). *Toxicol In Vitro* 2006;20(4):490–8.
- [146] Iwamuro S, Sakakibara M, Terao M, Ozawa A, Kurobe C, Shigeura T, et al. Teratogenic and anti-metamorphic effects of bisphenol A on embryonic and larval *Xenopus laevis*. *Gen Comp Endocrinol* 2003;133(2):189–98.
- [147] Kudo Y, Yamauchi K. In vitro and in vivo analysis of the thyroid disrupting activities of phenolic and phenol compounds in *Xenopus laevis*. *Toxicol Sci* 2005;84(1):29–37.
- [148] Sugiyama S, Miyoshi H, Yamauchi K. Characteristics of a thyroid hormone responsive reporter gene transduced into a *Xenopus laevis* cell line using lentivirus vector. *Gen Comp Endocrinol* 2005;144(3):270–9.
- [149] Nakamura K, Itoh K, Yaoi T, Fujiwara Y, Sugimoto T, Fushiki S. Murine neocortical histogenesis is perturbed by prenatal exposure to low doses of bisphenol A. *J Neurosci Res* 2006;84(6):1197–205.
- [150] Veldhoen N, Boggs A, Walzak K, Helbing CC. Exposure to tetrabromobisphenol-A alters TH-associated gene expression and tadpole metamorphosis in the Pacific tree frog *Pseudacris regilla*. *Aqua Toxicol* 2006;78(3):292–302.
- [151] Vasudevan N, Kow LM, Pfaff DW. Early membrane estrogenic effects required for full expression of slower genomic actions in a nerve cell line. *Proc Natl Acad Sci USA* 2001;98(21):12267–71.
- [152] Storey NM, Gentile S, Ullah H, Russo A, Muessel M, Erxleben C, et al. Rapid signaling at the plasma membrane by a nuclear receptor for thyroid hormone. *Proc Natl Acad Sci USA* 2006;103(13):5197–201.
- [153] Wong JK, Le HH, Zsarnovszky A, Belcher SM. Estrogens and ICI182,780 (Faslodex) modulate mitosis and cell death in immature cerebellar neurons via rapid activation of p44/p42 mitogen-activated protein kinase. *J Neurosci* 2003;23(12):4984–95.
- [154] Funakoshi T, Yanai A, Shinoda K, Kawano MM, Mizukami Y. G protein-coupled receptor 30 is an estrogen receptor in the plasma membrane. *Biochem Biophys Res Commun* 2006;346(3):904–10.
- [155] Pedram A, Razandi M, Levin ER. Nature of functional estrogen receptors at the plasma membrane. *Mol Endocrinol* 2006;20(9):1996–2009.
- [156] Steinmetz R, Gutierrez-Hartmann A, Bigsby RM, Ben-Jonathan N. Activation of the prolactin promoter in transfected GH3 cells by posterior pituitary cells. *Endocrinology* 1994;135(6):2737–41.
- [157] Steinmetz R, Brown NG, Allen DL, Bigsby RM, Ben-Jonathan N. The environmental estrogen bisphenol A stimulates prolactin release in vitro and in vivo. *Endocrinology* 1997;138(5):1780–6.
- [158] Pappas TC, Gametchu B, Watson CS. Membrane estrogen receptors identified by multiple antibody labeling and impeded-ligand binding. *Faseb J* 1995;9(5):404–10.
- [159] Watson CS, Pappas TC, Gametchu B. The other estrogen receptor in the plasma membrane: implications for the actions of environmental estrogens. *Environ Health Perspect* 1995;103(Suppl 7):41–50.