

Review

In vivo effects of bisphenol A in laboratory rodent studies[☆]

Catherine A. Richter^{a,*}, Linda S. Birnbaum^b, Francesca Farabollini^c, Retha R. Newbold^d,
Beverly S. Rubin^e, Chris E. Talsness^f, John G. Vandenberg^g,
Debby R. Walser-Kuntz^h, Frederick S. vom Saalⁱ

^a U.S. Geological Survey, Columbia Environmental Research Center, Columbia, MO 65201, United States

^b U.S. Environmental Protection Agency, Research Triangle Park, NC, United States

^c Department of Physiology, University of Siena, Siena, Italy

^d National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC, United States

^e Department of Anatomy and Cell Biology, Tufts University School of Medicine, Boston, MA, United States

^f Institute of Clinical Pharmacology and Toxicology, Charité Universitätsmedizin Berlin, Berlin, Germany

^g Department of Zoology, North Carolina State University, Raleigh, NC, United States

^h Department of Biology, Carleton College, Northfield, MN, United States

ⁱ Division of Biological Sciences, University of Missouri, Columbia, MO, United States

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Abstract

Concern is mounting regarding the human health and environmental effects of bisphenol A (BPA), a high-production-volume chemical used in synthesis of plastics. We have reviewed the growing literature on effects of low doses of BPA, below 50 mg/(kg day), in laboratory exposures with mammalian model organisms. Many, but not all, effects of BPA are similar to effects seen in response to the model estrogens diethylstilbestrol and ethinylestradiol. For most effects, the potency of BPA is approximately 10–1000-fold less than that of diethylstilbestrol or ethinylestradiol. Based on our review of the literature, a consensus was reached regarding our level of confidence that particular outcomes occur in response to low dose BPA exposure. We are confident that adult exposure to BPA affects the male reproductive tract, and that long lasting, organizational effects in response to developmental exposure to BPA occur in the brain, the male reproductive system, and metabolic processes. We consider it likely, but requiring further confirmation, that adult exposure to BPA affects the brain, the female reproductive system, and the immune system, and that developmental effects occur in the female reproductive system.

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Keywords: Behavior; Neuroendocrine; Endocrine disruptors; Immune system; Metabolism; Mouse; Rat; Reproduction

Abbreviations: 2F, located *in utero* between female siblings; 2M, located *in utero* between male siblings; ADI, acceptable daily intake; AhR, aryl hydrocarbon receptor; ARC, arcuate nucleus; AVPV, anteroventral periventricular preoptic area; BPA, bisphenol A; CrI:CD(SD), Charles River Laboratories Sprague–Dawley rat strain; DES, diethylstilbestrol; DRN, dorsal raphe nucleus; EDC, endocrine disrupting chemical; E2, 17 β -estradiol; ER α , estrogen receptor protein α ; ER β , estrogen receptor protein β ; Esr1, estrogen receptor gene 1 (α); Esr2, estrogen receptor gene 2 (β); GD, gestation day; HRT, hormone replacement therapy; IFN, interferon; IgE, immunoglobulin E; i.m., intramuscular; i.p., intraperitoneal; LD50, lethal dose, 50% of population; LH, luteinizing hormone; LoEC, lowest effective concentration; LOAEL, lowest observed adverse effect level; MPA, medial preoptic area; NMU, *N*-nitroso-*N*-methylurea; NTP, National Toxicology Program; PND, postnatal day; PIN, prostate interepithelial neoplasia; p.o., oral administration; RAR, retinoic acid receptor; RXR, retinoid X receptor; SERM, selective estrogen receptor modulator; s.c., subcutaneous; Th1, T helper lymphocyte 1; Th2, T helper lymphocyte 2; TH, tyrosine hydroxylase; VMH, ventromedial nucleus

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* Corresponding author at: 4200 New Haven Road, Columbia Environmental Research Center, USGS, Columbia, MO 65201, United States.

Tel.: +1 573 876 1841; fax: +1 573 876 1896.

E-mail address: Crichter@usgs.gov (C.A. Richter).

Contents

1.	Introduction	200
1.1.	Rationale for experimental design, analysis and reporting criteria	201
1.1.1.	Animal model	202
1.1.2.	Feed, water, and housing	202
1.1.3.	Method of dosing	202
1.1.4.	Design and analysis issues	202
1.2.	Definition of “low dose”	202
1.3.	Timing of exposure	203
2.	Developmental effects of BPA due to exposure during gestation through puberty	203
2.1.	Effects on neurotransmitters and their receptors, neuroendocrine function and hormone receptors in the brain in males and females	203
2.1.1.	Receptors	203
2.1.2.	Neuroendocrine effects	203
2.1.3.	Rapid signaling effects	210
2.1.4.	Brain structure	210
2.1.5.	Behavioral effects	210
2.1.6.	Sex differences	211
2.2.	Developmental effects on the female reproductive tract	211
2.2.1.	Timing of puberty	211
2.2.2.	Mammary gland	211
2.2.3.	Uterus and vagina	212
2.2.4.	Ovary, oocytes and fertility	212
2.3.	Developmental effects on the male reproductive tract	212
2.3.1.	Serum testosterone levels	212
2.3.2.	Prostate	213
2.3.3.	Testes, epididymis, sperm and seminal vesicles	213
2.4.	Developmental effects on metabolism	214
2.5.	Developmental effects on the immune system	214
3.	Effects of BPA exposure during adulthood	214
3.1.	Adult effects on the brain and behavior	214
3.2.	Adult effects on the female reproductive tract	215
3.3.	Adult effects on the male reproductive tract	215
3.4.	Adult effects on metabolism	215
3.5.	Adult effects on the immune system	216
4.	Comparison of findings of significant effects and no-significant effects in low dose BPA studies	216
4.1.	Strain differences	216
4.2.	BPA as a SERM: responses in different tissues and in different animal models	217
4.3.	Batch-to-batch variability in feed: impact on low dose endocrine disruptor research	217
4.4.	The use of positive controls	217
4.5.	The importance of dose	218
5.	Conclusions and levels of confidence for different outcomes	218
5.1.	Based on existing evidence, we are confident of the following	218
5.1.1.	Developmental effects on the brain and behavior	218
5.1.2.	Developmental effects on the male reproductive tract	218
5.1.3.	Developmental effects on enzyme activity, growth and metabolism	218
5.1.4.	Adult effects on the male reproductive tract	219
5.2.	We consider the following to be likely but requiring confirmation	219
5.2.1.	Developmental effects on the female reproductive tract	219
5.2.2.	Adult effects on the brain and behavior	219
5.2.3.	Adult effects on the female reproductive tract	219
5.2.4.	Adult effects on the immune system	219
	Acknowledgements	219
	References	219

1. Introduction

Bisphenol A (BPA) is used as the monomer to manufacture polycarbonate plastic, the resin that lines most food and beverage cans, dental sealants, and as an additive in other plastics

(Fig. 1, Table 1) [1]. BPA is one of the highest volume chemicals produced worldwide; global BPA production capacity in 2003 was 2.2 million metric tonnes (over 6.4 billion pounds), with a 6–10% growth in demand expected per year [2]. Heat and either acidic or basic conditions accelerate hydrolysis of the ester bond

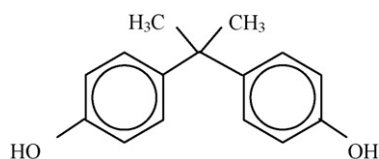


Fig. 1. Chemical structure of bisphenol A (BPA).

linking BPA monomers, leading to release of BPA and the potential for human and environmental exposure. Studies conducted in Japan [3] and in the USA [4] have shown that BPA accounts for the majority of estrogenic activity that leaches from landfills into the surrounding ecosystem.

BPA has been demonstrated in both *in vivo* and *in vitro* experiments to act as an endocrine disrupting chemical (EDC) (reviewed in [5,6]). There is extensive evidence that BPA is an estrogen-mimicking chemical, although recent findings have revealed that BPA is a selective estrogen receptor modulator (SERM), since BPA and the potent endogenous estrogen 17 β -estradiol (E2) do not always show identical effects, and in some studies BPA has been shown to antagonize the activity of E2 [7]. There is evidence that, similar to other estrogens, BPA can bind to androgen receptors and inhibit the action of androgen [8]. In addition, there is evidence for an anti-thyroid hormone effect of BPA [9]. However, effects of BPA mediated by binding to androgen and thyroid hormone receptors appear to require higher doses than those required to elicit estrogenic or antiestrogenic responses [7].

Chemicals classified as endocrine disruptors include not only hormone-mimics or antagonists that act via binding to receptors, but also chemicals that can interfere with hormone synthesis and clearance, as well as other aspects of tissue metabolism. Experiments have shown that BPA influences enzyme activity and thus metabolism in various tissues. Another mechanism of endocrine disruption is the alteration of hormone receptor expression, and experiments described below have shown that BPA alters hormone receptor numbers and hormone receptor gene activity in target tissues.

In this review, we summarize the recent literature on low dose effects of BPA in laboratory animals. The majority of the studies used rats and mice; only a few used other mammalian species. We conclude with a series of statements expressing our level of confidence concerning various effects of BPA in laboratory animals at low doses.

Table 1
Physical, chemical, and acute toxicological properties of bisphenol A (BPA) [1,199]

Molecular formula	(CH ₃) ₂ C(C ₆ H ₄ OH) ₂
Molecular weight	228.29
Water solubility	120–300 mg/l
log <i>k</i> _{ow}	3.40
Melting point	150–155 °C
Boiling point	220 °C (4 mmHg)
LD50 ^a , rat, oral	3300–4240 mg/kg [199]
LD50, mouse, oral	2500–5200 mg/kg [1]
LD50, fish (<i>Pimephales promelas</i>), 96 h	4.6 mg/l [1]

^a Acute lethal dose, 50% of population.

1.1. Rationale for experimental design, analysis and reporting criteria

Endocrinology experiments with laboratory animals are particularly vulnerable to confounding effects. Among other difficulties, treatment effects can be masked by hormonally active components of feed, water, or caging; and species and strains differ greatly in their sensitivity to different hormonally active compounds. Therefore, proper reporting of experimental design is critical to evaluation of studies in the literature. Below, we discuss several of the most important aspects of experimental design, which we considered when evaluating the studies discussed in this review.

1.1.1. Animal model

Published results must identify precisely the animal model and supplier being used. For example, Sprague–Dawley rats from different commercial breeders cannot be assumed to be the same, since it is an outbred stock. In particular, the outbred Sprague–Dawley CD rat from Charles River Laboratories [CrI:CD(SD)] has very low sensitivity to exogenous estrogens, and after more than 50 years of selective breeding by Charles River for large body size and litter size, it would be inappropriate to identify these rats as just Sprague–Dawley (Table 2). In contrast to the CrI:CD(SD) rat, male and female CD-1 (ICR) mice are highly sensitive to exposure to low doses of BPA during development as revealed by over 20 published studies (reviewed below) reporting significant effects of low doses of BPA in this outbred stock. This high sensitivity of the CD-1 mouse to BPA is predicted by the high responsiveness to positive control estrogens: E2, ethinylestradiol and diethylstilbestrol (DES), as revealed by both the *in vivo* studies discussed here and other studies of CD-1 cells and organs in primary culture [10–12].

The marked difference in sensitivity of different animal models used in toxicological, pharmacological and endocrinological research is just one of many reasons why it is essential that experiments include appropriate positive controls, which is discussed in more detail in following sections. With regard to examination of the *in vivo* estrogenic activity of BPA, which is the subject of this review, the sensitivity of the endpoint of interest in the chosen animal model should be characterized with a positive control such as E2 (appropriate if administration is by injection or subcutaneous capsule, due to very limited oral absorption of E2) or either DES or ethinylestradiol (appropriate if chemicals are administered orally, since they are orally active at very low doses). For example, in the CD-1 mouse, which is the animal model used by the U.S. National Toxicology Program, an appropriate positive control dose of ethinylestradiol or DES to detect a response to BPA within

Table 2
Lack of effects reported in low dose *in vivo* bisphenol A (BPA) research using the Charles River Laboratories Sprague–Dawley (CrI:CD(SD)) rat strain

Effect	0 (0%)
No effect	13 (100%)

the low dose range (below 50 mg/(kg day)) would be an oral dose not greater than 5 μ g/(kg day). This suggestion is based on numerous reports that for responses mediated by nuclear estrogen receptors, estimates of BPA potency in CD-1 mice range between 10 and 1000-fold less than either ethinylestradiol or DES, depending on the specific response being measured [10,12–16]. Also, doses of DES above 5 μ g/(kg day) can result in opposite effects relative to lower doses; this has been shown following developmental exposure for the prostate [16,17] and uterus [18]. High doses of these positive control estrogenic chemicals are thus not appropriate as a positive control for low dose effects of BPA or other estrogenic endocrine disrupting chemicals.

1.1.2. Feed, water, and housing

The exact feed used must be identified. Ideally, the estrogenicity of the feed should be characterized, since estrogenic components have been demonstrated to occur in both soy-based and non-soy-based animal feeds. There is also the possibility of variation in estrogenic activity between different lots of feed commonly used in toxicological research [19]. If possible, the same lot of feed (same mill date) should be used throughout an experiment.

The type of caging should be carefully selected to avoid estrogenic contamination of experimental animals. In particular, polycarbonate cages and water bottles should not be used, since they will leach uncontrolled concentrations of BPA to the experimental animals [20]. Polypropylene cages have been used successfully in studies of estrogens in mice, and polysulfone cages are available to replace polycarbonate; polysulfone is a co-polymer containing BPA and sulfone, but it is reported to be more resistant to degradation at high temperatures relative to polycarbonate [20]. Similarly, the source of drinking water must be free of BPA and other estrogens; reverse osmosis and carbon filtration is often necessary to achieve this requirement. In addition, if BPA is delivered in drinking water, the water must be free of chlorine or other reactive ions. Adding BPA to chlorinated water results in formation of tetrachlorobisphenol A.

1.1.3. Method of dosing

In all cases the precise method of dosing the animals and the time of dosing should be identified. Methods of administration of BPA include—(1) oral (p.o.): by gavage, by adding BPA to feed or drinking water, or by feeding the chemical in oil; (2) injection: subcutaneous (s.c.), intraperitoneal (i.p.), intracisternal, or intramuscular (i.m.) routes; and (3) implantation of Silastic[®] capsules or Alzet[®] minipumps that lead to steady-state exposures. The rationale for the dosing method must also be stated. For example, minipump implants model continuous exposure and avoid the first-pass metabolism of BPA in the liver that results from oral exposure.

The positive control chosen must be compatible with the selected route of exposure. For example, E2 has very low activity when administered orally. For studies in which BPA is administered orally, DES or ethinylestradiol are appropriate positive

controls. When BPA is administered in constant release capsules, E2 would be an appropriate positive control, while an estrogen agonist with a long half-life would be inappropriate. Route of administration influences the rate of metabolism of BPA, at least in adults [21], and there is some evidence for a higher contribution from ingestion of BPA in humans relative to inhalation or absorption through the skin [22]. However, the report that BPA levels in plasma collected from pregnant women in Germany show a range encompassing two orders of magnitude, between 0.1 and 10 parts per billion [23], suggests the likely possibility of variable exposure to multiple sources of BPA. Thus, although oral delivery appears to be most relevant for extrapolation to humans, all delivery methods may reveal effects of BPA.

1.1.4. Design and analysis issues

When animals are assigned to groups, the litter must be taken into account. It is well established that for both outbred stocks and inbred strains, the litter is a significant source of variation and needs to be accounted for in assigning animals to groups. Ideally, one animal per litter should be used. In cases when this is not possible, there are statistical methods (such as including litter as a main effect variable and dividing the *F* value for treatment effects by the *F* value for litter effects) that can be used when more than one animal per litter is used, or litter can be used as a covariate in ANCOVA. The method used to avoid confounding litter effects must be reported.

For all experiments, the positive and negative controls must be clearly identified. Experiments using a replicate block design should include a positive and a negative control in each block, if possible. Experiments showing no effect and lacking positive control data cannot be interpreted.

When taking measurements from adult animals, care must be taken to normalize the reproductive state of the experimental animals. Males should be singly housed for 2–4 weeks before collection, in order to avoid physiological differences arising from differences in dominance status of the males. Normalization of adult female reproductive status can be achieved by assaying their estrous cycles and collecting females at the same stage, or by using ovariectomized females with or without hormone replacement [24].

1.2. Definition of “low dose”

Low dose effects of environmental endocrine disrupting chemicals generally refer to effects being reported at doses lower than those used in traditional toxicological studies for risk assessment purposes. “Low dose” is also commonly used to refer to environmentally relevant doses, i.e., doses resulting in serum levels close to those observed in human serum. For BPA, prior to 1997, the lowest dose studied for risk assessment purposes was 50 mg/(kg day), which in the USA remains the currently accepted lowest observed adverse effect level (LOAEL) that was used to calculate the current EPA reference dose (and FDA acceptable daily intake or ADI dose) of 50 μ g/(kg day); this presumed “safe” dose is estimated by dividing the LOAEL by three 10-fold safety factors (i.e. by 1000) [25]. Thus, we included

Table 3

Published studies since 1997 (reviewed in detail in Table 4) that reported low dose effects of bisphenol A (BPA) in mice and rats resulting from exposure during development or during adulthood

Number of studies (total number of studies, including citations in other sections)	Observed adverse effects
51 (51)	Altered brain physiology, structure, and behavior
22 (27)	Altered male reproductive organs, including lower sperm production
17 (19)	Altered female reproductive organs, including accelerated puberty
8 (22)	Altered metabolism
7 (7)	Altered immune system

in our analysis studies dosing with less than 50 mg/(kg day) BPA (Tables 3 and 4).

1.3. Timing of exposure

Exposures to endocrine disruptors have different effects depending on the life stage of the exposed animals. Effects resulting from adult exposure are generally reversible and are termed “activational”. Effects resulting from exposure during organ development (beginning during prenatal development and continuing in postnatal life through puberty) may result in persistent alterations of the affected systems, even in the absence of subsequent exposure; these effects are termed “organizational” [26]. Some organizational effects are measurable immediately upon exposure and persist throughout the life of the animal [16]. Other organizational effects are undetectable at the time of exposure, but they become apparent in subsequent adulthood [26,27]. Windows of vulnerability, also known as critical periods, during which the developing system is most sensitive to exposure, are common features of organizational effects [28]. Exposures occurring outside the critical periods will not elicit organizational effects. There is evidence that organizational effects of estrogenic endocrine disruptors such as BPA are mediated by epigenetic alterations in DNA [29]. Organizational and activational effects on the same tissue often differ qualitatively as well as in duration and in the dose required to elicit effects. In this review, we will first discuss organizational effects of prenatal through pubertal BPA exposure, termed “developmental effects” and then activational effects of adult BPA exposure, termed “adult effects”.

2. Developmental effects of BPA due to exposure during gestation through puberty

Many studies have examined the effects of prenatal, neonatal (shortly after birth) and lactational (birth through weaning) exposure to low doses of BPA. These experiments involved examining effects of exposure to low doses of BPA during “critical periods” in the development of different tissues. These critical periods continue through puberty, the period of physiological transition to fertility.

2.1. Effects on neurotransmitters and their receptors, neuroendocrine function and hormone receptors in the brain in males and females

A large number of studies have involved examination of the effects of exposure to low doses of BPA during critical periods in brain development on subsequent brain structure, function and behavior.

2.1.1. Receptors

Increases in estrogen receptors α (ER α) and β (ER β) have been observed in diverse areas of the brain in response to developmental BPA exposure. BPA was administered to Wistar rats via minipumps at doses of 25 and 250 μ g/(kg day) from gestation day (GD) 8 through parturition [30]. The male offspring of these mothers were examined, and BPA was found to permanently up-regulate estrogen receptor 2 (β) (Esr2) mRNA levels in the preoptic area at 25 μ g/(kg day) [30]. Female offspring were not examined in this study [30]. Neonatal [postnatal days (PND) 1–5] injection of Fischer 344 rat pups with a dose of approximately 15 mg BPA/(kg day) resulted in increased estrogen receptor 1 (α) (Esr1) mRNA expression in the medial basal hypothalamus of females, and increased both Esr1 and Esr2 mRNA expression in the anterior pituitary in males [31]. In Sprague–Dawley rats (Harlan), fed 40 μ g BPA/(kg day) at early puberty, from PND 23–30, an increased number of ER α -labelled neurons was found at puberty (PND 37) in the arcuate nucleus (ARC) in males and females, and in the ventromedial nucleus (VMH) in females only. At maturity, more ER α -labelled neurons were found in the medial preoptic area (MPA) in females [32]. Esr1 and Esr2 mRNA expression were also increased in the dorsal raphe nucleus (DRN) of male ICR (CD-1) mice prenatally exposed to 2 μ g BPA/(kg day) via oral administration to the pregnant female on GD 11–17 [33]. In this study, serotonin, serotonin transporter, and serum testosterone were not altered [33]. BPA induced changes in somatostatin receptors in the brains of offspring born to Sprague–Dawley rats that received an oral dose of 400 μ g BPA/(kg day) during pregnancy and lactation. Exposed offspring were examined on PND 10 and 23 [34]. BPA also induces increased mRNA expression of the ligand-activated transcription factors aryl hydrocarbon receptor (AhR), retinoic acid receptor (RAR) α , and retinoid X receptor (RXR) α in embryonic brain [35,36].

2.1.2. Neuroendocrine effects

BPA-induced changes in function of the hypothalamus–pituitary–gonad axis have been observed in both males and females. In females, disruption of LH and estrous cyclicity have been reported. The female offspring of Sprague–Dawley rats exposed during gestation and lactation to approximately 1.2 mg/(kg day) of BPA in their drinking water exhibited disrupted, prolonged estrous cycles and decreased hypersecretion of LH in response to ovariectomy, suggesting lasting neuroendocrine effects of early BPA treatment [37]. Disruption of adult estrous cycles seen as an increase in the length of the estrus phase was also reported to occur in female offspring of pregnant Sprague–Dawley rats administered BPA orally at a dose of

Table 4
Published papers reporting biological effects in animal studies (excluding aquatic animals) for bisphenol A (BPA) in the low dose exposure range (<50,000 µg/kg BW/day), sorted by tissue or endpoint examined

Reference	In other sections	Animal	Sex	Exposure, vehicle	Time of exposure	Doses tested, µg/kg BW/day (*P<0.05)	Endpoints	Age at collection	Other chemicals tested
Effects on brain physiology									
Akingbemi [41]	Sex difference, testosterone, metabolism	Long Evans rat, CRL	M	Oral, oil oral gavage	PND 21–PND 35; GD 12–PND 21	2.4*, 10(*), 100,000, 200,000; 2.4*, 10*, 100,000*, 200,000; 2.4*	Serum LH, testosterone suppression, serum estradiol suppression, pituitary LHβ and ERα mRNA expression; body weight; seminal vesicle weight (see also <i>in vitro</i>)	PND 35; PND 90	
Aloisi [120] Ceccarelli [32]		Rat Sprague–Dawley rat, Harlan, Italy	F, M	Oral, oil Oral	Adult PND 23–30	40,000* 40*	ERα Hypothalamic ERα	Adult PND 27, PND 90	Ethinylestradiol
Evans [39]		Sheep, Poll Dorset	F	Injection, oil	4 weeks (prepuberty)–11 weeks	3500*	Tonic LH secretion; LH pulse frequency and amplitude	11 weeks	DES, octylphenol
Facciolo [34]		Sprague–Dawley rat (CRL, Italy)	F	Oral, oil	GD 0–PND 23	40, 400*	GABA _A receptors	PND 10, PND 23	
Funabashi [117]		Rat	F	Injection, s.c.	Adult	50,000*	Hypothalamic preoptic area progesterone receptors		Estradiol, butyl benzyl phthalate
Funabashi [119]		Rat	F	Injection s.c.	Adult	40,000*	Frontal and temporal cortex change in progesterone receptor mRNA	Adult	Octylphenol, nonylphenol
Funabashi [118]		Rat	F	Oral, oil	Adult	4, 40, 400*, 4000*	Hypothalamic progesterone receptors, POA and VMH	Adult	Estradiol
Kawai [33]		ICR mice	M	Oral	GD 11–17	2*	Change in ERα, and ERβ immunoactivity in dorsal raphe nucleus	PND 35, PND 63, PND 91	
Kawato [42]		Wistar rat	M	Oral, water	GD 11–PND 21	0.1*, 1, 10, 50 mg/l in drinking water	Brain steroidogenesis	4 weeks	
Khurana [31]		Fischer 344 rat	F, M	Injection, oil	PND 1–5	15,000*, 75,000*, 15,000*	Hyperprolactinemia; pituitary ERα, ERβ expression	PND 10–PND 30	DES, octylphenol
MacLusky [44]		Rat	F	Injection, oil	Adult	40*, 120*, 400*; 45*	Hippocampal pyramidal neuron synaptogenesis	Adult	17β-Estradiol, 17α-estradiol
Nikaido [15]	Puberty, metabolism	Crj:CD-1 (ICR) mouse	F	Injection, DMSO	GD 15–GD 18	500*, 10,000*; 500, 10,000*	Cycle length, diestrus, reduced corpora lutea, mammary gland development; age at vaginal opening	4, 8, 12, 16 weeks and weeks 9–11 examined for estrous cycles	DES, genistein, zearalenone, resveratrol
Ramos [30]	Prostate	Wistar-derived rat	M	Osmotic minipump	GD 7–22	25*, 250*	Ventral prostate, HPG axis, ERβ	PND 15, 30, 120	
Rubin [37]	Metabolism	Sprague–Dawley rat, Taconic	F	Oral, water	GD 6–PND 21	100*, 1200*	Body weight, cyclicity, LH	BW from PND 2–110, cycles 4 and 6 months	
Savabieasfahani [40]		Suffolk Ewes	F	s.c. injection in cottonseed oil into pregnant dams	GD 30–GD 90	5000*	Low birth weight, increased LH levels during first 2 months of postnatal life, extended breeding season, dampened magnitude of LH surge	6 weeks–40+ weeks of age	Methoxychlor
Steinmetz [121]		Rat	F	Implant	Adult	300*	Serum prolactin; PRF activity (see also <i>in vitro</i>)	Adult	Estradiol

Talsness [38]	Prostate, metabolism	Sprague–Dawley rat, Harlan–Winkelmann	F, M	Oral, oil	GD 6–GD 21	100*, 50,000*; 100*, 50,000	Reproductive system effects, body weight; estrus cycle; AGD in males	Adult, 3 months, PND 3, 15, 21	Ethinylestradiol
Zoeller [9]		Sprague–Dawley rat, Zivic Miller	F, M	Oral, feed, via treated wafer	GD 6-duration not defined	1000*, 10,000*, 50,000*	Serum thyroxin; dentate gyrus RC3/neurogranin expression	PND 4, 8, 15, and 35	
Zsarnovszky [43]		Sprague–Dawley rats	F, M	Injections into cerebellum	PND 4–PND 19 and adults 250–300 g	Concentrations of 10 ⁻¹² to 10 ⁻⁶ M BPA were injected into the cerebellum (3 µl in PND 4–10, 5 µl into older animals)	Rapidly increased pERK beginning on PND 8; co-administration with E2 inhibited rapid E2 induced ERK1/2 activation. Dose dependent	PND 4–PND 19 and adults	Cyclodextrin encapsulated estradiol
Effects on brain structure									
Kubo [45]	Sex difference	Wistar rat	F, M	Oral, water	Mating to weaning	1500*	Loss of sexual dimorphism in behavior and in size of locus coeruleus	6–12 weeks	
Kubo [46]	Sex difference, prostate	Rat	F, M	Oral, water	Mating to weaning	30*, 300*	Reversal in the sexually dimorphic volume of the locus coeruleus, behavioral changes	Postnatally, 6, 11–12 and 14 weeks	DES, resveratrol
Masuo [49]	Behavior	Wistar rat	M	Intra-cisternal injection, oil 10 µl into pups	PND 5	Approximately 2, 20*, 200*, 2000*; 2000*	Motor hyperactivity (spontaneous motor activity); midbrain gene expression	PND 56	Nonylphenol, octylphenol, DEHP
Nakamura [50]		ICR/Jcl mice	F, M	s.c. injection, oil	GD 0-collection	20*	Altered neuronal migration and gene expression	GD 10.5–GD 16.5	
Patisaul [48]	Sex difference	Sprague–Dawley rat, CRL	F, M	s.c. injection in sesame oil into pups	PND 1-2	Approximately 75,000*	Altered AVPV development, altered sexual dimorphism of dopamine (TH) neuron number and number of TH/ER expressing neurons	PND 19	
Rubin [47]		CD-1 mouse	F, M	Osmotic minipump	GD 8–PND 21	0.025, 0.250*	Dopamine neuron number in the AVPV reduced/loss of expected sexual dimorphism	PND 24–27	
Effects on behavior									
Adriani [64]		Sprague–Dawley rat (Italy)	F, M	Oral, oil	GD 0–PND 26	40*	Behavioral tests, M & F; amphetamine response, M	PND 30–35, PND 70	
Aloisi [56]		Sprague–Dawley rats, CRL	F, M	Oral, oil, trained to suck BPA from pipette	Dams exposed thru pregnancy and lactation. Two groups: placental exp suckling exp	40*	Pain behavior; formalin-induced nociception	22 weeks of age	
Della Seta [61]	Metabolism	Rat	F	Oral, oil	Adult	40*	Maternal behavior		
Della Seta [67]	Testosterone	Sprague–Dawley (Italy)	M	Oral, oil	PND 23–PND 30	40*	Decreased serum testosterone	PND 37, 45, >90	Ethinylestradiol
Dessi-Fulgheri [62]		Sprague–Dawley rat (Italy)	F, M	Oral, Oil	40, GD 0–PND 21; 400, GD 14–PND 6	40*, 400*, 40*, 400, 40, 400*	Play behaviors	PND 35, 45, 55	
Farabollini [51]		Sprague–Dawley rat (Italy)	F, M	Oral, oil	GD 0- GD 22; PND 1–PND 21	40*	Aggression, Sexual Behavior	PND 100	
Ishido [54]	Metabolism	Wistar rat	M	Intracisternal injection, oil 10 µl	PND 5	≈3, ≈30, ≈300*, ≈3000*; ≈3, ≈30, ≈300, ≈3000* (87 fmol–87 nmol BPA/rat) 0.02–20 µg/rat	Hyperactivity; reduced gene expression of dopamine receptor and dopamine transporter; reduced midbrain TH-IR	4–8 weeks	

Table 4 (Continued)

Reference	In other sections	Animal	Sex	Exposure, vehicle	Time of exposure	Doses tested, $\mu\text{g}/\text{kg BW}/\text{day}$ (* $P < 0.05$)	Endpoints	Age at collection	Other chemicals tested
Kawai [52]	Testosterone	CD-1 mouse	M	Oral, oil	GD 11–17	2*, 20*	Aggression, testis weight	8, 12, 16 weeks	
Laviola [66]	Sex difference	CD-1 mouse	F, M	Oral, oil, drink from syringe	GD 11–18	10*	Amphetamine-induced conditioned place preference	60 days	Methoxychlor
Masuo [53]		Rat		Intracisternal injection	Neonatal	$\sim 20 \mu\text{g}/\text{kg}^*$	Motor hyperactivity	4–5 weeks	
Mizuo [55]		ddY mice		Oral, feed	GD 0–PND 14	0.002, 0.5*, 2* mg/g in food	Enhanced reward effect and hyperlocomotion in response to morphine	Adult	
Negishi [59]		F344/N rat	M	Oral, oil (corn) gavage	GD 3–PND 20	100*	Behavioral alterations active avoidance test	8–24 weeks	Nonylphenol
Nishizawa [35,36]		Mouse	F, M	Oral, oil	GD 6–13, GD 6–17	0.02*, 2*, 200*, 2000*	Embryonic brain and gonad AhR expression; RAR α , RXR α expression		
Palanza [60]	Metabolism	CD-1 mouse, CRL	F	Oral, oil	GD 14–18	10*	Maternal behaviors	In adulthood	
Porrini [63]		Sprague–Dawley rat, CRL, Italy	F, M	Oral, peanut oil, micro-pipette	Daily from mating to parturition	40*	Socio-sexual behaviors	35, 45, 55 days	
Razzoli [122]		Gerbil	F	Oral, oil	Adult	2*, 20; 2*, 20*; and 2, 20*	Social investigative behavior, free exploratory tests		Ethinylestradiol
Ryan [57]	Puberty, metabolism	C57Bl6 mice	Ovx F	Oral, gavage needle into back of mouth	GD 3–PND 21	2, 200*	Accelerated puberty, altered anxiety related behaviors	Adult, 6 weeks +	Ethinylestradiol
Suzuki [65]		ddY mouse	M	Oral, in feed	GD 0–PND 21	0.002, 0.5, 2 mg/g feed, $\approx 300^*$, $\approx 75,000^*$, $\approx 300,000^*$	Dopamine D1 receptor-mediated enhanced induced abuse state	Not specified	
Effects on sex differences in the brain Carr [68]		Fischer 344 rat	F, M	Oral, oil gavage in pups	PND 1–PND 14	100, 250*; 100*, 250	Morris water maze; elimination of sex difference	Tests begin PND 34	Estradiol
Farabollini [58]		Sprague–Dawley rat (Italy)	F, M	Oral, oil	GD 0–PND 21	40*, 400*	Exploratory behavior, activity	PND 85	
Fujimoto [73]		Wistar rat	F, M	Oral, drinking water	GD 0–PND 1	15*	Sex differences in open field and forced swimming behavioral tests	7–9 weeks	
Funabashi [72]		Wistar rat	F, M	Oral, drinking water	Gestation and lactation, no GD identified	$\approx 2500^*$, (2500)	Sex differences in CRH neurons in bed nucleus of the stria terminalis	4–7 months	
Imanishi [70]		ICR mouse	F, M	Oral, oil	GD 6–GD 17	2*	Placental nuclear receptor gene expression, 9 of 20 examined; six non-nuclear receptor genes	GD 18	
Nishizawa [71]		ICR mouse	F, M	Oral, oil	GD 6-collection	2*	Embryonic brain and gonad retinoid receptor expression RAR α , RXR α	GD 12, 14, 16, or 18	
Shibata [69]	Metabolism	Wistar rat	M	Oral	2 or 4 weeks, adulthood	1500*	BPA and sex steroid metabolism in adult male	Adult	DES
Effects on puberty in females Honma [13]	Metabolism	ICR/Jcl mouse	F, M	s.c. injection, oil	GD 11–GD 17	2*, 20*; 2*, 20; 2, 20*	BW, estrous cycle length, male AGD, vaginal cytology; female AGD; age of vaginal opening, first estrus	Up to PND 120	DES

Howdeshell [78]	Metabolism	CF-1 mouse	F, M	Oral, oil	GD 11–GD 17	2.4*	Puberty, BW	Puberty	
Vandenberg [79]	Mammary gland	CD-1 mouse	F	Alzet osmotic pump	GD 8–18	0.25*	Mammary gland morphology	GD 18	
Effects on the mammary gland									
Colerangle [85]		Rat	F	Osmotic minipump	4 weeks old, administered 11 days	100*, 54,000*	Mammary gland differentiation	5.5 weeks	DES
Durando [82]		Wistar rat	F	Osmotic minipump	GD 7–22	25*	Mammary gland, cancer	PND 30, 50, 110, 180	
Markey [80]		CD-1 mouse	F	Osmotic minipump	GD 8–GD 19	25*, 250*	Mammary gland	PND 10, 1 month, 6 months	
Munoz-de-Toro [81]		Mouse	F	Osmotic minipump	GD 8 lactation	0.025*, 0.250*	Mammary gland morphogenesis, terminal end bud density	PND 30, 120	
Murray [83]		Wistar rat	F	Osmotic minipump		2.5*, 25*, 250*, 1000*	Mammary gland hyperplasia (carcinoma precursor)	PND 55, 95	
Effects on the uterus and vagina									
Markey [88]		Mouse	F	Osmotic minipump	GD 9-lactation	0.025*, 0.250*	Genital tract alterations; ER α and progesterone receptor up-regulation developmentally	Adult	
Nagel [173]		Mouse	F	Injection, oil	Adult	25 ($P < 0.06$), 791*, 25,000*	Uterus, gene expression		DES
Schönfelder [87]		Rat	F	Oral	GD 6–21	100*, 50,000*	Vaginal ER α expression, vaginal histology	Adult	Ethinylestradiol
Schönfelder [86]		Sprague–Dawley rat	F	Oral	GD 6–21	100*, 50,000*; 50,000*; 100*, 50,000*	Uterine epithelial histology; uterine ER α expression; uterine ER β expression	Adult	Ethinylestradiol
Suzuki [14]		Mouse	F	Injection, oil	GD 10–18	10,000*, 100,000*	Uterine and vaginal mitotic indices following prenatal exposure	PND 30	DES
Effects on the ovary, oocytes and fertility									
Al-Hiyasat [90]		Mouse	F	Oral, water	Adult for 28 days	5, 25*, 100*; 5, 25, 100*	Increased resorptions, uterine weight; ovarian weight	Adult	
Berger [92]		Mouse	F	Injection s.c.	GD 1–4	3, 375*; 10, 125*	Implantation rate; fecundity	GD 5, PND 1	
Hunt [89]		C57BL/6, mouse	F	Oral, oil	Peripubertal for 6–8 days	20*, 40*, 100*	Disruption of meiosis; aneuploidy	~30 days old	
Susiarjo [91]		C57BL/6, mouse	F	Oral, injection	GD 11–18	20*	Disruption of chromosomes	GD 18	
Effects on testosterone in males									
Takao [133]		Mouse	M	Oral, drinking water	Adult	≈120, ≈12,000*	Plasma free testosterone	Adult	
Effects on the Prostate									
Gupta [10]		Mouse	M	Oral, oil	GD 14–18	50*	Prostate weight, prostate AR (see also <i>in vitro</i>)	PND 3, 20, 60	DES, aroclor
Ho [29]		Sprague–Dawley rat	M	Injection s.c.	PND 1–5	10*	Prostate cancer (PIN lesions)	Adult	Estradiol
Nagel [94]	Metabolism	CF-1 mouse	M	Oral, oil	GD 11–GD 17	2*, 20*	Prostate weight	6 months	Octylphenol
Ramos [97]		Wistar rat	M	Osmotic pump, DMSO	GD 7–GD 22	25*, 250*	Ventral prostate	PND 30	
Stoker [100]		Wistar rat	M	s.c. injection, oil	PND 22–PND 32	50,000*	Serum prolactin, lateral prostate weight	PND 29, PND 120	Estradiol, Pimozide
Timms [16]		Mouse	M	Oral, oil	GD 14–18	10*	Fetal development of prostate and urethra	GD 19	Ethinylestradiol, DES
Effects on the testes, epididymis, sperm and seminal vesicles									
Aikawa [102]		SHN mouse	M	s.c. injection, oil	PND 1–PND 5	≈175*, ≈17,500*; ≈175, ≈17,500*	Abnormal sperm; decreased motility	10 weeks	Estradiol

Table 4 (Continued)

Reference	In other sections	Animal	Sex	Exposure, vehicle	Time of exposure	Doses tested, $\mu\text{g}/\text{kg BW}/\text{day}$ (* $P < 0.05$)	Endpoints	Age at collection	Other chemicals tested
Al-Hiyasat [129]	Metabolism	Swiss mouse	M	Oral	Adult, for 1 month	5*, 25* 100*	Reduced fertility, epididymidal sperm, body weight	Adult	
Chitra [131,132]	Metabolism	Wistar rat	M	Oral, oil	Adult, for 45 days	0.2*, 2*, 20*; 0.2*, 2*, 20*; 0.2*, 2*, 20*; 0.2*, 2*, 20*; 0.2*, 2*, 20*	Decreased testis, epididymis weight; increased ventral prostate weight; reduced sperm motility; sperm count; oxidative stress enzymes; increased H ₂ O ₂		
Chitra [132]		Wistar rat	M	Oral	Adult	0.2*, 2*, 20*	Decrease in sperm motility, decrease in oxidative stress enzymes	Adult	
Fisher [104]		Wistar rat	M	s.c. injection, oil	PND 2–PND 12	37,000*	Efferent duct epithelial height	PND 10, 18, 25, 35, and 75	DES, ethinylestradiol, genistein, octylphenol, parabens
Sakaue [127]		Rat	M	Oral, oil	Adult	0.2, 2, 20*, 200*, 2000*, 200,000*	Sperm production		
Takahashi [136]		Rat, mouse	M	Injection, propylene glycol	adult	2000, 20,000*	BPA low dose challenge after extended high-dose exposure		
Takao [134]		Mouse	M	Oral, drinking water	Adult	≈200, ≈20,000*	Testicular ER α , ER β		
Thuillier [188]		Sprague–Dawley rat, CRL	M	Gavage (BPA); s.c. injection (DES)	GD 14–GD 22	100, 1000*, 10,000*, 200,000*	Neonatal testicular gonocyte PDGF α , PDGF β	GD 21–PND 3	DES, genistein, coumestrol
Tohei [135]		Rat	M	Injection	Adult	3000*	Testes and serum hormones		
Toyama [103]		ICR mouse, Wistar rat	M	s.c. injection, oil	PND 2–PND 12	71, 714*, 3600*, 7100*; 180, 1800*, 18,000*	Spermatogenesis, mouse; rat	2–10 weeks	Estradiol, estradiol benzoate
Toyama [130]		Mice	M	Injection	Adult, for 6 days	20*, 200*	Abnormal sperm morphology;	Adult	
vom Saal [101]		CF-1 mouse	M	Oral, oil	GD 11–GD 17	2*, 20*; 2, 20*	Reproductive organs; sperm production	6 months	Octylphenol
Wang [105]		Sprague–Dawley rat, CRL	M	Oral, oil	GD 14–GD 22	1000, 10,000*, 200,000*; 1000*, 10,000, 200,000	Estrogen receptor-associated protein expr; Hsp90, p23	GD 21, PND 3, PND 21	DES, genistein, coumestrol
Wistuba [106]		Sprague–Dawley rat (Germany)	M	Oral gavage, Cornstarch suspension	GD 6–GD 21	100*, 50,000*	Sertoli cell number per testis	PND 290–370	Ethinylestradiol
Effects on metabolism									
Alonso-Magdalena [140]		OF1 mice	M	Injection, oil	Adult	10*	Insulin release, insulin sensitivity		Estradiol
Kabuto [141]		ICR mice	M	Injection i.p. for 5 days	Adult	25,000*, 50,000*	Antioxidant scavenging enzymes		
Kabuto [114]		Mouse	M	Oral, drinking water	GD 1-lactation	2.5–5*	Testis weight, brain weight, kidney weight, various oxidation markers	PND 28	
Lemmen [189]		Mouse	F, M	Injection, oil	GD 13.5	100, 1000*, 10,000*	Transgenic embryonic gene expression in whole embryo lysates		DES, estradiol propionate
Markey [110]		Mouse	F	Osmotic minipump	GD 8–19	25*, 250*	Estrus cycle alterations, blood-filled ovarian bursae, mammary gland budding	Adult	

Nunez [139]	Sprague–Dawley rat	F	Osmotic, pump	Adult	≈5000, 18,000*, 23,000*	Body weight, blood and tissue levels of BPA	Adult	
Takai [107]	B6C3F1 mouse	F, M	<i>Ex vivo</i> in culture medium	GD 2–4	0.1, 0.3, 1*, 3*, 10, 100,000* nM (0.023, 0.068, 0.23*, 0.68*, 2.3, 23,000* ppb)	Increased rate of development at 1 and 3 nM, decreased rate of development at 100,000 nM	Blastocyst	
Takai [108]	B6C3F1 mouse	F, M	During IVF in culture medium	GD 0–2	1 nM (0.228 ppb)*	Postnatal weight gain increase	Weaning	
Effects on the immune system								
Lee [145]	Mouse, BALB/c	F	Injection (i.p.)	Adult	25,000*	Increased IL-4 production, increased serum antigen-specific immunoglobulin E (IgE) levels	Adult	Nonylphenol
Sawai [142]	Mouse, C57BL/6, NZB/WF1	F, M	Oral, in feed	PND 35–42	2.5*	Decreased IFN γ and IL-10 secretion by splenic mononuclear cells; decreased IgG2a production; protection in lupus-prone mice (see also <i>In vitro</i>)	Adult	
Sugita-Konishi [147]	Mouse, BALB/c	F	Injection (s.c.), oil	Adult	5000*	Immune cells and functions, reduced IL-6 production and neutrophil phagocytic activity against bacterial infection	Adult	
Tian [144]	Mouse, BALB/cA	M	Oral, oil	Adult	≈370, 1800*, 9000*; ≈370, 1800, 9000*	IL-10 production; IL-4 production	Adult	
Yoshino [143]	Mouse, DBA/1J	F, M	Oral, oil	Adult	3, 30, 300*, 3000*; 3, 30*, 300*, 3000*	Increased serum anti-HEL IgG2a; augmentation of cytokine response	Adult	Estradiol
Yoshino [116]	Mouse, DBA/1J	F, M	Oral, oil	GD 0–GD 17	3, 30*, 300*, 3000*; 3, 30, 300*, 3000*	Increased serum anti-HEL IgG2a; T-helper cytokines (both Th1 and Th2) increased	Adult	
Yurino [148]	BWF1 mice		Silastic capsule	4 weeks	25 ng/ml in blood*	IgM antibodies	5 months	Estradiol DES

The information indicated in the column headings provides information in published studies. If information is not reported, this is indicated by “NR”. Day plug observed: GD 0. Mice GD 19: PND 1. Rats GD 22: PND 1. F: female; M: male; DES: diethylstilbestrol.

100 $\mu\text{g}/(\text{kg day})$ on GD 6–21 [38], and prolonged estrous cycles were observed in female offspring of CD-1 mice injected with a dose of 500 $\mu\text{g}/(\text{kg day})$ BPA on GD 15–18 [15]. Exposure via i.m. injection of prepubertal female Poll Dorset lambs to DES (0.005 mg/(kg day)) or BPA (1 mg/(kg day)) for 7 weeks beginning at 4 weeks of age (prior to puberty) had significant effects on LH secretion (a decrease in LH pulse frequency) that were similar, with the dose of DES being 200-fold lower than BPA [39]. Treatment of pregnant Suffolk Ewes with 5 mg/(kg day) of BPA dissolved in cottonseed oil via daily subcutaneous injections from GD 30–GD 60 resulted in marked effects in the female offspring. Levels of LH were increased during the first 2 months of postnatal life, breeding season was extended, and the magnitude of the LH surge was dampened [40].

In males, effects on LH, prolactin, and brain aromatase activity have been observed. Exposure of weanling males to 2.4 $\mu\text{g}/(\text{kg day})$ BPA for 15 days resulted in decreased serum LH and testosterone levels due to alterations in LH synthesis and secretion at the pituitary level; neuroendocrine effects in this study were observed following exposure of the weanling mice to BPA, but not following gestational and lactational exposure [41]. Injecting approximately 15 mg/(kg day) of BPA into neonatal Fisher 344 rat pups from postnatal days 1–5 resulted in an increase in serum prolactin levels (hyperprolactinemia) in both males and females on PND 30 [31]. Male offspring of pregnant and lactating rats exposed to 20 $\mu\text{g}/(\text{kg day})$ BPA in drinking water showed an increase in estrogen synthesis in hippocampal neurons due to an increase in aromatase activity [42].

BPA exposure also induces alterations in the hypothalamus–pituitary–thyroid axis. Dietary exposure to doses of 1, 10 or 50 mg/(kg day) of BPA in Sprague–Dawley rats from GD 6 through weaning caused a significant increase in serum total T4 in pups on PND 15, but serum thyroid-stimulating hormone was not different from controls. The expression of the thyroid hormone-responsive gene RC3/Neurogranin, measured by *in situ* hybridization, was significantly up-regulated by all BPA doses in the dentate gyrus. These findings suggest that BPA acts as an antagonist of TR β , which mediates the negative feedback effect of thyroid hormone on the pituitary gland, but that BPA is less effective at antagonizing TR α , leaving TR α -mediated events to respond to elevated plasma thyroid hormone levels [9].

2.1.3. Rapid signaling effects

BPA has effects similar to E2 (similar potency and efficacy) in stimulating rapid-response ERK1/2 activity in the cerebellar cortex of Sprague–Dawley rat pups at PND 5–19; treatment consisted of a 6-min intracerebellar injection followed by rapid fixation for immunohistochemical analysis for phosphorylation of ERK1/2. Significant effects of both BPA and E2 were observed at the lowest dose examined of 1 pM (0.228 ppt). Interestingly, in addition to acting as an estrogen, when co-administered in conjunction with E2, BPA was able to inhibit the rapid E2-induced ERK activity in the developing cerebellum, thus disrupting the action of E2 in a dose-dependent manner [43]. There is another report that BPA can act as an estrogen antago-

nist in the hippocampus when BPA is co-administered with E2 [44].

2.1.4. Brain structure

Developmental exposure to BPA resulted in a significant change in the locus coeruleus, where BPA at oral doses of 30 $\mu\text{g}/(\text{kg day})$ (in drinking water) and above reversed the normal sex differences in this brain structure and eliminated sex differences in behavior [45,46]. Pregnant and lactating CD-1 mice were exposed to very low doses of BPA, 0.025 and 0.250 $\mu\text{g}/(\text{kg day})$ BPA, via Alzet minipumps, and the brain of the offspring were examined at PND 24–27. The expected robust sex difference in the number of tyrosine hydroxylase (TH) positive neurons in the sexually dimorphic anteroventral periventricular preoptic area (AVPV) was apparent in the control offspring. However, in the offspring exposed to BPA, the sex difference in TH neuron number was undetectable due to a decline in TH neuron number in BPA exposed females. No significant difference in TH neuron number was noted in the males [47]. It is interesting to note that injection of much higher doses of BPA (approximately 300,000-fold higher than the highest daily dose administered to pregnant and lactating females in the study discussed above, 500 $\mu\text{g}/(\text{day pup})$ or approximately 75 mg/(kg day)) directly into rat pups on PND 1–2 also altered TH neuron number in the AVPV; however, the alteration was in a different direction from that reported with very low doses of BPA. With this much higher dose of BPA, the male offspring showed differences from controls, and the male AVPV resembled the female AVPV, whereas the females showed no significant effect of the BPA injection. These data suggest that at this high dose, BPA may have acted as an antiestrogen and interfered with the masculinization of TH neuron number [48]. The importance of dose is discussed in more detail below.

In a study with male Wistar rats that received a single intracisternal injection of approximately 20 μg BPA/kg dose on PND 5, BPA reduced the number of dopamine-containing neurons and resulted in changes in gene expression [49]. A 20 $\mu\text{g}/(\text{kg day})$ injection of BPA into pregnant ICR (CD-1) mice disrupted normal neocortical development in fetuses by accelerating neuronal differentiation/migration [50].

2.1.5. Behavioral effects

2.1.5.1. Aggression. BPA resulted in an increase in defensive aggression in male Sprague–Dawley rat offspring prenatally exposed to BPA (administered orally to mothers throughout gestation) at a dose of 40 $\mu\text{g}/(\text{kg day})$; no effect was found in the offspring of mothers treated during lactation [51]. In addition, increased aggressiveness (using a composite score of aggression) in male CD-1 mouse offspring occurred as a result of oral administration of 2 $\mu\text{g}/(\text{kg day})$ of BPA to pregnant females on GD 11–17 [52].

2.1.5.2. Activity level and reactivity to stimuli. Treatment of male Wistar rat pups at PND 5 with an intracisternal injection of 0.87 nmol (approximately 20 $\mu\text{g}/\text{kg}$) BPA resulted in hyperactivity at 4 weeks of age [49,53]. Ishido et al. reported that single intracisternal injections of approximately 15 μg BPA/kg

or 150 μg BPA/kg at PND 5 increased spontaneous motor activity at 4 weeks of age [54]. Prenatal and lactational exposure to BPA in the feed (approximately 3–300 mg/(kg day)) also resulted in an increase in activity with dose and altered the response to morphine [55]. BPA increased reactivity to painful or fear-provoking stimuli in the offspring of Sprague–Dawley rats fed 40 μg /(kg day) throughout pregnancy and lactation: a hyperalgesic effect was found following prenatal, but not postnatal exposure [56]. When pregnant and lactating C57BL/6 mice were administered an oral BPA dose of 2 or 200 μg /(kg day), BPA increased anxious behavior in a dose-dependent fashion when females were examined in adulthood. Significant effects were observed in animals exposed to 200 μg /(kg day) of BPA and to 5 μg /(kg day) of ethinylestradiol, which was used as a positive control for the study [57]. In contrast, Farabollini et al. reported that maternal exposure to 40 μg /(kg day) of BPA throughout gestation and lactation resulted in reduced anxiety in adult male offspring, and lowered motivation to explore in both male and female adult offspring [58].

2.1.5.3. Learning. BPA impaired learning of both passive and active avoidance tasks in offspring of Fisher 344 rats fed 100 μg /(kg day) of BPA during pregnancy and lactation [59].

2.1.5.4. Maternal behavior. Feeding pregnant CD-1 mice 10 μg /(kg day) decreased subsequent maternal behavior in female offspring, similar to behavioral effects seen with adult exposure [60,61].

2.1.5.5. Social interactions and response to addictive drugs. Prenatal and lactational oral exposure of Sprague–Dawley rats to 40 μg /(kg day) altered adult play and other socio-sexual behaviors in both males and females [62,63]. Prenatal and lactational exposure to BPA in the feed (approximately 3–300 mg/(kg day)) resulted in an altered response to morphine [55]. The behavioral response to amphetamine was enhanced in rats and mice at 40–300 μg /(kg day) [64,65]. However, at a prenatal dose of 10 μg /(kg day) of BPA, exposed adult female CD-1 mice lost their responsiveness to the reward pathways normally stimulated by amphetamine, possibly through effects on the dopamine system; in this study there was no effect on male amphetamine responsiveness, and there was no effect on activity levels [66].

2.1.5.6. Sexual behavior. An impairment in the timing of copulatory sequence was found in Sprague–Dawley male rats, perinatally exposed to BPA (40 μg /(kg day) fed to the mothers through gestation or lactation) [51] or treated during early puberty [67]. In animals exposed via oral administration perinatally, a reduced performance in terms of latency and frequency of intromission was observed [51]. Effects in the same direction were found with prepubertal exposure to BPA; this was in agreement with results obtained with the positive control ethinylestradiol [67].

2.1.6. Sex differences

One of the most interesting findings concerning organizational effects of BPA on the brain and behavior and other

aspects of physiology is the loss of sex differences that are typically observed in control males and females. Also intriguing is the effect of BPA on one sex but not the other [45,46,48,58,66,68–73]. The mechanisms responsible for different effects of BPA in males and females are not clear, although it is known that BPA metabolism is influenced by testosterone [74,75], and BPA modifies the metabolism of testosterone [41,76,77]. Thus, one possibility is that the sex-specific effects of BPA in the brain and other tissues are due to an interaction with background levels of gonadal steroids. The effects of BPA on metabolism are discussed in more detail in a companion paper in this issue (see: L. Vandenberg et al.).

2.2. Developmental effects on the female reproductive tract

2.2.1. Timing of puberty

Early onset of sexual maturation in females occurred at maternal doses between 2.4 and 500 μg /(kg day) in a number of different mouse strains [13,15,57,78]. Puberty in female rodents can be assayed by age at vaginal opening or by age at first ovulation, signaling the onset of fertility. The age at first ovulation can be detected by assessing the age at which the vaginal epithelium is first cornified, indicating that the female is in estrus, or by pairing prepubertal females with experienced males and monitoring the females' age at first parturition. Timing of puberty is linked to postnatal growth, since puberty is dependent on age, body size, and energy stores. A study with CF-1 mice showed that the response to prenatal exposure (via oral dosing of the mother) to a very low dose of BPA (2.4 μg /(kg day)) from GD 11–17 was influenced by the relative position of the fetus with respect to its female and male siblings in the uterus. The response to BPA was greatest in the females that were located *in utero* between female siblings (2F females) in terms of stimulating an increase in postnatal growth and accelerating the age at first vaginal estrus; postnatal growth was also stimulated in males that were located *in utero* between female siblings (2F males) but not in male offspring that were located *in utero* between male siblings (2M males). This finding is consistent with the observation that a very low maternal dose of BPA stimulated a change in the developing mammary gland in 2F female but not 2M female CD-1 mouse fetuses [79]. Taken together, these findings suggest that the endogenous concentrations of E2 and/or testosterone are significant factors in determining the response to BPA during fetal life in mice.

2.2.2. Mammary gland

Stimulation of mammary gland development in CD-1 mice was observed in the offspring of dams exposed to the very low maternal dose of 0.025 and 0.250 μg /(kg day) delivered tonically by an Alzet pump [80]. A significant increase in the percentage of ducts, terminal ducts, terminal end buds, and alveolar buds was observed in female offspring of BPA-treated dams at 6 months of age, collected during proestrus. At 6 months of age, the authors identified terminal end buds as appearing much less bulbous than terminal end buds observed during puberty. The treatment-induced changes in histo-architecture, coupled with an increased presence of secretory product within alve-

oli observed in the BPA-exposed offspring, resembled those of early pregnancy. Examination of BPA-exposed females (using the same paradigm) at 30 days of age [81] revealed an increase in mammary gland area and number of terminal end buds, as well as an increase of progesterone receptor-positive ductal epithelial cells that were localized in clusters, suggesting future branching points. These sites may be involved in the increase in lateral branching noted in the mammary glands of offspring born to BPA-exposed dams [80].

The female offspring of pregnant Wistar rats implanted throughout pregnancy with an Alzet minipump that released 25 $\mu\text{g}/(\text{kg day})$ of BPA were examined at various times up to 6 months of age [82]. At puberty, animals exposed prenatally to BPA showed an increased mammary gland proliferation/apoptosis ratio both in the epithelial and stromal compartments. BPA exposed animals showed an increased number of hyperplastic ducts with signs of desmoplasia, suggesting a heightened risk of neoplastic transformation. Administration of a sub-carcinogenic dose of *N*-nitroso-*N*-methylurea (NMU) to the female rats exposed prenatally to BPA increased the percentage of hyperplastic ducts and induced the development of neoplastic lesions. In a related report Wistar-Furth offspring born to mothers implanted with osmotic minipumps that released BPA at a dose of 2.5 $\mu\text{g}/(\text{kg day})$ during gestation and lactation revealed evidence of ductal hyperplasias at PND 50 and 95 that included increased expression of Ki67 and Esr1. Some of the ductal lesions were identified as carcinoma *in situ* [83].

The effect of exposure to 0.25 $\mu\text{g}/(\text{kg day})$ delivered to CD-1 mice by Alzet minipump from the evening of gestational day 8 throughout pregnancy was examined in female fetuses on GD 18 [79]. In unexposed fetuses, the mammary gland ductal tree was more developed in 2M females than in 2F females [79]; this result is counterintuitive, since prior research has shown that 2M female fetuses have elevated serum testosterone and lower serum E2 relative to the 2F female siblings [84]. BPA exposure increased mammary gland ductal area and ductal extension and eliminated intrauterine position differences; this was due to BPA stimulating a significant change in 2F females but not 2M females [79]. In the stroma, BPA exposure promoted maturation of the fat pad and altered the localization of collagen. Within the epithelium, BPA exposure led to a decrease in cell size and delayed lumen formation [79]. Pubertal exposure to 100 $\mu\text{g BPA}/(\text{kg day})$ also increased differentiation of mammary gland structures and increased proliferation of epithelial cells [85].

2.2.3. Uterus and vagina

Oral administration of 100 $\mu\text{g}/(\text{kg day})$ or 50 $\text{mg}/(\text{kg day})$ of BPA to pregnant Sprague–Dawley rats resulted in a significantly decreased uterine ER β protein expression in BPA-treated animals at both doses (relative to controls) when measured during the estrous phase of the cycle. Interestingly, a high dose of 200 $\mu\text{g}/(\text{kg day})$ ethinylestradiol caused a similar effect [86]. These same doses of BPA and ethinylestradiol caused striking changes in vaginal morphology during estrus, including a decrease in the thickness of the epithelial layer [87]. In addition,

Western Blot analysis indicated that following exposure to either dose of BPA, the full-length variant (64 kDa) of Esr1 was not expressed in the vagina of female offspring during estrus, whereas during the diestrus stage, ER α protein expression did not differ from the control group [87].

Studies in the CD-1 mouse have also revealed alterations in the uterus and vagina in offspring born to mothers treated with 0.025 and 0.25 $\mu\text{g BPA}/(\text{kg day})$ via Alzet minipumps [88]. These alterations include decreased vaginal wet weight, decreased volume of the endometrial lamina propria and increased protein expression of both ER α and progesterone receptor in the luminal epithelium and subepithelial stroma of the uterus. Consistent with these effects of BPA, studies have shown that exposure during early development (via injection on PND 1–5) to very low doses of DES (0.01 $\mu\text{g}/(\text{kg day})$) alters ER α receptor levels and morphology of the uterus in CD-1 mice [18].

2.2.4. Ovary, oocytes and fertility

Significant disruption of the alignment of chromosomes during meiosis was observed in developing oocytes due to leaching of BPA from polycarbonate drinking bottles at doses between 15 and 70 $\mu\text{g}/(\text{kg day})$; this finding leads to the prediction that exposure to BPA during the time that meiosis is reinitiated by the mid-cycle surge in luteinizing hormone (LH) can result in an increase in aneuploidy, which is one of the major causes of spontaneous abortion in humans [89]. As predicted by this finding, there was an increase in mortality of embryos that occurred at a maternal dose of 25 $\mu\text{g}/(\text{kg day})$ [90]. Subsequently, a defect in meiosis was induced in C57BL/6 mouse embryos by maternal oral or injected BPA at 20 $\mu\text{g}/(\text{kg day})$ by Hunt et al., and the effect was similar to the defect seen in untreated Esr2-knockout mice [91]. The impact of this effect would not be directly on the *in utero* exposed female, but on the embryos produced from her oocytes (F2 generation effect). Implantation of embryos is not affected at low BPA maternal doses (as low as 10 $\mu\text{g}/(\text{kg day})$), and is significantly decreased only at a maternal dose of approximately 70 $\text{mg}/(\text{kg day})$, which is just above the low dose range [92].

2.3. Developmental effects on the male reproductive tract

2.3.1. Serum testosterone levels

Male Long-Evans rats born to mothers exposed to 2.4 $\mu\text{g}/(\text{kg day})$ of BPA via feeding, from GD 12 through lactation had decreased levels of testicular testosterone, which was not due to a decline in serum LH levels but was consistent with a decline in the steroidogenic capacity of Leydig cells in the BPA exposed males [41]. Decreased serum testosterone in male CD-1 mice also occurred at a maternal dose of 2 $\mu\text{g}/(\text{kg day})$ given on GD 11–17 [52]. In Sprague–Dawley (Harlan) male rats, alterations in circulating testosterone levels were observed after BPA treatment with 40 $\mu\text{g}/(\text{kg day})$ at early puberty (PND 23–30): in this study testosterone was reduced in the juveniles (PND 37), and this decrement persisted in the adults [67].

2.3.2. Prostate

Development of the prostate gland from the urogenital sinus is dependent on systemic testosterone, which is metabolized to 5 α -dihydrotestosterone in the urogenital sinus mesenchyme. The mesenchyme directs the development of the epithelium into the glandular structure of the prostate [93]. An increase in adult prostate size in male offspring occurred when pregnant females were fed BPA at 2 or 20 $\mu\text{g}/(\text{kg day})$ on GD 11–17 in CF-1 mice [94], at 10 $\mu\text{g}/(\text{kg day})$ on GD 14–18 in CD-1 mice [16], and 50 $\mu\text{g}/(\text{kg day})$ on GD 16–18 in CD-1 mice [10]; in the experiments conducted by Timms et al. [16] and Gupta [10], DES caused the same effects as BPA at a maternal oral dose of 0.1 $\mu\text{g DES}/(\text{kg day})$. Gupta [10] also showed that the effect of BPA on the prostate was directly on prostate tissue by removing the prostatic region of the fetal urogenital sinus and examining the effect of BPA in organ culture; the lowest effective concentration (LoEC) of BPA that stimulated an increase in prostate growth was 50 pg/ml (ppt). DES also stimulated prostate growth in primary culture at a dose of 0.5 pg/ml (ppt) [11]. Richter et al. removed the prostatic region of the fetal urogenital sinus and examined the prostatic mesenchyme cells, which contain the androgen and estrogen receptors, in primary culture. With a constant physiological concentration of 5 α -dihydrotestosterone, E2 stimulated androgen receptor mRNA levels at 1 pM (0.27 pg/ml), while BPA caused the same effect at 1 nM (0.23 ng/ml), which is within the range of BPA detected in human fetal umbilical cord blood [23]. Maternal exposure to DES (0.1 $\mu\text{g}/(\text{kg day})$, p.o.), an increase in free serum E2 (of 0.1 pg/ml , via Silastic implant) and BPA (50 $\mu\text{g}/(\text{kg day})$, p.o.) all caused a permanent increase in prostatic androgen receptors in mice in addition to an increase in adult prostate weight, relative to negative controls [10,16,17].

In addition to effects on prostate size and androgen responsiveness, prenatal exposure to BPA may affect the development of prostate cancer in later life. Timms et al. [16] observed in GD 19 male CD-1 mouse fetuses that a maternal oral dose of 10 $\mu\text{g BPA}/(\text{kg day})$ on GD 14–18 stimulated an increase in the number of primary prostatic ducts as well as proliferation of basal cells (the progenitor cells thought to be responsible for the development of prostate cancer) in the dorsolateral, but not ventral, primary ducts. This is of interest in that a similar dose of BPA administered via injection to neonatal rats resulted in 100% of the subsequent adult males exhibiting prostate interepithelial neoplasia (PIN) lesions, which are pre-tumorous prostate cancer lesions [29]. Epigenetic changes occurring during development may be the basis for the adult altered susceptibility to disease [95]. BPA is also implicated as a factor in the disruption of therapy for human prostate cancer, since BPA can bind and activate a mutant form of the androgen receptor, T877A, found in some human prostate cancers, and thus stimulate proliferation of human prostate cancer cells in the absence of androgen [96].

The prostate in rodents is composed of distinct lobes that respond differently to endocrine disruptors. Differences in cell proliferation and differentiation markers in stromal cells were observed in ventral prostate following prenatal exposure to 25 $\mu\text{g}/(\text{kg day})$ of BPA in prepubertal Wistar rats [30,97]. This

finding is different from those reported by Timms et al. [98], in which male Sprague–Dawley rats exposed to the highest natural serum levels of E2 (due to developing between female fetuses [99]) showed enlargement of the dorsolateral prostate but not the ventral prostate. In another study, prepubertal male Wistar rats were injected with 50 $\text{mg BPA}/(\text{kg day})$ between PND 22–32. BPA induced a transient surge in prolactin (implicated in the regulation of prostate growth) and a subsequent increase in adult prostate size in later adulthood [100]. On PND 70, prostate weight was significantly increased and daily sperm production was decreased in Sprague–Dawley rats exposed during gestation via a maternal oral dose of 100 $\mu\text{g}/(\text{kg day})$ of BPA [38]. In contrast to these findings, DES (6.5 $\mu\text{g}/(\text{kg day})$) or BPA (30 and 300 $\mu\text{g}/(\text{kg day})$) administered via drinking water to pregnant and lactating Wistar rats did not induce significant effects on the ventral prostate (the only region examined) [46].

2.3.3. Testes, epididymis, sperm and seminal vesicles

In contrast to development of the prostate from the urogenital sinus, development of the epididymis, vas deferens and seminal vesicles from the Wolffian ducts is dependent on diffusion of testosterone from the testis rather than systemic testosterone, and does not involve metabolism of testosterone to 5 α -dihydrotestosterone. Thus, drugs and environmental chemicals can have quite different impacts on differentiation of the Wolffian duct vs. the urogenital sinus [93]. Adult CF-1 male offspring that had been exposed prenatally to a maternal oral BPA dose of 2 $\mu\text{g}/(\text{kg day})$ showed decreased weights of the epididymis and seminal vesicles, but increased weights of the prostate and preputial glands; a 20 $\mu\text{g}/(\text{kg day})$ dose of BPA resulted in a decrease in daily sperm production per gram testis [101]. Injection (s.c.) of BPA (50 $\mu\text{g}/\text{animal}$, about 15–20 $\text{mg}/(\text{kg day})$) for the first 5 days after birth resulted in a decrease in the percentage of moving sperm, an increase in the incidence of malformed sperm, and an increase in the number of ER α -positive cells in the epididymides of SHN strain mice at 10 weeks of age [102]. Abnormalities in the acrosomal granule and nucleus of step 2–3 spermatids were also observed in neonatal ICR (CD-1) mice and Wistar rats injected with 300 $\mu\text{g BPA}/(\text{kg day})$ or 1 $\mu\text{g E2}/(\text{kg day})$ [103]. Ectoplasmic specialization between the Sertoli cell and spermatids was also affected, and some specializations were partially or totally deleted [103]. Decreased epithelial height in the efferent ducts of the testes was observed in prepubertal Wistar rats exposed prenatally to 37 $\text{mg BPA}/(\text{kg day})$ [104].

Alterations in heat shock proteins in response to BPA were examined in testicular germ cells, since they are involved in the response to environmental stress and have also been implicated in developmental events. Hsp90 protein levels were significantly increased at maternal oral doses of 1 $\text{mg}/(\text{kg day})$ of BPA and 0.01 $\mu\text{g}/(\text{kg day})$ of DES [105]. A finding that contrasts with those described above occurred when pregnant Sprague–Dawley rats were fed BPA at 0.1 or 50 $\text{mg}/(\text{kg day})$. Spermatogenesis was qualitatively normal in all groups, but both doses of BPA

increased testicular weight and Sertoli cell number per organ [106].

2.4. Developmental effects on metabolism

The earliest reported exposure regime is direct dosing of pre-implantation mouse embryos at the two-cell stage [107]. In this experiment, development to the eight-cell stage and to the blastocyst stage was accelerated by exposure to 1–3 nM (0.23–0.69 ng/ml (ppb)) BPA, and was delayed by exposure to 100 μ M (23 μ g/ml) BPA. Both the acceleration and the delay of development to blastocyst were inhibited by co-treatment with 100 nM tamoxifen, which seems unexpected since that dose is 1000-fold less than the high BPA concentration. Also, the effects on embryo development were not seen with E2 over a dose range of 10 fM to 1 μ M [107]. When embryos treated with 1 nM BPA were implanted in a female and allowed to develop, the resulting pup weights at weaning were significantly increased compared to control pups treated with solvent only (0.1% ethanol) as embryos [108].

Increased postnatal growth in both male and female rats and mice occurred at maternal doses between 2.4 and 500 μ g/(kg day) [15,37,41,78,107,109,110]. Evidence is accumulating that during critical periods in development, estrogenic chemicals can have unexpected effects on the differentiation of adipocytes as well as postnatal growth [111]. Newbold et al. reported that neonatal exposure to a low dose (1 μ g/(kg day)) of DES stimulated a subsequent increase in body weight and an increase in body fat in mice [18]. In a related study a high 100 μ M dose of BPA stimulated an increase in the glucose transporter GLUT4 and glucose uptake into 3T3-F442A adipocytes in cell culture [112]. In a separate study of transgenic mice over expressing the GLUT4 gene, increased basal and insulin-induced glucose uptake was observed in whole body and in isolated adipocytes [113]. Whether the mouse 3T3 cell lines, which are relatively insensitive to estrogen, are an appropriate model to study the effects of BPA *in vitro* remains to be determined.

Some studies have found decreased body weight in response to developmental BPA exposures [13,38,94], and some have found no effects on body weight [54,57,114]. Recent research on the effect of the type of animal feed used in an experiment on postnatal growth suggests that whether or not an increase or decrease in body weight occurs may be related to the type of feed used [115]. In addition, the impact of exposure to BPA during pregnancy and/or lactation on the maternal behavior and lactational efficiency of mothers cannot be ignored [60,61]. For example, an experiment by Howdeshell et al., which reported increased body weight in response to prenatal BPA exposure [78], differed in design from an experiment conducted by Nagel et al., which reported decreased body weight [94]. Howdeshell cesarean delivered the pups and fostered them to untreated dams. In contrast, Nagel allowed prenatally treated mice to nurse their own offspring, leading to an opposite effect on adult body weight. These two studies used the same low (2 μ g/(kg day)) maternal dose of BPA, the same CF-1 strain of mice, and the same type of feed.

2.5. Developmental effects on the immune system

There has been only one study of the developmental effects of BPA on immune function. Prenatal BPA exposure appeared to increase all tested immune responses to soluble antigen in exposed offspring [116]. Following feeding of DBA/1J mice a 30 μ g BPA/(kg day) dose from day 0 through day 17 of gestation, adult male offspring produced increased antigen-specific IgG2a antibody. A higher prenatal dose of 300 μ g/(kg day) increased adult production of both T helper 1 interferon gamma (IFN- γ) and T helper 2 IL-4 cytokines in exposed male and female offspring [116]. The two cytokines tested have wide-ranging and distinct effects on immune function; dysregulation of cytokine production could have implications for inflammation or allergic responses.

3. Effects of BPA exposure during adulthood

BPA exposure at low doses has diverse activational effects. Some of these effects are predicted due to the affinity of BPA for ER α and ER β , while other effects diverge from those observed in response to activation of estrogen receptors.

3.1. Adult effects on the brain and behavior

Funabashi et al. [117–119] reported that a single injection of adult ovariectomized female Wistar rats with BPA at 400 μ g/kg–40 mg/kg increased progesterone receptor protein in the preoptic area, ventromedial hypothalamus and frontal cortex, as well as progesterone receptor mRNA in the preoptic area. Specifically, estrogen acts to induce progesterone receptor in the hypothalamus, and Northern blot analysis revealed an increase in progesterone receptor mRNA in the preoptic area and anterior pituitary of adult female rats acutely dosed with approximately 35 mg BPA/kg [117]. In this study, BPA caused responses similar to estrogen in the preoptic area and anterior pituitary, but not in the mediobasal hypothalamus [117]. A single dose of approximately 35 mg BPA/kg in adult female rats induced progesterone receptors in the frontal cortex, but repressed progesterone receptor expression in the temporal cortex [119].

Activational effects of BPA in the brain include an increase in ER α -expressing cells in the medial preoptic area of pregnant and lactating adult females and non-pregnant, cycling adult female Sprague–Dawley rats at a BPA dose of 40 mg/(kg day) administered orally for a period of 42 days, corresponding to the time from mating to weaning in the pregnant and lactating group. In contrast, a decrease in ER α -expressing cells was observed in the arcuate nucleus of pregnant and lactating females but not non-pregnant, cycling females in response to this treatment [120].

Injection of ovariectomized E2-treated female Sprague–Dawley rats with 40–300 μ g BPA/(kg day) resulted in a monotonic dose-related inhibition of E2-stimulated increase in hippocampal synapses, with all doses producing significant effects [44]. Thus, in the hippocampus, BPA acted to antagonize the action of E2 on synaptogenesis.

BPA induced a significant increase in serum prolactin levels in 8–10 week old ovariectomized Fisher 344 rats when administered at a low dose of 40–45 $\mu\text{g}/(\text{kg day})$ of BPA for a period of 3 days via Silastic capsules. In contrast, Sprague–Dawley rats were relatively insensitive to both BPA and E2 in this experiment, which was the first report that the model animal was an important factor in BPA studies [121].

Exposure of adult female Sprague–Dawley rats from the day of mating through lactation to 40 $\mu\text{g}/(\text{kg day})$ of BPA via feeding resulted in alterations in maternal behavior towards their young; specifically, grooming was reduced in BPA-exposed dams [61]. In Mongolian gerbils (Charles River, Italy), exposure to pairs of adult males and females via feeding (in oil) 2 or 20 $\mu\text{g BPA}/(\text{kg day})$ for 3 weeks increased their social interactions and reduced their exploratory behavior; similar effects were observed in response to 0.04 $\mu\text{g}/(\text{kg day})$ of ethinylestradiol [122].

3.2. Adult effects on the female reproductive tract

Acute exposure of C57BL/6 female mice to 20 through 100 $\mu\text{g}/(\text{kg day})$ of BPA resulted in a significant increase in meiotic abnormalities in the oocytes when exposure occurred during the peri-pubertal period, suggesting that BPA exposure would lead to aneuploidy; this abnormality was also observed in mice that were housed in polycarbonate cages and that were provided water in polycarbonate bottles that had been damaged by exposure to a harsh detergent during washing [89]. The effect of BPA on aneuploidy has also been examined in cell culture [123–126].

3.3. Adult effects on the male reproductive tract

A decrease in daily sperm production and fertility in male Sprague–Dawley rats was reported at oral doses between 20 and 200,000 $\mu\text{g}/(\text{kg day})$ due to adult exposure, and maximum suppression of sperm production occurred at 20 $\mu\text{g}/(\text{kg day})$. At doses below 20 $\mu\text{g}/(\text{kg day})$, daily sperm production was not significantly different from controls [127]. This suggests that there is a sub-population of cells that are impacted by BPA, and an approximately 40% decrease in daily sperm production is the maximum that occurs, regardless of the dose of BPA administered above 20 $\mu\text{g}/(\text{kg day})$. This finding is similar to data reported for prenatal exposure to ethinylestradiol, where maximum suppression of daily sperm production occurred at 2 $\text{ng}/(\text{kg day})$, with no further suppression occurring at higher doses [128].

Similar to the findings in rats, oral exposure of adult Swiss mice for 1 month to 25 and 100 $\mu\text{g}/(\text{kg day})$ resulted in a decrease in daily sperm production and epididymal sperm concentration, which was associated with a decrease in fertility. A dose of 5 $\mu\text{g}/(\text{kg day})$ also resulted in a decrease in the weight of the testes and seminal vesicles [129] (Note that the doses were incorrectly reported in the paper as $\text{ng}/(\text{kg day})$ instead of $\mu\text{g}/(\text{kg day})$.) A dose of 20 $\mu\text{g}/\text{kg}$ body weight of BPA was injected (s.c.) to adult ICR (CD-1) mice and Wistar rats for 6 days, and abnormalities were observed in the spermatids: acrosomal vesicles, acrosomal caps, acrosomes and nuclei of

the spermatids were severely deformed. The ectoplasmic specialization between the Sertoli cell and spermatids was also affected: incomplete specialization, redundant ectopic specialization, and aplasia were observed [130]. A significant decrease in testis and epididymal weight was also reported to occur in adult male Wistar rats exposed orally for 45 days to 0.2, 2 and 20 $\mu\text{g}/(\text{kg day})$, while an increase in ventral prostate weight occurred at all doses [131,132].

Successive i.p. administration of BPA to adolescent male C57BL/6 mice at a dose of 20 $\text{mg}/(\text{kg day})$ for 4 weeks decreased the prostate and seminal vesicle weights (but not testis or epididymis weights) and also decreased serum testosterone and both liver and kidney weights [133].

BPA at concentrations of approximately 0.2 and 20 $\text{mg}/(\text{kg day})$ was administered in the drinking water to young male C57BL/6 mice for 8 weeks beginning at 3 weeks of age (before the onset of puberty), and the number of ER β -containing cells and *Esr2* mRNA per testis were significantly decreased in the 20 $\text{mg BPA}/(\text{kg day})$ treated group compared with controls. In contrast, ER α -immunopositive cells and *Esr1* mRNA per testis were markedly increased in these males relative to controls [134].

An s.c. injection for 2 weeks of 3 $\text{mg}/(\text{kg day})$ dose of BPA significantly reduced testicular testosterone content, and serum testosterone, while plasma LH showed an increase in adult male Wistar rats [135]. Injection (i.p.) of BPA at a dose of 20 $\text{mg}/(\text{kg day})$ for 4 weeks decreased the prostate and seminal vesicle weights but not the testis or epididymis weights, and also decreased serum testosterone and both liver and kidney weight in Wistar rats [136]. Plasma free testosterone levels were dramatically decreased following 8 weeks of 12 $\text{mg}/(\text{kg day})$ of BPA treatment of adult male C57BL/6 male mice compared with control group, and morphologically abnormal multinucleated giant cells having greater than three nuclei were found in seminiferous tubules in the testis following 8-week BPA treatment at 120 $\mu\text{g}/(\text{kg day})$, while no controls showed this [133].

3.4. Adult effects on metabolism

There is considerable experimental evidence that in adult mice E2 acts via ER α to have an inhibitory effect on adipocyte number and lipogenesis, and removal of estrogen by ovariectomy or ER α via a genetic mutation also causes impaired glucose tolerance and insulin resistance in addition to increased fat mass [137,138]. Estrogen has central effects on food consumption and energy expenditure that also contribute to its overall inhibitory effects on adipose deposition in adults. BPA has also been reported to decrease body weight in adults [129,139]. These findings thus contrast numerous reports that low dose BPA or DES exposure during early development stimulates postnatal growth.

Very low doses of BPA stimulated rapid secretion of insulin in mouse pancreatic β cells in primary culture through a non-classical, non-genomic rapid estrogen-response system. In the same study prolonged exposure to a low oral dose of BPA (10 $\mu\text{g}/(\text{kg day})$) resulted in stimulation of insulin secretion in adult mice that was mediated by the classical nuclear estrogen

receptors; the prolonged hypersecretion of insulin was followed by insulin resistance [140].

Other effects on metabolic pathways have also been observed in response to BPA. A decrease in antioxidant enzymes occurred at the very low dose of 0.2 $\mu\text{g}/(\text{kg day})$ of BPA in adult Wistar rat males [131], and at a higher dose of 50 $\text{mg}/(\text{kg day})$ in ICR (CD-1) male mice [141]. In adult Wistar rats, oral administration of 1 $\text{mg}/(\text{kg day})$ of BPA reduced in adult males but not females the expression of UGT2B1 and other UGT isoforms that mediate the glucuronidation of BPA and sex hormones [69].

3.5. Adult effects on the immune system

BPA has been reported to modulate immune function at doses between 2.5 and 30 $\mu\text{g}/(\text{kg day})$ [142,143], including patterns of cytokine and antibody production, response to infection, and autoimmune disease progression. T helper lymphocytes are a source of cytokine families that stimulate inflammatory responses and resistance to intracellular infections (Th1 cytokines), or that shift the response to antibody production, resistance to extracellular organisms, and allergy (Th2 cytokines). Antigen-specific IFN- γ (Th1) secretion was increased in adult DBA1/J mice given 30 $\mu\text{g}/(\text{kg day})$ BPA p.o. for 20 days and sacrificed 24 h later [143]. In contrast, a shorter term oral dose of 2.5 $\mu\text{g}/(\text{kg day})$ BPA given PND 35–42 to C57BL/6 and autoimmune-prone NZB/WF1 mice significantly decreased the production of IFN- γ by splenocytes stimulated with 4 $\mu\text{g}/\text{ml}$ of the T lymphocyte mitogen ConA, up to 5 weeks post treatment [142]. A single oral dose of approximately 9 mg BPA/kg just prior to infection with the parasite *Trichinella spiralis* increased production of both IL-4 and IL-10 by lymph node cells stimulated with parasite antigen [144]. Addition of BPA at doses ranging from 0.1 to 30 μM to cultures of lymphocytes from *Leishmania major*-infected BALB/c or C57BL/6 mice did not increase IFN- γ production [144]. Other studies also report an increase in the Th2-associated cytokine, IL-4, following BPA exposure. Injection (i.p.) of 25 $\text{mg}/(\text{kg day})$ BPA every other day for 1 week in adult KLH-primed BALB/c mice resulted in significant increase of IL-4 production in CD4+ T cells and of serum antigen-specific immunoglobulin E (IgE) levels [145]. Antigen specific spleen cells of adult BPA-exposed DBA1/J mice (300 $\mu\text{g}/(\text{kg day})$ p.o. for 20 days) produced an elevated IL-4 response [143]. Thus, most models indicate that BPA may enhance or shift the pattern of cytokine production following antigen stimulation. Skewing of the Th1/Th2 cytokine profile by EDCs has been associated with allergy and asthma [146].

Exposure to BPA has also been associated with modulation of innate immune system cell function. For example, administration of 5 $\text{mg}/(\text{kg day})$ s.c. to adult BALB/c mice for 5 days decreased innate host defense to bacterial infection. Upon challenge with i.p. *E. coli* injection, both neutrophil phagocytosis and IL-6 production were significantly reduced [147].

Autoimmune diseases, particularly those mediated by an antibody response to host tissues, are more common in females; estrogens and prolactin are believed to act as drivers for differential disease development and progression in individuals with

an underlying genetic susceptibility to autoimmunity. However, BPA exposure modulates the course of glomerulonephritis in the NZB/WF1 mouse model of systemic lupus erythematosus. An oral dose of 2.5 $\mu\text{g}/(\text{kg day})$ given on PND 35–42 to female NZB/WF1 mice decreased the production of IgG2a antibody by splenocytes and delayed the onset of glomerulonephritis as measured by albuminuria [142]. Yurino et al. used Silastic implants to administer BPA, E2, or DES to ovariectomized, 4 week old, female NZB/WF1 mice [148]. Serum concentrations of 30 ng/ml BPA were measured 4 months after implantation. At 5 months of age, both DES- and E2-treated mice showed an increase in IgG anti-DNA antibody and prominent deposition of immune complexes in the glomeruli, both indicators of disease. In contrast, although BPA, DES, and E2 increased IgM-class autoantibodies, there was no evidence of glomerular immune complex deposition and no increase in serum anti-DNA IgG antibody in the BPA-exposed mice.

In summary, while it is well accepted that estrogen alters immune function, the interaction is complex [149]. Estrogen has been reported to both decrease and increase immune function, depending on dose, including conflicting reports on the production of the T helper 1 cytokine, IFN- γ [150,151]. The effects of BPA exposure on the immune system may be critically dependent on the timing of BPA exposure. Estrogen receptor expression by lymphocytes is dependent upon the age and strain of the animal; in addition, recent evidence suggests the spleen undergoes significant molecular remodeling during puberty, resulting in both age and gender-dependent differences in immune gene expression [152]. Nevertheless, studies conducted by Yoshino et al. [116,143] indicate similar dose-associated, gender-independent immune system effects in 8-week-old offspring of BPA-exposed dams and animals exposed as adults. These results suggest quantitative, rather than qualitative, differences in lifestage-dependent immune system sensitivity to BPA.

4. Comparison of findings of significant effects and no-significant effects in low dose BPA studies

As of the end of October 2006 we are aware of 27 *in vivo* studies reporting no significant effects in response to low doses of BPA. The variables that account for most of the studies that find no significant effects have recently been reviewed [5,153]. Below we discuss a number of these variables.

4.1. Strain differences

The major factor that accounts for 13 studies that draw the conclusion of no effect of low doses of BPA is the use of a strain of rat sold by Charles River Laboratories, referred to as the CD-SD rat [CrI:CD(SD)] (Table 2). According to Charles River [154], rats were purchased by Charles River from Sprague–Dawley in 1950. This colony was continuously subjected to selective breeding for rapid postnatal growth and large litter size for over 40 years. The CrI:CD(SD) rat strain is insensitive to BPA, and 0 of 13 studies report low dose effects [155–167]. In addition, this rat strain is insensitive to

the drug ethinylestradiol at doses ($\sim 0.5 \mu\text{g}/(\text{kg day})$) used in oral contraceptives [163,167]. As shown above, a large number of studies show that significant low dose effects of BPA have been observed in the original Sprague–Dawley rat. However, a number of studies have reported that the inbred Fisher 344 rat is more sensitive to low dose effects of BPA relative to the original Sprague–Dawley rat [121,168,169]. Wistar rats also appear to be more sensitive than Sprague–Dawley rats to the uterotrophic effects of BPA [168,170]. One assessment of the uterotrophic response of immature Sprague–Dawley rats revealed a response following 3 days of exposure to 300 mg/(kg day) of BPA. In the same study, a significant response to ethinylestradiol was noted at 0.3 ug/(kg day) [171]. Why the Sprague–Dawley rat does not respond to low doses of BPA in the uterotrophic bioassay or in the vaginal stimulation bioassay has been investigated, and the lack of responsiveness appears to be due to factors after binding to estrogen receptors [169].

The importance of the strain of animal used in low dose research was acknowledged in the 2001 Low Dose National Toxicology Program (NTP) report. The NTP panel emphasized the need to test for the sensitivity of any animal model by including a positive control, such as the well characterized estrogenic drugs DES or ethinylestradiol, and stated [172, p. vii]: “Because of clear species and strain differences in sensitivity, animal model selection should be based on responsiveness to endocrine active agents of concern (i.e., responsive to positive controls), not on convenience and familiarity.”

4.2. BPA as a SERM: responses in different tissues and in different animal models

A difference in the response of tissues to low doses of BPA is shown in articles that have been published reporting that low doses of BPA do not stimulate a typical uterotrophic (increase in uterine weight) response in mice and rats, while low dose effects are observed in many other endpoints. For example, effects on uterine weight have been observed at relatively high doses of BPA (25–40 mg/(kg day)), while changes in uterine gene activity occurred at lower doses of 10 mg/(kg day) in B6C3F1 mice and 0.8 mg/kg in transgenic ERIN mice [173–175]. In prepubertal CD-1 mice, a dose of 100 mg/(kg day) injected s.c. for 3 days was required to stimulate an increase in uterine weight [176]; however, 5 mg/(kg day) was sufficient to cause a significant increase in the height of uterine epithelial cells in this strain. Similar findings regarding the inability of low doses of BPA to stimulate a uterotrophic response in prepubertal CD-1 mice were reported in another study [177], which, in comparison to the B6C3F1 mouse data, provides further evidence for strain differences in sensitivity to BPA.

The prepubertal uterotrophic assay is thus a relatively insensitive endpoint for studies of BPA, since the dose required to stimulate a uterotrophic response (one of the most commonly used bioassays in toxicology to assess estrogenic activity), is markedly higher relative to doses that cause significant effects in CD-1 mice in the prostate [10,16], testes [52], mammary glands [79–81,88], and the brain and behavior [47,52,60,66]. All of these studies report significant effects in response to develop-

mental exposure of CD-1 mice to doses of BPA at and far below the reference dose of 50 $\mu\text{g}/(\text{kg day})$ (there are over 20 low dose BPA studies published reporting significant effects of BPA in CD-1 mice). As a specific example, when the magnitude of the response to BPA in mammary gland and uterus are compared, the mammary gland appears to be a more sensitive target for BPA action, but clearly, *in utero* exposure to low doses of BPA does have lasting effects on the uterus in CD-1 mice [88].

4.3. Batch-to-batch variability in feed: impact on low dose endocrine disruptor research

The other major variable that has been implicated in contributing to discrepancies in the outcome of experiments on low dose effects of BPA is the type of feed used. Two workshops on this issue were held in 2005 and 2006 to address this issue, and a manuscript reviewing the conclusions from these workshops is being prepared. The consensus was that there is a critical need for researchers to better understand the potential for components of commercial feed to impact research outcomes. Importantly, no one type of feed was deemed appropriate for the many different types of research conducted with laboratory rats and mice, and both soy-based and non-soy-based feeds were reported to contain variable amounts of estrogenic contaminants that were not soy phytoestrogens [178]. The major concern was that feed manufacturers should make every effort to reduce the batch-to-batch variability in feed components that can lead to phenotypic variation in control animals.

Findings also presented at the workshops emphasized the critical importance of including appropriate positive controls in experimental studies. With regard to BPA for example, the inclusion of an appropriate positive control (based on experimental objectives and techniques, such as route of administration) allows one to determine whether or not the test system has been rendered insensitive to any estrogenic chemical or drug when drawing conclusions concerning the potential safety of estrogenic chemicals such as BPA. A specific example was provided by the finding of Thigpen et al. that a commonly used feed in toxicological studies, PMI 5002, showed significant batch-to-batch variability in phytoestrogens that related to an inability to detect significant effects of the potent estrogenic drug diethylstilbestrol (DES) with some batches of this feed [19]. Some studies that reported finding no significant effects at low doses of BPA [166,179] used PMI 5002 feed, raising concerns that effects of DES would not have been found with the batches of this feed that were used. In fact, 0.1 $\mu\text{g}/(\text{kg day})$ DES (administered orally to pregnant CF-1 mice) was used as a positive control in one study [179], and DES-exposed animals did not differ from negative controls on any outcome.

4.4. The use of positive controls

The NTP panel commented in its 2001 report on the issue regarding: “a study in which the positive control does not produce the expected positive response. The prudent course of action in such cases may be to declare the study inadequate and repeat it, regardless of the experimental outcome in the test

groups” [172, pp. 5–10]. The NTP panel went on to note that: “For those studies that included DES exposure groups, those that showed an effect with BPA showed a similar low dose effect with DES (e.g. prostate and uterus enlargement in mice), while those that showed no effect with BPA also found no effect with DES.” In many studies in which no statistically significant effect of low doses of BPA were reported, no positive control was included [155,156,159,160,166,180–185]. In particular, a positive control is the only way to distinguish whether an experiment has been compromised by the presence of an estrogenic contaminant in animal feed, water, or housing.

Three studies that concluded that there was no effect of low doses of BPA on the male [179,186] or on both the male and female [109] reproductive system in mice suffered from a lack of statistically significant effect of the positive control, DES. One study did report some effects at a relatively high (15 $\mu\text{g}/(\text{kg day})$) dose of DES and no significant effects at any dose of BPA [161]. As discussed previously, this high dose of DES is not a valid positive control in experiments that fail to show effects of low doses of BPA. In summary, experiments reporting only the absence of statistically significant effects of low doses of BPA that do not include a positive control, or that fail to show significant effects of the positive control, cannot be interpreted. Without positive control data obtained concurrently with the BPA data, there is no way to determine the reason that significant effects were not observed for any test chemical.

There are now numerous studies that have been published showing similar effects of low doses of BPA and DES, the most commonly used positive control for examining estrogenic effects of BPA. For many outcomes, BPA has been reported to have the same efficacy but a 100–1000-fold lower potency relative to DES [10,13–16,46,69,105,148,187–189].

There are some studies showing that, particularly for rapid responses mediated by receptors associated with the cell membrane [190–192], BPA and DES (as well as E2) have equal potency. This subject is covered in detail in the report from the panel concerned with molecular mechanisms. As discussed above, there is evidence that BPA is a SERM, and thus, while there are studies showing similar effects of BPA and endogenous E2 or estrogenic drugs such as DES, there are also studies showing qualitative differences in the responses of BPA and other estrogens.

4.5. *The importance of dose*

There have been many published *in vivo* and *in vitro* mechanistic studies providing examples of effects of BPA that are observed at a low dose, but are not observed at higher doses. This type of dose–response relationship is known as an inverted-U-shaped curve. In toxicology, it was long assumed that the only valid dose-related effect is a monotonic dose–response relationship. However, as toxicologists have investigated more sub-lethal endpoints and more extensive dose ranges, non-monotonic dose–response relationships have been encountered with increasing frequency. The molecular basis for such findings is beginning to be understood in terms of the stimulation and inhibition of unique sets of genes as one moves across the

dose–response curve [193,194]. These effects are likely to be mediated by different receptors [for estrogens, ER α , ER β , ERR γ and membrane-associated receptors [43,195,196], and both positive and negative feedback effects on estrogen receptors and other receptors [6,7]]. Several examples of unique effects of low doses of BPA have now been reported and are available for review [45,46].

5. Conclusions and levels of confidence for different outcomes

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

The criterion for an outcome being assessed as achieving this level (we are confident) is that multiple independent studies have shown the same or similar outcomes.

5.1.1. *Developmental effects on the brain and behavior*

There is extensive evidence for effects of exposure during critical periods to low doses of BPA on subsequent brain structure and chemistry, receptors for hormones and neurotransmitters, and behavior. We can thus state with confidence that low doses of BPA during development have persistent effects on brain structure, function and behavior in rats and mice.

It is likely that due to species and strain differences, effects of BPA on specific brain structures, functions and behaviors may show differences. However, some effects appear consistent. For example, a number of studies have shown that expected differences between males and females are not observed in animals exposed to BPA during development. In some experiments this outcome has been due to males showing a more feminine phenotype and females showing a more masculine phenotype [47,73], while in other experiments, the loss of the sex difference was due to a measurable change in only one sex [197]. Further research may reveal whether estrogenic effects dominate when low doses of BPA are administered and whether other effects of BPA such as antiestrogenic or antiandrogenic effects are likely to be observed in the presence of higher doses of BPA.

5.1.2. *Developmental effects on the male reproductive tract*

There is extensive evidence that BPA impacts the reproductive system in male rats and mice, although there appear to be species and strain differences in the sensitivity of specific outcomes to BPA. The evidence supports an effect on the testes, with subsequent changes in testosterone secretion and sperm production. Impacts on other reproductive structures have been reported in a number of independent studies, including the epididymis and epididymal sperm, prostate, and seminal vesicles. These findings are consistent with effects of low doses of positive control chemicals, such as DES and ethinylestradiol.

5.1.3. *Developmental effects on enzyme activity, growth and metabolism*

There is extensive evidence for “programming” effects of BPA on subsequent activity of enzymes in tissues and thus metabolic processes. An increase in postnatal growth rate due

to developmental exposure to low doses of BPA has been shown in many studies, and this finding is consistent with the effect of developmental exposure to a low, but not high, dose of DES.

5.1.4. Adult effects on the male reproductive tract

There is a significant amount of evidence that adult exposure to BPA has adverse consequences for testicular function in male rats and mice. This is not surprising as estrogen, while essential for normal epididymis function, has inhibitory effects on the brain–pituitary–gonadal axis in males, and it is well documented that elevated E2 inhibits spermatogenesis and testicular testosterone secretion [1].

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

The criterion for achieving this 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.

5.2.1. Developmental effects on the female reproductive tract

There is extensive evidence for effects of BPA on development of the mammary gland. Studies in both rats and mice have shown effects of developmental exposure to BPA on mammary gland morphology that may predispose animals to develop cancer. These findings have not yet been repeated in multiple independent laboratories.

5.2.2. Adult effects on the brain and behavior

There are a number of studies that have found a variety of significant outcomes of adult exposure to BPA. Given the more extensive literature showing developmental effects, there is no reason to expect that adult effects will not also occur. However, effects in the adult may require higher doses of BPA exposure or longer periods of BPA exposure.

An important unanswered question and research need is whether long-term adult exposure to BPA may have adverse consequences, as has been recently found to be the case for hormone replacement therapy (HRT), which is now thought to increase the risk for a number of diseases [198]. The finding that BPA can stimulate proliferation of human cancer cells in the absence of androgen is also cause for concern.

5.2.3. Adult effects on the female reproductive tract

While a number of studies have found significant effects of BPA on the female reproductive system, not as much research has been conducted as for the male reproductive system. For example, effects on meiosis in oocytes need to be confirmed by additional studies. The recent report that maternal exposure to a very low dose of BPA also disrupts meiosis in the embryonic oocyte during formation of the primary follicles adds to our concern.

5.2.4. Adult effects on the immune system

There is extensive evidence that BPA modulates both T helper 1 and T helper 2 cytokine production and alters antibody production.

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