Analysis of the most appropriate regulatory management option (RMOA)

Substance Name: Bisphenol B

EC Number: 201-025-1

CAS Number: 77-40-7

Authority: FR- MSCA

Date: September 2019

Cover Note

Bisphenol B is identified in the TEDX list and in the US –EDSP list as an agonist of the estrogen receptors. It has been selected to be evaluated within a yearly evaluation French program on potential EDs (French Strategy on Endocrine Disruptors, SNPE 2018) although it is not widely produced or used (less than one ton/ year) and not registered in EU under REACH (ECHA website, 2018). However, it is found in biomonitoring studies conducted outside EU probably because it is a potential substitute of Bisphenol A (BPA, CAS 80-05-7), substance used widely to make certain plastics and resins since the 1960s, receipts, food packaging, which has been recently identified in Europe as a Subtance of Very High Concern for its endocrine disrupting properties for human and the environment. Many manufacturers have started to use other chemicals as substitutes for BPA, although many voices have question on whether these substitute are safer than BPA.

Comment are welcome until November 27th 2019.

DISCLAIMER

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1 IDENTITY OF THE SUBSTANCE

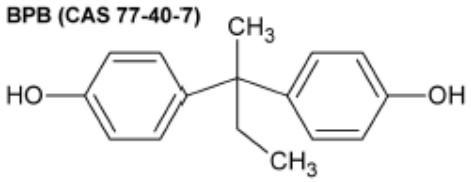
1.1 Other identifiers of the substance

Table 1: Other substance identifiers

EC name (public):	201-025-1
IUPAC name (public):	2,2-Bis(4-hydroxyphenyl)butane 4,4'-(1-Methylpropylidene)bisphenol 4-[2-(4-hydroxyphenyl)butan-2-yl]phenol
Index number in Annex VI of the CLP Regulation:	none
Molecular formula:	C16H18O2
Molecular weight or molecular weight range:	242.318
Synonyms:	Bisphenol B

Type of substance ☒Mono-constituent ☐Multi-constituent ☐UVCB

Structural formula:



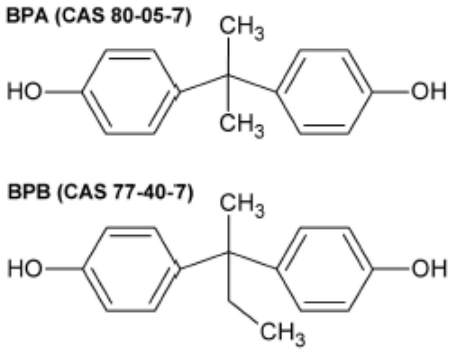
Other relevant information about substance composition:

No other relevant information as the substance is not registered.

1.2 Similar substances/grouping possibilities

BPB is structurally similar to BPA (CAS 80-05-7).

Structural formula:



2 OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

There is no completed or ongoing processes on BPB (Table 2).

Table 2: Completed or ongoing processes

RMOA	☐ Risk Management Option Analysis (RMOA) other than this RMOA	
REACH Processes	Evaluation	☐ Compliance check, Final decision
		☐ Testing proposal
		☐ CoRAP and Substance Evaluation
	Authorisation	☐ Candidate List
		☐ Annex XIV
	Restri -ction	☐ Annex XVII[1]
Harmonised C&L	☐ Annex VI (CLP) (see section 3.1)	
sses unde r other EU	☐ Plant Protection Products Regulation Regulation (EC) No 1107/2009	

[1] Please specify the relevant entry.

	☐ Biocidal Product Regulation Regulation (EU) 528/2012 and amendments
Previous legislation	☐ Dangerous substances Directive Directive 67/548/EEC (NONS)
	☐ Existing Substances Regulation Regulation 793/93/EEC (RAR/RRS)
(UNEP) Stockholm convention (POPs Protocol)	☐ Assessment
	☐ In relevant Annex
Other processes/ EU legislation	☐ Other (provide further details below)

3 HAZARD INFORMATION (INCLUDING CLASSIFICATION)

3.1 Classification

3.1.1 Harmonised Classification in Annex VI of the CLP

None

3.1.2 Self classification

According to the classification provided by companies to ECHA in **CLP notifications** this substance causes serious eye irritation, is harmful if swallowed, may cause long lasting harmful effects to aquatic life and causes skin irritation. (cf. https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.000.933)

Aggregated GHS information from 3 notifications provided by 25 companies to the ECHA C&L Inventory. Each notification may be associated with multiple companies.

H302 (96%): Harmful if swallowed [Warning Acute toxicity, oral]
H319 (100%): Causes serious eye irritation [Warning Serious eye damage/eye irritation]
H413 (92%): May cause long lasting harmful effects to aquatic life [Hazardous to the aquatic environment, long-term hazard]

Information may vary between notifications depending on impurities, additives, and other factors. The percentage value in parenthesis indicates the notified

classification ratio from all companies (Only Hazard Codes with percentage values above 10% are shown when majority companies gave hazard codes).

Precautionary Statement Codes: P264, P270, P273, P280, P301+P312, P305+P351+P338, P330, P337+P313, and P501 (The corresponding statement to each P-code can be found here).

3.1.3 Proposal for Harmonised Classification in Annex VI of the CLP

None

3.1.4 CLP Notification Status

Table 3: CLP Notifications

	CLP Notifications[2]
Number of aggregated notifications	3
Total number of notifiers	25

3.2 Human health data

Human and environmental hazard properties presented are based on available data from scientific literature and from the chemical safety report (CSR).

3.2.1 Biomonitoring and human exposure data

- **Human biomonitoring data:**

Among the available biomonitoring studies, those from Andra et al. (2015), Nielen et al. (2004), Ohkuma et al. (2002) and Mouneimne et al. (2017) did not assess internal exposure to BPB. To the best of our knowledge, only one study analyzed BPB in human blood sera (Cobellis et al. 2009) and 3 studies analyzed Bisphenol B (BPB) in human urines (Yang et al. 2014a, Cunha and Fernandes 2010, Heffernan et al. 2016). The studies testing BPB in urine concerned 94 Chinese participants living near a bisphenol AF manufacturing plant, 20 Portuguese volunteers, and 30 Australian pregnant women, respectively. BPB was only detected in 2 out of the 20 Portuguese participants and was neither detected in the Chinese nor in Australian participants (LOD ranging from 0.04 to 0.26 ng/mL).

The analytical methodologies used to quantify BPB in urines in the 3 studies presented sensitivities close to that of the method reported for BPA. For BPA, the analytical sensitivity was in the range of acceptable standards and the percentage of positive detection as well as the range of concentration was in line with values

[2] C&L Inventory database, http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database (accessed 1 September 2019)

reported in the literature and considered reliable. Regarding the analytical methods that were used in the three studies, no major bias explaining the low rate of detection for BPB was noticed.

Assuming comparable metabolism between BPA and BPB, the absence of BPB in most of the urine samples tested in these studies would therefore be attributed to the absence of exposure or to low levels of exposure resulting in undetectable urinary concentrations. For the two urine samples where BPB was detected, the concentration was in the same order of magnitude to that of BPA (i.e. between 0.3 and 10 ng/mL for geometric mean).

The study analyzing plasma (Cobellis et al. 2009) was based on samples collected from 69 fertile women. This study reported lower detection frequency for BPB (16 subjects = 27.6%) than BPA (30 subjects = 51.7%). The concentration ranges of BPA and BPB were comparable (from 0.8 to 12ng/mL).

Conclusion

The studies mentioned above suggest that BPB can be detected in both urine and serum in the same order of magnitude than BPA although retrieved less oftenly than BPA. However, those data are too limited to be considered representative of the general population and thus, to draw solid conclusions on the frequency and the concentrations of BPB in these matrices.

Furthermore, it is worth noting that BPB is included in the list of HBM4EU priority substance group "bisphenols"[3]. HBM4EU is a joint effort of 28 countries, the European Environment Agency and the European Commission, co-funded under Horizon 2020. Running from 2017 to 2021, HBM4EU aims to generate knowledge provide better evidence of the actual exposure of citizens to chemicals. More information on human exposure to BPB in Europe is expected in the upcoming years.

- **Food exposure data:**

Among the currently published data related to the presence of BPB in media such as food products, seventeen studies were identified. Three studies were classified as not directly relevant for food contamination as they were focused on compartments/products other than food such as food packaging material (Lin et al. 2015), sewage sludge (Yu et al. 2015) or personal care products (Liao and Kannan 2014). The following analysis is then based on the fourteen remaining studies.

The examined studies primarily describe some proposed analytical methods, with various performances, to measure BPB and other BPs in various food matrices. None of them reported representative and quantitative consolidated food exposure data that could be used as a basis for a rigorous risk assessment. However, the reported quantified levels of BPB demonstrate a the presence of BPB in some particular products, linked to an external migration from food packaging material. In addition, the observed detection rate was rather low. Altogether, recommendations could be formulated to further investigate BPB occurrence in food, including:

[3] https://www.hbm4eu.eu/the-substances/bisphenols/.

- The need to develop more sensitive analytic methods permitting to better characterized the lowest concentration levels detected for instance in beverages,
- The need to confirm the nature of the most relevant marker(s) of exposure to BPB in food for distinguishing BPB of an animal origin (possible biotransformation product) from BPB as a result of an external contamination from the packaging material (with the measure of the parent compound as a marker of exposure),
- The need to conduct more exhaustive and representative screening based on a well-designed sampling,
- The need for a more documented relationship between the precise nature and composition of food packaging material and possible release of BPB, and more globally for a more in-depth investigation of the different sources of external exposure to BPB.

- **Indoor dust:**

Among the currently published data two studies reported external exposure data for BPB and other BPs determined in indoor dust: Liao et al. (2012a) and Wang et al. (2015). The analytical methodologies for BPB detection, identification and quantification globally appear of good level.

The results indicate a detectable level of BPB in very few (1-5%) of the analyzed samples (440 in total), with upper values in the range of several µg/kg to several 10's µg/kg. These data show a very limited exposure to BPB through indoor dust both in terms of detection frequency and measured concentrations, in particular when compared to similar data reported for BPA and other substitutes including BPF or BPS.

However, it must be emphasized that these available data concern mainly non-European countries, so that a first inventory of BPB levels in indoor dust/air is still missing for EU in general and for France in particular. The diversity of possible sources of exposure and industrial uses would justify the collection of similar data for France and EU to better assess the current need of further investigations on risk assessment.

3.2.2 Toxicokinetics

No relevant human or experimental data are available on absorption, distribution and elimination of BPB. Very few data are available on the biotransformation of BPB. The findings from Yoshihara et al. (2004) are reported below.

Yoshihara et al. published original data on the biotransformation of BPB and BPA incubated with liver S9 fractions from Wistar rats as well as information on the structure and estrogenic potency of both BPB and BPA metabolites, especially the BPA dimer (isopropenylphenol dimer, namely MBP) suggested by Yoshihara's previous work.

BPA, BPB or BPA+BPB incubations (100 µM) were analyzed by HPLC, and the different HPLC profiles showed several picks corresponding to metabolites. Some of them were identified by LC/MS/MS and/or GC/MS. For BPA, 2 metabolites, namely 3OH-BPA and BPA-o-quinone, produced by rat liver S9, were identified, as well as an isopropenylphenol dimer, less polar than BPA. Regarding BPB metabolism, the authors' interpretation is less detailed, mentioning "similar HPLC

peaks". Again, 2 metabolites, less polar than BPB, were detected and LC/MS/MS fragmentations suggest dimers of isobutenylphenol. Interestingly, co-incubation of BPA + BPB with rat liver S9 showed, in addition to the metabolites detected previously, new dimers produced by recombination of radical fragments, isobutenylphenol from BPB and isopropenylphenol from BPA by carbon–phenyl bond cleavage.

Regarding the endocrine activity, after BPA or BPB or BPA+BPB incubations, estrogenicity of eluates from each extract was determined with the YES assay. The results suggested that the biotransformation of BPA, BPB and BPA+BPB generated metabolites exhibiting an estrogenic activity. Nevertheless, several technical points were not taken into consideration by the authors to validate their results. The study lacks of appropriate control. For instance regarding incubations of BPA and BPB with male Wistar rat liver S9 fractions, metabolites were extracted by SPE methodology using a Sep-Pak Plus C18 cartridge without checking any estrogenic potency coming from the cartridge. Regarding the cell culture, the authors did not mention the steroid content of the fetal bovine serum, which may also be a source of estrogenicity.

Although this work suggests the formation of new dimers produced by recombination of radical fragments, isobutenylphenol from BPB and isopropenylphenol from BPA by carbon–phenyl bond cleavage, important data and confirmation by other scientific teams are missing, rendering difficult to validate the authors' interpretation.

3.2.3 Acute toxicity

No relevant experimental data on BPB are available.

3.2.4 Hypersensitivity, allergy and intolerance

- **Studies in Humans or in animals**:

No relevant human or experimental data are available neither for the cutaneous route nor for the respiratory tract (dusts or vapours).

- **In Silico Profiling:**

Some QSAR modeled data are available in Rosenmai et al. (2014). Two QSAR modeling tools, were applied namely, MultiCASE [4](version 2.4.1.4) and Leadscope[5] (version 3.04-10). BPB was predicted negative for skin irritation, negative for respiratory sensitization and positive for skin sensitization (see **Table**

[4]MultiCASE is a statistical model system that aims to discover fragment combinations, called biophores/biophobes for active/inactive molecules, relevant for an observed effect. Further MultiCASE identifies modulators, such as physiochemical properties, which may affect the probability of a fragment being a biophore/biophobe. Warnings are given in predictions if a fragment is not represented in the training set, or if a contradictory modulator is present in a prediction. Warnings were considered an indication that the molecule was outside the model applicability domain. MultiCASE predictions are reported as positive or negative.

[5] Leadscope uses a library of 27,000 structural features typically found in small drug molecules and eight calculated molecular descriptors for QSAR modeling.

4 below). For further details on the applied QSAR models, please refer to Rosenmai et al. (2014) Supplementary materials 1 and to Dybdahl et al. (2012), Jensen et al. (2008, 2011), Jonsdottir et al. (2012), and Vinggaard et al. (2008).

Table 4: QSAR predictions for tests compounds as BPB and other bisphenols (BPA, BPE, BPF, BPS) and HPP (4-cumylphenol). Quoted from Rosenmai et al., 2014.

			BPA	BPB	BPE	BPF	BPS	HPP
Metabolism[a]	CYP2D6	Substrates	0.69	0.69	0.69	0.69	0.34	0.74
		Inhibitors	0.64	0.73	0.64	0.62	0.16	0.67
	CYP3A4	Substrates	0.82	0.83	0.80	0.80	0.36	0.84
		Inhibitors	0.59	0.62	0.58	0.57	0.37	0.55
	CYP2C9	Substrates	0.64	0.65	0.64	0.62	0.73	0.70
		Inhibitors	0.27	0.33	0.26	0.25	0.29	0.26
	PXR binding[b]		0.64	0.75	0.60	0.53		0.74
Endocrine disruption	ER binding[b]		*		*	*	*	*
	Estrogenicity reporter gene[b]		*		*	*	*	*
	Antiandrogen[b]		*			*		
Reprotoxicity	Teratogenicity FDA TERIS							
Genotoxicity	Ashby structural alerts for DNA reactivity		*					
	Reverse, mutation test, Ames[b]		*				*	
	Chromosomal aberrations in CHO[b]		*					
	Chromosomal aberration in CHL[b]							
	Mouse lymphoma[b]		*					
	HGPRT/CHO[b]							
	UDS Rat hepatocytes[b]							
	SHE cell transformation[b]		*					
	Rodent dominant lethal[c]							
	Drosophila melanogaster SLRL[c]		*					
	SCE Mouse[c]							
	Mouse micronucleus[c]		*					
	COMET assay[c]							
Cancer	Carcinogenicity	Male rats (AF1)	*					
		Female rats (AF2)	*					
		Male mice (AF3)	*					
		Female mice (AF4)	*					
	RCA overall QSAR call (AF1-4)							
Sensitization	Skin		*					
	Respiratory							
Irritation	Skin		*				*	

Notes. Color code: red, positive; green, negative; white, out of domain. (*) Included in the training set of the model and the experimental result is indicated. *a*Leadscope model, $p \geq 0.7$ and $p \leq 0.3$ is a positive and negative prediction, respectively*; b*In vitro*; c*In vivo*.

3.2.5 Genotoxicity

- **Experimental studies:**

A mutagenicity assay (*umu test*), was conducted on *Salmonella typhimurium* TA1535 with or without metabolic activation. BPB as well as BPA, BPF, BPP and BPS were tested from 0.1 to 100 mg/L. No significant increase in β-galactosidase activity was observed with all the tested bisphenol compounds with or without metabolic activation (Chen et al. 2002) showing no mutagenic potential.

- **In Silico Profiling:**

BPB was mostly predicted negative for genotoxicity or was out of domain of the MultiCASE (version 2.4.1.4) and Leadscope (version 3.04-10), according to Rosenmai et al. (2014) (see **Table 4** below).

3.2.6 Short , long-term studies and Carcinogenicity

- **Experimental studies:**

No relevant experimental data on BPB are available.

- **In Silico Profiling:**

Some QSAR modeled data with MultiCASE (version 2.4.1.4) and Leadscope (version 3.04-10). are available in Rosenmai et al. (2014). BPB was predicted positive for carcinogenicity in male mice. However, the prediction for BPB was based on only 5 chemicals containing the biophore of which two where tested marginally positive including BPA. The overall Research Collaboration Agreement (RCA) QSAR cancer call were negative for all compounds, except BPB which was out of domain. An estimate of the carcinogenic potency in rodents (TD50) indicated very low potency (>1000 mg/kg/day) for all compounds (data not shown in Rosenmai et al. 2014).

3.2.7 Reproduction/developpement toxicity

- **In Silico Profiling**

Some QSAR modeled data are available in Rosenmai et al. (2014) with MultiCASE (version 2.4.1.4) and Leadscope (version 3.04-10). BPB was predicted negative in a QSAR model for human teratogenicity (see **Table 4** – FDA TERIS). It should noted that QSAR results cannot exclude possible *in vivo* effects due to metabolite formation, new chimeric molecules (e.g. metabolites combinaison) and other effects not taken into account in QSAR model.

- **Studies in humans and in animals:**

There are no relevant human or experimental data available.

3.3 Environment data

3.3.1 E-Fate

As specified by several suppliers, BPB is in a form of white to low brown powder with a molar mass of 242.32 g/mol (www.sigmaaldrich.com, www.us.vwr.com). According to EPIsuite, BPB has the following predicted properties: a melting point of 139.43°C, a boiling point of 375.14°C, a water solubility of 29.23 mg/L, and a low volatile vapour pressure (3.3 10^{-5} Pa at 25°C). According to HENRYWIN model of EPIsuite, BPB exhibits a Henry's law constant value of 2.73 10^{-4} Pa m^3/mol suggesting a low probability of partitioning from the aqueous system to the atmosphere. In the atmospheric compartment, BPB is predicted to undergo reactions with hydroxyl radicals with an estimated half-life of 1.57 hours.

According to EPIsuite, BPB has a logKoc derived from LogKow of 3.54 and estimated from molecular connectivity index of 4.86. **These results suggest that BPB has a tendency to adsorb to suspended solids and to accumulate and to be less mobile in sediment and soils.** The BIOWIN degradation models were run to estimate BPB biodegradation. According to Biowin 2 (non-linear model) and Biowin 6 (MITI non linear model), BPB does not biodegrade fast (p=0.41) but ultimate biodegradation could range between weeks to months (Biowin3). In addition, aerobic biodegradation pathways were not identified by *in silico* prediction using pathway prediction system of EAWAG-BBD tool. The few information available in the literature suggest **a possible biodegradation of BPB in water, in sediment** (Ike et al. 2006, Chang et al. 2014) or by specific microorganisms (Sakai et al. 2007, Lobos et al. 1992), albeit total mineralisation is not reported. For instance, results from a microcosm study showed that BPB concentration in sediment decreased by 60% under anaerobic conditions after 80 days (Ike et al. 2006). According to level III fugacity model (EPIsuite), the half-life of BPB is 37.5 days in water, 75 days in soil and 337.5 days in sediment.

Considering experimental and predicted information, there is an alert on P/vP properties of BPB in sediment based on P criteria under REACh regulation (vP > 180 days).

Regarding bioconcentration, BPB has an estimated logKow of 4.13 and an estimated BCF in fish of 248.1 (EPIsuite). Considering the worst-case scenario of no biotransformation, a BCF of 1391 is estimated which is under the limit of 2000 set for B criteria under REACh. Wang et al. (2017) assessed bioaccumulation factor (BAF) for several bisphenols in aquatic organisms of Taihu Lake in China. BPB was detected in 64.7% of aquatic organisms but not in water, impeding the BAF to be calculated. Based on the correlation between LogKow and logBAF of the 7 other bisphenol derivatives published in Wang et al. (2017), a logBAF of 1.64 could be extrapolated for BPB, that is lower than the logBAF of 2.23 estimated by EPISuite.

According to BCF estimation, BPB is not likely to fulfil the B criteria under REACh. However, the few biomonitoring data available suggest that BPB might bioconcentrate in aquatic organisms. Thus, more experimental data are required.

3.3.2 Occurrence data

BPB was measured in municipal STP influents in India (Karthikraj and Kannan 2017) and in industrial STP effluents in Slovenia applying different waste water treatments (Česen et al. 2018) with a mean concentration of 2.5 ng/L and 8.46 ng/L, respectively. STP removal efficiency ranged between 38.6 % to 100% in India and 6.39% to 98.9% in Slovenia. In Slovenia, low removal rate of BPB (< 50%) were observed for WWTP applying constructed wetlands, membrane biological treatment or biofiltration treatment (Česen et al. 2018). No BPB was detected in sewage sludge of Indian and Chinese STP (Sun et al. 2017, Song et al. 2014, Karthikraj and Kannan 2017, Sun et al. 2018) and was exceptionally detected in one sample during a USA nationwide study at 1.1 ng/g dw (Yu et al. 2015). Interestingly, not all BPB derivatives had similar behaviour in STP, some being preferentially biodegraded, other removed by adsorption on sludge or being both biodegraded and adsorbed (Sun et al. 2017, Česen et al. 2018).

In aquatic ecosystem, BPB belongs to the least frequently detected bisphenols, as reviewed in Chen et al. (2016), and Noszczyńska and Piotrowska-Seget (2018). BPB was not detected in sediment or surface water in China (Jin and Zhu 2016, Yang et al. 2014b, Zheng et al. 2015, Wang et al. 2017), Japan, Korea, USA and India (Yamazaki et al. 2015, Liao et al. 2012b) except in one sediment sample (10.6 ng/g dw, Liao et al. 2012b). Contrasting with previous results, two recent studies investigating BPs occurrence in Taihu freshwater lake in China report its detection in almost all water and sediment samples (Yan et al. 2017, Liu et al. 2017). The mean concentration of BPB ranged between 7.3 ng/L (Yan et al. 2017) and 19.8 ng/L (Liu et al. 2017) in water and between 1.2 ng/g dw (Yan et al. 2017) and 2.12 ng/g dw (Liu et al. 2017) in sediment. During previous sampling campaigns done in the same lake in 2013 (Jin and Zhu 2016) and in 2015 (Wang et al. 2017), no BPB was detected in water or sediments, suggesting a recent local increase in BPB release into the environment.

Conclusion:

BPB occurrence in the aquatic ecosystem has been poorly investigated. Albeit not frequently detected, recent studies suggest an increased occurrence in STP and freshwater ecosystem. Thus, additional monitoring

data is needed to assess the environmental contamination by BPB, especially in Europe.

3.3.3 Ecotoxicity

No long-term toxicity data are available in the scientific literature. The short-term toxicity data, summarized in Tab. 1, are based on acute toxicity tests on crustacean (*Daphnia magna*) and fish (Japanese medaka and zebrafish). In *Daphnia magna* acute immobilisation test, a 48h-LC50 of 5.5 mg/L is reported (Chen et al. 2002), in agreement with the Danish QSAR predictions ranging between 0.92 and 7.78 mg/L (EPIsuite). In fish, Yokota et al. (2008) report an 96h-LC50 of 6.1 mg/L on medaka larvae and a 14-d EC50 of 7.4 mg/L on medaka embryo hatching rate. These values are above the 96h-LC50 on fathead minnow predicted by the Danish QSAR (2 to 3 mg/L) and by ECOSAR (0.695 mg/L). BPB was about twice more toxic than BPA in both crustacean and fish tests (**Table 5**). In algae, the estimated 96-LC50 is 0.964 mg/L for green algae (ECOSAR prediction) and the 72h-LC50 on pseudokirchneriella reached up to 19.14 mg/L.

Conclusion:

Considering estimated and experimental data, BPB is not likely to fulfil the T criteria. In the CLP classification, if the P criteria is confirmed (not readily biodegradable) and considering estimated logKow and acute toxicity on *Daphnia magna*, BPB could be classified as Aquatic Chronic 2 (H411).

Table 5: Summary of acute toxicity data on BPB

Species	Study principle	Life stage	Parameter	Results	BPA/BPB ratio	Reference
Daphnia magna	OECD 202 (acute immobilisation)	Adult	24h LC50 48h LC50	9 mg/L 5.5 mg/L	2.7 1.8	(Chen et al. 2002)
Japanese medaka	acute toxicity	24h-old larvae	96h LC50	6.1 mg/L	2.3	(Yokota et al. 2008)
		24h-old embryos	14d EC50 hatching	7.4 mg/L	2.0	
			NOEC hatching	5.93 mg/L	1.1	
			LOEC hatching	8.89 mg/L	1.4	
Zebrafish	Acute toxicity	6 hpf to 120 hpf	NOEC mortality LOEC mortality	1.5 mg/L 15.5 mg/L	1 1	(Truong et al. 2014)

3.4 Endocrine disrupting properties

3.4.1 Information sources and strategy for endocrine disruptor identification

An overview of *in vitro* and *in vivo* results extracted from literature search according to EATS and non-EATS criteria is provided in **Table 6** and

Table 7, respectively, as defined in the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 abd (EC) No 1107/2009.

Table 6: Overview of endpoints and studies investigating BPB endocrine activity and effects. Results are ordered by endocrine pathway and OECD

levels. The number represents the available results for a given endpoint. Adapted from Brown et al. (2017).

	In vitro Level 2									*In vivo* Level 3			*In vivo* Level 3/4	
	ER binding	AR binding	ER Transactivation assay	AR transactivation assay	Cell proliferation	Steroidogenesis	ER gene expression	AR gene expression	TR gene expression	Uterotrophic assay	Hershberger assay	Fish short term gene expression	Short term fish fecundity assay + gene expression	*In vivo* short term and chronic toxicity assay on testicular fonctions
ER +	2		9		6		2			1		1	1	
ER -			5											
AR +		1		3							1			1
AR -				4										1
T									2					
S						2								1

Table 7: Summary of *in vitro* results related to non-EATS mediated effects.

	AhR	PXR	Progesterone (PgR) binding	Glucocorticoid (GR) binding	SHBG (sex- hormone - binding globulin) binding assay	Adiponectin
AhR	1					
PXR		1				
PgR			1			
GR				2		
SHBG					1	
Adiponectin						1

In addition, ToxCast database[6] was screened for results on endocrine activity of BPB. Over the 801 assays performed, a selection of assays was done based on the EATS and non-EATS criteria mentioned previously which lead to 132 results. A summary of ToxCast screening results is presented in **Table 8**.

Table 8: Summary of ToxCast assays related to EATS and non-EATS criteria in which BPB was active (October 2018).

Intended target	Nbre of positive results/nb assays	Intended target	Nb of positive results/nb assays

[6] https://actor.epa.gov/dashboard/, accessed on October 8th 2018

ER	18/24	PXR	4/5
AR	8/13	RXR	1/4
Thyroid (ThR, TPO, TR)	3/6	LXR	1/6
PR	2/2	CAR	0/2
GR	2/6	AhR	1/3
Metabolism (Cyp family)	3/5	PPAR	4/15
Insulin receptor	0/2	Vitamin D receptor	1/1
		Oxytocin R	0/1

3.4.2 *In vitro* data (OECD level 2)

3.4.2.1 Androgen pathway

Overall, 7 studies that investigated the androgenic and anti-androgenic properties of BPB *in vitro* were analysed. The main results are summarized below.

- **AR binding:**

BPB binding capacity to AR has been investigated in the study by Fang et al. (2003) and in several assays included in the ToxCast database and in Sipes et al. (2013). The results indicate that BPB is able to competitively bind AR from different species (human, rat, chimpanzee) in the µM range (2.2 to 37.5 µM). **BPB and BPA yield similar results** when both chemicals were tested.

- **AR agonism:**

Among results reported in the literature, no hAR agonism was observed in either human cells (Wang et al. 2014), yeast cells (Conroy-Ben et al. 2018, Wang et al. 2014) or mouse NIH3T3 cells (Kitamura et al. 2005). In ToxCast Database, BPB induced an increase in reporter gene activity in only 1 out of 5 *in vitro* assays**. As observed for BPA, BPB is unlikely to have agonist effects**.

- **AR antagonism:**

BPB had antagonistic effects in most *in vitro* studies on hamster cells (Rosenmai et al. 2014), human cells (ToxCast Database, Wang et al. 2014), yeast cells (Conroy-Ben et al. 2018) or mouse cells (Kitamura et al. 2005). IC50 reported ranged from 0.93 µM to 64.24 µM.

Conclusion:

The data available indicate that BPB can competitively bind AR and induce anti-androgenic effects in vertebrate cells.

3.4.2.2 Estrogen pathway

Overall, 17 studies investigating the estrogenic activity of BPB *in vitro* were analysed. The main results are summarized below.

- **ER binding** :

Blair et al. (2000) assessed the capacity of BPB to competitively bind ER in rats using a preparation of uterine cytosol. They showed that BPB could bind rat ER with an IC50 of 1.08 µM and a relative binding affitiny (RBA) to E2 of 0.086, indicating that BPB had about a 10-time lower binding affitiny than E2 to rat ER.

As part of ToxCast project, Sipes et al. (2013) analysed over 970 chemicals across 331 assays. They showed that BPB could bind to human ER from breast cancer cells, to bovine ER from uterus membrane and to recombinant mouse ERα ligand binding domain (LBD) with an IC50 ranging from 0.023 µM to 0.43 µM. In both Sipes et al. (2013) and Blair et al. (2000), **BPB showed a higher affinity toward ER than BPA.**

- **Promoter occupancy:**

Two studies assessed the capacity of BPB to induce the receptor occupancy of prolactin gene (PLR) promoter in Hela cells stably transfected with GFP-tagged hERα or hERβ (Stossi et al. 2014, Ashcroft et al. 2011). After 30 min exposure, BPB induced a dose-dependent increase in promoter occupancy of both ERs, with ERβ showing a higher affinity to PLR promoter (EC50: 0.161 µM) compared to ERα (EC50: 1.8 µM). BPB effects remained weak compared to E2 (e.g. EC50: 0.85 nM for hERα). The overall response profile of **BPB was similar to BPA**, but BPB showed slightly higher capacity to induce PLR promoter occupancy than BPA.

- **ER transactivation in reporter gene assays:**

BPB induced an estrogenic response with an EC50 of 5 µM and 0.59 µM in the transactivation assay based on yeast cells stably transfected with human hERα (Wang et al. 2014) or medaka mERα (Yokota et al. 2008), respectively. The study by Okuda et al. (2011) and Hashimoto et al. (2001) showed that S9 fraction activation increased the estrogenic activity of BPB in yeast cells, suggesting that metabolism could contribute to increase the estrogenic response. For the reporter gene assays based on human cancer cells expressing human or rat ERα (Mesnage et al. 2017, Yamasaki et al. 2002, Kitamura et al. 2005, Rosenmai et al. 2014, Wang et al., 2014), BPB induced ER transactivation with an EC50 ranging from 0.07 to 0.3 µM**. Across all reporter gene assays, the measured estrogenic activity of BPB was similar or even higher than that observed for BPA**, although 4 to 5 times less potent than the reference E2. In addition, the capacity of BPB to decrease E2-induced hERα transactivation in breast cancer and yeast reporter gene assays was investigated (Wang et al. 2014, Kitmura et al. 2005, (Okazaki et al. 2017). **BPB had no anti-estrogenic activity on reporter gene and a similar response was reported for BPA in these studies**.

- **ER-regulated gene expression:**

Three studies investigated gene expression profile of MCF-7 cells following either single (Okazaki et al. 2017, Mesnages et al. 2017) or dose-dependent exposure (Rivas et al. 2002) to BPB. Mesnage et al. (2017) assessed the transcriptome profile of BPB and several BP derivatives in MCF-7 cells after 48h of exposure using microarray analysis. They identified that BPB at 0.24 µM altered the expression of several genes involved in the etiology of breast cancer and hormone-induced proliferative effect with a similar profile than that obtained with BPA. The study by Rivas et al. (2002) focused on the induction of pS2 gene expression and protein levels in MCF-7 cells and showed that BPB induced significant increase in pS2 mRNA and protein levels from 1 µM.

- **Proliferative assays:**

Five studies investigated the proliferative effects of BPB on ER positive MCF-7 cells and show that **BPB is able to induce a dose-dependent increase in cell proliferation** (Pisapia et al. 2012, Mesnage et al. 2017, Stossi et al. 2014, Rivas

et al. 2002, Hashimoto et al. 2001). Mesnage et al. (2017) report an AC50 of 0.24 µM for BPB, close to BPA response (0.36 µM). An increased proliferation was also observed in the ER positive T47D cancer cells stably transfected with ERE-luciferase transgene, but not in the ER negative MDA-MB-231 cell lines, suggesting that ER, indicating that BPB proliferative effect was mediated by hERα (Mesnage et al. 2017). **GPER signaling pathway:**

The BPB nongenomic estrogenic effects were investigated in human breast cancer SKBR3 cells that express G-coupled protein ER (GPER) but not ER (Cao et al. 2017). Using SKBR3 cell-based fluorescent competitive binding assay, the authors report that BPB binds GPER with an IC50 of 3.3 µM and a RBA affinity of 8.8%, that is much higher than that of BPA (1.1%). Based on these results, BPB had a much higher binding affinity toward GPER than toward ERα (< 0.5 %), indicating that at low concentrations, BPB may preferably activate extragenomic signaling pathways. The authors further showed that binding to GPER resulted in increase calcium mobilization (LOEC: 10 nM), cAMP production (LOEC: 10 nM) and cell migration (LOEC: 100 nM). These effects were abolished by pre-treatment of the cells with GPER-selective inhibitor 15, confirming the GPER-mediated effects of BPB. BPA induced a similar response than BPB.

Conclusion:

The available information demonstrates the capacity of BPB to competitively bind to ER of several vertebrates species including human, rat, and mouse. Binding to ER leads to activation of ER signaling pathway as evidenced by ER transactivation in reporter cell lines, increased promoter occupancy and induction of ER-regulated gene expression, and eventually, evoked physiological cell response (e.g. proliferation). In addition, a recent study showed that BPB could bind extragenomic GPER with a relative binding affinity (8.8%) higher that that of ER (<1%). Activation of GPER signaling in breast cancer cells leads to increase calcium mobilization and cAMP production at 10 nM and further favored cell migration. The *in vitro* results show that both ER nongenomic and genomic signaling pathways are activated by BPB, with similar or higher sensitivity than BPA.

3.4.2.3 Thyroid pathway

The *in vitro* activity of BPB on thyroid pathway was assessed in rat pituitary GH3 cells (Kitamura et al. 2005, Lee et al. 2018, Lee et al. 2017) and in rat thyroid follicular FRTL-5 cells, as well as in assays from ToxCast database. The main results are summarized below.

Kitamura et al. (2005) assessed the capacity of BPB to modulate the thyroid hormone-dependent production of growth hormone in GH3 cells exposed for 48h. The authors report that BPB did not modulate growth hormone release in these cells up to 100 µM, in absence or presence of T3. In another study, BPB induced proliferation of GH3 cells without T3 (LOEC: 0.1 µM) and in co-exposure with T3 (LOEC: 1 µM) after 96h of exposure. Cells co-exposed to T3 were less responsive to BPB proliferative effects, as observed with the higher LOEC reported.

The expression of thyroid hormone related gene expression was investigated in rat GH3 cells and in rat thyroid follicular FRTL-5 cells. In GH3 cells, BPB exposure led to a down-regulation of trα, trβ and dio2 expression at the highest concentration tested (41 µM). Compared to BPA, BPB did not decrease tshβ expression. However, BPB dit not induce significant changes in mRNA levels of genes involved in thyroid hormone production in FRTL-5 cells, as observed for

BPA and all other BP derivatives tested. Among ToxCast assays, no agonist activity toward TR was reported, but BPB decreased TR transactivation in 1 out of 2 assays (IC50: 53.87 µM), and decreased thyroperoxidase (TPO) activity in two assays (IC50 of 1.47 and 148.41 µM).

Conclusion:

The few available information on BPB thyroidal activity indicate that BPB might impact growth of GH-3 cells (Lee et al., 2018) but the underlying mechanism remains unclear (Kitamura et al., 2005). However, BPB might interfere with TH-related gene expression at high concentrations (Lee et al., 2017), and modulate tyroperoxidase activity (ToxCast database).

3.4.2.4 Steroidogenesis

The steroidogenesis activity of BPB was assessed in human adrenal cortico-carcinoma (H295R) cells (Wang et al., 2014, Rosenmai et al. 2014). **These two assays show that BPB affects steroidogenesis into the direction of decreased androgen levels (androstenedione and testosterone) and elevated estrone levels.** A decrease of cortisol is retrieved in these two assays associated or not with a decrease of 11-deoxycorticosterone and deoxycortisol. Increased progestagen levels are only observed by Wang et al. (2014).

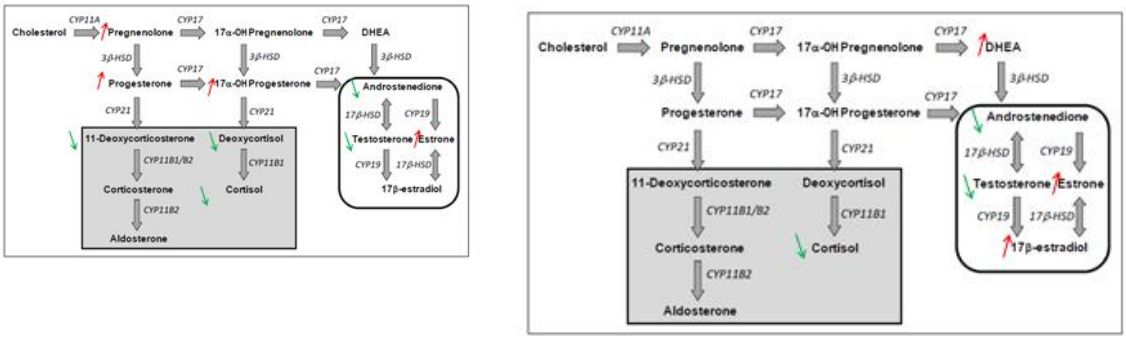


Figure 1: H295 R steroidogenesis assay results from Wang et al. (2014) on the left hand side and from Rosenmai et al. (2014) on the right hand side. Activation is highlighted with red arrows and deactivation with green arrows.

Overall, these *in vitro* results suggest that the observed effects were caused by specific interactions, and were not a result of a general down- or upregulation of steroidogenesis. The specific interactions of BPA within steroidogenesis have previously been investigated in the H295R assay (Zhang et al. 2011). Exposure to BPA was suspected to cause an increase in progesterone and a decrease in androgen levels through inhibition of the CYP17 lyase reaction and to increase estrogen levels through inhibition of metabolism of estrogens (Zhang et al. 2011). Overall, the results of these two steroidogenesis studies are in accordance with previous BPA's findings suggesting that one or both of the specific interactions of BPA may be applicable for BPB.

The study by Desdoits-Lethimonier et al. (2017) reports the effects of BPB and other bisphenols on human adult testicular explants. The amount of testosterone secreted in the medium was reduced with explants exposed for 24 or 48h to 0.1 µM BPB only whereas reduced testosterone secretion was reduced with BPA at 10 µM. An increase of INSL3 secretion was detected only with 0.001 µM after 24h of exposure to BPB or BPA. Lastly BPB and other bisphenols did not affect significantly the amount of inhibin B secreted in the medium. These episodically

hormonal secretion might be explained with the high variability within the individual values.

Conclusion:

These two H295R assays show that BPB affects steroidogenesis by decreasing androgen levels (androstenedione and testosterone) and increasing estrone levels, combined with a decrease of cortisol. Overall, the results of these two steroidogenesis studies are in accordance with previous BPA's findings suggesting that one or both of the specific interactions of BPA may be applicable for BPB. The study by Desdoits-Lethimonier et al. (2017) shows occasional positive or negative changes in hormonal secretions of the Leydig cells in cultured adult human testes exposed to BPB. The high variability within the individual values and the limited number of independent experiments make these results to be interpreted with caution.

3.4.2.5 Others endocrine pathways

Interactions with several CYP enzymes as well as two receptors, **AhR** and **PXR**, associated with metabolism were investigated *in vitro* by Sui et al. (2012) and by Rosenmai et al. (2014), respectively.

In the AhR reporter gene assay, no AhR activation was observed with BPB whereas its activation was shown with BPA at high concentrations (Rosenmai et al. 2014). Sui et al. (2002) show, in a reporter gene assay, that BPB is a potent agonist for human PXR receptor (hPXR) but not for mouse PXR (mPXR). Activation of hPXR was dose-dependent and BPB was more potent than BPA as hPXR agonists at a low concentration (5 µM), and had comparable agonistic effects at high concentrations (10 and 25 µM). Lastly, consistent with the reporter assays, BPB significantly induced PXR target gene expression namely, CYP3A4, UGT1A1, and MDR1 in a dose-dependent manner in human intestine epithelial cell line (LS180 cells). PXR and AhR activation induce the expression of enzymes involved in the metabolism of xenobiotics but also of endogenous hormones. Previous findings from Zhang et al. (2010) indicate that PXR activation has been associated with decreased androgen levels. Thus, activation of hPXR by BPB may add to the overall endocrine potential by increasing or decreasing the removal of endogenous hormones *in vivo* causing disruption of homeostasis.

Verma et al. (2018) investigated the *in silico* binding of several bisphenol analogs on different enzymes involved in the glucocorticoid biosynthetic pathway (GBP). This study clearly indicates the potential of BPB to bind to 3β and 17β-HSD with a docking score of -7.793 versus - 7.384 with trilostane. Interestingly, BPB was also shown to possess higher binding affinity (-5,929) compared to anastrazole (-5.626), an established inhibitor of CYP19A1. Lastly, BPB also showed comparable docking efficiency (-7.933) with Canrenone (-8.847), a known inhibitor of CYP21A2.

Sharma et al. (2018) studied binding efficiency of bisphenol analogues including BPA and BPB with human PPARs and retinoid X receptors (RXRs) which act as transcription factors and regulate genes involved in glucose, lipid, and cholesterol metabolism and adipogenesis. **BPB showed a stronger binding affinity with RXR compared to BPA**. In comparison, BPA showed a stronger binding affinity with hPPARβ than hPPARα with the D score of - 7.463 which was very close to the D score of one of the known binders of hPPARβ, retinoic acid (- 7.833).

In a **progesterone** (PgR) induction assay, BPB significantly increased the PgR levels at the highest tested dose level (10 µM) *versus* untreated human MCF-7 cells (Sipes et al. 2013). In a competitive binding assay, Guan et al. (2017) and Zhang et al. (2017) show a **glucocorticoid** (GR) agonistic activity of BPB at quite similar levels than BPA (IC50: 14.8 µM BPB versus 18.8 µM BPA).

In another competitive binding assay using human pregnancy plasma, Hong et al. (2015) measured the **binding to the human sex hormone-binding globulin** (SHBG) for BPB and BPA. SHBG is the major transport protein in serum that can bind androgens and estrogens and hormone molecules to target tissues and cells. Sequestration of an androgen or estrogen in the serum can alter the chemical elicited AR- and ER-mediated responses. In this assay, BPB exhibits binding activities with an IC50 10 µM *versus* 15 µM with BPA.

Lastly, the impact of BPB on **adiponectin production and secretion** in 3T3-L1 adipocytes and whether BPA acts through Akt signaling were investigated by Kidani et al. (2010). As BPA, BPB decreased the amounts of intracellular and medium adiponectin. The order of the potential to decrease the amount of intracellular adiponectin was BPB>BPA>BPE>BPF whereas the amount of adiponectin in the medium was similar for BPB and BPA. **These results indicate that BPB and BPA inhibit adiponectin production** in cells, resulting in reduced secretion of adiponectin. In those experiments neither the PPARγ antagonist (GW9662) nor the ER antagonist (ICI 182,780) can reverse the inhibitory effect of BPA on adiponectin production, thus indicating that BPA inhibits adiponectin production via an alternative mechanism that does not involve PPARγ nor the classical nuclear ER receptors in 3T3-L1 adipocytes. Adiponectin is known to increase insulin sensitivity and low plasma adiponectin levels in obesity might contribute to insulin resistance. Without taking into consideration the dose levels used (80 µM for BPB and from 20 to 80 µM for BPA), this study indicates that BPB and BPA may increase insulin resistance.

3.4.2.6 Conclusion of *in vitro* data

The *in vitro* estrogenic activity of BPB have been evaluated in depth. The results showed that BPB binds the estrogen receptors and induces estrogen pathway with a similar or higher potency than BPA. Albeit less investigated, the results on the androgen pathway indicate that BPB can bind the AR and induced an anti-androgenic response in most vertebrate cell lines. In addition, BPB was shown to interfere with the steroidogenesis, resulting in decreased concentrations of testosterone and cortisol and increased concentrations of estrone. In constrast, information on thyroid pathway are scarce and do not allow to draw firm conclusions.

Many data on non-EATS parameters were analysed. They suggest BPB capability to interfere with additional targets such as GR, PR, SHBG, PXR or adiponectin production.

3.4.3 *In vivo* mechanistic data (OECD level 3)

3.4.3.1 Human health data

Two *in vivo* mechanistic studies investigated the estrogenic and (anti)androgenic properties of BPB.

The study by **Yamasaki et al. (2002)** reports the estrogenic activity of 23 compounds, including BPB in an **immature rat uterotrophic assay** (OECD TG 440). BPB and 22 other chemical substances such as BPA and BPF were injected subcutaneously on the dorsal surface of Crj:CD (SD) rats at doses of 2, 20 and 200 mg/kg/day (dissolved in olive oil) from postnatal day 20 (PND 20) to PND 22, i.e. for 3 days (6 animals per group). A control group received only olive oil and positive control groups received 0.2, 2 and 20 µg/kg bw/day of ethynyl estradiol, 2, 20 and 200 mg/kg bw/day estrone or 17α-estradiol.

Watery uterine contents were detected in rats given BPB at 200 mg/kg/day and also in animals treated with estrone from 2 mg/kg/day, 17α-estradiol from 20 mg/kg/day or with ethynyl estradiol from 2 µg/kg bw/day. The uterine blotted weight (absolute value) was not significantly increased with 2 and 20 mg/kg bw/day BPB. With 200 mg/kg/day BPB, its weight was 257% as compared with controls *versus* 197% for 200 mg/kg/day BPA and 308% and 315% for 2 and 20 µg/kg/day ethynyl estradiol respectively. The blotted uterus of the animal treated with 20 mg/kg bw/day BPB or BPA weighted 146% and 141% as compared with controls respectively, but the difference was statistically significant only for BPA and not for BPB. For all the treatments, the relative weight changes were essentially the same as the blotted weight.

The uterus is an estrogeno-dependent tissue that responds to estrogens through two pathways. An initial response is an increase in weight due to water imbibition, then followed by a weight gain due to tissue growth. The effects observed in the study of Yamasaki et al. (2002) are therefore consistent with an estrogenic effect of BPB and BPA.

Lastly, it should be noticed that dams and pups were housed in polycarbonate pens until weaning (PND 17). Then, immature rats were housed individually in stainless steel, wire-mesh cages. Estrogenic properties of the diet were not characterised in this study. However, the contamination of the animal by estrogenic compound, if it exists, was probably negligible since the blotted uterus of controls weighed 30 mg and OECD 440 considers that results should be considered as suspicious if this weight is above 40 mg.

Conclusion:

This study shows that subcutaneous BPB treatment of immature Crj:CD (SD) rat by 200 mg/kg/day BPB from PND 20 to PND 22 increases watery uterine content and blotted uterine weight indicating that BPB has an estrogeno-mimetic activity in an immature uterotrophic assay.

Yamasaki et al. (2003) studied the ED properties of BPB and 29 other chemicals **in a Hershberger assay**. The tested compounds were dissolved in olive oil and orally administered (*via* a stomach tube) to castrated male Brl Han: WISTJcl (GALAS) rats for 10 consecutive days beginning on PND 56, 14 days after castration. The following dose levels were tested: 50, 200 or 600 mg/kg/day associated or not with 0.2 mg/kg bw/day testosterone propionate (TP) at 0.2 mg/kg per day administered by subcutaneous injection (6 animals per group). A control group received only olive oil.

A significant decrease in body weight (≈8%) and a reduction of spontaneous locomotion were observed after treatment with 600 mg/kg bw/day with BPB or BPB plus TP. No abnormalities were observed with BPA and BPA plus TP.

Table 9: Summary on the effect of BPB on the Hershberger's test on adult castrated male Wistar Jcl rats. TP: testosterone propionate. Results are given as relative organ weight. (Yamasaki et al., 2003)

	Positive control (TP)	**BPB**	**BPB+ TP**
Ventral prostate	↑ (x5)	=	↑↑ 200-600 mg/kg bw/d (x6.5-x8)
Seminal vesicles	↑ (x6.5)	=	↑↑600 mg/kg bw/d (x10)
Glans penis	↑ (x2)	=	↑600 mg/kg bw/d (x2.5)
BC/LA	↑ (x2)	↓ 200 -600mg/kg/d Not dose-dependent (x0.8)	↑ 600 mg/kg bw/d (x2.5)
Cowper's glands	↑ (x4.5)	=	↑↑600 mg/kg bw/d (x6)

The main results are presented in **Table 9**. The bulbocavernosus /levator ani muscle (BC/LA) weights decreased by (18%) after exposed to 200 mg/kg/day of BPB. The variability in the treated group was high. Lastly, no other modification of the examined organ weights (ventral prostate, seminal vesicle and Cowper's gland) at 50, 200 et 600 mg/kg/day were observed with BPB only. Taken together, these data suggest that BPB does not exhibit an androgen agonistic property.

In the presence of TP, a significant increase of the ventral prostate weight from 200 mg/kg bw/day compared to TP alone was observed with a dose-response relationship. Furthermore with 600 mg/kg/day BPB, the weights of all other examined sexual organs (glans penis, Cowper's gland, seminal vesicle and BC/LA) were significantly increased by 13 to 57%. This suggests that the administration of BPB exacerbated the effect of TP. It seems specific to BPB since it was not observed with BPA and BPF in this study.

Conclusion:

Overall, using the Hershberger assay, this study indicates that BPB administered alone in castrated rats did not exhibit androgenic properties at dose levels from 50 to 600 mg/kg bw/day. An anti-androgenic effects at 200 and 600 mg/kg bw/day was observed in one (BC-LA muscle) out the five examined androgen-dependent sexual organs, and further studies evaluating the anti-androgenic effect of BPB are required before any definitive conclusion can be made.

Nevertheless, rats given 600 mg/kg bw/day BPB plus TP (testosterone propionate) exhibit significant increases in the weights of all the five androgen-dependent targets. This suggests that BPB increases either TP availability or the action of TP. This effect would be specific of BPB since it was not observed with BPA and BPF. However, whether BPB exhibits an androgen agonist effect would need further experimental support.

3.4.3.2 Environmental health data

The study by **Yamaguchi et al. (2015)** reports the **estrogenic activity of several bisphenols, including BPB, on the medaka** (*Oryzias latipes*). Four-month-old male medaka were incubated at 25°C for 8 h to BPB at 0.5, 5 and 50 µM (purity> 97%, nominal concentrations), to E2 positive control (3.7 nM) and to BPA (5 and 50 µM). Hepatic estrogen-responsive gene expression levels of vtg1,

vtg2, chgH, chgL and ERα were assessed. For each liver sample, the mRNA expression levels were normalized to β actin mRNA expression.

The expression of hepatic estrogen-responsive genes vtg1, ChgH, ChgL and ERα was upregulated by BPB at the concentrations of 5 and 50 µM (except for ChgH at concentration 50 µM). However, the response was not monotonic as the maximum expression level was measured at 5 µM. The LOEC observed (5 µM) was lower than what was obtained with BPA (50 µM), but nearly 100 times higher than what was observed with E2 control.

Conclusion:

This study indicates that BPB treatment of 4 month-old male medaka exposed for 8 h upregulated hepatic estrogen-responsive gene expression, indicating that BPB has an estrogeno-mimetic activity in male fish (which is more potent that the one induced by BPA).

3.4.3.3 Conclusion of *in vivo* mechanistic data

Both studies investigated BPB estrogenic activity in rodent (uterotrophic assay) and fish (gene expression in male medaka liver) and confirmed the estrogenic activity of BPB observed *in vitro*. Regarding the androgen pathway, the Hershberger assay indicated some anti-androgenic activity and further studies evaluating the anti-androgenic effect of BPB are required before any definitive conclusion can be made. Lastly, the results suggest BPB capacity to promote propionate testosterone response.

3.4.4 *In vivo* adverse effect data (OECD level 3/4)

3.4.4.1 Human health data

Two recent studies on rodent investigated the effect of BPB on the testicular fonctions using *in vitro* and *in vivo* methods. Both originated from the same group, Ullah and colleagues, and focused on the effects of several bisphenol derivatives on male rat reproductive fonctions.

The study by **Ullah et al. (2018a)** reports the effects of BPA and three of its analogs (BPB, BPF and BPS) **on testicular functions using *in vitro* and *in vivo* approaches**. Sprague-Dawley adult male rats were housed in steel cages. Endocrine disrupting properties of the diet were not characterised in this study.

In the *in vitro* experiment, the testes were cut into five equal parts that were cut into slices and deposited in tubes containing the culture medium added with 0, 1, 10 or 100 ng/ml of BPB (1 ng/mL = 0.004 µM). After 2 hours of incubation, there was a trend of increased concentrations in reactive oxygen species (ROS) in the tissue exposed to BPB and BPA, which was statistically significant for 10 but not for 100 ng/ml BPB. Intratesticular testosterone concentration was unaffected by the exposure to BPB and BPA. Some limitations were identified regarding the conditions of culture which were not a good quality enough to guarantee the survival of the testicular cells. These data are not integrated in the weight of evidence analysis.

For the *in vivo* approach, Sprague-Dawley adult male rats aged between PND70 to 80 were exposed orally to 0, 5, 25 and 50 mg/kg/day bisphenols (7 animals per group) repetitively for 28 days. After treatments with 50 mg/kg/day BPB, the testicular concentrations in ROS and peroxided lipids (LPO) were increased and

the activities of some antioxidant enzymes such as peroxidase (POD) were decreased. Plasma and intratesticular testosterone concentrations were significantly reduced after all the treatment with BPB. However, this effect was not dose dependent. Lastly, histology of the testis and the epididymis showed changes in the group treated with the higher doses of BPB and BPA (50 mg/kg bw/day) as compared with controls. The height of the seminiferous tubules was quantified and exhibited a significant decrease, thus indicating that spermatogenesis was impaired. Qualitative observations showed reductions in the number of elongated spermatids/sperms in the lumen of the seminiferous tubules and of the epididymis. However, this observation was not quantified, therefore, it is difficult to assess the significance of this finding. Overall, BPA exerted similar effects as BPB excepted that the reduction of intratesticular testosterone level was dose-dependent and observed from 25 mg/kg bw/day.

Conclusion:

Overall, this study suggests that BPB reduces testicular testosterone production from the lower used dose (5 mg/kg bw/day) and alters spermatogenesis with 50 mg/kg bw/day in the adult rat. This may be the result, at least in part, of an increase in oxidative stress. BPA exerts similar or lower effects than BPB.

The study **Ullah et al. (2018b)**, reports the effects of **chronic exposure to low doses of BPA and three of its analogues (BPB, BPF and BPS) on pituitary - testicular activities.**

Sprague-Dawley adult male rats were housed in steel cages and were fed soy-free and alfalfa food and water in polysulfone bottles. Animals on PND23 received drinking water containing 0, 5, 25, 50 µg/L BPB or three other bisphenols, BPA, BPF, BPS) for 48 weeks (7 animals per group). Daily water intake was not monitored, but as a rat drinks around 10 mL/day/100 g body weight, one could estimate that they received around 0, 0.5, 2.5 and 5 µg/kg/day bisphenol. At the end of the treatment, the relative weights of the testis, the epididymis, the seminal vesicle were decreased with the highest dose of BPB (50 µg/L). The reduction of prostate weight was not statistically significance.

The testicular concentrations in reactive oxygen species (ROS) and peroxided lipids (LPO) were increased for 50 µg/L BPB. The activities of antioxidant enzymes were decreased: catalase (CAT) and peroxidase (POD) for 25 and 50 µg/L and superoxide dismutase (SOD) for 50 µg/L BPB. After treatment with BPB, there were dose-related trends towards decreased testosterone, LH and FSH concentrations and an increase in estradiol concentration in plasma. As compared with controls, all these changes were statistically significant with 50 µg/L BPB. Sperm production was examined. In the cauda epididymis, the motile sperm percentage were reduced by 3% after exposure to 50 µg/L BPB, but the viable sperm percentage was unaffected. Sperm count in the testis showed that the daily sperm production was dose-dependently reduced (statistically significance for 50 µg/L BPB, with 9 % of reduction). In the same way, the sperm number was also dose-dependently decreased in the caput epididymis (significance from 25 µg/L BPB) and in the cauda epididymis (significance for 50 µg/L BPB and BPA).

Testicular histological analyses were performed. The height of seminal epithelium was dose-dependently decreased (statistically significance for 50 µg/L BPB, with a reduction by 16 %) without changes in the diameter and the relative area of seminiferous tubules. The numbers of spermatogonia, spermatocytes and spermatids were statistically reduced after chronic exposure to 50 µg/L BPB in the

drinking water. Histological examination of the caput and cauda regions of epididymis did not exhibit changes in the tubular diameter and the epithelial height. Lastly BPA exerted, at the same dose levels, similar effects as BPB.

Some limitations in the study were observed regarding the methodology used to measure estradiol levels, that was judged not sensitive enough to detect such small variations of estradiol. In addition, the results on LH and FSH are also very consistent from one group to another, which is surprising when measuring pulsatils hormones.

Although the methodology followed in this chronic study (i.e. in intact animals, longer period of exposure and low dose levels) is not comparable to the Hershberger assay (in castrated animals, with a shorter period of exposure and at high dose levels), it should be noticed that the hormonal and histological effects observed in Ullah et al., 2018b are consistent with the anti-androgenic effect observed in one of the androgen-dependent sex accessory tissues namely BC/LA in the Hershberger assay.

Conclusion:

Taken together, these data evidence that a chronic exposure to low doses of BPB through drinking water alters the male reproductive system in the adult rat. These hormonal and histological effects are consistent with some of the anti-androgenic effect observed in castrated animals in the Hershberger assay. Both endocrine and autocrine/paracrine testicular functions were disrupted. BPB acted on the gonadotropic hypothalamo-pituitary system. BPB-induced changes in testicular hormonal production observed here are in accordance with those observed *in vitro* (Wang et al. 2014, Rosenmai et al. 2014). Lastly BPB and BPA had the same qualitative and quantitative effects in this study. It should be noted that the results reported for BPA are in contradiction with some of the existing and new literature (e.g. Dere et al. 2018). The influence of parameters varying among these studies, such as the route of exposure for example, should be further investigated.

3.4.4.2 Environmental health data

Yang et al. (2017) report the results of **fecundity test on zebrafish with BPB based on the OECD 230 guideline.** Six male and female zebrafish aged 4-month-old raised at 28°C in 10 L aquariums were exposed to BPB at concentrations of 0, 0.001, 0.01, 0.1 and 1 mg/L (purity > 98%, nominal concentrations) during 21 days. No E2 positive control was used.

The results obtained showed a range of significant effects. The hepato-somatic index of the 0.1 and 1 mg/L exposure groups were significantly higher than that of the control group, in both male and female zebrafish. The gonado-somatic index of the group exposed to 1 mg/L was significantly decreased in both male and female zebrafish. Histological analyses of the gonads showed an alteration of the testis tubules and a decrease of the amount of mature spermatids after exposure to 0.1 and 1 mg/L. In female, one ovary did not develop any post-vitellogenic oocyte at 1 mg/L exposure to BPB. The eggs production of parental fish, hatching and survival rates of their offspring were significantly decreased at 1 mg/L. Some malformations (e.g. abnormal curvature of larvae) in F1 generation were also noticed for the group treated with the higher dose.

Analyses of circulating hormones in **male fish** showed dose-dependent responses of testosterone (T), estradiol (E2) and progesterone (P). Significant decrease in T and significant increase in P concentrations were measured from the group

exposed to 0.1 mg/L, while an increase in plasmatic E2 content was already significant from 0.01 mg/L. Exposure of **female fish** resulted in decreasing T concentration for the 1 mg/L exposure group only, and in increasing E2 concentration for 0.01, 0.1 and 1 mg/L exposure groups.

Transcription of target genes regulating HPG axis and steroidogenesis were affected in both males and females when exposed to BPB, but the magnitude of the effect was more important in male fish. Significant and dose-dependent induction of gnrhr1, gnrhr2, fshβ, lhβ, ERα, cyp19b was measured in exposed-males brain while only few genes were significantly repressed at the maximal dose in female brain. In testis, a dose-dependent induction of fshr, lhr, cyp11a, 3βhsd and cyp19a gene expression was reported while cyp17 and 17βhsd transcript levels decreased (only at the maximum exposure dose). Significant induction of hepatic vtg in male liver indicates a marked estrogenic effect as early as 0.1 mg/L.

Conclusion:

Exposure of zebrafish during 21 days to high concentration of BPB (1 mg/L) impaired the reproductive function of zebrafish, reducing the egg number, the hatching rate and survival of the embryos (F1 generation). These alterations were concomitant to malformation of testis and ovary, modification of T and E2 levels, and to altered expression of key genes involved in HPG axis and steroidogenesis. Alterations of genes and hormones levels were more important in male than in female fish. In addition, hepatic vitellogenin expression was upregulated in male zebrafish exposed from 0.1 mg/L, indicating that BPB possesses estrogenic activity. The increase in VTG observed is coherent with the one reported by Yamagushi in medaka after short term exposure to BPB.

3.4.4.3 Conclusion on *in vivo* adverse effects (OECD levels 3/4)

Only 3 studies have investigated BPB adverse effects in vertebrates: two studies in rodent and one in fish. They all evidenced the effect of BPB on male reproductive system (altered spermatogenesis) and changes in hormones levels (decrease in T and increase in E2 levels). In zebrafish, BPB decreased fish fecundity at the highest concentration tested, as observed with the reduced egg numbers, hatching rate and survival.

3.4.5 Analysis of BPB mode of action

The available data of BPB adverse effects are scarce, considering both vertebrates or invertebrates. Only three *in-vivo* studies on intact organisms were found in the literature search. Yang et al. (2017) showed that BPB significantly reduced fecundity of adult fish exposed for 21 days and decreased embryos hatching and survival of F1 generation. Ullah et al (2018a, 2018b) showed that spermatogenesis is impaired in rat exposed to 50 mg/kg/day for 28 days or an estimated dose of 50 µg/kg/day for 48 weeks. Regarding endocrine mode of action, decreased fecundity of fish and spermatogenesis of rat could result from disruption of several endocrine pathways.

Although many interactions are involved in the regulation of spermatogenesis, *in vitro* and *in vivo* data point at least toward two main endocrine disruption pathways involved in BPB-induced gametogenesis disruption: (1) a decrease of androgenic action and/or (2) an increase of estrogenic action. Each of these two endocrine disruptions can originate from changes in hormonal production and/or in hormonal action.

The *in vitro* data on steroidogenesis and *in vivo* data in rat and fish demonstrate the capacity of BPB to decrease testosterone cellular levels (Rosenmai et al. 2014, Wang et al. 2010), plasmatic levels (Ullah et al. 2018a and 2018b, Yang et al. 2017), or intra-testicular levels (Ullah et al. 2018a). High levels of testosterone are required for spermatogenesis (review in Shiraishi and Matsuyama (2017)), thus, it can be hypothesized that BPB-induced spermatogenesis disruption is the consequence of a decreased of effective intra-testicular T concentrations.

Based on the *in vitro* and *in vivo* mechanistic data presented above, it is however not clear whether BPB negatively acted on spermatogenesis and testosterone levels via an anti-AR mode of action. Four out of five *in vitro* reporter gene assays showed BPB antagonist capacity (IC50 in the µM range), but the Hershberger assay gave unclear results (Yamasaki et al. 2003).

All the available *in vitro* data on steroidogenesis (Rosenmai et al. 2014, Wang et al. 2010) and *in vivo* data in rat and fish showed an increase in estrogens levels concomitantly to a decrease in testosterone (T)levels (Yang et al. 2017, Ullah et al. 2018a and 2018b). In addition, a large body of *in vitro* data showed that both ER genomic and extra-genomic signalling pathways are activated by BPB. In fish, the increase in levels of VTG expression in liver and the increase in ER-regulated cyp19a1b expression in brain of male zebrafish support the *in vitro* estrogenic activity of BPB (Yang et al. 2017, Yamaguchi et al. 2015). As estrogens are key regulators of male physiology in vertebrates (Cooke et al. 2017), it can be hypothesized that BPB acts via an estrogenic mode of action to alter spermatogenesis. Further investigations in rodent would be needed to know whether BPB acts on organs expressing aromatase (such as testis or the brain).

In addition, the *in vivo* data showed a decreased in LH and FSH plasmatic levels in rats (Ullah et al. 2018b), and LH- and FSH-related gene expression in brain and gonads of male zebrafish (Yang et al. 2017). LH and FSH are key regulators of spermatogenesis (O'Donnell et al. 2017). These results suggest that the alteration of spermatogenesis and the T levels might result from an indirect action via the hypothalamus-pituitary axis.

3.4.6 Overall conclusion on ED properties

The World Health Organization (WHO) defines an endocrine disruptor chemical (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" (WHO/IPCS 2002). Thus, the definition of EDC is based on three elements that must be identified concomitantly, i.e. (1) an adverse effect, (2) an endocrine activity and (3) a plausible mechanistic link between these two observations. In this report, these three elements were assessed 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 BPB 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 rats data were used in the same line of evidence to strengthen the weight of evidence assessment considering both the human and environmental health together. From a regulatory point of view, they are usually evaluated

separately (e.g. BPA identification as SVHC, European Chemical Agency 2017a, European Chemical Agency, 2017b). However, we considered the integrated approach relevant in 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 was shown to have clear estrogenic effects in fish and rats. Furthermore, BPB exposure lead to higher estrogen and lower androgens levels both in *in vitro* and *in vivo* studies. To a lesser extent, BPB may antagonize androgens actions, although this effect was not firmly confirmed in the Hershberger assayAltogether, these mechanistic data are consistent with the alteration of spermatogenesis, which could be disturbed by decreased testosterone levels and increased level of estrogens or estrogeno-mimetic chemicals (Akingbemi 2005, Delbès et al. 2005, Leavy et al. 2017). Thus, so far, BPB fulfils the criteria defining an EDC.

In addition, the comparison of BPB and BPA endocrine activities brings additional arguments for 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. In both *in vivo* studies in rats by Ullah and colleagues, BPB treatment resulted in lower seminal vesicle and epididymis weights, a lower height of epithelium in testicular tissues and less spermatocytes and spermatids. These changes were similar or even slightly more pronounced as compared with the same BPA treatment (Ullah et al. 2018b, Ullah et al. 2018a).

BPB is currently not registered under REACH regulation. The opportunity to identify BPB as an ED based on article 57 of REACH (SVHC) has been discussed at the ED expert group (EDEG) 13. Experts were not convinced that the quantity and level of information available on the single substance would be sufficient for identifying BPB as an ED based on article 57 of REACH (SVHC). The opportunity to identify BPB as a presumed ED has not been discussed at the EDEG. Therefore, the EDEG encouraged to strengthen the case with data of other bisphenols up to a grouping approach on the bisphenol family considering the limited information on the single substance. **Considering EDEG comments and although reviewing BPA endocrine properties is beyond the scope of this report, ANSES further considers the results obtained with BPA.**

Based on available scientific information, the European chemical agency (ECHA) has identified BPA as reprotoxic chemical of category 1B based on reprotoxic effects in both males and females (CLP dossier, European Chemical Agency 2014). The CLP dossier concluded that BPA induced negative effects on the plasma testosterone levels, on the organs of the reproductive tract and on the 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 are 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 are likely to contribute to the divergent sensitivities reported (National Toxicology Program 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 and SVHC-ED for the environment (European Chemical Agency 2017). The dossier reported a clear estrogen agonist activity of BPA in fish, also evidenced with 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 are demonstrated with BPB in the 21-day reproductive study in zebrafish (Yang et al. 2017). In addition, BPA exposure resulted in a lower sperm volume and/or 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 less spermatozoa (Metcalfe et al. 2001), supporting the likelihood of similar effects between both bisphenols in fish.

Overall, the existing information on BPB's estrogenic activity and inhibition of testosterone production is 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, the Industry might invest in BPB as a substitution for BPA, with possible detrimental consequences. In this context, the ANSES EDC Working Group and the ANSES scientific expert committee on REACh considers 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.

4 INFORMATION ON (AGGREGATED) TONNAGE AND USES[7]

4.1 Tonnage and registration status

Table: Tonnage and registration status

From ECHA dissemination site	
Registrations	☐ Full registration(s) (Art. 10) ☐ Intermediate registration(s) (Art. 17 and/or 18)
Total tonnage band for substance (excluding volume registered under Art 17 or Art 18, or directly exported)	Choose the appropriate option from this dropdown menu.

[7] *Please provide here the date when the dissemination site was accessed.*

The substance is not registered.

4.2 Overview of uses

The substance is not registered.

Table: Uses

	Use(s)
Uses as intermediate	/
Formulation	/
Uses at industrial sites	/
Uses by professional workers	/
Consumer Uses	/
Article service life	/

4.3 Additional information

In the US, according to HSDB (2013)[8], bisphenol B may be used in the manufacture of phenolic and polycarbonate resins that may be release into the environment through various waste streams. In addition, bisphenol B can be released from resin linings used as corrosion inhibitors to coat cans in the food industry, as discussed in part 3.2.1.

Furthermore, bisphenol B is in the 'List of Indirect Additives Used in Food Contact Substances' maintained by the U.S. Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN), in Section 175.300 'resinous and polymeric coating'.

[8] Hazardous Substances Data Bank. https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~EHIoRZ:1

5 JUSTIFICATION FOR THE REGULATORY MANAGEMENT OPTION

5.1 Need for (further) regulatory management

Table: SVHC Roadmap 2020 criteria

	Yes	No
a) Art 57 criteria fulfilled?	X, maybe within a family approach	
b) Registrations in accordance with Article 10?		x
c) Registrations include uses within scope of authorisation?		x
d) Known uses not already regulated by specific EU legislation that provides a pressure for substitution?		x

Bisphenol B is registered by the US FDA as an indirect additive used in some food contact resinous and polymeric coatings. BPB may constitue an alternative for BPA and BPS even if the substance is not yet registered in the EU.

In light of the consistent body of evidences on BPB endocrine activity and the adverse effects observed in vertebrates, BPB is currently not foreseen as a safe BPA alternative. The identification of BPB as an ED would be recommended to prevent its use and importation in Europe and to protect our industries to invest in unsustainable BPA alternatives. The ED EG recommends to consider BPB within a grouping approach on the bisphenol family.

5.2 Conclusions on the most appropriate (combination of) regulatory management options

The existing information on BPB's estrogenic activity and inhibition of testosterone production is similar to BPA's endocrine activity. It emerges from the available studies that BPB has similar or even greater effects than BPA, especially regarding the estrogenic activity. In this regard, even if BPB is not yet registered, this substance is currently not foreseen as a safe BPA alternative. Indeed, because of its structural similarity to BPA, BPB might be seen by the industry as a potential alternative to BPA. Therefore, in light of the consistent body of evidences on BPB endocrine activity and its similarity to BPA's endocrine activity based on existing information and the adverse effects observed in vertebrates, the identification of BPB as an ED is recommended to prevent its use and importation in Europe and to protect our industries from investing in unsustainable BPA alternatives. A grouping approach of bisphenols is considered relevant to avoid any regrettable substitution. **In view of the information**

currently available on BPB and regarding the above-mentioned context, the ANSES EDC Working Group and the ANSES scientific expert committee on REACh consider that the current information available is sufficient for regulating BPB for its endocrine properties, and thus, seek to protect human health and wildlife, while avoiding a regrettable substitution. ANSES therefore considers necessary to identify BPB as an ED and thus as an SVHC.

In addition, it is worth to note that information on levels of exposure to BPB in Europe is expected in the upcoming years from the EU-wide biomonitoring project HBM4EU that includes BPB within the priority substance group "bisphenols" monitored in European citizens[9]. Moreover, in France the national strategy on endocrine disruptors (SNPE2) aims to evaluate the knowledges on chemicals which are likely endocrine disruptors. Therefore, within the framework of the French strategy on ED 2, ANSES will assess 3 chemicals per year under REACH in 2019 and 2020 and 6 chemicals from 2021 onwards in order to propose the identification of these chemicals as ED.

[9]https://www.hbm4eu.eu/the-substances/bisphenols/.

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7 ABBREVIATION LIST

AR: Androgen receptor
ARh: Aryl hydrocarbon receptor
3β/17β-HSD: 3β/17β-hydroxysteroid dehydrogenase
BP: bisphenol
BPA: bisphenol A
BPB: bisphenol B
BPE: bisphenol E
BPF: bisphenol F
BPS: bisphenol S
CAR: Constitutive Androstane Receptor
CAT: Catalase
D4A: androstenedione
DHEA: dehydroepiandrosterone
DMSO: dimethyl sulfoxide
Dex-fl: dexamethasone fluorescein
E1: estrone
E2: estradiol
EC50: the exposure concentration leading to 50% of the maximum response.
Emax maximum efficacy values, which describe the observed maximum change in response compared with control.
ER: Estrogen receptor
ERR: Estrogen receptor related
FP: fluorescence polarization
GBP: Glucocorticoid biosynthetic pathway
GPER: G protein-coupled receptor
GR: glucocorticoid
HeLa: human cervical carcinoma cell line (HeLa229)
HPG: hypothalamic-pituitary gonadal
HPP 4-cumylphenol
LPO: lipid peroxides
MCF-7:
MOA: mode of action
MTT: 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
OHF: hydroxyflutamide
PND: postnatal day
PPAR peroxisome proliferator-activated receptors (PPARs)
RAR: retinoic acid receptor
ROS: reactive oxygen species
RXRs: retinoid X receptors
SHBG: Sex-hormone-binding globulin
SOD: superoxide dismutase
TCDD: 2,3,7,8-Tetrachlorodibenzo-p-dioxin
TP: testosterone propionate
U2-OS: human osteosarcoma cell line