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Evidence for Bisphenol B Endocrine Properties: Scientific and Regulatory Perspectives

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Background: The substitution of bisphenol A (BPA) by bisphenol B (BPB), a very close structural analog, stresses the need to assess its potential endocrine properties.

Objective: This analysis aimed to investigate whether BPB has endocrine disruptive properties in humans and in wildlife as defined by the World Health Organization (WHO) definition used in the regulatory field, that is, *a*) adverse effects, *b*) endocrine activity, and *c*) plausible mechanistic links between the observed endocrine activity and adverse effects.

Methods: We conducted a systematic review to identify BPB adverse effects and endocrine activities by focusing on animal models and *in vitro* mechanistic studies. The results were grouped by modality (estrogenic, androgenic, thyroid hormone, steroidogenesis-related, or other endocrine activities). After critical analysis of results, lines of evidence were built using a weight-of-evidence approach to establish a biologically plausible link. In addition, the ratio of BPA to BPB potency was reported from studies investigating both bisphenols.

Results: Among the 36 articles included in the analysis, 3 subchronic studies consistently reported effects of BPB on reproductive function. In rats, the 28-d and 48-week studies showed alteration of spermatogenesis associated with a lower height of the seminiferous tubules, the alteration of several sperm parameters, and a weight loss for the testis, epididymis, and seminal vesicles. In zebrafish, the results of a 21-d reproductive study demonstrated that exposed fish had a lower egg production and a lower hatching rate and viability. The *in vitro* and *in vivo* mechanistic data consistently demonstrated BPB's capacity to decrease testosterone production and to exert an estrogenic-like activity similar to or greater than BPA's, both pathways being potentially responsible for spermatogenesis impairment in rats and fish.

Conclusion: The available *in vivo*, *ex vivo*, and *in vitro* data, although limited, coherently indicates that BPB meets the WHO definition of an endocrine disrupting chemical currently used in a regulatory context. https://doi.org/10.1289/EHP5200

Introduction

Since the 1960s, bisphenol A (BPA) has been widely used in the production of a variety of polymers such as polycarbonate plastics, epoxy resins, or thermal papers and is therefore found in a wide range of consumer products, including plastics, receipts, and food packaging (ANSES 2011). Over the last decades, concerns on reproductive, metabolic, and developmental effects have led regulatory bodies worldwide to ban BPA from baby bottles (Government of Canada 2010; EC 2011). Further restrictions have been implemented for BPA's use in food packaging (EC 2018) and in thermal papers (EC 2016a). In 2017, BPA was recognized as an endocrine disrupting chemical (EDC) and a substance of very high concern (SVHC) in the European Union (EU) for both human health (ECHA 2017a) and for the environment (ECHA 2017b), limiting its importation and use on the European market. To meet the regulatory agencies' restrictions on BPA uses, the plastics industry has gradually replaced this substance with some structural analogs, although many voices have questioned whether these substitutes are indeed safer than BPA (Gao et al. 2015; Eladak et al. 2015; Kinch et al. 2015). Concern on some widely used substitutes have been substantiated, such as bisphenol S and bisphenol F (reviewed by Rochester and Bolden 2015), leading to further regulatory evaluation of their endocrine properties in the EU (ECHA 2018c). However, the health and environmental hazards of many other BPA analogs have not been addressed so far, albeit their endocrine activity might be similar to that of BPA (NTP 2017; Perez et al. 1998).

Bisphenol B (BPB) shares a strong structural similarity with BPA. It differs from BPA only by an additional methyl group on the central carbon (Figure 1). BPB is identified in The Endocrine Disruptor Exchange list (TEDX 2018) of potential EDCs, and *in vitro* results of the U.S. EPA Endocrine Disruptor Screening Program (EDSP; U.S. EPA 2018) indicate an agonist activity toward the estrogen receptor (ER). BPB is currently registered by the U.S. Food and Drug Administration (FDA) as an indirect food additive used in food-contact resinous and polymeric coatings (FDA 2018) but not in the EU under the European regulation on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH; ECHA 2018b). This means that BPB is produced or put on the European market at <1 ton/y. Therefore, no EU registrant has a legal obligation to produce toxicological or ecotoxicological data (EC 2006). Nevertheless, BPB has been detected in several European food products such as various canned foods (Cunha et al. 2011; Grumetto et al. 2008; Fattore et al. 2015; Alabi et al. 2014) and in commercial milk samples (Grumetto et al. 2013).

Compared with BPA, there is limited data on human exposure levels to BPB. The biomonitoring data indicate that BPB was detected in the same order of magnitude to BPA in the urine of Portuguese volunteers (Cunha and Fernandes 2010) and in the serum of endometriotic women in Italy (Cobellis et al. 2009),

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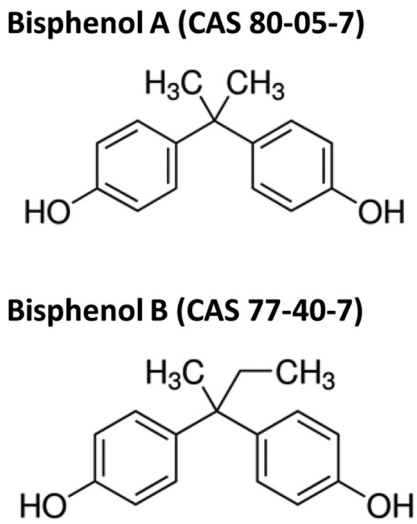

Figure 1. Chemical structures of bisphenol A (BPA) and bisphenol B (BPB).

although in a lower percentage of individuals screened. In contrast, BPB was not detected in urine samples of Australian pregnant women (Heffernan et al. 2016), nor of Norwegian mother–child pairs (Sakhi et al. 2018) or Chinese residents (Yang et al. 2014a). In the environment, BPB is one of the least investigated and detected bisphenols (reviewed by Noszczyńska and Piotrowska-Seget 2018). Mean total BPB concentrations of 2.5 ng/L and 8.46 ng/L were measured in municipal sewage treatment plants (STP) influents in India (Karthikraj and Kannan 2017) and in industrial STP effluents in Slovenia (Česen et al. 2018), respectively. BPB was quantified in 1 sediment sample in Korea at 10.6 ng/g of 172 samples collected in Japan, Korea, and the United States (Liao et al. 2012). BPB was not detected in surface water in Japan, Korea, or India (Yamazaki et al. 2015) or in different areas of China such as the Liaohe River basin (Jin and Zhu 2016), Beijing (Yang et al. 2014b), and the Jiuxiang river in Nanjing (Zheng et al. 2015). It was also not detected in the Taihu water source up to 2016 (Wang et al. 2017; Jin and Zhu 2016); however, two recent studies reported its quantification in almost all water and sediment samples of the same Chinese lake with mean concentrations in the low nanograms per liter (water) or nanograms per gram (sediment) (Yan et al. 2017; Liu et al. 2017). Information on BPB levels in European freshwater ecosystems is currently lacking.

There are currently no mandatory regulatory requirements to assess the endocrine properties of industrial chemicals such as BPB. The challenge posed by BPA's analogs lies in assessing their endocrine disrupting potential based on the available toxicological data. This is of particular importance to avoid industrial investment in unsafe substitutes and to prevent human and environmental health consequences. The World Health Organization (WHO) defines an EDC as "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" (Damstra et al. 2002). This definition, which is also the basis of the EU criteria for EDC (EC 2016b; Slama et al. 2016), involves three elements that must be identified concomitantly: an adverse effect, a modulation of endocrine functions, and a plausible mechanistic link between the endocrine activity and the adverse effect. The relationship between these keystones necessary to identify an EDC has been long studied and debated in Europe (Munn and Goumenou 2013). In 2018, the European Commission published a guidance (EDC guidance; ECHA and EFSA 2018) based on the WHO and International Programme on Chemical Safety (WHO/IPCS) definition and the Organisation for Economic Co-operation and Development (OECD) conceptual framework for testing and assessment of endocrine disrupters (guidance document no. 150; OECD 2018). First developed to support the regulatory requirement to identify and regulate EDCs covered by the plant protection products and the biocidal products regulations, this EDC guidance provides a unique methodological approach to evaluate endocrine properties. Integrating these methodologic reflections, the objective of this work was to perform a systematic review of the existing scientific literature to assess BPB endocrine disruptive properties according to the WHO/IPCS definition, cconsidering both human health and wildlife.

Methods

Context

As part of the French National Strategy on Endocrine disruptors (Ministries of Health and Ecological Transition 2014), BPB has been put on the list of compounds to be evaluated by the dedicated group of experts on EDC [i.e., the French Agency for Food, Environmental and Occupational Health Safety (ANSES) EDC working group]. This collective expert assessment undertaken at our agency enabled transparent and multidisciplinary discussions and debates on scientific data and regulatory decisions (see https://www.anses.fr/en/content/expert-committees-and-working-groups for more information). We conducted a systematic review on BPB endocrine disruptive properties by focusing on animal and *in vitro* mechanistic studies, but also on human epidemiological and case studies. However, biomonitoring and *in silico* data were not included in the review. This analysis was performed following the principles displayed in the EDC guidance developed by the European Chemical Agency (ECHA) and the European Food Safety Authority (EFSA), with the support of the Joint Research Centre (JRC), recently published to identify EDC under the plant protection products and the biocidal products regulations (ECHA and EFSA 2018). The EDC guidance provides a tiered approach to assess the adversity of chemicals on vertebrates, and to link it with an estrogenic (E), androgenic (A), thyroid hormone (T), or steroidogenesis-related (S) mode of action (the so-called EATS modalities). The evidence is first assembled by using a systematic review and weight-of-evidence approach. Then, the EATS-mediated adversity and the endocrine activity are assessed. If sufficient evidence is gathered, a mode of action is postulated and the plausible biological link discussed. The detailed methodology is presented in the following sections.

Research Question

A Population, Exposure, Comparator and Outcome (PECO) statement was developed to answer the question "Do the BPB endocrine properties meet the WHO definition of an endocrine disruptor?" (Table 1). The systematic review focused on studies investigating BPB effects for several levels of doses or concentrations, in *in vitro*, *ex vivo*, and experimental vertebrate models because they are relevant for human (mammals such as dogs, rodents, rabbits) and wildlife (e.g., fish, amphibians, birds, and reptiles), as well as human epidemiological and case studies, when available.

Search Design and Data Collection

The systematic searches were performed on 5 September 2018 in PubMed and Scopus databases without limitations on year of publication. We applied a single concept strategy search to

Table 1. Population, Exposure, Comparator and Outcome (PECO) key information: definition and associated search terms.

	Definition
Population	*In vitro*, *ex vivo*, and experimental animal studies on vertebrates relevant for human health (e.g., mammals such as dogs, rodents, and rabbits) and wildlife (e.g., fish, amphibians, birds, reptiles) and human epidemiological and case studies [as defined in the EDC guidance (ECHA 2018c)][a]
Exposure	Bisphenol B (CAS 77-40-7)
Comparator	Exposed groups vs. vehicle-treated controls
Outcome	Chemically induced endocrine activity or adverse effects related to EATS modalities (e.g., testis weight, hormone levels), or not specific to EATS modality (e.g., fertility)

Note: CAS, Chemical Abstracts Service; EATS, estrogenic, androgenic, thyroid hormone, and steroidogenesis-related; EDC, endocrine disrupting chemical.
[a]Human and wildlife biomonitoring studies and *in silico* data were not included.

retrieve all relevant information on BPB by using its Chemical Abstracts Service Registry Number (CASRN; CAS 77-40-7), scientific chemical names, and common names (e.g., "bisphenol derivative" or "bisphenol substitute"), as recommended in the EDC guidance (ECHA and EFSA 2018). The literature search strategy is presented in Table S1.

Studies were included in this systematic review when they met all of the following criteria: *a*) peer-reviewed research articles or primary reports of research findings that presented original data; *b*) exposure to one or various BPB doses; *c*) endocrine activity or adversity assessed in *in vitro, ex vivo*, or *in vivo* studies in vertebrate species (Table 1); and *d*) English-language articles. Accordingly, the exclusion criteria were as follows: *a*) no original data (e.g., review article) or abstract only, *b*) lack of exposure to BPB; *c*) lack of measurement of endocrine activity or adversity; *d*) *in silico* data, human or environmental biomonitoring studies; and *e*) full text not available in English. The relevance filtering was first based on title and abstract screening, and, second, on full-text screening. When checking title and abstract was insufficient to decide if the paper was relevant and should be included in the review, full-text screening was applied (e.g., BPB not explicitly mentioned in the abstract). Two reviewers (C.B. and H.S.) shared the two screening phases, and resolved any conflicts or discrepancies by complementary full-text screening and by discussion.

In addition to the systematic literature search and screening, ToxCast (Chen et al. 2017) and EDSP (U.S. EPA 2018) databases were queried for BPB bioactivity results using the CASRN to identify high-throughput *in vitro* screening assays that measured endocrine activity. The endocrine activity of each assay was defined by modality, that is, estrogenic, androgenic, thyroid hormone, or steroidogenesis-related (i.e., EATS) or non-EATS (others) endocrine activity based on selected criteria presented in Figure 2. Cross references of peer-reviewed research articles and gray literature (e.g., reports by national agencies) were also included in the review.

Initial Analysis of the Results

The following information was extracted from studies included in the review: author names, publication year, study design (biological model, type of treatment, exposure duration, range of concentrations tested) and the response observed. In addition, the ratios of BPA to BPB half maximal effective concentration (EC_{50}) or half maximal inhibitory concentration (IC_{50}) was reported from studies investigating both bisphenols to allow comparison of potency. Results on endocrine activity were grouped by EATS or non-EATS (others) modalities. In the next step, the data were grouped into three categories following OECD conceptual framework (OECD 2018) and EU EDC guidance (ECHA and EFSA 2018): *a*) *in vitro* mechanistic parameters (OECD Level 2); *b*) *in vivo* mechanistic parameters (OECD Level 3); and *c*) parameters providing information on adversity (OECD Levels 3, 4, and 5). OECD Level 2 and 3 data are mainly informative of endocrine activity, whereas Level 4 and 5 data provide information on adversity. All the results were combined by modality and parameter categories. Based on the adverse effects identified, results were further integrated into lines of evidence, defined as a "set of relevant information grouped to assess a hypothesis," using a weight-of-evidence approach (ECHA and EFSA 2018).

Assessment of the Evidence

Evaluation of study quality was performed using the Toxicological data Reliability Assessment Tool (ToxRTool) for all studies investigating adverse effects and for the mechanistic studies included in the lines of evidence (Schneider et al. 2009). The tool

EATS endocrine activity parameters	Non-EATS (others) endocrine activity parameters
Androgen receptor (AR)	Adiponectin
Estrogen receptor (ER α/β) and ER-related processes (e.g. cell proliferation of ER-expressing cells)	Aryl hydrocarbon receptor (AhR)
Estrogen receptor related (ERR)	Constitutive androstane receptor (CAR)
G-coupled estrogen receptor (GPER)	CYP1A, CYP3A4, CYP2A13
Hormones involved in hypothalamic-pituitary gonadal (HPG) axis (LH, FSH, GnRH)	Farnesoid X receptor (FXR)
Hormones involved in the steroidogenesis	Glucocorticoid receptor (GR)
Enzymes involved in the steroidogenesis	Insulin and insulin receptor
Thyroid hormones/ receptor (TR) and TR-related processes	Leptin
Thyroid peroxidase (TPO)	Liver X receptor (LXR)
	Oxytocin and oxytocin receptor
	Peroxisome proliferator-activated receptor (PPAR)
	Pregnane X receptor (PXR)
	Progesterone receptor (PR)
	Progesterone receptor like (PR-l), Prolactin
	Retinoic acid receptor (RXR)
	Sex-hormone-binding globulin (SHBG)
	Vitamin D receptor (VitD R)

Figure 2. Estrogenic, androgenic, thyroid hormone, and steroidogenesis-related (EATS) and non-EATS (others) endocrine activity parameters selected for the systematic review analysis. The parameters were selected based on recommendations of the endocrine disrupting chemical (EDC) guidance (ECHA and EFSA 2018) and of the French Agency for Food, Environmental and Occupational Health Safety (ANSES) EDC working group.

comprises 21 criteria for *in vivo* studies and 18 criteria for *in vitro* studies that cover information on the test substance, the test system, the study design, results, and plausibility of results. All criteria are answered either by 0 or by 1, and some selected criteria are deemed indispensable for evaluating the reliability of the study, namely: information on the identity and purity of the test substance, concentrations/doses tested, frequency and duration of exposure, time point of observation, species studied, inclusion of negative and positive controls, administration route, number of animals per group, and adequacy of the study design. The total number of criteria met enables the assignment of Klimisch Categories 1 (reliable without restrictions), 2 (reliable with restrictions), or 3 (not reliable) (Klimisch et al. 1997). The limitations of *in vitro* and *in vivo* studies identified were reported along with the results of the systematic review. All relevant studies were included and when the reliability was questionable (i.e., ToxR score of 3), the limitations were discussed as part of the weight-of-evidence approach. Teams of regulators and researchers of the EDC working group with relevant expertise in the field assessed *in vivo* studies investigating BPB adverse effects and discussed the biological link for the mode of action postulated (R.H., Ce.M., and C.B. for human health data, N.P.H., Ch.M., and H.S. for environmental data).

Results

The systematic search and screening resulted in the identification of 494 unique documents that described studies of BPB in experimental animals, and in *ex vivo* or *in vitro* models as presented in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart (Figure 3). This included 484 articles from PubMed and Scopus databases and 10 documents identified by screening cross-references and gray literature. After title, abstract, and full-text screening, 36 articles fulfilled the inclusion criteria according to the PECO statement. The relevant studies are listed in Excel Table S1, and the detailed results from literature searches of all databases are provided in Excel Tables S2–S5. The majority of studies focused on BPB mechanistic effects, and only 3 studies investigated BPB adverse effects in intact organisms. An overview of the *in vitro* and *ex vivo* mechanistic information is presented in Figure 4. There were 801 assay results on BPB bioactivity available in the ToxCast database, among which, 132 met the definitions of EATS and non-EATS (others) endpoints (Figure 2; see also Excel Table S3). In addition, 33 bioactivity results retrieved from EDSP database on E, A, and T modalities were included in the analysis (see Excel Table S4).

Adverse Effects of BPB

Three recent *in vivo* studies identified adverse effects of BPB in vertebrates: two studies in rats from the same laboratory (Ullah et al. 2018a, 2018b) and one in zebrafish (Yang et al. 2017). The line of evidence for BPB adverse effect is presented in Table 2.

BPB and the male reproductive system in rodents. The two studies on male rats were conducted to compare the ability of several bisphenols (including BPA and BPB) to disturb male reproductive function (Ullah et al. 2018a, 2018b). In these papers, limitations in the description of the experimental procedure were identified. The modalities of the oral administration method performed was missing in the paper by Ullah et al.

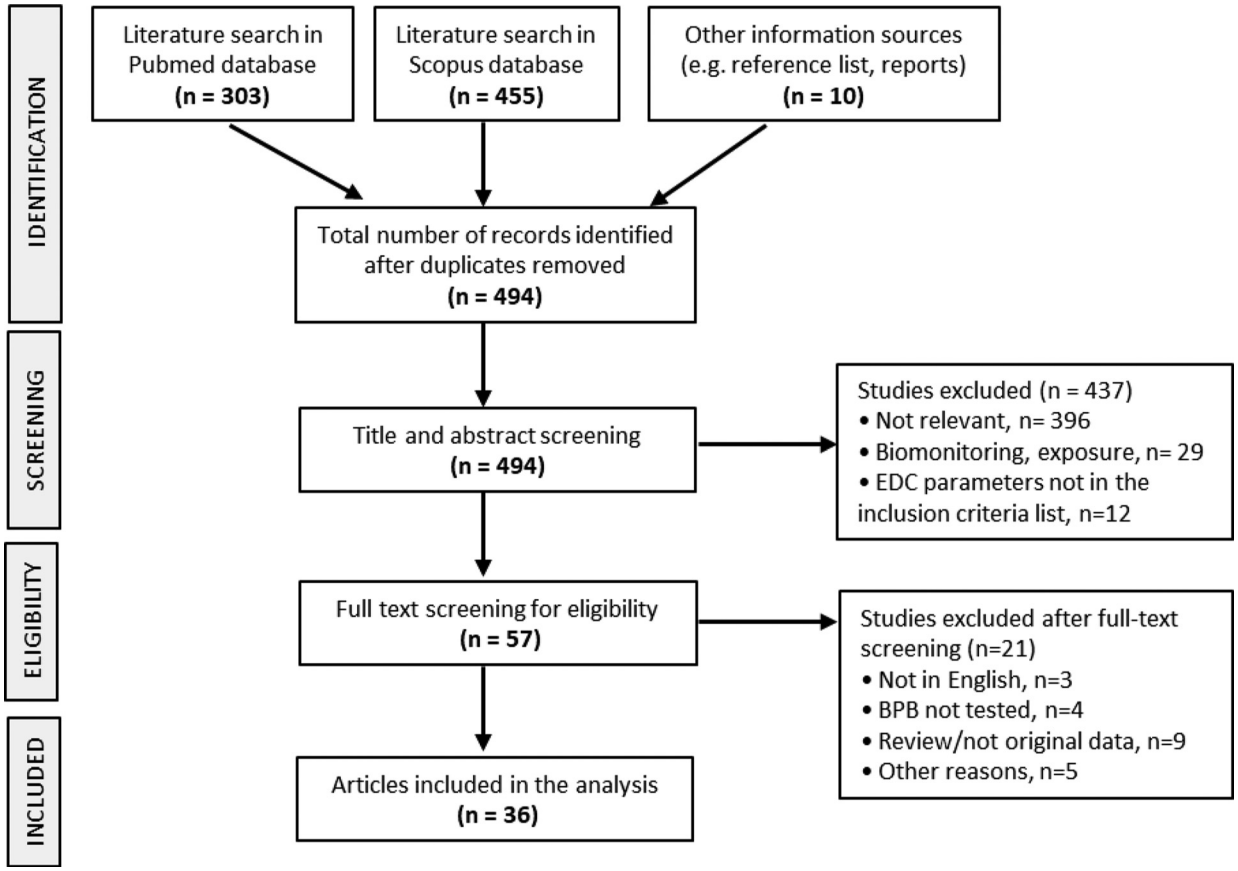

Figure 3. PRISMA flow diagram followed for studies selection. BPB, bisphenol B; EDC, endocrine disrupting chemical; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

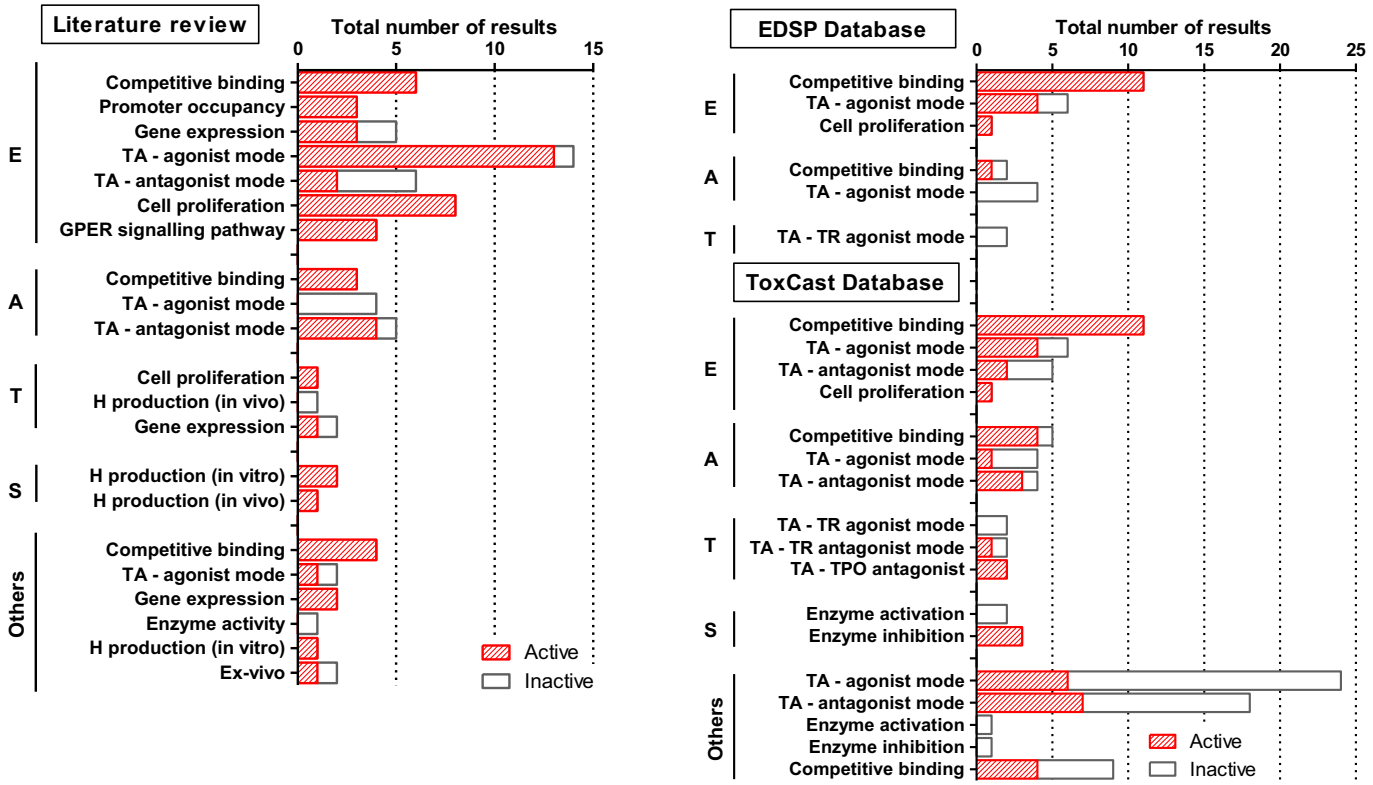

Figure 4. Summary of *in vitro* and *ex vivo* endocrine activity results identified from (A) literature search and (B) database screening. The results are classified by modality [estrogenic, androgenic, thyroid hormone, and steroidogenesis-related (EATS), or others non-EATS] and end point category. (All the data are presented in Excel Tables S2–S4.) The total number of results refers to the number of experiments investigating a given end point (e.g., several end points may have been investigated within a given study). A, androgenic; E, estrogenic; H, hormone; S, steroidogenic; T: thyroidal; TA, transactivation assay; TPO, thyroid peroxydase; TR, thyroid hormone receptor.

(2018a), and the histopathological evaluation was not sufficiently described. A low number of animals per group was used by Ullah et al. (2018b), and information on the sensitivity of hormonal assays and the number of replicates were lacking. Information on the CASRN and purity of the test chemical were not mentioned in either of the two papers by Ullah et al. (2018a, 2018b). Although to be taken with caution, these studies present a consistent set of data and were included in the weight-of-evidence approach.

In the first study, male Sprague-Dawley rats (70–80 postnatal days [PND 70–80] of age) were orally exposed to BPB at 0, 5, 25, and 50 mg/kg BW/d (7 animals/group) for 28 d (Ullah et al. 2018a). Effects on the testis morphology were evidenced with exposed rats exhibiting a statistically significant lower height of the seminiferous epithelium (−19%) compared with concurrent controls. The qualitative histological evaluation of the testis showed that exposed animals had fewer spermatids and sperm in the lumen of the seminiferous tubules compared with concurrent control animals, with only very few tubules and no elongated spermatids at the dose of 50 mg/kg BW/d.

In a follow-up study, Ullah et al. (2018b) reported a more documented analysis of the effects of a chronic exposure to low dose of BPB on testicular functions. PND-23 male Sprague-Dawley rats received drinking water containing 0, 5, 25, and 50 μg/L BPB for 48 weeks. A daily intake of 0, 0.3, 1.5, and 3 μg/kg BW/d can be roughly estimated from an average water daily intake of 6 mL/d per 100 g BW. However, it must be noted that the ingested BPB dose decreased over time given that the rats drink 10–14 mL/d per 100 g BW at PND 23 and 2–3 mL/d per 100 g BW at postnatal week 46 (Holdstock 1973). At the end of the treatment, rats exposed to 50 μg/L BPB had a statistically significant lower relative weight of the testis, epididymis, and the seminal vesicle. Effects on sperm parameters were evidenced with a dose-dependent smaller daily sperm production statistically significant at 50 μg/L (reduction of 9%) and a lower sperm number in the caput epididymis (statistically significant from 25 μg/L onward) and in the cauda epididymis (statistically significant at 50 μg/L). In the cauda epididymis of rats exposed at 50 μg/L, sperm number and the motile sperm percentage were statistically significantly lower, whereas the viable sperm percentage remained similar to control levels. In this group, rats exhibited a statistically significantly lower height of seminal epithelium (−16%), without differences in the diameter and the relative area of seminiferous tubules. In addition, rats in the high-dose group had statistically significantly fewer spermatogonia, spermatocytes, and spermatids. Taken together, these data evidenced that a chronic exposure to BPB at low doses through drinking water altered the testis function of adult rat. Importantly, BPA response was assessed in the two papers by Ullah et al. (2018a, 2018b), and BPA and BPB had similar qualitative and quantitative effects.

BPB adverse effects on fish reproduction. Yang et al. (2017) reported the results of a high quality and reliable fecundity study on zebrafish (*Danio rerio*) based on the OECD 229 and 230 technical guidelines with additional evaluation of endocrine parameters. Six 4-month-old male and six 4-month-old female zebrafish were exposed over 21 d to BPB at concentrations of 0, 0.001, 0.01, 0.1, and 1 mg/L (nominal concentrations). The study showed that zebrafish exposed to BPB had a dose-dependently impaired reproductive function (i.e., male and female), evidenced by a lower number of eggs laid, and a smaller hatching rate and embryo survival, reaching statistical significance in the high-dose

Table 2. Line of evidence for BPB reproductive dysfunction in fish and male rats.

End point	Biological model	Exposure	Parameter	Effect dose	ToxR score[a]	Reference
Testes histology	Male SD rats	28 d	[b]	50 mg/kg BW/d: fewer secondary spermatocytes, tubules, and elongated spermatids in the lumen	1	Ullah et al. 2018a
Testes histology	Male SD rats	48 weeks	LOAEL	0.025 mg/L: fewer spermatogonia, spermatocytes, and spermatids number	3	Ullah et al. 2018b
Testes histology	Male zebrafish	21 d	[b]	1 mg/L: alteration of testis tubules, decrease of mature spermatids	1	Yang et al. 2017
Sperm parameters	Male SD rats	48 weeks	LOAEL	0.025 mg/L: lower sperm number in the caput epididymis 0.05 mg/L: lower sperm motility, daily sperm production, sperm number in the cauda epididymis No differences in the amount of viable sperm	3	Ullah et al. 2018b
Testes histology, seminiferous tubules	Male SD rats	28 d	LOAEL	50 mg/kg BW/d: lower epithelial height No difference in the area of interstitium, nor on diameter of seminiferous tubule	1	Ullah et al. 2018a
Testes histology, seminiferous tubules	Male SD rats	48 weeks	LOAEL	0.05 mg/L: lower epithelial height No difference in the area of interstitium, nor on diameter of seminiferous tubule	3	Ullah et al. 2018b
Gonado–somatic index	Male SD rats	48 weeks	LOAEC	0.05 mg/L: lower	3	Ullah et al. 2018b
Gonado–somatic index	Male zebrafish	21 d	LOEC	1 mg/L: lower	1	Yang et al. 2017
Gonado–somatic index	Female zebrafish	21 d	LOEC	1 mg/L: lower	1	Yang et al. 2017
Hepato–somatic index	Male zebrafish	21 d	LOEC	0.1 mg/L: higher	1	Yang et al. 2017
Hepato–somatic index	Female zebrafish	21 d	LOEC	0.1 mg/L: higher	1	Yang et al. 2017
Fecundity	Adult zebrafish	21 d	LOEC	1 mg/L: lower	1	Yang et al. 2017
Hatching rate F1 generation)	Adult zebrafish	21 d	LOEC	1 mg/L: lower	1	Yang et al. 2017
Survival (F1 generation)	Adult zebrafish	21 d	LOEC	1 mg/L: lower	1	Yang et al. 2017

Note: BPB, bisphenol B; gonado–somatic index, [gonad weight/body weight] × 100; hepatosomatic index, [liver weight/body weight] × 100; LOAEC, lowest observed adverse effect concentration; LOAEL, lowest observed adverse effect level; LOEC, lowest observed effect concentration; SD, Sprague-Dawley.
[a]ToxR score refers to the study quality using Klimisch category (1, 2, or 3), which was assessed using the ToxRTool, which considers the test substance, test system, study design, results, and plausibility of results (Schneider et al. 2009).
[b]Qualitative assessment only, without statistical analyses reported.

group (reduction of about 50% compared with concurrent control animals). Some malformations of the F1 generation (e.g., abnormal curvature of larvae) were also reported in this group. Exposed male and female zebrafish had a statistically significantly higher hepato-somatic index in the 0.1 and 1 mg/L-exposure groups, and a statistically significantly lower gonado–somatic index in the 1 mg/L-exposure group. At 0.1 and 1 mg/L, the authors reported a histological testicular disorganization with the presence of an acellular area and a trend toward fewer mature spermatids, although not quantified. In the females, one fish exposed to 1 mg/L lacked post-vitellogenic oocytes.

BPB Endocrine Activity

All 36 studies included in the review provided *in vitro* or *ex vivo* and *in vivo* mechanistic information on BPB potential to interact with the endocrine system by modulating estrogenic, androgenic, steroidogenesis-related, thyroid hormone or others endocrine activities (see Excel Tables S2–S5). As illustrated in Figure 4, most of the studies focused on BPB estrogenic activity. For instance, 46 *in vitro* results for the E modality were gathered in the systematic review, whereas the other endocrine activities remained significantly less investigated (12, 4, 3, and 12 results for the A, T, S, and other modalities, respectively). In agreement with the WHO/IPCS definition, an endocrine activity must be identified to support the adverse effect, that is, the alteration of male reproductive function in rats and fish by BPB. Consistent with this definition of an adverse effect, only the acknowledged key factors regulating male reproductive function and spermatogenesis are discussed below: modulation of estrogenic, androgenic, and steroidogenesis-related endocrine activities.

Estrogenic endocrine activity. The *in vitro* estrogenic activity of BPB was investigated in 22 studies included in this systematic review and in many assays of ToxCast and EDSP databases (see Excel Tables S2–S4). The line of evidence for BPB estrogenic activity considering both *in vitro* and *in vivo* mechanistic data is presented in Table 3 and summarized in Figure 4. BPB competitively bound to ER of several species including human, rat and mouse (Sipes et al. 2013; Blair et al. 2000; Zhang et al. 2018). In the *in vitro* studies using hERα transactivation assays, the reported EC_{50} values ranged between 0.59 and 5 μM in yeast-based reporter assays and between 0.07 and 0.3 μM in vertebrate reporter cell lines. No anti-estrogenic activity was reported in ERE-promoters and luciferase transgene assays (Wang et al. 2014; Kitamura et al. 2005; Okazaki et al. 2017). Only one study testing a single concentration of BPB (10 μM) during a short exposure period of 30 min in human cells expressing the green fluorescent protein (GFP) reporter gene under the control of a prolactin promoter reported an anti-estrogenic activity (Stossi et al. 2014). The estrogenic activity of BPB was confirmed in human mammary MCF-7 cell lines expressing ERα by the induction of ER-regulated gene expression, such as *pS2* and progesterone receptor (*PgR*) (Rivas et al. 2002; Mesnage et al. 2017). Exposure to BPB resulted in a higher promoter occupancy of prolactin gene, with hERβ having a stronger array occupancy compared with hERα (Stossi et al. 2014; Ashcroft et al. 2011). In addition, human cells exposed to BPB had a higher expression of genes involved in hormone-induced proliferative effects and in pathogeny of breast cancer (Mesnage et al.

2017). The proliferative effect of BPB was confirmed in ER-positive human breast cancer cell lines (Pisapia et al. 2012; Mesnage et al. 2017; Stossi et al. 2014; Rivas et al. 2002; Hashimoto et al. 2001). Interestingly, Cao et al. (2017) showed in a recent study that BPB bound with a higher relative binding affinity the extragenomic human G-coupled protein ER (hGPER) (8.8% compared with estradiol) than the hER (<1%). Activation of hGPER signaling in breast cancer cells lead to a statistically significantly higher calcium mobilization and cAMP production in cells treated with 10 nM BPB, and further favored cell migration (Cao et al. 2017). Pretreatment of the cells with the hGPER-selective inhibitor G15 abolished these responses, confirming the hGPER-mediated effects of BPB. Many studies investigated BPA and BPB concomitantly, which allowed us to perform a direct comparison of potency in each study by calculating the ratio of EC_{50} (or IC_{50}) of BPA to BPB. Overall, 20 results reported in 15 different papers compared the *in vitro* estrogenic activity of BPA and BPB (Table 3). When considering all these results, the ratios between BPA potency and that of BPB ranged from 0.7 (Rosenmai et al. 2014) to over 100 (Cao et al. 2017), with a median of 4. This result indicates that, in most assays, BPB potency should be considered similar to or even greater than that of BPA.

In addition to the *in vitro* findings, three studies provided *in vivo* information on BPB estrogenic activity. In an uterotrophic assay, immature Crj:CD Sprague-Dawley rats treated subcutaneously with 200 mg/kg BW/d BPB from PND 20 to PND 22 had more watery uterine content and a greater blotted uterine weight, which suggests that BPB has an estrogeno-mimetic activity (Yamasaki et al. 2002). BPB estrogenic activity was also observed in the 4-month old male medaka exposed to BPB (0.5, 5, and 50 μM) for 8 h (Yamaguchi et al. 2015). In fish exposed from 5 μM, the authors reported a statistically significantly higher expression of the hepatic estrogen-responsive genes vitellogenin-1 (*vtg1*), choriogenin-L (*ChgL*) and ERα. Interestingly, the lowest observed effect concentration (LOEC) observed for BPB (5 μM) was lower than that obtained with BPA (50 μM). Last, a higher expression of hepatic vitellogenin protein was observed in male zebrafish exposed for 21 d from 0.1 mg/L along with a dose-dependent higher RNA expression of ERα (LOEC of 0.01 mg/L) and ER-regulated *cyp19a1b* (LOEC of 0.001 mg/L) in the exposed male brain (Yang et al. 2017).

Androgenic and anti-androgenic endocrine activity. Compared with the estrogenic activity, there are fewer results on BPB androgenic and anti-androgenic properties. The line of evidence for BPB anti-androgenic activity is presented in Table 4. BPB binding capacity to the androgen receptor (AR) has been investigated in the study by Fang et al. (2003) and in several assays included in the ToxCast database (e.g., Sipes et al. 2013). The results indicated that BPB competitively bound AR of human, rat, and chimpanzee with IC_{50} values from 2.2 μM to 36.65 μM. In transactivation assays, no agonist activity was evidenced, except for one positive hit in the ToxCast database, which had a very high EC_{50} value (0.1 mM), raising the question of the biological relevance of this isolated result. On the contrary, BPB had AR antagonistic activity in almost all vertebrate and yeast reporter gene assays with IC_{50} values ranging from 0.93 μM to 64.24 μM (Kitamura et al. 2005; Rosenmai et al. 2014; U.S. EPA 2018; Wang et al. 2014; Conroy-Ben et al. 2018). Overall, the ratio of BPA to BPB IC_{50} for all six *in vitro* findings presented in Table 4 ranged between 1 and 3.9, with a median of 2.1, indicating similar activities between both bisphenols.

Yamasaki et al. (2003) studied the *in vivo* androgenic and anti-androgenic properties of BPB in a Hershberger assay using castrated male rats exposed for 10 consecutive days. BPB administered alone did not exhibit androgenic properties at dose levels from 50 to 600 mg/kg BW/d. A statistically significant anti-androgenic effect was observed in one endpoint (bulbocavernosus/levator ani muscle), whereas there were no differences in the four other examined androgen-dependent sexual organs in the 200 and 600 mg/kg BW/d-exposure groups. In contrast, surprisingly, co-exposure of castrated rats to BPB and testosterone propionate (TP) resulted in a higher ventral prostate weight from the dose of 200 mg/kg BW/d, and a higher weight of all the five androgen-dependent targets at the highest dose, as compared with TP alone. These results suggest that BPB could induce either a higher TP internal exposure or activity. In addition, this effect would be specific to BPB, given that it was not observed with BPA.

Steroidogenesis-related endocrine activity. The line of evidence for BPB steroidogenesis-related activity is presented in Table 5 and includes steroidogenic enzyme activity but also steroid hormones levels, levels of precursors of steroid hormones, gene transcripts involved in steroidogenesis, and hormones regulating the steroidogenesis. *In vitro* data on BPB steroidogenesis-related activity are limited to two studies using H295R assays (Wang et al. 2014; Rosenmai et al. 2014) and one ToxCast assay result (see Excel Table S3). Both studies on H295R cells showed that cells exposed to BPB in the low micromolar range had lower androstenedione, testosterone, and cortisol levels and higher estrone levels. A higher estradiol concentration was observed in only one study (Rosenmai et al. 2014). In addition, differences in testosterone and cortisol precursor levels were noticed in both studies. Interestingly, BPA had similar effects as BPB on most investigated hormone concentrations (such as testosterone, androstenedione, cortisol, and estrone), except for 17-hydroxy progesterone. Cells treated with BPB had lower 17-hydroxy progesterone levels, whereas BPA exposure resulted in a higher hormonal level. *In vitro*, *cyp19a1* gene expression in the aromatase inhibition assay was lower after BPB exposure, but at relatively high concentrations (IC_{50} of 44.27 μM), well above the cytotoxicity limit (6.03 μM), raising the question of the relevance of the result (see Excel Table S3). In an *ex vivo* study, adult human testes explants were exposed to BPB for 24 or 48 h (Desdoits-Lethimonier et al. 2017), and inconstant lower testosterone secretions were observed. However, these data must be interpreted with caution because of the high variability of the results and the limited number of independent experiments.

Importantly, the abovementioned *in vivo* assays performed in rodents and fish reported steroidogenic effects of BPB consistent with the *in vitro* findings. Exposure of male rats to BPB by the oral route resulted in a statistically significant lower intratesticular testosterone concentrations at all doses, although not dose related (Ullah et al. 2018a). There is no explanation provided by the authors for the lack of dose–response curve for testosterone levels. In the second study performed by the same team, rats exposed to low doses of BPB in drinking water for 48 weeks had a 22% lower testosterone plasma level in the 50 μg/L-exposure group (Ullah et al. 2018b). In the same study, BPA was shown to exert, at the same dose level, similar effects as BPB. In male and female zebrafish, exposure to BPB for 21 d (0.001–1 mg/L in water) resulted in a dose-dependent lower body-homogenate concentration of testosterone (Yang et al. 2017). This response was statistically significant from 0.1 mg/L in male and at 1 mg/L in female fish. In the high-dose–exposure group, male fish had a lower testosterone level (lower by 33%), whereas the estradiol levels were higher in both sexes (LOEC of 0.01 mg/L), and up to 42% higher at the highest concentration (Yang et al. 2017). In addition, male fish had less progesterone at 0.1 mg/L and 1 mg/L. In testis, *cyp11a*, 3β-*hsd* and *cyp19a1a* gene expression levels were statistically significantly higher (LOEC of 0.1 mg/L), whereas *star* and *cyp17*

Table 3. Line of evidence for BPB estrogenic activity.

End point	Biological model	Test duration	Parameter	Effect dose	BPA/BPB[a]	Reference
In vitro endocrine activity						
Rat ER—binding	Uterine cytosol	—	EC_{50}	1.05 μM	11.1	Blair et al. 2000
Rat ER—binding	Uterine cytosol	—	RBA	0.086	—	Perez et al. 1998
Mouse ERα-LBD—binding	Recombinant	—	EC_{50}	0.023 μM	4.8	Sipes et al. 2013
Bovine ER—binding	Uterus membrane	—	EC_{50}	0.43 μM	1.5	Sipes et al. 2013
hERα—binding	Breast cancer cells	—	EC_{50}	0.30 μM	2.7	Sipes et al. 2013
hERα-LBD—binding	Recombinant	—	EC_{50}	1.45 μM	4.9	Zhang et al. 2018
hGPER—binding	SKRB3 cells	—	EC_{50}	3.3 μM	7.7	Cao et al. 2017
hERα—PRL promoter occupancy	HeLa cells	30 min	EC_{50}	1.8 μM	2.3	Ashcroft et al. 2011
hERα—PRL promoter occupancy	HeLa cells	30 min	EC_{50}	Weak agonist activity	—	Stossi et al. 2014
hERβ—PRL promoter occupancy	HeLa cells	30 min	EC_{50}	0.161 μM	4.5	Stossi et al. 2014
hERα—TA agonist activity	Yeast cells	24 h	EC_{50}	5 μM	4	Wang et al. 2014
hERα—TA agonist activity	YES assay	24 h	EC_{50}	1.73 μM	19.5	Conroy-Ben et al. 2018
Medaka ERα—TA agonist activity	Yeast cells	4 h	EC_{50}	0.59 μM	1.5	Yokota et al. 2008
hERα—TA agonist activity	Yeast cells	4 h	[b]	Estrogenic activity	—	Chen et al. 2002
hERα—TA agonist activity	Yeast cells	4 h	[b]	Estrogenic activity	—	Hashimoto et al. 2001
rat ERα—TA agonist activity	HeLa cells	24 h	EC_{50}	0.167 μM	14.7	Yamasaki et al. 2002
hERα—TA agonist activity	MVLN cells	24 h	LOEC	1 μM	—	Rivas et al. 2002
hERα—TA agonist activity	T47D-KBluc cells	24 h	EC_{50}	0.3 μM	1.3	Mesnage et al. 2017
hERα—TA agonist activity	U2OS cells	24 h	EC_{50}	0.12 μM	2.3	Wang et al. 2014
hERα—TA agonist activity	BG1-luc42E cells	22 h	EC_{50}	0.12 μM	0.7	Rosenmai et al. 2014
hERα—TA agonist activity	MFC-7 cells	24 h	EC_{50}	0.07 μM	9	Kitamura et al. 2005
hERα—TA agonist activity	HeLa cells	30 min	[b]	Agonist activity	—	Stossi et al. 2014
hERβ—TA agonist activity	HeLa cells	30 min	[b]	No agonist activity	—	Stossi et al. 2014
pS2 mRNA and protein level	MCF-7 cells	144 h	LOEC	1 μM: higher	—	Rivas et al. 2002
pS2 protein level	MCF-7 cells	144 h	LOEC	1 μM: higher	—	Perez et al. 1998
Cell proliferation	T47D cells	144 h	[b]	Greater	—	Mesnage et al. 2017
Cell proliferation	T47D cells	80 h	AC_{50}	0.283 μM	1.4	Rotroff et al. 2013
Cell proliferation	MCF-7 cells	144 h	[b]	Greater	—	Hashimoto et al. 2001
Cell proliferation	MCF-7 cells	96 h	[b]	Greater	—	Pisapia et al. 2012
Cell proliferation	MCF-7 cells	144 h	AC_{50}	0.24 μM	1.5	Mesnage et al. 2017
Cell proliferation	MCF-7 cells	144 h	RPE	92.96%	—	Stossi et al. 2014
Cell proliferation	MCF-7 cells	144 h	RPE	88%	—	Perez et al. 1998
Cell proliferation	MCF-7 BUS cells	144 h	LOEC	0.1 μM	—	Rivas et al. 2002
hGPER signaling—Ca^{2+} mobilization	SKRB3 cells	<30 min	EC_{50}	1.7 μM	4.4	Cao et al. 2017
hGPER signaling—cAMP production	SKRB3 cells	<30 min	EC_{50}	0.0975 μM	>100	Cao et al. 2017
hGPER signaling—cell migration	SKRB3 cells	48 h	LOEC	0.1 μM	—	Cao et al. 2017
In vivo endocrine activity						
cyp19a1b mRNA in brain	Male zebrafish	21 d	LOEC	0.01 mg/L: higher	—	Yang et al. 2017
cyp19a1b mRNA in brain	Female zebrafish	21 d	LOEC	No effect	—	Yang et al. 2017
ERα mRNA in brain	Male zebrafish	21 d	LOEC	0.1 mg/L: higher	—	Yang et al. 2017
ERα mRNA in brain	Female zebrafish	21 d	LOEC	0.1 mg/L: lower	—	Yang et al. 2017
ERβ2 mRNA in brain	Adult zebrafish	21 d	LOEC	No effect	—	Yang et al. 2017
VTG protein in liver	Male zebrafish	21 d	LOEC	0.1 mg/L : higher	—	Yang et al. 2017
vtg1 mRNA in liver	Male medaka	8 h	LOEC	1.2 mg/L: higher	—	Yamaguchi et al. 2015
vtg2 mRNA in liver	Male medaka	8 h	LOEC	No effect	—	Yamaguchi et al. 2015
Chg-L mRNA in liver	Male medaka	8 h	LOEC	1.2 mg/L: higher	—	Yamaguchi et al. 2015
Chg-H mRNA in liver	Male medaka	8 h	LOEC	1.2 mg/L: higher	—	Yamaguchi et al. 2015
Uterine blotted weight	Immature female rat	3 d	LOEC	200 mg/kg BW/d: greater	—	Yamasaki et al. 2002
Estradiol level (plasma)	Male SD rats	48 weeks	LOAEC	0.05 mg/L: higher	—	Ullah et al. 2018b
Estradiol level (body)	Male zebrafish	21 d	LOEC	0.01 mg/L: higher	—	Yang et al. 2017
Estradiol level (body)	Female zebrafish	21 d	LOEC	0.01 mg/L: higher	—	Yang et al. 2017
LH level (plasma)	Male SD rats	48 weeks	LOAEC	0.05 mg/L: greater	—	Ullah et al. 2018b
FSH level (plasma)	Male SD rats	48 weeks	LOAEC	0.05 mg/L: greater	—	Ullah et al. 2018b

Note: Only studies that tested multiple concentrations are included in the table; all the results are presented in Excel Tables S2–S5. —, Not applicable; AC_{50}, half maximal activity concentration; BPA, bisphenol A, BPB, bisphenol B, Chg, choriogenin; cyp19a1b, aromatase B, EC_{50}, half maximal effective concentration; ER, estrogen receptor; FSH, follicle stimulating hormone; GPER, G-coupled estrogen receptor; hER, human estrogen receptor; IC_{50}, half maximal inhibitory concentration; LBD, ligand binding domain; LH, luteinizing hormone; LOAEC, lowest observed adverse effect concentration; LOEC, lowest observed effect concentration; PLR, prolactin; RBA, relative binding affinity; RPE, relative proliferative effect; SD, Sprague-Dawley; TA, transactivation assay; VTG, vitellogenin.

[a]BPA/BPB ratio calculated with IC_{50} or EC_{50} values, when both chemicals were tested within the same study and showed activity in the same direction.

[b]Qualitative assessment only, no parameter calculated.

(LOEC of 1 mg/L) and 17β-*hsd* (LOEC of 0.1 mg/L) transcript levels were lower. In male brain, *cyp19a1b* gene expression level was statistically significantly higher from 0.01 mg/L.

Biological Plausible Link between Adversity and Endocrine Activity

In order to define an EDC in accordance with the WHO/IPCS definition, an endocrine mode of action must be postulated to link the endocrine activity of a chemical to its identified adverse effect. The most plausible modes of action of BPB leading to altered reproductive function of rats and fish in relation to its identified endocrine activity are presented below.

Are BPB adverse effects mediated by ER? In the rat, ERα is expressed in Leydig, Sertoli, and some germ cells, whereas ERβ is expressed in all somatic testicular cells and in various germ cell types (Chimento et al. 2014; Cooke et al. 2017). Importantly, although spermatogenesis requires estrogens, an excess in estrogens

Table 4. Line of evidence for BPB anti-androgenic activity.

End point	Biological model	Test duration	Parameter	Effect dose	BPA/BPB[a]	Reference
In vitro endocrine activity						
hAR—binding	LnCAP cells	—	IC_{50}	2.2 μM	3.9	Sipes et al. 2013
Rat AR—binding	Recombinant	—	IC_{50}	21 μM	2.2	Sipes et al. 2013
Rat AR—binding	Recombinant	—	IC_{50}	37.5 μM	2	Fang et al. 2003
Rat AR—binding	Recombinant	—	RBA	0.0082	—	Fang et al. 2003
hAR—antagonist activity	NIH3T3 cells	24 h	IC_{50}	1.7 μM	2.5	Kitamura et al. 2005
hAR—antagonist activity	U2OS cells	24 h	IC_{50}	0.93 μM	—	Wang et al. 2014
hAR—antagonist activity	Yeast cells	24 h	IC_{50}	No antagonism	—	Wang et al. 2014
hAR—antagonist activity	Yeast cells	24 h	IC_{50}	10 μM	1	Conroy-Ben et al. 2018
hAR—antagonist activity	CHO cells	20 h	IC_{50}	3.4 μM	1.1	Rosenmai et al. 2014
In vivo endocrine activity						
Bulbo-cavernosus/levator ani muscle weight	Castrated male SD rats	10 d	LOEC	BPB: 200 mg/kg BW/d: lower BPB + TP: 600 mg/kg BW/d: higher	—	Yamasaki et al. 2002
Ventral prostate weight	Castrated male SD rats	10 d	LOEC	BPB: no effect BPB + TP: 200 mg/kg BW/d: higher	—	Yamasaki et al. 2002
Seminal vesicle, Cowper's gland, glans penis weight	Castrated male SD rats	10 d	LOEC	BPB: no effect BPB + TP: 600 mg/kg BW/d: higher	—	Yamasaki et al. 2002
Testosterone level (plasma)	Male SD rats	48 weeks	LOAEC	0.05 mg/L: lower	—	Ullah et al. 2018b
Testosterone level (body)	Male zebrafish	21 d	LOEC	0.1 mg/L: lower	—	Yang et al. 2017
Testosterone level (body)	Female zebrafish	21 d	LOEC	1 mg/L: lower	—	Yang et al. 2017
LH level (plasma)	Male SD rats	48 weeks	LOAEC	0.05 mg/L: lower	—	Ullah et al. 2018b
FSH level (plasma)	Male SD rats	48 weeks	LOAEC	0.05 mg/L: lower	—	Ullah et al. 2018b

Note: Only studies that tested multiple concentrations are included in the table; all the results are presented in Excel Tables S2–S5. —, Not applicable; AR, androgen receptor; BPA, bisphenol A; BPB, bisphenol B; FSH, follicle stimulating hormone; IC_{50}, half maximal inhibitory concentration; LH, luteinizing hormone; LOAEC, lowest observed adverse effect concentration; LOEC, lowest observed effect concentration; RBA, Relative binding affinity, SD, Sprague-Dawley; TP, testosterone propionate.
[a]BPA/BPB ratio calculated with IC_{50} or EC_{50} values, when both chemicals were tested within the same study and showed activity in the same direction.

and the activation of ER can lead to an alteration of spermatogenesis and disruption of testicular functions (Akingbemi, 2005; Bernardino et al. 2018; Delbès et al. 2005; Leavy et al. 2017).

As presented in Table 3 and Figure 4, the many *in vitro* results are converging to indicate BPB interaction with either or both ERα and ERβ signaling of human, rodent, and fish. This estrogenic activity is consistent with the higher uterine weight of treated animals in the immature rat uterotrophic assay (Yamasaki et al. 2002). Estrogen receptors are well preserved among vertebrates such as between fish and human (Matthews et al. 2000). BPB estrogenic activity was also evidenced in fish by induction of vitellogenin in male medaka (Yamaguchi et al. 2015) and in male zebrafish (Yang et al. 2017). In addition, expression of the ER-regulated *cyp19a1b* gene was higher in the brain of exposed male zebrafish (Yang et al. 2017), which supports the estrogenic effect of BPB in fish (Diotel et al. 2010). Furthermore, BPB may act in the testis via the noncanonical GPER, as shown for BPA in zebrafish (Fitzgerald et al. 2015). Indeed, Cao et al. (2017) showed that BPB bound to hGPER and activated extra-genomic pathways. In rodents, GPER is expressed in variety of testicular cell types including germ, peritubular, Leydig, and Sertoli cells and its activation alters testicular functions (Lucas et al. 2010; Vaucher et al. 2014). Altogether, these data indicate that BPB may exert a negative effect on spermatogenesis via a direct action on either or both ER and GPER.

Are BPB adverse effects mediated by AR? Spermatogenesis requires high levels of testosterone within the testis, which are 50 to 1,000 times higher than in the systemic circulation (reviewed by Shiraishi and Matsuyama 2017). Produced exclusively by the Leydig cells, testosterone acts as a paracrine factor to stimulate spermatogenesis via the Sertoli cells and the myoid cells ancillary. As presented in Table 4, an anti-androgenic activity was evidenced in most *in vitro* reporter gene assays, whereas the only *in vivo* study was not conclusive. Indeed, in the Hershberger assay, an anti-androgenic effect was only observed in one of five androgen-dependent sexual organs, whereas a puzzling pro-androgenic response was observed in co-exposure of BPB with TP (Yamasaki et al. 2003). In conclusion, further information is warranted to know whether BPB acts negatively on spermatogenesis by reducing the effect of testosterone in the testis through an AR antagonist action.

Are BPB adverse effects associated to steroidogenesis alteration? BPB exposure resulted in a lower testosterone production in all *in vitro* studies and *in vivo* studies in rat and fish, providing converging evidence (Table 5). However, because the experimental design of each study was different, it is difficult to compare the doses at which this effect occurred. In particular, the reduction in plasma and intratesticular testosterone levels observed in rats exposed orally for 28 d was similar whatever the dose of BPB (Ullah et al. 2018a). In addition, the *cyp17* and *17β-hsd* genes, involved in testosterone production, were less expressed in adult male zebrafish testis (Yang et al. 2017), which suggests that the alteration of spermatogenesis may be the consequence of altered steroidogenesis in testis.

BPB may also act by increasing estrogen levels. As observed for testosterone production, all studies are remarkably convergent. *In vitro*, a higher level of either or both estrone and estradiol levels in H295R assays was measured after exposure to BPB (Rosenmai et al. 2014; Wang et al. 2014). A similar higher estradiol level was observed *in vivo* in fish homogenate (Yang et al. 2017) and seemed to occur in rat plasma (Ullah et al. 2018b). In male zebrafish exposed to BPB, *cyp19a1a* (aromatase A) and *cyp19a1b* (aromatase B) gene expressions, which are enzymes converting androgens into estrogens (Diotel et al. 2010), were higher in testis and brain, respectively. Thus, these results support the high circulating level of estrogens in fish. In the rat testis, aromatase is expressed in Sertoli and Leydig cells and in various germ cell types (reviewed by Carreau et al. 2007). Additional investigations are needed to understand the specific mechanism of action of BPB on aromatase expression and activity in fish and rats.

In addition, BPB could impair testis function by acting directly on the hypothalamus–pituitary axis, as suggested by the differences observed in LH, FSH, and GnRH receptors transcript levels in exposed zebrafish (Yang et al. 2017) or in FSH and LH

Table 5. Line of evidence for the alteration of steroidogenesis by BPB.

End point	Biological model	Test duration	Parameter	Effect dose	BPA/BPB[a]	Reference
In vitro and *ex vivo* endocrine activity						
11-deoxycortisol	H295R cells	48 h	LOEC	3 μM: lower	—	Wang et al. 2014
Cortisol	H295R cells	48 h	LOEC	10 μM: lower	—	Wang et al. 2014
Cortisol	H295R cells	48 h	IC_{50}	11.8 μM	0.93	Rosenmai et al. 2014
Testosterone	H295R cells	48 h	LOEC	10 μM: lower	—	Wang et al. 2014
Testosterone	H295R cells	48 h	IC_{50}	18.8 μM	0.3	Rosenmai et al. 2014
Testosterone	Adult human testis explants	24 h and 48 h	LOEC	Significantly lower at 0.1 μM only	—	Desdoits-Lethimonier et al. 2017
Testosterone	Adult rat testis explants	2 h	LOEC	No effect	—	Ullah et al. 2018a
Androstenedione	H295R cells	48 h	LOEC	10 μM: lower	—	Wang et al. 2014
Androstenedione	H295R cells	48 h	IC_{50}	16 μM	0.19	Rosenmai et al. 2014
DHEA	H295R cells	48 h	LOEC	10 μM: lower	—	Wang et al. 2014
DHA	H295R cells	48 h	EC_{50}	3.8 μM	Opposite response	Rosenmai et al. 2014
11-deoxycorticosterone	H295R cells	48 h	LOEC	30 μM: lower	—	Wang et al. 2014
Corticosterone	H295R cells	48 h	IC_{50}	4.5 μM	—	Rosenmai et al. 2014
Progesterone	H295R cells	48 h	LOEC	3 μM: higher	—	Wang et al. 2014
Progesterone	H295R cells	48 h	—	No effect	—	Rosenmai et al. 2014
17-hydroxyprogesterone	H295R cells	48 h	EC_{50}	8.2 μM	Opposite response	Rosenmai et al. 2014
Pregnenolone	H295R cells	48 h	LOEC	10 μM: higher	—	Wang et al. 2014
Estrone	H295R cells	48 h	LOEC	1 μM: higher	—	Wang et al. 2014
Estrone	H295R cells	48 h	EC_{50}	17.4 μM	0.41	Rosenmai et al. 2014
Estradiol	H295R cells	48 h	LOEC	No effect	—	Wang et al. 2014
Estradiol	H295R cells	48 h	EC_{50}	13.6 μM	1.0	Rosenmai et al. 2014
In vivo endocrine activity						
Estradiol level (plasma)	Male SD rats	48 weeks	LOAEC	0.05 mg/L: higher	—	Yang et al. 2017
Estradiol level (body)	Male zebrafish	21 d	LOEC	0.01 mg/L: higher	—	Yang et al. 2017
Estradiol level (body)	Female zebrafish	21 d	LOEC	0.01 mg/L: higher	—	Yang et al. 2017
Testosterone level (plasma)	Male SD rats	48 weeks	LOAEC	0.05 mg/L: higher	—	Ullah et al. 2018b
Testosterone level (body)	Male zebrafish	21 d	LOEC	0.1 mg/L: lower	—	Yang et al. 2017
Testosterone level (body)	Female zebrafish	21 d	LOEC	1 mg/L: lower	—	Yang et al. 2017
lhr mRNA	Male zebrafish, testes	21 d	LOEC	0.01 mg/L: higher	—	Yang et al. 2017
fshr, cyp11a, 3β-*hsd, cyp19a1a* mRNA	Male zebrafish, testes	21 d	LOEC	0.1 mg/L: higher	—	Yang et al. 2017
17β-*hsd* mRNA	Male zebrafish, testes	21 d	LOEC	0.1 mg/L: lower	—	Yang et al. 2017
star, cyp17 mRNA	Male zebrafish, testes	21 d	LOEC	1 mg/L: lower	—	Yang et al. 2017
cyp19a1b mRNA	Male zebrafish, brain	21 d	LOEC	0.01 mg/L: higher	—	Yang et al. 2017
*gnrh3, gnrhr1, gnrhr2, fsh*β, *lh*β mRNA	Male zebrafish, brain	21 d	LOEC	0.1 mg/L: higher	—	Yang et al. 2017
gnrh2, gnrhr4 mRNA	Male zebrafish, brain	21 d	LOEC	No effects	—	Yang et al. 2017
gnrhr2 and *fsh*β mRNA	Female zebrafish, brain	21 d	LOEC	1 mg/L: lower	—	Yang et al. 2017
*gnrh2, gnrh3, gnrhr1, gnrhr4, lh*β, *cyp19a1b* mRNA	Female zebrafish, brain	21 d	LOEC	No effects	—	Yang et al. 2017
fshr mRNA	Female zebrafish, ovaries	21 d	LOEC	0.1 mg/L: lower	—	Yang et al. 2017
lhr mRNA	Female zebrafish, ovaries	21 d	LOEC	1 mg/L: lower	—	Yang et al. 2017
star, cyp11a, 3β-*hsd, cyp17,* 17β-*hsd, cyp19a1a* mRNA	Female zebrafish, ovaries	21 d	LOEC	No effects	—	Yang et al. 2017

Note: Only studies that tested multiple concentrations are included in the table; all the results are presented in Excel Tables S2–S5. —, Not applicable; BPA, bisphenol A; BPB, bisphenol B; *cyp*, cytochrome P450; DHA, dehydroandrosterone; DHEA, dehydroepiandrosterone; EC_{50}, half maximal effective concentration; *fsh*β, follicle stimulating hormone subunit β; *fshr*, follicle stimulating hormone receptor; *gnrh,* gonadotropin-releasing hormone; *gnrhr*, gonadotropin-releasing hormone receptor; *hsd*, hydroxysteroid dehydrogenase; IC_{50}, half maximal inhibitory concentration; *lh*β, luteinizing hormone subunit β; *lhr*, luteinizing hormone receptor; LOAEC, lowest observed adverse effect concentration; LOEC, lowest observed effect concentration; SD, Sprague-Dawley.

[a]BPA/BPB ratio calculated with IC_{50} or EC_{50} values, when both chemicals were tested within the same study and showed activity in the same direction.

[b]Qualitative assessment only, no parameter calculated.

[c]Opposite response means that cells exposed to BPA had a lower hormone level compared with control, thus no ratio could be calculated.

plasma levels in exposed rats (Ullah et al. 2018b). However, these measurements require additional studies to be confirmed and to uncover the BPB mode of action upon the gonadotropic hypothalamus–pituitary axis.

Discussion

BPB Endocrine Properties and the WHO Definition of EDC

The WHO defines an EDC as "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" (Damstra et al. 2002). Thus, the definition of EDC is based on three elements that must be identified concomitantly, that is, *a*) an adverse effect, *b*) an endocrine activity, and *c*) a plausible mechanistic link between these two observations. In this review, we assessed these three elements to answer the question, "Does BPB have endocrine disruptive properties complying with the WHO/IPCS definition?" Taken together, the 36 studies selected for their relevance to the question converge to show that BPB has the capacity to interfere with the estrogen signaling pathway, to reduce testosterone production, and to alter spermatogenesis in rats and zebrafish, and eventually to impair fish reproduction.

Fish and rat data were used in the same line of evidence to strengthen the weight of evidence assessment considering both human health and wildlife together. From a regulatory point of view, they are usually evaluated separately (e.g., BPA identification

as SVHC [ECHA 2017a, 2017b]). However, we considered the integrated approach relevant in the case of BPB evaluation because of the conservation in specific endocrine targets among mammals and fish, such as the estrogen receptors (Matthews et al. 2000), and the few data available. In addition, the consistent adverse effects and endocrine activity observed in these two species reinforced the relevance of this approach. BPB exposure altered the reproductive functions of both fish and rats, although no clear dose–response was observed on plasma and intratesticular testosterone levels in rats at the doses tested by Ullah et al. (2018a). The available data did not include a measurement of fertility in rodents that might strengthen the evaluation of BPB effects. However, the fact that no sperm was observed in the seminiferous tubules of rats treated with high doses of BPB informs on the probable effect on human fertility (Ullah et al. 2018a). Furthermore, one fish study reported a reduction of fertility in adult zebrafish exposed to BPB (Yang et al. 2017). In both *in vitro* and *in vivo* studies, BPB was shown to have clear estrogenic effects in fish and rats (Table 3). Furthermore, BPB exposure lead to higher estrogen but lower androgen levels (Table 5). To a lesser extent, BPB may antagonize androgen actions, although this effect was not firmly confirmed in the Hershberger assay (Table 4). Altogether, these mechanistic data are consistent with the alteration of spermatogenesis, which could be disturbed by decreased testosterone levels and increased level of estrogens or by estrogeno-mimetic chemicals (Akingbemi, 2005; Delbès et al. 2005; Leavy et al. 2017). Thus, so far, BPB fulfills the criteria defining an endocrine disrupting chemical.

Comparison of BPB with BPA Endocrine Properties

The comparison of BPA and BPB endocrine activities brings additional arguments for the EDC properties of BPB. Whenever they were tested in the same *in vitro* study, BPB had similar or even greater effects than BPA, especially regarding the estrogenic activity. Thus, the median of BPB estrogenic potency was four times higher than that of BPA when considering all the *in vitro* results (Table 3; see also Excel Table S2). In the two *in vivo* studies in rats by Ullah et al. (2018a, 2018b), BPB treatment resulted in lower seminal vesicle and epididymis weights, a lower height of epithelium in testicular tissues and fewer spermatocytes and spermatids. These changes were similar or even slightly more pronounced as compared with similar BPA treatment at 5–50 mg/kg (Ullah et al. 2018a) or 5–25 μg/L (Ullah et al. 2018b). Furthermore, the doses tested in Ullah et al. (2018b) were much lower than the starting point for the derived no-effect level derivation of BPA (8,960 μg/kg BW/d; ECHA 2017). The data available so far on human exposure levels to BPB, although consistent with BPA, are much more limited, which makes the comparison of hazard and exposure data complicated.

BPA endocrine properties have been extensively studied in the scientific literature, and reviewing these data is beyond the scope of the present paper. Nevertheless, based on available scientific information, the ECHA has identified BPA as a reprotoxic chemical of category 1B based on reprotoxic effects in both males and females (Classification Labelling and Packaging dossier; ECHA 2014). The CLP dossier concluded that BPA induced negative effects on plasma testosterone levels, on the organs of the reproductive tract, and on sperm production and quality, although some divergences were noticed considering the effective BPA concentrations. Similarly, in two recent studies published after ECHA evaluation and performed by NIEHS/NTP/FDA, effects of BPA on testis and epididymis morphology in rats were reported but only at the highest dose tested (Dere et al. 2018; Delclos et al. 2014). The animal strain, route of exposure, and protocols used likely contributed to the divergent sensitivities reported (NTP 2001).

There is no study comparing BPA and BPB adverse effects in fish within the same study design. However, BPA endocrine properties in fish have been reviewed recently for the identification of BPA as an EDC for the environment (ECHA 2017b). The dossier reported a clear estrogen agonist activity of BPA in fish, also evidenced in the present review for BPB by induction of vitellogenin in male fish (Yamaguchi et al. 2015; Yang et al. 2017). In addition, zebrafish exposed to BPA had a lower egg production and a smaller hatching rate and embryo survival (Segner et al. 2003; Chen et al. 2017). Similar effects on fecundity and embryo development were demonstrated with BPB in the 21-d reproductive study in zebrafish (Yang et al. 2017). In addition, BPA exposure resulted in either or both a lower sperm volume and motility in adult zebrafish (Chen et al. 2017), brown trout (Lahnsteiner et al. 2005), and goldfish (Hatef et al. 2012), and exposed Japanese medaka had fewer spermatozoa (Metcalfe et al. 2001), supporting the likelihood of similar effects between both bisphenols in fish.

BPB and the Regulatory Challenge of EDC Identification

In a regulatory context, this review on BPB endocrine properties raises the question of the level of evidence and the set of data needed to define a compound as an EDC. Chemicals are regulated differently depending on their uses, leading to specific testing requirements and consequences. For instance, in EDC identification at the EU level in REACH regulation, as well as in plant protection products and biocidal products regulations, each requires an evaluation based on hazard data, which originates mainly from the standardized test protocols described in the OECD guidance document no. 150 (ECHA and EFSA 2018). In the present review, the vast majority of data came from the scientific literature. Although some scientific studies were based on standardized protocols (e.g., Yamasaki et al. 2002, 2003), most research articles did not comply with OECD standards. Nevertheless, many research articles adhered to rigorous practices and thus provided key information on sensitive endocrine targets, which may not be included in any standardized tests because science on EDC evolves rapidly. For instance, Yang et al. (2017) extended the OECD guideline to evaluate hormone levels and gene expression, which allowed supporting strongly BPB endocrine mechanism of action in fish. On the other hand, data provided by the uterotrophic and Hershberger assays play a major role in EDC evaluation (ECHA and EFSA 2018), whereas these assays are known to have a relatively low sensitivity (Varayoud et al. 2017; Heneweer et al. 2007). Opinions may diverge on the relative importance of regulatory tests compared with results from the scientific literature, as observed (Myers et al. 2009). However, it may be considered that all sound scientific studies should be used and adequately weighted to draw regulatory conclusions.

There is increasing expectations from the scientific community to avoid unsafe BPA substitutions as concern on EDC properties arise from other analogs. In a recent prioritization exercise of potential EDC, the Danish Environmental Protection Agency identified bisphenol AF as an EDC complying with the WHO definition with a strong level of confidence (Hass et al. 2018). In the EU, industrial chemicals are covered by the REACH regulation, which includes EDC identification under Article 57 (EC 2006). The major BPA substitute, bisphenol S, is currently under evaluation for its EDC properties (ECHA 2019). Since its implementation in 2009, only eight industrial chemicals have been identified as EDC for the environment (regulatory term meaning EDC for wildlife), four for human health, and two for both human health and the environment (ECHA 2018a). Most of them are well-known chemicals such as BPA, phenols derivatives, or phthalates and have benefited from a large amount of

data given that they have been evaluated by scientists and regulators for many years, or even decades (Sumpter and Johnson 2008). However, the number of chemicals regulated has grown significantly over the last decade, and regulators are expected to tackle less-documented suspected EDCs.

Conclusion

In this review, we report that existing information on BPB's estrogenic activity and inhibition of testosterone production are similar to BPA's endocrine activity. This endocrine mode of action is consistent with the alteration of the male reproductive system observed in fish and rats, effects that are also reported with BPA. More information on BPB endocrine properties may become available in the coming years due to growing concern. However, in the meantime, industrial interests might invest in BPB as a substitution for BPA, with possible detrimental consequences. In this context, the authors think that the current information available should be considered as sufficient for regulating BPB for its endocrine properties and, thus, seek to protect human health and wildlife, while avoiding a regrettable substitution.

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