Substance Name:
4,4'-(1-methylpropylidene)bisphenol;
(bisphenol B)
EC Number: 201-025-1
CAS Number: 77-40-7

MEMBER STATE COMMITTEE SUPPORT DOCUMENT FOR IDENTIFICATION OF

4,4'-(1-METHYLPROPYLIDENE)BISPHENOL; (BISPHENOL B)

AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS ENDOCRINE DISRUPTING PROPERTIES (ARTICLE 57(F) - ENVIRONMENT), ENDOCRINE DISRUPTING PROPERTIES (ARTICLE 57(F) - HUMAN HEALTH) PROPERTIES

Adopted on 3 June 2021

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List of abbreviations

AC50: concentration require to activate by 50%
AhR: aryl hydrocarbon receptor
AR: androgen receptor
ART: assisted reproductive technologies
BAF: bioaccumulation factor
BC/LA: bulbocavernosus /levator ani muscle
BCF: bioconcentration factor
BPs: bisphenols
BPA: Bisphenol A
BPAF: Bisphenol AF
BPB: Bisphenol B
BPF: Bisphenol F
BPAP: Bisphenol AP
BPP: Bisphenol P
BPS: Bisphenol S
BPZ: Bisphenol Z
C&L: classification and labelling
cAMP: Cyclic adenosine monophosphate
CAR: Constitutive androstane receptor
CAT: catalase
Chg: choriogenin
CIK: *Ctenopharyngodon idella* (grass carp) kidney cells
CYP3A4: cytochrome P450 monooxygenase involved in the metabolism of sterols, steroid hormones, retinoids and fatty acids
CYP17: 17α-hydroxylase
Cyp19a1: aromatase
Cyp19a1b: aromatase B
CYP21A2: 21-hydroxylase
Dio2: thyroxine deiodinase, type II
dpf/h: days post fertilisation/hatch
DT50: degradation half-life time
DT90: Degradation time for 90% of the substance
Dw: dry weight
DWTP: drinking water treatment plant
EATS Estrogen/Androgen/Thyroidal/Steroidogenesis (modalities)
EAWAG-BBD: database about information on microbial biocatalytic reactions and biodegradation pathways
E2: 17β-Estradiol
EC20: 20% effective concentration
EC50: half maximal effective concentration
EC ED EAG: Expert Advisory Group of the European Commission on Endocrine Disruptor
ECHA: European Chemical Agency
ED: endocrine disruptor
EDC-WG: ANSES' Thematic Working group on Endocrine Disruptors
EE2: 17α-Ethinylestradiol
EFSA: European Food Safety Authority
ELoC : equivalent level of concern
ER: estrogen receptor
ERE: estrogen response element
ERRγ: estrogen-related receptor γ
FSH: follicle stimulating hormone
GC: gas chromatography
GD: gestation day / guidance document
GM: geometric mean
GPER: G-coupled estrogen receptor
GR: glucocorticoid receptor
GREB-1: gene regulated by estrogen in breast cancer
GSI: Gonadosomatic index (gonadal weight/body weight x 100)
hAR: human androgen receptor
HBM4EU : European Human Biomonitoring Initiative
HeLa: Human cervical epithelial cancer cells

hER: human estrogen receptor
HSI: Hepatosomatic index
Hpf: hours post fertilisation
HPG: hypothalamic-pituitary-gonadal axis
HPLC: high performance liquid chromatography
HPT: Hypothalamic-pituitary-thyroid (axis)
HSD: Hydroxysteroid dehydrogenase
IAM-LC: Immobilised artificial membrane liquid chromatography
IC50: Concentration required to inhibit the cell viability by 50%
INSL3: Insulin-like 3 protein
IP: intraperitoneal
JRC : Joint Research Centre
LBD: ligand binding domain
LC: liquid chromatography
LC50: concentration inducing 50% lethality
LH: luteinising hormone
LOAEC: lowest observed adverse effect concentration
LOEC: Lowest Observed Effect Concentration
LOD: limit of detection
LOQ: limit of quantification
LPO: Lipid peroxidation
LXR: Liver X receptor
MBBR: Moving bed bioreactor
MCF-7: breast cancer cell line (Michigan Cancer Foundation-7)
MDL: Method detection limit
MDR1: multi-drug resistance 1
MeOH: methanol
MoA: Mode of Action
MR: Mineralocorticoid receptor
MS: mass spectrometry
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
nd: not detected
NICEATM: NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NOAEL: No Observed Adverse Effect Level
NOEC: No Observed Effect Concentration
NTP: National Toxicology Program
P: progesterone
PBT: persistent, bioaccumulative and toxic
PLR: prolactin
PND: Post natal day
PNW: Post natal week
POD: Peroxide dismutase
PPARγ: peroxisome proliferator-activated receptor-γ
PgR: progesterone receptor
PVC: Polyvinyl chloride
PXR: Pregnane X receptor
QSAR: Quantitative Structure-Activity Relationship
RAC: Risk Assessment Committee
RAR: retinoic acid receptor
RBA: Relative binding affinity
REP: Relative estrogenic potency
ROR: RAR-related orphan receptor
ROS: Reactive oxygen species
RPE: relative proliferative effect
RXR: Retinoid X receptor
SASR: Suspended activated sludge reactor
SD: Sprague-Dawley
SHBG: sex hormone-binding globulin
SOD: Super oxide dismutase
SPM: suspended particulate matter
SULT: sulfotransferase
SVHC: substance of very high concern
T: testosterone
TA: Transactivation assay
$T_{1/2}$: Half-life time
T3 : 3,5,3’-triiodo-L-thyronine
T4: L-thyroxine

TBARS: Thiobarbituric acid reactive substances
TGT: Technical Guidance Document on Risk Assessment
TH: thyroid hormone
ToxRTool: Toxicological data Reliability assessment Tool
TP: testosterone propionate
TPO: thyroperoxidase
TR: thyroid hormone receptor
TSH: thyroid-stimulating hormone (thyrotropin)
TTR: Transthyretin receptor
UGT: UDP-glucuronyl transferases
UGT1A1: UDP-glycosyltransferase 1 polypeptide A1
UHPLC–MS/MS : ultra-high performance liquid chromatography tandem mass spectrometry
US: United States of America
US EPA: Environmental Protection Agency of the United States of America
UTDB: Uterotrophic database
UV: ultraviolet
UVC/BDD: ultraviolet C /boron doped diamond
VDR: Vitamin D receptor
vPvB: very persistent and very bioaccumulative
VTG: vitellogenin
WHO/IPCS: International Program on Chemical Safety of the World Health Organisation
WWTP: Wastewater treatment plant
YES: yeast estrogen screen

IDENTIFICATION OF A SUBSTANCE OF VERY HIGH CONCERN ON THE BASIS OF THE CRITERIA SET OUT IN REACH ARTICLE 57

Substance Name: 4,4'-(1-methylpropylidene)bisphenol (bisphenol B; BPB)

EC Number: 201-025-1

CAS number: 77-40-7

- According to Article 57(f) of the REACH Regulation 4,4'-(1-methylpropylidene)bisphenol, referred to hereinafter as bisphenol B and BPB, is identified as a substance of equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of Regulation (EC) No 1907/2006 (REACH).

Summary of how the substance meets the criteria set out in Article 57 of the REACH Regulation[1]

Adverse effects

Consistent adverse effects are observed in rodents and fish exposed to bisphenol B (**BPB**). The observed adverse effects in mammalian vertebrates are reduced sperm count and quality consistently observed in several reliable studies in two species (rats and mice). In fish, adverse effects include an altered hepato-somatic index and gonado-somatic index in male and female zebrafish. Qualitative observations of altered testis tubules and a decreased amount of mature spermatids in males also provide supportive evidence. BPB was demonstrated to significantly reduce fecundity of adult fish exposed for 21 days and to decrease embryo hatching and survival of F1 generation in a reliable study. Supportive evidence is provided by the induction of malformations (no detailed information) in zebrafish in one study. **BPB therefore induces adverse effects on the male reproductive system in rodents and fish.**

Estrogenic activity

BPB exposure leads to higher estrogen and lower androgen levels in both *in vitro* and *in vivo* studies in rodents and fish. Additionally, *in vitro* data unambiguously show the estrogenic activity of BPB: competitively binding to **ER**[2] of several vertebrate species (e.g. human, bovine, rat, mouse and medaka in the µM range), activation of ER signalling pathway (e.g. ER transactivation in reporter cell lines, increased promoter occupancy and induction of ER-regulated gene expression) and physiological cell response (e.g. proliferation) with similar or higher potency than 4,4'-isopropylidenediphenol (bisphenol A; **BPA**). This estrogeno-mimetic activity of BPB is also supported by the results of immature rat uterotrophic assays with increase in watery uterine content and blotted uterine weight. This effect was similar to BPA, but with a slightly higher magnitude for BPB. In fish, the increase in levels of **VTG**[3] gene expression in the liver of male medaka and male zebrafish, and the increase in ER-regulated cyp19a1b expression in the brain of male zebrafish also strongly support the estrogenic activity of BPB.

BPB was therefore shown to have clear estrogenic effects in rats and fish.

Other potential modes of action

[1] The shaded text is a copy and paste of summary in section 6.5.7 and of the conclusion of section 7.2 without bibliographic references and with additional explanation of acronyms.
[2] oestrogen receptor
[3] vitellogenin

BPB was shown to bind the **AR**[4] and to induce an anti-androgenic response in most vertebrate cell lines including in human cells but this effect was not confirmed in the Hershberger assay. Therefore, **BPB possibly has anti-androgenic effects.**

The *in vivo* data also showed a decrease in **LH**[5]- and **FSH**[6]-related gene expression in brain and gonads of male zebrafish and a decrease in plasma LH and FSH levels in rats, suggesting an action of BPB *via* the hypothalamic-pituitary axis. It is however not known whether it may be a cause, a consequence or a specific mode of action in addition to estrogenic and possible anti-androgenic effects.

Oxidative stress was reported in several rodent studies and may also have an impact on the testis. It is however not known whether it may be a consequence or a specific mode of action in addition to estrogenic and possible anti-androgenic effects.

Plausibility of the link between effects and endocrine activity

BPB may have multiple modes of action that interact or superimpose and are difficult to distinguish from each others. The estrogenic effects of BPB is established in fish and rats and anti-androgenic effects are suggested. Estrogenic and anti-androgenic modes of action are known to be involved in the regulation of spermatogenesis and are closely inter-related. Considering the concomitant decrease in plasma testosterone levels and the increase in plasma estradiol levels, the link between these endocrine activities and the adverse effects on the male reproductive system in rodents and fish is highly plausible.

Relevance of effects and endocrine modes of action

In the present assessment, the *in vivo* available evidence on rodents shows that BPB can affect the male reproductive system. These observed adverse effects in mammalian vertebrates are considered relevant for effects on human health and on mammalian wildlife species in the environment (such as mice, rats) and supportive for non-mammalian vertebrate species (fish, amphibians) with respect to the underlying mode of action and adverse effects.

Supportive evidence from BPA

The link between the observed effects and these specific endocrine activity is supported by the data on BPA, as BPB and BPA share very similar structures, adverse effects and modes of action. BPA has been identified already as SVHC due to its endocrine disrupting properties relevant for human health and the environment. It should be noted that considering the extremely large database available for BPA, it was decided to focus the SVHC identification for BPA due to its endocrine properties for human health on the endpoints having the strongest plausible link at the time of the identification. Male reproduction was not included. However, the effects of BPA on male reproduction are acknowledged, in addition to female reproduction, in the justification to classify BPA as Repro 1B for reproduction. In contrast, the endpoints included in the BPA SVHC identification for human health are largely not investigated for BPB. However, when data are available, they provide indications of a similar effect of BPB to BPA for female reproduction and metabolic effects. This support the consistency of effects between BPB and BPA.

Conclusion on endocrine disrupting properties

Overall, BPB has estrogen agonist properties and induces adverse effects on the male reproductive system in rodents and fish that are plausibly mediated by this endocrine activity.

Supportive evidence is provided by the consideration that BPB possibly has androgen-antagonist properties. This endocrine activity could also plausibly contribute to the

[4] androgen receptor
[5] luteinising hormone
[6] follicle stimulating hormone

adverse effects on the male reproductive system in rodents and fish.

The effects on rodents are relevant for human health and the effects in fish and rodents are relevant for the environment as an effect on the reproductive function can have consequences at a population level.

Therefore, there is scientific evidence that BPB fulfils the definition of an endocrine disruptor relevant for environment and human health.

The effects of BPB due to its endocrine disrupting properties are considered to be of equivalent level of concern to substances listed in Article 57 points (a) to (e). The concern is substantiated by the severity and irreversibility of the effects on organisms and populations that may have long term consequences, the large variety of species that may be adversely affected and the difficulties to quantify a safe level of exposure with regard to the endocrine mediated effects. An equivalent level of concern is also supported by the potential for combined exposure with other bisphenols that share similar modes of action. The assessment shares similar lines of argumentation as for previous SVHC identifications of BPA for its ED properties, for which a considerable amount of data is available. Due to the very close structural similarity between BPB and BPA, commonalities of effects and of modes of action, the main arguments justifying the equivalent level of concern of BPA are also relevant to BPB.

In conclusion, there is scientific evidence that BPB causes probable serious effects to the environment and human health due to its endocrine disrupting properties which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of the REACH Regulation.

Registration dossiers submitted for the substance? No

Justification

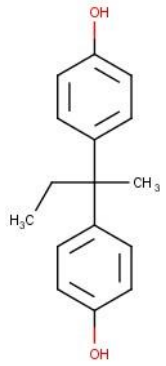
Identity of the substance and physical and chemical properties

1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	201-025-1
EC name:	4,4'-(1-methylpropylidene)bisphenol
CAS number (in the EC inventory):	77-40-7
CAS number: **Deleted CAS numbers:**	77-40-7
CAS name:	Phenol, 4,4'-(1-methylpropylidene)bis
IUPAC name:	4,4'-butane-2,2-diyldiphenol 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:	$C_{16}H_{18}O_2$
Molecular weight range:	242.318
Synonyms:	Bisphenol B BPB 2,2-Bis(4-hydroxyphenyl)butane p,p'-sec-butylidenediphenol p,p'-Dihydroxy-2,2-diphenylbutane 4,4'-(1-Methylpropylidene)diphenol 4,4'-(2,2-Butanediyl)bisphenol 4,4'-(Methylethylmethylene)bisphenol Phenol, 4,4'-sec-butylidenedi- 4,4'-sec-Butylidenediphenol Bis(4-hydroxyphenyl)methylethylmethane Butane, 2,2-bis(4-hydroxyphenyl)- 2,2-Bis(p-hydroxyphenyl)butane

Structural formula:



1.2 Composition of the substance

Name: 4,4'-(1-methylpropylidene)bisphenol (bisphenol B; BPB)

Substance type: mono-constituent

Further information on the substance composition is not available as the substance is not registered.

The identification of BPB as an SVHC is based on the properties of the main constituent only. The other constituents and impurities are not relevant for the identification of BPB as an SVHC.

1.3 Identity and composition of degradation products/metabolites relevant for the SVHC assessment

Not relevant for this report.

1.4 Identity and composition of structurally related substances

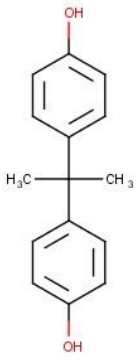
Comparison with 4,4'-isopropylidenediphenol (bisphenol A; BPA) is used in this report as a supporting element.

Table 2: Structurally related substance identity

EC number:	201-245-8
EC name:	4,4'-isopropylidenediphenol
SMILES:	CC(C)(C1=CC=C(O)C=C1)C1=CC=C(O)C=C1
CAS number (in the EC inventory):	80-05-7
CAS number:	80-05-7
CAS name:	Phenol, 4,4'-(1-methylethylidene)bis
IUPAC name:	2,2-bis(4-hydroxyphenyl)propane
Index number in Annex VI of the CLP Regulation	604-030-00-0
Molecular formula:	$C_{15}H_{16}O_2$
Molecular weight range:	228.28 g/mol
Synonyms:	Bisphenol A; Phenol, 4,4'-isopropylidenedi- (8CI); (4,4'-Dihydroxydiphenyl)dimethylmethane; 2,2-Bis(4-hydroxyphenyl)propane; 2,2-Bis(p-hydroxyphenyl)propane; 2,2-Di(4-hydroxyphenyl)propane; 2,2-Di(4-phenylol)propane; 2,2-Bis(4-hydroxyphenyl)propane; 4,4'-(1-Methylethylidene)bisphenol; 4,4'-(Propane-2,2-diyl)diphenol; 4,4'-Isopropylidenebis[phenol]; 4,4'-Methylethylidenebisphenol; Bis(4-hydroxyphenyl)dimethylmethane; Bis(p-hydroxyphenyl)propane; Diphenylolpropane; Isopropylidenebis(4-hydroxybenzene); p,p'-Bisphenol A; p,p'-Dihydroxydiphenylpropane; p,p'-Isopropylidenebisphenol; p,p'-Isopropylidenediphenol; β,β'-Bis(p-hydroxyphenyl)propane

Substance type: mono-constituent

Structurally related substance(s) formula:



1.5 Physicochemical properties

Table 3: Overview of physicochemical properties

Property	Value	Reference/source of information
Physical state at 20°C and 101.3 kPa	white to low brown powder	Sigma Aldrich[7]
Melting/freezing point	120.5° - 139.43 °C	Haynes 2010, EPIsuite (experimental and weighted value)
Boiling point	375.14 °C (pressure not specified)	EPIsuite (modelled)
Vapour pressure	3.3E-05 Pa at 25 °C	EPIsuite (modelled)
Water solubility	29.23 mg/L at 25 °C (pH not specified)	EPIsuite (modelled)
Partition coefficient n-octanol/water (log value)	Log Kow: 4.13 (temperature and pH not specified)	EPIsuite (modelled)

[7] www.sigmaaldrich.com

2. Harmonised classification and labelling

No harmonised classification for BPB.

Self-classifications are reported in the ECHA's C&L Inventory[8] with 3 notifications provided by 40 notifiers. Information may vary between notifications depending on impurities, additives, and other factors.

The following self-classification is notified by the majority of notifiers (38/40):

- Acute Tox 4 - H302 : Harmful if swallowed
- Eye Irrit 2 - H319 : Causes serious eye irritation
- Aquatic Chronic 4 - H413 : May cause long lasting harmful effects to aquatic life

[8] C&L Inventory database, http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database (accessed on 3 March 2020)

3. Environmental fate properties

This report focuses on endocrine properties of BPB. Environmental fate properties are described for information, as some elements can be considered in the discussion of the equivalent level of concern for the SVHC identification (see section 7).

It is not the intention of this report to provide conclusions on these properties.

3.1 Degradation

3.1.1 Abiotic degradation

The physical and chemical properties of BPB suggest that hydrolysis and photolysis under environmental conditions are negligible. Nevertheless, available data (see below) tend to show that under specific conditions abiotic degradation of BPB can occur.

Luo et al., 2019, investigated the UVC-assisted electrochemical degradation of BPs including BPB and BPA in water. The elimination of BPs was highly sensitive to UVC/BDD (boron-doped diamond) electrolysis, with more than 99% of the substrates removed after 120 min of electrolysis following pseudo first order kinetics and with degradation rates of BPA > BPB. The degradation of BPB progressed via hydroxylation, ketonisation and three alkyl-cleavage transformation products generation (similar to the electrochemical decomposition pathway of BPA). Before electrochemical degradation, BPB and BPA at 10 mg/L led to 80% luminescence inhibition of *Vibrio fischeri.* It dropped significantly to 12.4% inhibition after 120 min of electrochemical degradation.

Kovačič et al., 2019, investigated the hydrolysis, adsorption, biological treatment (see section 3.1.2.3) and UV photolysis of 18 BPs including BPB and BPA under laboratory conditions. BPB, just like BPA, was shown to be stable in the hydrolysis experiment (>90% at the end of exposure time of 48 h). Adsorption to biomass seems one primary mechanism for BPB removal from wastewater, as was the case for BPA. The photolysis assay showed that BPB removal follows pseudo-first-order kinetics, with a rate constant of 0.028 min^{-1} and a half-life of 24.76 min (51% BPB remaining after 240 min UV irradiation), whereas BPA shows a rate constant of 0.002 min^{-1} and a half-life of 346.57 min. The removing efficiency of BPB is higher than BPA.

Vela et al., 2018b, investigated the photodegradation of BPs at pilot plant scale by photolysis (with and without oxidant) or photocatalysis with different forms of TiO_2. The degradation rates obtained were significantly lower after 240 min in photolysis (remaining BPB was 48 ± 5% (45% for BPA)) compared to photocatalysis (remaining BPB was 0.002 to 0.004 ± 0.001% for BPB and BPA depending on the form of TiO2 used). Mineralisation was not complete at the end of exposure. Toxicity was then assessed with *V. fischeri* bioluminescence inhibition assay (UNE-EN-ISO 11348-3). Toxicity to *V. fisheri* decreased from 67 ± 7% to 48 ± 8% (photolysis) or to 19 ± 5% (TiO_2 P25) at the end of the treatments. All compounds followed an apparent first-order degradation curve and the degradation rate was in the order: BPB > BPA. The BPB DT_{90} was 501 min with TiO_2 P25 and 1001 min with TiO_2 vlp 7000.

In a second study (Vela et al., 2018a), they investigated the photodegradation of substances including BPA and BPB in laboratory and at pilot plant scale under natural sunlight in June 2016. The efficiency of the process was significantly slower at the pilot scale with natural light than those observed under laboratory conditions mainly due to the presence of interfering substances like some anions and cations and dissolved organic matter. Toxicity was then assessed with *V. fischeri* bioluminescence inhibition assay (UNE-EN-ISO 11348-3). Toxicity to *V. fischeri* decreased from 70% to 45 ± 8% (photolysis) and to 11 ± 5% (photocatalysis) at the end of the experiment. The kinetics of disappearance followed an apparent first-order degradation curve. The DT_{50} of BPB was 5 min and the DT_{90} was 18 min (7 and 24 min for BPA, respectively) (Vela et al., 2018a).

3.1.2 Biodegradation

The BIOWIN degradation models were run to estimate BPB biodegradation. According to Biowin 2 (non-linear model) (p=0.41) and Biowin 6 (MITI non-linear model) (p=0.16), BPB does not biodegrade fast. Ultimate biodegradation could range between weeks to months (Biowin3). In addition, aerobic biodegradation pathways were not identified by *in silico* prediction using the pathway prediction system of EAWAG-BBD tool. Nevertheless, a possible biodegradation of BPB by specific microorganisms was suggested in the literature (Sakai et al. 2007, Lobos, Leib, and Su 1992).

3.1.2.1 Biodegradation in water

Frankowski et al., 2020, evaluated the biodegradation of BPs including BPB and BPA with river water and activated sludge from two wastewater treatment plants (WWTPs), one from a rural area and one from a city area. Primary biodegradation of BPB was found to be minimal in all tests. It was below 10% after 52 days in river water (<20% for BPA), without significant biodegradation, and 40% for the two inoculum from WWTPs (100% for BPA). Biodegradation started immediately for the city sample, increased linearly to 40% until day 20 and remained stable then until the end of the assay. For rural area, the biodegradation is lower and is following the same pattern. This highlights the fact that WWTPs dealing with urban wastewater are adapted to this type of chemical and have the ability to degrade them to some extent.

Ike et al., 2006, investigated the biodegradation of BPs including BPB and BPA in water under aerobic conditions. River water samples were collected from three sites (upstream, midstream and downstream) of four rivers running over Osaka providing 24 samples. Microorganisms were collected by membrane filtration and dispersed into artificial river water resulting in 10-times concentrated samples. These microorganisms were aerobically incubated at 28°C under agitation (rotary shaking at 120 rpm) to BPs, resulting in a final TOC concentration of 10 mg/l. Control and blank were prepared in parallel. At the end of the assays (23 days), BPB was not degraded in 50 % of the microcosms and incomplete primary degradation occurred for the other 50% under aerobic conditions. The aerobic degradability of BPB with river water microbes was lower than that of BPA (complete primary degradation in 19/24 microcosms with complete mineralisation in 2 microcosms and 5 microcosms with no degradation).

3.1.2.2 Biodegradation in sediment

Chang et al., 2014, investigated the aerobic degradation of BPA, BPB and other BPs in river sediment collected from heavily contaminated streams of the Erren River in Taiwan. The degradation rates in the sediment were BPA > BPB, with a DT50 of 6.3 days for BPB and 5 days for BPA.

Ike et al., 2006, investigated the biodegradation of BPs including BPB and BPA in sediment under anaerobic conditions. Sediment sampled originates from Inukai Pond in the Suita Campus of Osaka University (Osaka, Japan). They were centrifuged, washed three times with phosphate buffer, and resuspended to obtain 2-times condensed inoculum. Final concentration of BPs were 10mg/L in a 25 ml sealed bottle where headspace was totally replace by N_2 gas and performed at 28°C. Analyses of degradation product were performed by HPLC. Under these anaerobic conditions, all the tested BPs were biodegraded to a certain extent, although the degradation proceeded very slowly compared with the aerobic degradation in water previously described. BPA and BPB showed a long lag period of 50–60 days, before the anaerobic degradation started, and the degradation was about 40–60% (80 days). BPB was found to be the most recalcitrant bisphenol in this study.

3.1.2.3 Biodegradation in simulated Drinking/Waste Water Treatment Plant (D/WWTP)

Kovačič et al., 2019, investigated the hydrolysis, adsorption, UV photolysis (see section 3.1.1) and biological treatment of 18 BPs including BPB and BPA under laboratory conditions. For the biological treatment experiment, the removal efficiency of BPB was 92% ± 11 or 95% ± 3 after 48h depending on two types of wastewater treatment tested in this experiment.

Moreover, removal efficiency of BPB in drinking water treatment plants (DWTPs) across China was respectively 77.8% and 85.5% for the two detected sources (for BPA, average value of 90.9%; range from 65.8% to 100%) (Zhang et al., 2019a).

Česen et al., 2018a, investigated the occurrence and source of BPs in 18 wastewater samples collected at five Slovene WWTPs during August and October 2015. WW inflows from industrial, commercial and residential sources (see 3.2.1 for occurrence data). Poor removal of BPB was observed (6.39 - 38.7%) in small WWTPs (900–55,000 population equivalent), where Membrane Bioreactor, Moving Bed Biofilm Reactor or conventional treatments with constructed wetland technologies are used, suggesting that BPB was poorly removed. On the other hand, conventional treatment at another WWTP resulted in high removal of BPB (>96%), suggesting that this type of treatment is a more suitable alternative for BPB removal.

BPB removal efficiency ranged between 38.6 % to 100% in Indian municipal WWTPs (Karthikraj and Kannan 2017) and between 6.39% to 98.9% in Slovenia (Česen et al. 2018). In Slovenia, low removal rate of BPB (<50%) was observed for WWTP applying constructed wetlands, membrane biological treatment or biofiltration treatment.

3.1.3 Summary and discussion of degradation

The physical and chemical properties of BPB suggest that abiotic degradation via hydrolysis and photolysis is negligible. The available data show that BPB, just like BPA, is stable in hydrolysis experiment. BPB can be rapidly removed from waters by abiotic degradation when using enhanced physico-chemical degradation technique such as UV-assisted and natural light photocatalysis and is therefore rapidly degradable under these conditions.

According to level III fugacity model (EPIsuite), the estimated half-life of BPB is 37.5 days in water, 75 days in soil and 337.5 days in sediment. Considering the fragmented 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).

The few information available in the literature suggest a possible biodegradation of BPB in water, in sediment (Ike et al. 2006, Chang, Liu, and Liao 2014) or by specific microorganisms (Sakai et al. 2007, Lobos, Leib, and Su 1992). Besides biodegradation, adsorption onto sludge is one of the most crucial parameters affecting removal efficiency, given that BPB has a tendency to adsorb onto sludge. The data highlight that BPB may be difficult to biodegrade in natural water and sediment under environmental conditions.

No information is available on environmental half-life in waters, sediments or soils under standard test guideline conditions and no conclusion is possible on the persistence of BPB.

3.2 Environmental distribution and occurrence data

According to HENRYWIN model of EPIsuite[9], 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 log Koc derived from LogKow of 4.13 and estimated from molecular connectivity index of 4.86. The estimated log Koa was 13.43, indicating that BPB could potentially bioaccumulate in air-breathing organisms. These results suggest that BPB has a tendency to adsorb to suspended solids, to accumulate and to be less mobile in sediment and soils.

3.2.1 Environmental occurrence data - WWTP, source or drinking water and seawater

BPB was measured in Indian municipal WWTPs (Karthikraj and Kannan 2017) and in Slovenian and Croatian municipal and industrial WWTPs (Česen et al. 2019; 2018a) with a detection rate of 60 to 100% in India, 8.3% to 67% in Slovenia and Croatia and with a mean concentration of 2.5 ng/L, 8.46 ng/L and 27.1 ng/L in India, Slovenia and Croatia, respectively.

BPB was also measured in 46 samples of fresh sludge from WWTPs of six geographical regions of China (North China, Northeast China, Eastern China, Central South China, Southwest China and Northwest China) and from 15 cities of Henan province (Zhu et al., 2019a, Pang et al., 2019). The mean concentration of BPB was of 1.38 ng/g dw and a maximum concentration of 3.55 ng/g dw with a detection rate of 6.52% in sludge from WWTPs. It ranged from not detected (nd) to 5.23 ng/g dw (mean value of 0.38 ng/g dw) with a detection rate of 33% in sewage sludge from WWTPs of cities. The average contribution of BPB to total BP concentrations was 0.688% (BPA = 85.8%) for fresh sludge sampled in the 46 WWTPs and 0.2 % for WWTPs samples of the Henan province, respectively. Significant correlations were found between BPB and WWTP characteristics, namely BPB is positively correlated with the ratio of treatment capacity to populations served (Pang et al., 2019).

BPB in sewage sludge was exceptionally detected in one sample during a USA nationwide study at 1.1 ng/g dw (Yu et al. 2015).

BPB was not detected in the influent, primary effluent, final effluent and sludge from two WWTPs (Albany, New York State, USA; Xue and Kanan, 2019) and in sewage sludge of Indian, Slovenian and Chinese WWTPs (Sun et al. 2017, Song et al. 2014, Karthikraj and Kannan 2017, Sun et al. 2018). BPs considered in the studies had different behaviour in WWTPs, some being preferentially biodegraded, others removed by adsorption on sludge or being both biodegraded and adsorbed (Sun et al. 2017, Česen et al. 2018a).

Moreover, BPB was recently detected in source water and drinking water from 20 drinking water treatment plants (DWTPs) across China (March-November 2017). BPB was detected in 10% of the 20 source water samples with mean concentration of 1.0 ng/L (nd–14.3 ng/L) (BPA = 80% detection frequency and mean concentration of 12.8 ng/L (nd–34.9 ng/L)). In finished drinking water, BPB was also detected in 10% of the 20 samples with mean concentration of 0.2 ng/L (nd–3.2 ng/L) (BPA = 40% detection frequency and mean concentration of 1.6 ng/L (nd-6.5 ng/L)) (Zhang et al., 2019a).

In aquatic ecosystem, BPB was the least frequently detected bisphenol, 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, Lu, et al. 2014, Zheng et al. 2015, Wang et al. 2017), Japan, Korea, USA and India (Yamazaki et al. 2015, Liao, Liu, Moon, et al. 2012) except in one sediment sample (10.6 ng/g dw, Liao et al. 2012a).

[9] US EPA. 2020. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.1. United States Environmental Protection Agency, Washington, DC, USA.

Contrasting with previous results, 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.

BPB was also detected in seawater from 25 sites in the Pearl River Estuary in south China collected in December 2017. BPB median concentrations were 1.51 ng/L in seawater (0.17-13.1 ng/L min-max, with 100% detection), 73.3 ng/L in suspended particulate matter (SPM) (ND-97.1 ng/L min-max, with 44% detection) and 3.94 ng/L in the total system (0.36-18.1 min-max, with 100% detection) (Zhao et al., 2019b). BPB was found in 0% of surface sediment samples and BPA in 97.9% of surface sediment (GM: 1.87, range: nd-116 ng/g) collected from the Bohai Sea and Yellow Sea in northern Chinese coastal areas. BPB was not frequently detected in sediment cores (detection rate (DR) comprised between 0-16% for surface sediment and three sediment cores extracted from the inner shelf of Yellow Sea, the central and inner shelves of northern part of East China Sea using a gravity corer, nd-20.9 ng/g) (Liao et al., 2019).

Jurek and Leitner, 2018, determined the occurrence of BPs in paper products (various cellulose, paper and board samples (virgin fibre and recycled fibre)) from different European paper and board manufacturers collected in 2015. BPB was always below the LOQ (0.40 to 1.32 µg kg−1, depending on paper type).

Regarding dissemination in the environment, BPB was demonstrated to adsorb on PVC (Polyvinyl chloride) microplastics with pseudo-second order kinetics, increasing with increasing concentration of PVC, with a maximum adsorption efficiency of 0.22 ± 0.01 mg/g and a 68% efficiency adsorption alone and 41% when in mixture with other BPs (Wu et al., 2019).

Data on occurrence in environmental species are presented in sections 3.4.1 and 3.4.2 below. In addition, data on occurrence in humans are presented in section 3.2.4 below.

3.2.2 Occurrence in food and food contact material

Among recent literature available studying the occurrence of BPB, the following studies did not find the presence of BPB:

- in food samples (González et al., 2020a, Wang et al., 2019a, Zhang et al., 2019b, Zhou et al., 2019, Van Leeuwen et al., 2019, Tu et al., 2019, Gallo et al., 2019, Cao et al., 2019, Dong et al., 2018, Regueiro and Wenzl, 2015, Cao and Popovic, 2015),
- in beverages and drinks (Zhou et al., 2019, Zhao et al., 2019a, Zhang et al., 2019b, Wang et al., 2019a, Ding et al., 2019, Cirillo et al., 2019, Cao and Popovic, 2018, Amini et al., 2018, Gallo et al., 2017, Cheng et al., 2017, Cunha et al., 2012)
- and in food contact materials (Wang et al., 2019b, Van Leeuwen et al., 2019, Hwang et al., 2018, Lin et al., 2015).

These studies were mostly conducted in Asia (China and Korea) and in Europe (The Netherlands, Italy, Portugal), Iran and Canada.

Alabi et al., 2014, investigated the occurrence of BPs in canned foodstuffs (n=14) from local supermarkets in Córdoba, **Spain,** in June 2013, revealing the presence of BPB in 3 samples (21%) with levels ranging from 25-40 µg/kg (BPA in 12 samples with levels ranging from 13 to 242 µg/kg).

Fattore et al., 2015, monitored the occurrence of BPs in canned tuna (n=33) from **Italian** markets. BPB was detected in 4/33 (12%; 67% for BPA) samples with concentration ranging from 19.1 to 145.9 µg/kg (25.4 – 187 µg/kg for BPA).

Liao and Kannan 2014, investigated the occurrence of BPs in 289 food and beverages samples collected from nine cities in **China**, including Baoding (*n* = 28), Beijing (31), Harbin (41), Jinan (41), Jinchang (36), Liuzhou (49), Qinghuangdao (37), Shanghai (14) and Tianjin (12) from July

to September 2012. BPB was detected between 0% (fish and seafood, milk and milk products, fruits, vegetables, cookies and snacks and beverage) to a maximum of 9% in cooking oils and eggs (0.390 ng/g and 0.034 ng/g maximum concentration).

Grumetto et al., 2013, investigated the occurrence of BPs in milk (n=68) from **Italian** markets. BPB was detected in 6/68 (9%; 29.4% for BPA) samples with concentration ranging from 16 to 67 µg/L (14 – 481 µg/L for BPA).

Cunha et al., 2011 quantified simultaneously BPA and BPB in liquid food matrixes (canned beverages, 30 samples in total and powdered infant formula) in **Portugal**. BPA was detected in 21 of 30 canned beverages (ranging from 0.03 to 4.70 µg/l with a LOD of 5.0 ng/l and a LOQ of 10.0 ng/l) and in two of seven powdered infant formula samples (0.23 and 0.40 µg/l with a LOD of 60.0 ng/l and a LOQ of 200 ng/l) collected in Portuguese local supermarkets. BPB was only detected in canned beverages, being positive in 15 of 30 samples analysed (concentrations ranging from 0.06 to 0.17 µg/l with a LOD of 2.0 ng/l and a LOQ of 7.0 ng/l).

Cunha et al., 2012 determined the occurrence of BPA and BPB in canned seafood samples in **Portugal** (total=47) revealing the presence of BPA in more than 83 % of the samples with levels ranging from 1.0 to 99.9 µg/kg, while BPB was found in only one sample at 21.8 µg/kg.

Cunha and Fernandes 2013, investigated the occurrence of BPs in canned vegetables and fruits from local markets in Oporto Metropolitan Area (North **Portugal**) revealing the presence of BPA in more than 87% of the samples with levels ranging from 3.7 to 265.6 µg/kg, while BPB was detected in only two samples, one canned fruit and one canned vegetable with levels of 3.4 µg/kg and 3.0 µg/kg, respectively.

García-Córcoles et al., 2018 determined the occurrence of seven BPs in baby food samples (powdered milk, cereals with milk, juices, yoghurt and homogenised fruit, meat and fish) from different brands in Granada, **Spain**. BPB was detected and quantified in five samples (detection rate: 33%) at concentrations ranging from 1.1 to 8.5 ng/g (LOD: 0.3 ng/g and LOQ: 0.9 ng/g). BPA was detected and quantified in only one sample.

González et al., 2020b, assessed the exposure of an adult population to nine BP analogues including BPB and BPA through a duplicate diet study. Up to 40 canned and non-canned food samples were purchased from Tarragona (Catalonia, **Spain**). BPB was detected in four samples, corresponding to a 13% detection rate. Both pairs of canned and non-canned chicken and olive oil samples had BPB above their corresponding LOD. For chicken, the concentration of BPB in fresh samples was slightly higher than that found in the canned chicken (4.19 vs 3.86 µg/kg, respectively). In contrast, canned olive oil showed a higher concentration than non-canned olive oil (1.25 vs 0.85 µg/kg, respectively). At the same time, a duplicate diet study was performed to assess exposure to BPs of an adult cohort of 26 individuals (average body weight of 68 kg). They were divided into two groups: a potential high-BPA diet (i.e. based on canned food) and a BPA-free diet (i.e. based on fresh food and food products packed in glass containers or other BP-free materials). Each food item was stored appropriately for further analysis. For BPB, the canned group had an intake of 0.31 and 0.15 µg/day (day 1 and day 2) for each day. Similarly, the non-canned group had intakes of 0.31 and 0.14 µg/day, respectively. With respect to BPB, 0.007 µg/kg bw was the estimated exposure for both diet groups. For BPA, the canned group had an intake of 15.7 and 9.26 µg/day for each day. In contrast, the non-canned group had intakes of 2.20 and 0.92 µg/day, respectively. Two-day diet total BPA exposure was estimated to be 0.37 and 0.05 µg/kg bw for canned and non-canned diet, respectively.

Grumetto et al., 2008, investigated the occurrence of BPA and BPB in canned peeled tomatoes (n=42) from **Italian** markets. BPB was detected in 9/42 (21%; 52% for BPA) samples with concentration ranging from 27.1 to 85.7 µg/kg (20.5 – 115.3 µg/kg for BPA).

Russo et al., 2019a, investigated the occurrence of BPs from January to October 2018 in 52 beverages (39 beers and 13 energy drinks) all packed in aluminium cans and from different brands retailed **in Italy** both from local supermarkets and Internet stores. Both beers and energy drinks were from various countries (beers: 10 from Italy, 8 from Germany, 5 from Poland, 4 from the Netherlands, 2 from Ukraine, 2 from Russia, 2 from Slovenia, 1 from Belgium, 1 from

Denmark, 1 from Japan, 1 from Romania, 1 from the United States, and 1 from the UK; energy drinks: 9 from the United States, 2 from Italy, 1 from Austria, 1 from Japan). BPB was found in 21 beer samples (53%) (from <LOQ to 48 ng/mL) and only 1 energy drink (183 ng/mL).

Wang et al., 2019b, determined the concentration of BPs in food-contact plastic material made of polyethylene, real samples of freshness protection packages and preservative film comprising 24 preservative film and 6 freshness protection packages from local markets in **Beijing.** BPB was detected in 13 samples (54%), among which 9 samples could be quantified ranging from 0.34 to 0.71 µg/g in preservative films. BPB was quantified in 3 samples of freshness protection package (50%) with mean value of 0.44 µg/g.

3.2.3 Other occurrence data

- **Indoor dust**

Two studies reported concentrations of BPB and other BPs in indoor dust: Liao, Liu, Guo, et al. (2012) and Wang et al. (2015). Samples were collected in 12 countries including Greece and Romania in the EU. 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. Detection of BPB in indoor dust was very limited both in terms of detection frequency and measured concentrations when compared to similar data reported for BPA.

- **Personal care products / Feminine hygiene products**

Gao and Kannan, 2020 report no BPB exposure via 77 feminine hygiene products (pads, panty liners, tampons, wipes, bactericidal creams and solutions, and deodorant sprays and powders) collected in the Albany area of New York State in the **United States.**

- **Dental sealants**

Xue and Kannan, 2019 detected BPB in some (no numeric data provided in the publication) of the 70 sealants collected from the **U.S.** market from June to August 2015.

- **Medical material**

Zhang et al. 2019b, determined concentration of BPs in soaking solution, pacifier, sodium chloride injection (0.9%) and glucose injection (5%) used in the Liaoning Province Tumor Hospital **in China** by UHPLC–MS/MS. BPB was only detected in a pacifier with a concentration of 1.89 µg/kg.

- **Textiles**

Li and Kannan, 2019 detected BPB in 59.5% of 74 pantyhose samples collected from 6 countries (China, Japan, Korea, Portugal, Chile, USA). The mean concentration was 409 ng/g (range: <1.3 – 7230 ng/g).

3.2.4 Human biomonitoring data

Human biomonitoring studies are available on BPB, including 13 studies on human urine (Yang, Guan, et al. 2014, Cunha and Fernandes 2010, Heffernan et al. 2016), 7 studies on blood/plasma (Cobellis et al. 2009), 3 studies in cord serum, amniotic fluid and placenta (Ihde et al., 2018, Van Overmeire et al., 2019 and Zhang et al., 2020a) and 1 study on saliva (Russo et al., 2019b).

Among the 13 studies that focused on urinary analysis, nine studies (Duan et al., 2018; González

et al., 2019; Heffernan et al., 2016; Husøy et al., 2019; Sakhi et al., 2018; Shang et al., 2019; Yang et al. 2014a; Yao et al., 2018 and Zhang et al., 2020a) did not detect BPB. These studies were conducted in Europe (Spain and Norway) and in Australia, Canada and China. Among the four studies which detected BPB in urine (Asimakopoulos et al. (2016) in Saudi Arabia; Cunha and Fernandes, 2010 in Portugal; Ihde et al. (2018) in the US; Philips et al., 2018 in the Netherlands), the detection frequency was in the range of 10% to 57% with median value of 0.12 ng/ml, and up to 1.28 ng/ml in Ihde et al., 2018. Among these studies, the LOD and LOQ were of quite similar order with the exception of Husøy et al. (2019) where the LOD and LOQ were 30 ng/ml and 100 ng/ml respectively- much higher than the other studies. Lastly, it should be noted that only few details are given - in particular, it is not known whether morning spot sampling was used.

Among the 8 studies that focused on blood or plasma analysis, 2 studies did not detect BPB or detected BPB at a low detection rate (<5%) in maternal plasma (Jin et al., 2018 in China and Zhang et al., 2020a in China). Among the six studies which detected BPB (Cobellis et al. 2009 in Italy, González et al., 2019 in Spain, Li et al., 2020 in China, Russo et al., 2019b in Italy, Tan et al., 2019 in China, Shen et al., 2019 in China), the detection frequency was in the range of 3% to 60% with concentrations ranging from 0.8 ng/ml up to 144.71 ng/ml. Among these studies, the LOD and LOQ were of quite similar orders and much lower than the LOD and LOQ from González et al. (2019) of 760 ng/ml and 2500 ng/ml respectively.

Other fluids such as human cord blood, placenta, amniotic liquid and saliva were investigated by Ihde et al. (2018) in the US, Van Overmeire et al., 2019 in Belgium, Zhang et al., 2020a in China and Russo et al., 2019b in Italy. Ihde et al., 2018 detected BPB in cord blood samples with a detection frequency of 3.3% among a population of 30 mother-child pairs. Russo et al., 2019b detected BPB with a detection frequency of 40% in saliva of patients undergoing orthodontic treatments (population of 5 patients undergoing orthodontic treatment in Italy) with concentration values ranging from 4.04 to 144.71 ng/ml (LOD of 1.16 ng/ml and a LOQ of 3.88 ng/ml).

3.2.5 Summary and discussion of environmental distribution and occurrence data

BPB occurrence in the environment has been poorly investigated in Europe. Albeit not frequently detected, recent studies suggest an increased occurrence in WWTPs and freshwater ecosystems, with detection even in remote areas.

BPB has been detected in human food samples in several studies mainly conducted in Europe and Asia. It is reported in canned food in particular, but also in non-canned food (egg and oils in Liao and Kannan 2014; milk in Grumetto et al., 2013; baby food in García-Córcoles et al., 2018; chicken and oil in González et al, 2020b). Indoor dust data are very limited and indicate a low BPB detection rate. BPB was also detected in dental sealant and in pacifiers. However, only few data are available and relate mainly to non-European countries.

The available human biomonitoring studies suggest that BPB can be detected in both urine and serum in the same order of magnitude as BPA although detected less often than BPA. BPB was also detected in maternal plasma and in human cord blood. However, those data are too limited to be considered representative of the general population and thus not sufficient 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"[10]. 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 to provide better evidence of the actual exposure of citizens to chemicals. More information on human exposure to BPB in Europe is expected in the

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

upcoming years.

Additional monitoring data is also needed to assess the environmental contamination and occurrence of BPB, especially in Europe. BPB detection and occurrence are low, in particular compared to BPA that is used at a larger scale, but they are increasing and may reflect an increase in use.

3.3 Data indicating potential for long-range transport

Recent data demonstrates the presence of BPB in biota of remote areas. BPB has been detected in eggs of Arctic char (*Salvelinus alpinus*), kittiwakes (*Rissa tridactyla*) and glaucous gull (*Larvus hyperborus*) from a Svalbard island in a monitoring report of the Norwegian Polar Institute (Lucia et al., 2016). BPB has been detected in cod, and blood samples and eggs of herring gull in a series of monitoring reports of an Urban Fjord in Norway (Ruus et al., 2016 and 2017). Here, BPB, just like BPA, was among the most quantitatively abundant compounds found in seabird eggs (Lucia et al. 2016). Hence, BPB can reach habitats from various sources, and can be present in surface waters and other compartments. Many organisms may therefore be exposed more or less continuously to BPB and potentially cannot avoid exposure.

3.4 Bioaccumulation

3.4.1 Bioaccumulation in aquatic organisms (pelagic & sediment organisms)

Wang et al., 2020, evaluated the toxicokinetics and bioconcentration of eight BPs in mixture, including BPA and BPB (each BP at 50 nM) in the common carp (*Cyprinus carpio*) according to OECD TG 305. The eight BPs were not detected or close to the LODs in the carp tissues of the control group and they were all detected at significant levels in the various tissues of the carp on the second day upon exposure. The BP_{total} concentrations in the whole body of carp and various tissues increased continuously but did not reach equilibrium within 28 days exposure. The BP_{total} concentrations decreased gradually during the 40 days depuration period. The contribution of conjugated BPA in the whole body of carp was 72.7%, comparable to data available in literature, and 62% for BPB. Based on the BP_{total}, the $t_{1/2}$ was 10.76 ± 1.75 days for BPB and 5.98 ± 0.32 days for BPA. For BPB, kinetic BCF in free form range from 2.5 in the blood to 309.3 L/kg in the kidney and 29.1 L/kg in the whole body. The estimated kinetic BCF of BPB based on the total concentrations at the end of exposure was 80.2 L/kg for BPB, lower than the value estimated in this study by the author by using EPIWEB 4.1[11] (245.47) possibly because accumulation did not reach a steady state.

3.4.2 Field data

Tian et al., 2019, investigated the presence of BPs in northern pike (*Esox lucius*) collected in late May to early June 2014 and 2015 from the St. Lawrence River, Canada, 4 km upstream (n = 12) and 4 km downstream (n = 14) of the point of discharge of a major primary WWTP. None of the ten BPs were detected in the muscle tissues of the 26 northern pike collected.

Zhu et al., 2019b, determined the occurrence of 45 substances in bovine urine samples collected from three countries: China (Tianjin; n = 100), India (Mettupalayam, Tamil Nadu; n = 45), and the United States (Murray, Kentucky; n = 38) between March and November 2018. The selected sites were rural and agricultural areas with no point sources in the vicinity. The bovines from China were zero-grazed (housed permanently in shelters) and fed with commercial feed, in contrast to India and the United States where bovines were allowed to graze in open pastures/ grasslands and fed with a combination of grain and grass. BPB was found sporadically and at low concentrations whereas BPA was found in >70% of the urine samples analysed. The bovine

[11] US EPA. 2020. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.1. United States Environmental Protection Agency, Washington, DC, USA.

urinary distribution of BPs among the three countries was similar.

Zhao et al., 2019b, determined the occurrence, distribution, bioaccumulation, and ecological risk of 19 substances including BPB and BPA in organism samples (marine organisms (n=21), including shellfish species (n=11) and fish species (n=10)) from 25 sites in the Pearl River Estuary in south China collected in December 2017 (see 3.2.1 for occurrence data). In marine organisms, BPB measured median concentration was 12.3 ng/g (nd - 161 ng/g min-max, 36.4% detection). Highest concentrations of BPB were found in *Moerella iridescens* (161 ng/g) and Flower screw (66.6 ng/g) (shellfish). For BPB, the calculated logarithm of bioaccumulation factors (log BAF) was between 1.42 and 4.49 with a median value of 3. More especially, the values for Flower screw, *Moerella iridescens* and Sea crab were determined to be 12700 (log BAF = 4.11), 30800 (log BAF = 4.49) and 5200 (log BAF = 3.72), respectively, with a median value for all biota of 2360 (log BAF = 3.25) and a mean value of 6700 (log BAF = 2.92). Based on the observed logBAF, BPB has the potential to bioaccumulate. For BPA, median and mean values were respectively 23 (log BAF = 1.36) and 715 (log BAF = 1.42).

Liao and Kannan, 2019, investigated the species-specific accumulation and temporal trends of BPs and benzophenones in mollusc samples collected from coastal areas of five cities along the Bohai Sea from 2006 to 2015 (except for 2008). BPB was detected in <5% of the samples ranging from nd-65.3 ng/g dw. BPA and Bisphenol F (BPF, EC no 219-578-2, EC name 2,2'-methylenediphenol) collectively accounted for >90% of all BPs in molluscs.

3.4.3 Summary and discussion of bioaccumulation

Regarding bioconcentration, BPB has an estimated logKow of 4.13, a higher value than measured logKow 3.4 for BPA, and it indicates a potential for BPB to bioaccumulate. BPB has an estimated BCF in fish of 248.1 (EPIsuite), higher than the BCF determined for BPA with a BCF for fish estimated to be ≤ 73.4 (ECHA, 2017b). Considering the worst-case scenario of no biotransformation, a BCF of 1391 was estimated which is under the limit of 2000 set for the B criterion under REACh. The rare available experimental data provide BCF values ranging from 2.5 to 309 L/kg depending on organ consideration. The estimated kinetic BCF for BPB based on the total concentrations at the end of exposure was 80.2 L/kg for BPB. Based on this data, 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. The calculated logBAF for BPB is between 1.8 and 3.7, and the observed log BAF can reach 4.49 for *Moerella iridescens,* in accordance with the log BAF of 2.23 estimated by EPISuite, indicating that BPB can bioaccumulate, even to a high amount in some organisms. Moreover, BPB has an estimated log Kow of 4.13 and an estimated logKoa of 13.43, indicating that BPB has the potential to biomagnify in terrestrial food chains and air-breathing marine wildlife as well as in humans. **Overall, environmental data suggest a slightly higher bioaccumulation potential compared to BPA, although more experimental data are required to fully characterise the bioaccumulation potential of BPB.**

4. Human health hazard assessment

Only toxicokinetic information is presented here and data relevant for the identification of endocrine properties of BPB are presented in the corresponding section 6 below.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Non-human and human information

No relevant *in vivo* experimental data are available on absorption, distribution and elimination of BPB. Based on the knowledge on other bisphenols and the structural similarity of BPB with BPA, an efficient oral absorption (>90%) (with possible enterohepatic recirculation) and excretion within two days is expected and dermal absorption is expected to occur (ANSES, 2013). The detection of BPB in maternal plasma, human cord blood and urine (see chapter 3.2.4) shows that this substance is absorbed, systemically available and eliminated, as parent molecule or its metabolites, via the kidneys. Very few data are available on the biotransformation of BPB. However, based on the knowledge on other bisphenols and the structural similarity of BPB with BPA, urinary elimination of BPB either as free BPB and glucuronidated or sulfo-conjugated metabolites may be anticipated (Gramec Skledar et al., 2016; Taylor et al., 2011; (Zalko et al., 2003). Glucuronidation by UDP-glucuronyl transferases (UGT) results in the monoglucuronide conjugate, which is a major metabolite of BPS (Gramec Skledar et al., 2016) and BPA (Hanioka et al., 2008; Kurebayashi et al., 2010). Other BPA metabolites, presumably also BPB metabolites given structural similarities between the two substances, include the monosulphate conjugate produced by sulfotransferases (SULT) (Yalcin et al., 2016), and various sulphate/glucuronide diconjugates (Inoue et al., 2016; Yalcin et al., 2016).

Once the bisphenols are absorbed, they reach the systemic circulation and are distributed in different tissues (Waidyanatha et al., 2018). The binding of bisphenols to plasma proteins modulates the biodistribution process ((Toutain and Bousquet-Mélou 2004). The fraction unconjugated and unbound to plasma proteins, i.e. the active form of bisphenols, is responsible for their toxicity. Two studies have investigated the interaction of bisphenols with proteins. One demonstrates *in vitro* and *in silico* that BPA and BPB interact similarly with plasma proteins (human serum albumin and alpha1-acid glycoprotein) (Grumetto et al., 2019) while a molecular docking study indicates a stronger affinity of BPB than BPA to bovine serum albumin (Ikhlas et al., 2019).

Regarding metabolisation, Yoshihara et al. (2004) 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) suggested by Yoshihara's previous work.

BPA, BPB or BPA+BPB incubations (100 µM) were analysed by HPLC, and the different HPLC profiles showed several peaks corresponding to metabolites. Some of them were identified by LC/MS/MS and/or GC/MS. For BPA, 2 metabolites produced by rat liver S9, namely BPA catechol (3OH-BPA) and BPA o-quinone, 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 suggested dimers of isobutenylphenol. 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.

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 appropriate controls. Regarding incubations of BPA and BPB with male Wistar rat liver S9 fractions, metabolites were extracted by solid-phase extraction 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.

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. The difference in radical structure originate from the structural difference between BPB and BPA (one additional methyl) and does not suggest different metabolisation pathways for BPB and BPA. Finally, it should be highlighted that these results have not been confirmed in other studies. It is therefore difficult to evaluate and further validate the authors' interpretation.

Biomonitoring studies are described above in section 3.2.4. BPB has been identified in urine, but data do not inform on potential metabolites in humans.

Conclusion on toxicokinetics

No relevant experimental data are available on absorption, distribution and elimination of BPB. Very few data are available on the biotransformation of BPB. However, based on the knowledge on bisphenols once they are absorbed, they reach the systemic circulation and are distributed in different tissues including the target organs. The binding of bisphenols to plasma proteins modulates the biodistribution process and the fraction unconjugated and unbound to plasma proteins, i.e. the active form of bisphenols, underlying their toxicity. Lastly, based on the knowledge on other bisphenols and the close analogy of BPB with BPA, urinary elimination of BPB either as free BPB and glucuronidated or sulfo-conjugated metabolites may be anticipated. Based on the detection of BPB in human urine, serum, maternal plasma and human cord blood (see section 3.2.4), those human biomonitoring data are in agreement with previous knowledge on bisphenols and their urinary elimination. The experimental design of those human biomonitoring studies did not distinguish the metabolites and parental form of BPB since only total BPB was measured. Lastly, no human or experimental studies assessed the bioaccumulation properties of BPB.

5. Environmental hazard assessment

5.1 Environmental toxicity

This report focuses on the endocrine disrupting properties of BPB. Environmental toxicity data are summarised for information but it is not the intention of this report to provide conclusions on these properties.

There is one long term study reported in the literature, namely a study combining the OECD 21-day short-term fecundity assay and the OECD 21-day fish assay.

Yang et al. (2017) report the results of a fecundity test on zebrafish with BPB based on the OECD 230 guideline (ToxRtool score 1). Six male and female zebrafish aged 4 months 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) over 21 days (two replicates). The fish were maintained in a 16:8 light/dark cycle and were fed twice a day with fresh Artemia (sp. Nauplii). The quality criteria seems to have all been met (mortality < 10%, temperature stability, fish are actively spawning, no data on oxygen levels and substance concentration variations reported). The results obtained showed a range of significant effects. The gonado-somatic index of the group exposed to 1 mg/L was significantly decreased in both male and female zebrafish. Additionally one female did not develop any post-vitellogenic oocyte at 1 mg/L exposure to BPB in one of her ovaries. The egg 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 the F1 generation were also noticed for the group treated with the higher dose. The results of this study are also detailed in section 6.4.1. Based on these results, an NOEC of 0.1mg/L can be estimated.

The short-term toxicity data available, summarised in Table 4, are based on acute toxicity tests on crustacea (*Daphnia magna*) and fish (*Japanese medaka* and zebrafish (*Danio rerio*)). In a *Daphnia magna* acute immobilisation test, a 48h-LC_{50} of 5.5 mg/L is reported (Chen, Ike, and Fujita 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 a 96h-LC_{50} of 6.1 mg/L on medaka larvae and a 14-d EC_{50} of 7.4 mg/L on medaka embryo hatching rate. These values are above the 96h-LC_{50} on fathead minnow predicted by ECOSAR[12] (0.695 mg/L). Catron et al., 2019, investigated the host developmental toxicity of BPA and its alternatives in a zebrafish (*Danio rerio*) light/dark behavioral assay and how BPs alter microbiota and modulate secondary adverse behavioral effects. The estimated 10 day AC_{50} value (mortality and abnormality concentration) for developmental toxicity was 5.8 µM (1.4 mg/L). The determined NOEC in the behavioural zebrafish assays was 5.1 µM (1.2 mg/L) (see section 6.3.1 for more detailed description of the study in relation to ED properties). BPB was about three times more toxic than BPA in fish tests (Table 4). In algae, the estimated 96h-LC_{50} is 0.964 mg/L for green algae (ECOSAR prediction) and the 72h-LC_{50} on *pseudokirchneriella* reached up to 19.14 mg/L (Danish QSAR predictions).

Table 4: 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, Ike, and Fujita 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	

[12] US EPA. 2020. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.1. United States Environmental Protection Agency, Washington, DC, USA.

Species	Study principle	Life stage	Parameter	Results	BPA/BPB ratio	Reference
			NOEC hatching LOEC hatching	5.93 mg/L 8.89 mg/L	1.1 1.4	
Zebrafish (*Danio rerio*)	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)
Zebrafish (*Danio rerio*)	Developmental toxicity assay	1 dpf to 10 dpf	AC50 Mortality/Abnormality NOEC Mortality/ Abnormality	1.4 mg/L 1.2 mg/L	3.7 2.3	(Catron et al., 2019)

Conclusion:

Based on the combined OECD 229 and OECD 203 performed on zebrafish, a NOEC of 0.1 mg/L can be estimated..

6 Assessment of endocrine disruption potential

6.1 General approach for the assessment of endocrine properties

6.1.1 Framework of the evaluation

To evaluate whether or not BPB fulfils the WHO/IPCS definition (WHO/IPCS, 2002) of an endocrine disruptor as interpreted by the EC ED EAG (JRC, 2013), both *in vitro* data and *in vivo* data were taken into account, in order to demonstrate:

- Adverse effects
- Endocrine mode of action
- Plausible biological link between adverse effects and endocrine mode of action
- Environmental relevance and human relevance, respectively

As highlighted in EDC guidance developed by ECHA and the European Food Safety Authority (EFSA) to identify EDC under the plant protection products and the biocidal products regulations and published in 2018 (ECHA & EFSA, 2018), the 'endocrine mode of action' in the second bullet point should be interpreted as 'endocrine activity', i.e. the substance has the potential to alter the function(s) of the endocrine system.

Information from level 1 of the OECD conceptual framework (OECD, 2018), *in vitro* and mechanistic information obtained from *in vivo* studies, were used to demonstrate the endocrine modes of action and pathways. The assessment of *in vivo* data focuses on the question whether adverse effects can be inferred to originate from the presumed modes of action or to be a consequence of general systemic toxicity.
As the aim of the current assessment is an evaluation of the endocrine properties of BPB, the focus was on all available studies and endpoints relevant for identifying or explaining endocrine properties.

The structure and the assessment of data is mainly based on the OECD Revised Guidance Document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption (OECD, 2018).

Two different aspects are assessed separately:

- Evidence for endocrine activity
- Effects on apical endpoints that provide evidence that a substance exerts adverse effects owing to its endocrine activity.

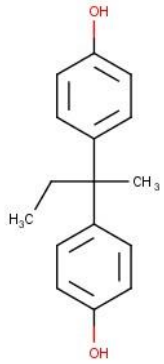
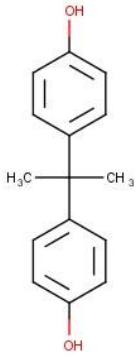
6.1.2 Comparison with BPA

Bisphenol A has been identified as an SVHC due to its endocrine properties for human health in June 2017 (ECHA, 2017a) based on evidence on the alteration of female reproductive function, mammary gland development, cognitive function and metabolism with disruption of the estrogenic pathways being the main mode of action. It should be noted that considering the extremely large database available for BPA, it was decided to focus the SVHC identification for BPA due to its endocrine properties for human health on the endpoints having the strongest plausible link at the time of the identification. Male reproduction was not included. However, the effects of BPA on male reproduction are acknowledged, in addition to female reproduction, in the justification to classify BPA as Repro 1B for reproduction (ECHA, 2014).

In addition, BPA has been identified as an SVHC due to its endocrine properties for the environment in December 2017 (ECHA, 2017b) based on estrogen-agonist effects in fish and in amphibians, thyroid antagonist-effects in amphibians with support from thyroidal MoA in fish, possible endocrine-related effects in some invertebrate taxa, as well as evidence of endocrine disruptive properties on mammalian vertebrate species demonstrated in the previous SVHC dossier.

BPB has a strong structural similarity to bisphenol A (BPA) with only one additional methyl group (see Table 5 below). The National Toxicology Program (Pelch et al., 2017) has shown the structural similarity between BPA and some of its analogues that were tested in Tox21, indicating that BPB is the most structurally similar analogue to BPA according to the Tanimoto coefficient with the same pattern of effect as BPA (Pelch et al., 2017).

Table 5: Comparison of structure and main physico-chemical properties of BPB and BPA

	Bisphenol B	Bisphenol A
Structural formula		
Molecular weight (g/mol)	242.318	228.28
Water solubility (mg/L at 25°C)	29.23	300
Partition coefficient n-octanol/water (log value)	4.13	3.4

When BPB and BPA were both tested in the same study, results of BPA are reported in this report together with those of BPB in order to allow comparison of the properties of both bisphenols.

6.1.3 Information sources and strategy for endocrine disruptor identification

Systematic review

The systematic review was performed following the principles illustrated in the EDC guidance (ECHA & 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 partly identified using systematic review and then assembled and weighed. 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.

A systematic review was conducted up to 5 September 2018 and was further completed with a scientific literature search up to January 2020. The systematic review on BPB endocrine disruptive properties was focused on animal and *in vitro* mechanistic studies, but also on human epidemiological and case studies. The systematic search was performed in PubMed and Scopus databases without limitations on year of publication. The complementary scientific literature search was conducted in PubMed database. Bibliographic monitoring after January 2020 up to October 2020 have not led to the identification of new data recently published that would impact the assessment and in particular there was no new *in vivo* data investigating effects that were relevant for the ED assessment.

In both of these literature searches, a single concept strategy search was applied to retrieve all relevant information on BPB by using its Chemical Abstracts Service Registry Number (CAS No 77-40-7), scientific chemical names, and common names (e.g., "bisphenol derivative" or "bisphenol substitute"), as recommended in the EDC guidance (ECHA & EFSA, 2018). Studies were included in the systematic review based on their relevance 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; and d) English-language articles. 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 distinct reviewers shared the two screening phases during the systematic search and resolved any conflicts or discrepancies by complementary full-text screening and by discussion. For further details on the methodology followed, please refer to Serra *et al*., 2019.

In addition to the systematic literature search and screening, ToxCast[13] and EDSP (US EPA 2018) databases were queried for BPB bioactivity results using the CASRN to identify high-throughput *in vitro* screening assays that measured endocrine activity. Cross references of peer-reviewed research articles and grey literature (e.g., reports by national agencies) were also included in the review.

No registration dossier is available for BPB as the substance is not registered in Europe.

Assessment of the Evidence

The present analysis was performed in collaboration with the ANSES Thematic Working group on Endocrine Disruptors (EDC-WG)[14]. The studies were considered on the basis of their relevance (see criteria of selection based on relevance above), reliability and adequacy for the analysis and were qualitatively weighted based on expert judgement to produce a conclusion on the selected adverse effects and their ED MoA.

Experimental data were compared to each other with specific consideration given to the periods of exposure. Experimental data on all species (i.e. fish and rodents) were used in the same line of evidence to strengthen the weight of evidence assessment considering both the human and environmental health together. The integrated approach is 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). The conclusion of the WoE for each effect was based

[13] https://actor.epa.gov/dashboard/, accessed on October 8th 2018
[14] https://www.anses.fr/en/content/endocrine-disruptors

on the combination of experimental *in vivo* and *in vitro* data, when available.

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. ToxRtool[15] was developed by the European Commission's Joint Research Centre in 2009 (Schneider et al. 2009, Segal *et al*., 2015) and builds on Klimisch categories by providing additional criteria and guidance for assessing the reliability of toxicological studies. It is applicable to various types of experimental data, endpoints and studies (study reports, peer-reviewed publications).

All studies selected as relevant 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-WG 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. All the relevant available studies on BPB have been analysed without any restriction to specific levels of doses and both "low doses" as well as "standard doses" for regulatory testing have been considered as relevant for the identification of adverse effects and the understanding of the MoA. It is, however, recognised that the MoA may have a different pattern and modulations across the whole range of doses.

Similarly, although not considered as relevant for the identification of an adverse effect, studies performed in non-intact animals (i.e. ovariectomised or castrated animals) were included for the understanding of the MoA. Studies conducted *via* non-physiological routes such as the intraperitoneal (IP) route were considered as supportive evidence for adverse effect identification and were considered relevant for MoA understanding.

Analysis of the Results

The data were grouped into three categories following the conceptual framework of the OECD Revised Guidance Document 150 (OECD 2018) and the EU EDC guidance (ECHA & EFSA, 2018): a) *in vitro* and *ex vivo* mechanistic parameters in section 6.2 (OECD Level 2); b) *in vivo* mechanistic parameters in section 6.3 (OECD Level 3); and c) parameters providing information on adversity in section 6.4 (OECD Levels 3, 4, and 5). Human epidemiological data are also presented in section 6.4. OECD Level 2 and 3 data are mainly informative of endocrine activity, whereas Level 4 and 5 data provide information on adversity. 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 & EFSA, 2018).

The analysis on how the data fulfils the WHO/IPCS definition of an endocrine disruptor for BPB is presented in section 6.5.

6.2 *In vitro* information indicative of endocrine activity (OECD level 2)

Forty-four studies gathered in the literature and included in the review provided *in vitro* or *ex vivo* mechanistic information on the capacity of BPB to interact with the endocrine system. Most of the studies focused on BPB estrogenic activity. For instance, 27 *in vitro* results for the E modality were gathered in the literature search, whereas the other endocrine activities remained by far less investigated (11, 4, 4, and 17 results for the A, T, S, and other modalities, respectively). The results presented are statistically significant or positive following criterion of estrogenic activity.

[15] https://eurl-ecvam.jrc.ec.europa.eu/about-ecvam/archive-publications/toxrtool

6.2.1 Estrogen pathway

Overall, 27 studies investigating the estrogenic activity of BPB *in vitro* were analysed. The main results are summarised below and further discussed in section 6.5.2 below.

- **ERα and ERß 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 can displace 17β-Estradiol (E2) from its binding site with an half maximal inhibitory concentration (IC50) of 1.08 µM and a relative binding affinity (RBA) to E2 of 0.086%, indicating that BPB had about a 1000-time lower binding affinity than E2 to rat ER. As part of the 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 IC50 ranging from 0.023 µM to 0.43 µM. BPB has a weak relative potency compared to diethylstilbesterol or 17α-Ethinylestradiol (EE2). Binding of BPB with ERα and ERß was also observed by Liu et al., 2019 in radiolabelled ligand binding assay or with human nuclear receptor with an IC50 of 0.215 µM and 0.073 µM, respectively. **BPB showed a higher affinity than BPA toward ERα** (Sipes et al. (2013), Blair et al. (2000), Zhang et al. (2018), Liu et al. (2019)) **and toward ERß** (Liu et al. (2019))**.**

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

BPB induced an estrogenic response in the transactivation assay based on yeast cells stably transfected with human hERα (Chen et al. (2002), with an EC50 evaluated from 1.73 to 5 µM (Conroy-Ben et al. 2018, Van Leeuwen et al., 2019, Wang et al., 2014)), rat ERα (Yoshihara et al., 2004) or on medaka ERα with an EC50 of 0.59 µM (Yokota et al. 2008). 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), BPB induced ERα transactivation with an EC50 ranging from 0.07 to 0.3 µM**.** In a reporter gene assay based on CHO-K1 cells, BPB induced hERα and hERß transactivation with an EC50 of 0.07 µM and 0.05 µM, respectively (Kojima et al., 2019). BPB had a higher potency to induce hERß than hERα transactivation (see Kojima et al., 2019). **Across all reporter gene assays, the measured estrogenic activity of BPB was similar or even higher than that observed for BPA**, with BPB being 4 to 5 times more potent than BPA (Kojima et al., 2019) although nearly 500 times less potent than the reference E2 positive control. Pelch et al., 2019 showed that the ERα/ERE-mediated activity of BPB and BPA was blocked by the ER antagonist ICI 182,780 (ICI) indicating the specificity of the ERα/ERE-mediated mechanism. In addition, the capacity of BPB to decrease E2-induced human ERα transactivation was investigated in several reporter gene assays either in breast cancer, HELN, CHO-K1 or yeast assays (Wang et al. 2014, Grimaldi et al., 2019, Kojima et al., 2019, Kitamura et al. 2005, (Okazaki et al. 2017). **No anti-estrogenic activity was observed in reporter gene assays and a similar response was reported for BPA in these studies**.

- **ER-regulated gene expression:**

Three studies investigated the gene expression profile of MCF-7 cells following either single (Okazaki et al. 2017, Mesnage 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 BPs in MCF-7 cells after 48h of exposure using microarray analysis. They identified that 0.24 µM BPB altered the expression of several genes involved in breast cancer and hormone-induced proliferative effects with a similar profile to that obtained with BPA. These results were supported by those of Pelch et al., 2019 which showed a significant increase in the expression of GREB1, a gene regulated by estrogen in breast cancer. 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 a significant increase in pS2 mRNA and protein levels from 1 µM.

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). Lastly, Pelch et al., 2019 showed that **BPB significantly** induced **expression of PgR in ERα positive MCF7 cells.** These results may be a consequence of ER activation in MCF7 cells as the PGR gene is a ER regulated gene in these cells.

- **Promoter occupancy:**

Two studies assessed the capacity of BPB to induce the receptor occupancy of prolactin (PLR) gene promoter in Hela cells stably transfected with hERα or hERβ (Stossi et al. 2014, Ashcroft et al. 2011). Exposure to BPB resulted in a higher promoter occupancy of prolactin gene, with hERβ having a stronger array occupancy (EC50: 0.161 µM) compared with hERα (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.

- **Proliferative assays:**

Five studies investigated the proliferative effects of BPB on hERα and hERß positive MCF-7 cells and T47D cancer cells and showed 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 the BPA response (0.36 µM). An increased proliferation was also observed in the hERα and hERß positive T-47D cancer cells stably transfected with ERE-luciferase transgene, but not in the ER negative MDA-MB-231 cell lines, suggesting that the BPB proliferative effect was mediated by hERα (Mesnage et al. 2017). BPB was also reported as positive in a human uterine adenocarcinoma cell line (Ishikawa)-based assay (IKA assay) (Beames et al., 2019). According to Zhu et al. (2020), at low concentrations, BPB and BPA appeared to stimulate the growth of *Ctenopharyngodon idella* (grass carp) kidney (CIK) cells due to their estrogenic activity. When concentration were increased, BPB and BPA showed significant cytotoxicity in a concentration-dependent manner. After treatment at these higher bisphenols concentrations, CIK cells number was reduced and their morphology changed. The cells became round-like and shrank in size and underwent abscission. Regarding combined toxicity, BPB and BPA expressed additiveseffects.

- **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 IC_{50} of 3.3 µM and an RBA affinity to E2 of 8.8%, which is higher than that of BPA (1.1%). Based on these results, BPB had a much higher binding affinity toward GPER than toward ERα, indicating that at low concentrations, BPB may preferably activate extragenomic signaling pathways which are rapid cellular and physiologic responses, inconsistent with the time frame of transcriptional mechanisms. The authors further showed that binding to GPER resulted in an increase in calcium mobilisation (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 to BPB on calcium mobilisation. **Thus, the potency of BPB to bind to GPER is higher than that of BPA and BPB is able to activate the GPER signaling pathway.** Activation of GPER signaling in breast cancer cells leads to increased calcium mobilisation and cAMP production at 10 nM and further favors cell migration.

- **ERα localization pattern and ERα signals:**

In BPB treated mouse oocytes, an alteration in the distribution of ERα was shown with a significant aggregation of ERα on the spindle (Zhang et al. 2020). ERα signals in control-oocytes diffused in the cytoplasm while ERα accumulated separately around the chromosome and showed a spindlelike pattern in oocytes exposed to BPB. In addition, fluorescence intensity analysis showed that ERα signals were sharply increased in the BPB-exposed oocytes spindle in comparison to control oocytes (57.56 ± 2.209, n = 34 control vs. 5.54 ± 6.005, n = 33; $p<0.01$). These data suggest that BPB exposure disrupted the localization patterns of ERα. This study has been submitted through, and added further, to the public consultation and consequently has not been thoroughly reviewed.

Conclusion:

The available *in vitro* information demonstrates the capacity of BPB to competitively bind to ERα and ERß of several vertebrate species including in the human, rat, and mouse. Binding to ER leads to activation of the ER signaling pathway as evidenced by ER transactivation in reporter cell lines, increased promoter occupancy and induction of ER-regulated gene expression, and eventually, related physiological cell responses (e.g. proliferation). A recent study showed that BPB could bind extragenomic GPER with a relative binding affinity (8.8%) higher than that of ER (<1%). In addition, a recent study showed that BPB disrupted the localization patterns of ERα in mice oocytes and sharply increased ERα signals in BPB-exposed oocytes spindle.

The *in vitro* results show that both ER nongenomic and genomic signaling pathways are activated by BPB, with similar or higher sensitivity than BPA.

6.2.2 Androgen pathway

Overall, 11 studies that investigated the androgenic and anti-androgenic properties of BPB *in vitro* were analysed. The main results are summarised below.

- **AR binding:**

BPB binding capacity to the androgen receptor (AR) has been investigated in the study by Fang et al. (2003) and Pelch et al., 2019 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) with an IC50 in the µM range (2.2 to 37.5 µM). **BPB and BPA yielded similar results** when both chemicals were tested in the same experiment.

- **AR agonism:**

Among the results reported in the literature, no human AR agonism was observed in either human cells (Wang et al. 2014), Grimaldi et al., 2019), mouse NIH3T3 cells (Kitamura et al. 2005), hamster CHO-K1 cells (Kojima et al., 2019), yeast cells (Conroy-Ben, Garcia, and Teske 2018, Wang et al. 2014) or with human nuclear receptor in a radiolabelled ligand binding assay (Liu et al., 2019). In the 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 toward the hAR**.

- **AR antagonism:**

BPB had antagonistic effects in most *in vitro* studies on yeast cells (Conroy-Ben, Garcia, and Teske 2018), mouse (Kitamura et al. 2005), hamster (Rosenmai et al. 2014, Kojima et al., 2019), and human cells (ToxCast Database, Wang et al. 2014, Grimaldi et al., 2019, Pelch et

al., 2019). The IC50 reported ranged from 0.93 µM to 64.24 µM. These results were expressed at non cytotoxic concentrations. When a significant cytotoxicity was observed, the corresponding IC50 are not reported. Overall, the ratio of BPA to BPB IC50 for all nine *in vitro* findings ranged between 1 and 3.9, with a median of 2.1, **indicating similar or higher activity of BPB compared with that of BPA.**

Conclusion:

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

6.2.3 Thyroid pathway

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

Kitamura et al. (2005) did not show modulation of the thyroid-hormone dependent production of growth hormone in GH3 cells exposed for 48h (no further data reported). Lee et al., 2018 assessed the capacity of BPB to modulate the thyroid-hormone dependent production of growth hormone in GH3 cells exposed for 48h or 96h. Lee et al., 2018 reported that BPB did not modulate growth hormone release in these cells up to 1 µM, in absence or presence of T3 after 48h of exposure. While after 96h of exposure, BPB induced proliferation of GH3 cells without T3 (LOEC: 0.1 µM) and with T3 (LOEC: 1 µM). 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 (Lee et al., 2017). In GH3 cells, BPB exposure led to a down-regulation of trα, trβ and dio2 expression at the highest concentration tested (0.04 µM). Compared to BPA, BPB did not decrease tshβ expression. However, BPB did not induce significant down-regulation changes of genes involved in thyroid hormone production in FRTL-5 cells, as observed for BPA and all other BPs 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). The value for TR antagonist activity (IC 54 uM) is close to the IC50 for the cell viability of the same assay (78.3 uM, reported in ToxCast database), suggesting that the inhibition observed might not be specific (not TR-mediated).

Conclusion:

The available information on BPB thyroidal activity indicates that BPB might impact the thyroid hormone-dependent production of growth hormone in rat GH3 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 the highest tested concentrations (Lee et al., 2017), and may modulate thyroperoxidase activity (ToxCast database).

6.2.4 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 in the direction of decreased androgen levels (androstenedione and testosterone) and elevated estrone levels (**Figure 1**). A decrease of cortisol is found in these two assays associated or not with a decrease of 11-deoxycorticosterone and deoxycortisol. Increased progesterone and 17α-OH progesterone levels are only observed by Wang et al. (2014).

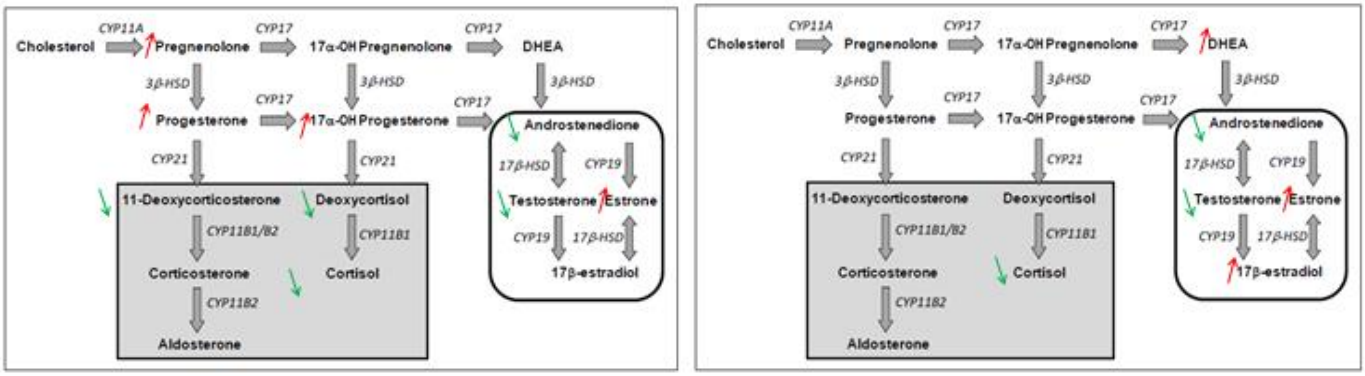


Figure 1: H295 R steroidogenesis assay results for BPB extracted from Wang et al. (2014) on the left hand side and from Rosenmai et al. (2014) on the right hand side.
Activation is highlighted with red arrows and deactivation with green arrows.

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 with 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 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 significantly reduced with explants exposed for 24 or 48h to 0.1 µM BPB (but not with 0.001, 0.01, 1 and 10 µM) whereas testosterone secretion was significantly reduced with BPA only at the highest dose of 10 µM. A significant increase of INSL3 secretion was detected only with 0.001 µM BPB or BPA after 24h of exposure. Lastly BPB and other bisphenols did not affect significantly the amount of inhibin B secreted in the medium. These episodic hormonal secretions might be explained by the high variability within the individual explants. However, these data must be interpreted with caution because of the high variability of the results and the limited number of independent experiments.

Conclusion:

The two H295R assays performed with BPB 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 are in agreement with previous BPA findings, suggesting that one or both of the specific interactions of BPA may be applicable for BPB.

6.2.5 Non-EATS modalities

Metabolism and obesity

Kidani et al. (2010) investigated the impact of BPB and BPA on **adiponectin production and secretion** in 3T3-L1 adipocytes at 80 µM. As with BPA, BPB decreased the amounts of intracellular and medium adiponectin. The decrease in the amount of intracellular adiponectin was higher for BPB than for BPA (-89% and -57%, respectively) whereas the decrease in the amount of adiponectin in the medium was similar for BPB and BPA (-43% and -58%, respectively). **These results indicate that BPB and BPA inhibit adiponectin production** in cells, resulting in reduced secretion of adiponectin. Further experiments conducted on BPA only showed that 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. **This study indicates that BPB and BPA may increase insulin resistance.**

In addition, Ramskov Tetzlaff et al., 2019 investigated the effect of BPA analogues including BPB in the 3T3-L1 mouse model of adipocytes. Lipid accumulation was significantly enhanced for BPB from 1 µM and only at the 10 and 20 µM concentrations for BPA. For BPB, the strongest lipid accumulation was seen at 5 µM (150%), whereas the most pronounced effect for BPA was observed at 10 or 20 µM. Biomarkers of terminal differentiation such as leptin and adiponectin releases were measured. The authors indicated enhanced releases with BPB and BPA but data are less than conclusive given the high variability between samples. Indeed, each triplicate is shown which highlights the variability between triplicates and no statistical analysis has been made. In addition, data are expressed in percentage of control. It is therefore not possible to determine if leptin levels are in the range generally detected in 3T3-L1 cells. Regarding adiponectin, the authors indicate that adiponectin was as well increased by BPA and BPB (and by the other bisphenol analogues studied) but to a lesser extent than the increase observed for leptin; however, data are not shown. Gene expression analysis of Cidec, Lpl and Fabp4, known to be upregulated during adipocyte differentiation and robustly expressed in mature adipocytes, and of Nr1c3 (PPAR γ) the master regulator of adipogenesis indicated possible trends for enhanced gene expression in response to BPB exposure. Lpl was the only marker, which mRNA levels significantly enhanced in response to BPB. Exposure to BPA and other analogues caused no significant effects on any of the genes analysed. The positive control rosiglitazone also had little effect on these markers. Fabp4 was the only gene significantly enhanced in response to rosiglitazone, indicating that the 3T3-L1 cells may not have been properly cultured. Finally, the PPAR γ transactivation assays were not conclusive. Indeed, rosiglitazone which is a powerful agonist of PPAR γ had no significant effects except at the highest dose of 30 µM, indicating lack of sensitivity of the assay.

Although BPB induced lipid accumulation, the study does not allow to draw any conclusion because of lack of significant data yielded on leptin and adiponectin releases, gene expression profiling on adipogenic markers and PPAR γ transactivation assays. **Effects of BPB on lipid accumulation were however observed at much lower concentrations (from 1 µM) compared to BPA (10 µM).**

To conclude, there are evidences that BPB induced lipid accumulation at low concentrations in the micromolar range and that it can increase insulin resistance as previously described for BPA. However, available published data do not allow to conclude on modes of action and if PPAR γ the master gene of adipogenesis is involved.

Other 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), Rosenmai et al. (2014) and Grimaldi

et al., 2019, 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. (2012) show, in a reporter gene assay, that BPB is a potent agonist for human pregnane X receptor (hPXR) but not for mouse PXR (mPXR). Activation of hPXR was dose-dependent and **BPB was a more potent hPXR agonist than BPA at a low concentration** (5 µM) and had comparable agonistic effects at high concentrations (10 and 25 µM). These results were supported by those of Grimaldi et al., 2019 and Kojima et al., 2019 showing activation of hPXR in a reporter human cell line HG5LN and monkey cell line COS-7 cells with an EC50 value of 22.1 µM for BPB *versus* 93.7 µM for BPA and 9.8 µM for BPB *versus* no activity for BPA, respectively. Additionally, Liu et al., 2019 observed a binding of BPB with hPXR with an IC50 in the 5 µM range for BPB and BPA in a radiolabelled ligand binding assay. 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 activations 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 analogues on different enzymes involved in the **glucocorticoid biosynthetic pathway**. 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, an established inhibitor of 17β-HSD. BPB was also shown to possess higher binding affinity (-5,929) compared to anastrazole (-5.626), an established inhibitor of CYP19A1 (aromatase). Lastly, BPB also showed comparable docking efficiency (-7.933) with Canrenone (-8.847), a known inhibitor of CYP21A2 (21-hydroxylase).

In a reporter gene assay based on monkey kidney cells (COS-7) expressing human constitutive androstane receptor (**CAR**) ((Mesnage et al. 2017, Yamasaki et al. 2002, Kitamura et al. 2005, Rosenmai et al. 2014), BPB behaves as a CAR inverse agonist with an IC20 of 2.9 µM *versus* 7.3 µM with BPA. 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). These results were not confirmed by the recent work from Liu et al., 2019 where binding with RXRα, RXRß and RXRγ was not observed with BPB or with BPA.

In a radiolabelled ligand binding assay with human nuclear receptor, Liu et al., 2019 observed a binding of BPB with **the progesterone receptor (PgR)** (IC50 of 7.520 µM with BPB *versus* >10 µM with BPA). In a reporter gene assay based on human cancer cells (HELN cells) expressing a chimeric PgR (Mesnage et al. 2017, Yamasaki et al. 2002, Kitamura et al. 2005, Rosenmai et al. 2014), neither BPB nor BPA showed PgR agonistic activities. The majority of the tested bisphenols including BPB antagonised $R5020^{16}$-induced luciferase expression, R5020 being a progesterone receptor agonist. Their IC50 values varied between 5.6 and 29 µM with BPB showing an IC50 value of 12.1 ± 3.3 whereas BPA did not display measurable antiprogestative activity using this reporter cell line. Lastly, as mentioned above in relation to ER-regulated gene expression, BPB significantly induced **expression of PgR in ERα positive MCF7 cells** (Sipes et al. 2013, Pelch et al., 2019)

In a competitive binding assay, Guan et al., 2017 and Zhang et al., 2017 showed a **glucocorticoid (GR)** affinity of BPB at quite similar levels to BPA (IC_{50}: 14.8 and 18.8 µM for BPB and BPA, respectively). Whereas in a reporter cell line expressing **GR,** BPB and BPA did not exhibit agonistic activity on GR (Grimaldi et al., 2019 and Kojima et al., 2019). A weak inhibitory effect on the GR transcriptional activity was observed with BPB (IC50: 9.3 µM *versus* no activity

for BPA) in Kojima et al., 2019. Lastly in a radiolabelled ligand binding assay with human nuclear receptor Liu et al., 2019 showed a moderate binding of BPB with GR (IC50 of 0.37 µM with BPB *versus* 1.73 µM with BPA).

In a reporter gene assay based on human cancer cells expressing human **ERRγ** ((Mesnage et al. 2017, Yamasaki et al. 2002, Kitamura et al. 2005, Rosenmai et al. 2014), BPB induced ERRγ transactivation with an EC50 of 3.22 µM which was much higher than BPA at 0.97 µM. Thouennon et al., 2019 support these previous results with an ERRγ transactivation with BPB (EC50 of 528 nM) and BPA (EC50 of 174 nM) and an affinity constant (Kd) of 569 nM *versus* 99 nM for BPB and BPA, respectively. Liu et al., 2019 observed an extremely highly active binding of BPB with human nuclear receptor ERRγ in a radiolabelled ligand binding assay with IC50 of 0.008 *and* 0.005 µM for BPB and BPA respectively. ERRγ has been shown to control many specific genetic programs in both normal and cancer cells (Misra et al., 2017, Deblois and Giguere, 2013). ERRγ-regulated genes are involved in oxidative metabolism in skeletal and cardiac muscles (Alaynick et al., 2007, Wang et al., 2015b), ion homeostasis (Luo et al., 2013), insulin signalling (Kim et al., 2011), and gluconeogenesis (Kim et al., 2012). Hence, the relationship that exists between ERRγ and metabolism strongly suggests that this receptor might play a major role in EDC-induced metabolic diseases.

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.

In a mineralocorticoid (**MR**)-reporter cell line (Grimaldi et al., 2019), BPB did not show agonistic mineralocorticoid activity. The EC50 value of aldosterone for MR was 1 nM. Most of the tested bisphenols displayed antagonistic activities on MR with an IC50 value of 2.74 ± 0.24 for BPB *versus* 2.94 ± 0.94 for BPA.

Lastly, Liu et al., 2019 in a radiolabelled ligand binding assay with human nuclear receptor did not show binding of BPB nor BPA with other known human nuclear receptors, such as the three RAR-related orphan receptors **RORα, RORß, and RORγ**, the three retinoid X receptors **RXRα, RXRß, and RXRγ** or Vitamin D receptor (**VDR**).

6.2.6 Conclusion of the *in vitro* data

The *in vitro* estrogenic activity of BPB has been evaluated in depth.

Estrogenic modality: The results showed that **BPB binds the estrogen receptors (ERα and ERβ) and induces estrogen** pathways **with a similar or higher potency than BPA**.

Androgenic modality: Albeit less investigated, the results on the androgen pathway indicate that **BPB can bind the AR and induce an anti-androgenic response in most vertebrate cell lines**.

Thyroid modality: Information on thyroid pathways are scarce and do not allow to draw firm conclusions.

Steroidogenic modality: **BPB interferes with steroidogenesis, resulting in decreased concentrations of testosterone and cortisol and increased concentrations of estrogens.**

Many data on non-EATS pathways were analysed. They suggest **BPB capability to interfere with additional targets such as PgR and MR (as full antagonists), GR (as agonist), PXR (as antagonist), SHBG or adiponectin production**. Effects were similar to BPA with the exception of AhR activation observed for BPA and not for BPB and of the interactions with PgR

reported for BPB and not for BPA. The potency of BPB was generally similar or higher than BPA, with the only exception of the **activation of ERRγ which was slightly weaker with BPB**.

6.3 *In vivo* mechanistic data with regard to an endocrine mode of action (OECD level 3)

6.3.1 Fish data

The study by Yamaguchi et al. (2015) (ToxRtool score 1) reports the **estrogenic activity of several bisphenols, including BPB, on medaka** (*Oryzias latipes*). Four-month-old male medaka were exposed 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 expression of the estrogen-responsive genes vtg1, vtg2, chgH, chgL and ERα was assessed.

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 only). However, the response was not monotonic as the maximum expression level was measured at 5 µM. The gene expression for vtg1 was increased by a factor of 0.5 and 700 at 5 and 50 µM of exposure for BPA, of 0.5, 30 and 15 after exposure to 0.5, 5 and 50 µM for BPB and of 2400 for E2 exposure. For vtg2, gene expression was increased by a factor of 0.3 and 200 at 5 and 50 µM for exposure to BPA, of 1, 25 and 0.5 after exposure to 0.5, 5 and 50 µM for BPB and of 2000 for E2 exposure. For ChgH, gene expression was increased by a factor of 1 and 40 at 5 and 50 µM for exposure to BPA, of 0.6, 30 and 6 after exposure to 0.5, 5 and 50 µM for BPB and of 500 for E2 exposure. For ChgL, gene expression was increased by a factor of 1.2 and 15 at 5 and 50 µM for exposure to BPA, of 1.5, 17 and 3 after exposure to 0.5, 5 and 50 µM for BPB and of 90 for E2 exposure. For ERα, gene expression was increased by a factor of 1.2 and 300 at 5 and 50 µM for exposure to BPA, of 1.3, 13 and 4 after exposure to 0.5, 5 and 50 µM for BPB and of 400 for E2 exposure. At 5 µM of exposure, BPB express more potency in regard to up-regulation of all hepatic estrogen-responsive genes in comparison to BPA. 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 positive control.

Conclusion:

The study by Yamaguchi et al. (2015) indicates that exposure of 4-month old male medaka to BPB for 8 h upregulated hepatic estrogen-responsive gene expression, indicating that BPB has an estrogeno-mimetic activity in male fish which is more potent than the one induced by BPA.

6.3.2 Rodent data

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

The study by Yamasaki et al. (2002) (ToxRtool score 1) reports the estrogenic activity of 23 compounds, including BPB in an immature rat uterotrophic assay (OECD TG 440). BPB was injected subcutaneously on the dorsal surface of Crj:CD (SD) rats at doses of 2, 20 and 200 mg/kg bw/day (dissolved in olive oil) for 3 days from postnatal day 20 (PND 20) to PND 22 (6 animals per group). BPA was also tested. A control group received only olive oil and positive control groups received 0.2, 2 and 20 µg/kg bw/day of ethinylestradiol, 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 bw/day and also in animals treated with estrone from 2 mg/kg bw/day, 17α-estradiol from 20 mg/kg bw/day or with ethinylestradiol 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 bw/day BPB, its weight

was 257% as compared with controls *versus* 197% for 200 mg/kg bw/day BPA and 308% and 315% for 2 and 20 µg/kg bw/day ethinylestradiol, respectively. For all the treatments, the relative weight changes were essentially the same as with 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 animals by estrogenic compounds, 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.

Another *in* vivo uterotrophic assay is available, referenced by NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) in UTDB (*in vivo* uterotrophic database) (see: https://ehp.niehs.nih.gov/doi/10.1289/ehp.1510183 and Kleinstreuer et al., 2016) (ToxRtool score 2). Results from NICEATM are quoted in this manually curated database: BPB was injected subcutaneously in immature rats (strain not specified) at three dose levels from 20 to 200 mg/kg bw/day for 3 days from PND 19 to PND 22 (6 animals per group). A control group received the vehicle (no more information given) and another group received 17-ß-estradiol. An increase of the relative and absolute blotted and wet uterine weights was reported (1.5-fold increase) from 20 mg/kg bw/day (NICEATM, 2016).

Yamasaki et al. (2003) studied the ED properties of BPB and 29 other chemicals in a Hershberger assay (ToxRTool Score 1). The test 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 bw/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 6: 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 and 600mg/kg bw/day Not dose-dependent (x0.8 at both doses)	↑ 600 mg/kg bw/d (x2.5)
Cowper's glands	↑ (x4.5)	=	↑↑600 mg/kg bw/d (x6)

The main results of the Yamasaki's study are presented in **Table 6**. The bulbocavernosus /levator ani muscle (BC/LA) weights decreased by 18% after exposure to 200 and 600 mg/kg bw/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) were observed with BPB

only. Taken together, these data do not suggest that BPB exhibits an androgen agonistic property. The same conclusion was reached for BPA.

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 bw/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% compared to TP alone. This suggests that the administration of BPB exacerbated the effect of TP. It seems specific to BPB since it was not observed with BPA in this study. This significant increase in the weights of all the five androgen-dependent targets compared with TP alone suggests that BPB increases either TP availability or the action of TP. This effect would be specific to BPB since it was not observed with BPA but is described by only one paper and it must be confirmed by other data before speculating on explanations.

Conclusion:

Two uterotrophic assays show that subcutaneous BPB treatment of immature rats from PND 19/20 to PND 22 increases watery uterine content and blotted uterine weight from 20 mg/kg bw/day (NICEATM study) or at 200 mg/kg bw/day (Yamasaki et al., 2002) indicating that BPB has an estrogeno-mimetic activity in immature rat uterotrophic assays.

A Hershberger assay indicates that BPB administered alone in castrated rats does not exhibit androgenic properties at dose levels from 50 to 600 mg/kg bw/day. An anti-androgenic effect at 200 and 600 mg/kg bw/day was observed in one (BC-LA muscle) out of the five examined androgen-dependent sexual organs when exposed to BPB only. The anti-androgenic effect cannot be seen when BPB was co-administered with TP and the interpretation of these results is uncertain.

6.3.3 Conclusion of the *in vivo* mechanistic data

Studies investigating BPB estrogenic activity in rodents (uterotrophic assays) and fish (gene expression in male medaka liver) confirm the estrogenic activity of BPB observed *in vitro*. Regarding the androgen pathway, no androgenic effects of BPB alone were observed in the Hershberger and no clear conclusions can be drawn regarding its anti-androgenic effect.

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

6.4.1 Fish data

Yang et al. (2017) report the results of a fecundity test on zebrafish with BPB based on the OECD 230 guideline (ToxRtool score 1). Six male and female zebrafish aged 4-months 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) over 21 days (two replicates). No E2 positive control was used. The fish were maintained in a 16:8 light/dark cycle and were fed twice a day with fresh Artemia (sp. Nauplii). During this exposure period, eggs were collected 1 h post-fertilization and cleaned with fresh water. The fertilized eggs were incubated at 28 °C in clean water until 6 days post-fertilization.The validity criteria set out in OECD 229, 230 and 234 seems to have all been met (mortality < 10%, temperature stability, fish are actively spawning, size and weight of fish were measured for the determination of somatic index and no deviation inside control were reported, no effect of solvent was reported and no data on oxygen levels and substance concentration variations reported).

The results obtained showed a range of significant effects. The hepato-somatic index of the 0.1 and 1 mg/L exposure groups was 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, although not quantified, 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. Additionally one female did not develop any post-vitellogenic oocyte at 1 mg/L exposure to BPB in one of her ovaries. The egg production of parental fish, and hatching and survival rates of their offspring were significantly decreased at 1 mg/L. Some malformations (e.g. abnormal curvature of larvae) in the 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-male 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 gene expression in male liver indicates a marked estrogenic effect as early as 0.1 mg/L.

Catron et al. (2019) (ToxRtool score 1) investigated the developmental toxicity, the behavioural toxicity and the alteration of the fish microbiota of BPB and its alternatives in Zebrafish (*Danio rerio*). Chemical exposures started on day 1 until 6 dpf (static exposure). Then chemical exposure solutions were renewed daily from 6 to 9 dpf with an 80% media change. Larvae were qualitatively assessed for mortality and malformations including pericardial edema, yolk sac edema, curved body axis, shortened trunk, head/jaw abnormalities, and swim bladder inflation on day 10 (n=3 replicate flasks with 15 larvae per flask). For behaviour testing, locomotor activity based on dark/light phases was recorded (20 min in the dark (0 lux), 20 min testing period (10 min light phase (5.0 lux) and 10 min dark phase (0 lux)). The estimated AC_{50} (abnormality concentration) for developmental toxicity was 5.8 µM (1.4 mg/L) for BPB and 21.5 µM for BPA (4.9 mg/L) (no further information on the type of abnormalities). The determined NOEC in the developmental zebrafish assays was 5.1 µM (1.2 mg/L) for BPB and 11.5 µM (2.6 mg/L) for BPA. BPA induced shift in family taxa of microbiota, but BPB did not alter relative abundances of all bacterial families (which may be difficult to detect due to important variation in DMSO control). Behavioural assessments in zebrafish larvae exposed to BPs showed no significant changes in locomotor activity throughout the 20 min testing period. Zebrafish developmental toxicity and microbiota disruption data were compared to *in vitro* ER potency from ToxCast ESR1 (11 assays) and literature data and visualised using the Toxicological Prioritisation Index (ToxPi) tool (version 2.0, http://toxpi.org/). The data produced in the study highlighted that microbial disruption was inversely related to developmental toxicity and estrogenicity. Bisphenol AF (BPAF; EC No 216-036-7, EC name 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol) and BPB, the two chemicals with highest potency for zebrafish developmental toxicity (NOEC of 1.8 µM (0.6 mg/L) and 5.1 µM (1.2 mg/L), respectively) and ER activity (ToxPi scores of 0.903 and 0.785, respectively), failed to significantly alter zebrafish microbial communities. BPA was ranked as the third most potent chemical (ToxPi score 0.721) and significantly disrupted zebrafish-associated microbiota.

Conclusion:

Exposure of zebrafish during 21 days to a 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 the HPG axis and steroidogenesis. Alterations of genes and hormone levels were more important in male than in female fish. In addition, hepatic vitellogenin gene expression was upregulated in male zebrafish exposed from 0.1 mg/L, indicating that BPB possesses estrogenic activity. The observed increase in VTG gene expression is coherent with the one reported by Yamaguchi et al. (2015) in medaka after short term exposure to BPB. Ultimately, BPB was demonstrated to induce abnormalities and death in zebrafish with an AC_{50} of 1.4 mg/L.

6.4.2 Rodent data

6.4.2.1 Male reproductive system

Five studies from 2 research laboratories examined the effects of BPB on male reproductive function: Ullah et al. (four studies numbered 1, 2, 4, 5 in Table 7 below) and Ikhlas (one study numbered 3 in Table 7). Four studies also used BPA in the same protocols (# 1, 2, 4, 5) allowing comparison with this substance already identified as ED. The studies are presented below by period of exposure.

Fetal life exposure

In Ullah et al., 2019a (ToxRTool score 2) referenced **# 1** in Table 7, bisphenols including BPB and BPA were administered in drinking water at concentrations of 5, 25, and 50 µg/L from GD 1 to GD 21 to pregnant Sprague-Dawley rats. Since an adult pregnant rat drinks about 12 ml/100 g body weight/d, it can be estimated that the daily BPB intake was about 0.6, 3 and 6 µg/kg bw/day respectively in the 3 treated groups. Observations were performed in male F1 on PND16 and on PND 80 using 8 males per group. Cages were made of glass and feed was soy and alfalfa free.

No signs of systemic toxicity were reported on mothers during the pregnancy. An increase of the bodyweight gain in dams was reported with BPB (and BPA) although non-statistically significant. Body weight of the male offspring was significantly increased with BPB (and BPA) at 50 µg/L at PND 80 but not at PND 16. No effect was observed on litter size. Adrenal and liver weight did not change throughout groups.

On PND16, a slight increase of the absolute weight of seminal vesicle (+5.1% and +2.6%), prostate (+5.2 and +9.4%), was observed at 50 µg/L for BPB and BPA respectively. These effects were not statistically significant. A slight increased body weight (+4.6% and +7.2%) was observed at the same dose level of 50 µg/L for BPB and BPA respectively. A decrease of the bulbourethral gland weight (-9.7% and -10.8%) was observed whereas bulbocavernosus muscles weight was increased with BPB (+2.9%) and decreased with BPA (-5%). Overall, BPB (and BPA) exposure did not affect significantly any parameter of the androgen-dependent development (ano-genital distance, nipple involution and weights of prostate, epididymis, seminal vesicle, bulbourethral gland and bulbocavernosus muscles) on PND16.

On PND 80, the activity of anti-oxidant enzymes in the testis were decreased from 25 (peroxidase) or 50 µg/L BPB (catalase and superoxide dismutase) and at 50 µg/L with BPA. Oxidative stress measured by reactive oxygen species and lipid peroxidation was increased with both bisphenols at 50 µg/L. A decrease in plasma testosterone (-30.3 %), LH (-29.3% and -30.5%) and FSH (-61.7% and -59.6%), and an increase in plasma œstradiol (+279% and +283%) were observed at 50 µg/L for BPB and BPA respectively. With 50 µg/L BPB or BPA, the absolute weight of seminal vesicle (-3.4% and -5.1%) was statistically significantly decreased but not that of prostate (-15.1% and -18.9%) whereas body weight (+9.9% and +9.4%) was statistically significantly increased.

Regarding spermatogenesis, histological morphometric data of the testis report an increase in seminiferous epithelium height for both BPB and BPA (+3.4% and +3% respectively) This is not a classical observation since spermatogenesis alterations rather exhibit a diminution of this height. Relative interstitial and seminiferous spaces were reduced in the range from -15.4% to -16.1% and from -4.1 to to -4.6% for BPB and BPA respectively. This is puzzling since testis is formed only by these two compartments. Lastly, a decrease in different cell types (spermatogonia (-6.8% and -7.8%)and spermatocytes (-7% and -6.5%)) within the seminiferous tubule is observed at 50 µg/L for BPB and BPA respectively. Taken together, these histological observations do not provide a consistent picture and this morphometric analysis is not conclusive.

However, clear alterations of spermatogenesis were evidenced by sperm analysis that demonstrated changes in multiple endpoints: significant decreases in daily sperm production at 50 µg/L (-16.4% and -16.2%), in sperm number within caput/corpus at 25 µg/L (-3% and -3.8%) and 50 µg/L (-3% and -0.4%) (but no effect within cauda epididymis) and in sperm motility at 50 µg/L (-6.7% and -2.9%) for BPB and BPA, respectively.

In conclusion, this study clearly shows that exposure to BPB during fetal life provokes alterations in the quality and the number of sperms at adulthood similarly to BPA.

Pubertal and adult exposure

In Ullah et al., 2018a (ToxRTool score 2) referenced **# 2** in Table 7, Sprague-Dawley adult male rats were housed in cages made of steel and given free soy 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 including BPA) for 48 weeks (7 animals per group). As a young adult rat drinks around 12 mL/day/100 g body weight, they received around 0, 0.6, 3 and 6 µg/kg bw/day bisphenols. However, it is known that the daily water intake referred to body weight changes as a function of the age (Holdstock, 1973). Thus, the above estimated doses must be roughly doubled when considering first stages of exposure (PND23) and reduced by half for the end of exposure (51 week-old). Thus, animals received from 1.2 to 0.3, from 6 to 1.5 and from 12 to 3 µg/kg bw/day from PND23 to PNW51 for the 3 treated groups, respectively.

At the end of the treatment, body weights of male rats were slightly increased (+1.4 and +1.5%) at the highest dose (50 µg/L in drinking water) of BPB and BPA respectively. No information is reported on the liver or adrenal weight. Decreased relative weights of the testis (-5.2% and -4.9%), the epididymis (-2.5% for both substances), and the seminal vesicle (-6.8% and -7.1%) were observed with the highest dose of BPB and BPA. The reduction in relative prostate weight by 2.2% for both substances did not reach statistical significance. The gonadosomatic index (GSI) which is equal to gonadal weight/body weight x 100, showed significant reduction (by -5.8% and -7.3%) with BPB and BPA at 50 µg/L.

The testicular concentrations of reactive oxygen species (ROS) and peroxided lipids (LPO) were increased for 50 µg/L BPB and BPA. 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 and BPA.

There were dose-related trends that reach statistically significance at 50 µg/L for BPB and BPA towards decreases in testosterone (-22.1% and -18.8%), LH (-17.3% and -15.1%) and FSH (-26.6% and -25.3%) concentrations and toward an increase in estradiol (+61.9% and +49.5%) concentration in plasma after treatment with BPB and BPA respectively.

Sperm production was examined. In the cauda epididymis, the motile sperm percentage was significantly reduced by 2.9 and 2.5 % after exposure to 50 µg/L BPB and BPA respectively, but the viable sperm percentage was unaffected. Sperm count in the testis showed that the daily sperm production was dose dependently reduced (statistically significant for 50 µg/L BPB and BPA, with a reduction by 9 %). In the same way, the sperm number was also dose-dependently decreased in the caput epididymis (significance from 25 µg/L BPB and BPA, with a reduction by 3.4 and 4.3% for BPB and by 3.7% for BPA)and in the cauda epididymis (significance for 50 µg/L BPB and BPA, with a reduction by 2.9 and 2.5%).

Testicular histological analyses were performed. The height of seminal epithelium was dose-dependently decreased (statistically significant for 50 µg/L BPB and BPA, with a reduction by 16 % and 14% for BPB and BPA, respectively) without changes in the diameter or in the relative area of seminiferous tubules. At 50 µg/L BPB and BPA, there was also a statistically decrease of the numbers of spermatogonia by 6.7 and 7.7%, spermatocytes by 6.9 and 6.4% and spermatids by 4.6 and 4.5%.

Lastly histological examination of the caput and cauda regions of epididymis did not exhibit changes in the tubular diameter and the epithelial height.

Taken together, **these data clearly evidence that a chronic exposure to low doses of BPB alters the reproductive function in the male adult rat. Both endocrine and exocrine testicular functions were disrupted. BPB and BPA also acted on the hypothalamic-pituitary-gonadal system, with modifications of LH and FSH levels.** Oxidative stress was also observed. BPB-induced changes in testicular hormonal production observed here are coherent 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.

In Ikhlas and Ahmad, 2020 (ToxRTool score 2) referenced **# 3** in Table 7, Swiss albino mice fed with standard animal diet were intraperitoneally (IP) injected with BPB on PND 35/42. Then, they were again injected 2 or 4 times at 7-day intervals. Animals were sacrificed 2 days after the last injection *i.e.* 16 (3 injections) or 30 (5 injections) days after the first injection. The given doses were equal to 5%, 10% and 15% the LD50 of BPB. In this paper, the LD50 was found to be equal to 250 mg/kg with this protocol of administration of BPB in using Dixon's up and down method (Dixon 1965). Consequently, the given doses were 12.5, 25, 37.5 mg/kg in each injection.

No indication is given about a potential general toxicity or on body weight changes. An increase in oxidative stress markers was evidenced in spermatozoa: significant increase in ROS concentrations after three 37.5 mg/kg BPB injections and after five 25 and 37.5 mg/kg injections, decrease in reduced glutathione, and increase in peroxided lipids after five 37.5 mg/kg BPB injections.

After 3 and 5 injections of 25 or 37.5 mg/kg BPB, the following significant changes were observed:

- increased DNA damage in sperm evaluated by the comet assay,
- the sperm viability estimated by the percentage of cells which exclude Trypan Blue was decreased (by half at the highest dose),
- the percentage of sperm with abnormal morphology was increased (x2 with the highest dose),
- the immaturity of the sperm evaluated by the remaining histones, was increased (x3 with the highest dose),
- the motility evaluated using the OpenCASA software was decreased for 8 out of the 10 measured parameters. As an example, the beat frequency of the flagellum was reduced by half at the highest dose.

Lastly after 3 and 5 injections of 37.5 mg/kg BPB, a decrease in the sperm number in the cauda epididymis was observed.

In conclusion, this study reports numerous and well-performed analyses of the sperm characteristics. They clearly show an adverse effect of BPB on sperm count and quality

after weekly intraperitoneal injections of high doses of BPB. No indication about a potential general toxicity is given.

Adult exposure

In **Ullah et al., 2018b** (ToxRTool score 2) referenced **# 4** in Table 7, Sprague-Dawley adult male rats were housed in cages made of steel. Endocrine disrupting properties of the diet were not characterised in this study. Animals on PND 70-80 were exposed orally for 28 days to 0, 5, 25 and 50 mg/kg bw/day BPA, BPB, BPF or Bisphenol S (BPS; EC No 201-250-5; EC Name: 4,4'-sulphonyldiphenol) (7 animals per group).

No effect was observed on body weight and on testis weight. After treatments with 50 mg/kg bw/day BPB and BPA, the testicular concentrations of ROS and LPO were significantly increased. The activities of some antioxidant enzymes such as POD were significantly decreased (all doses of BPB and 50 mg/kg bw/day of BPA).

Plasma and intratesticular testosterone concentrations were reduced after all the treatments with BPB with a reduction by 29 to 35% for plasmatic concentration and by 18.9 to 22.3% for intratesticular concentration and BPA with a reduction by 32.9 to 36.1% for plasmatic concentration and by 17 to 22.8% for intratesticular concentrations (except the intratesticular level with 5 mg/kg bw/day BPA). The effect was not dose-dependent (except the effect of BPA on intratesticular testosterone concentration with a reduction by 6.5, 17 and 22.8%).

Lastly, histology of the testis showed changes in the group treated with the highest dose of BPB and of BPA as compared with controls. Qualitative observations showed reductions in the number of elongated spermatids/sperm in the lumens of the seminiferous tubules. However, these changes have not been quantified. Importantly, the height of the epithelium of the seminiferous tubules was significantly decreased by 18.8 and 16.9% for BPB and BPA respectively, thus confirming that spermatogenesis is impaired. No significant effect on diameter and area of the seminiferous tubule was observed.

An *in vitro* experiment was also conducted in this study. The adult 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 bisphenols (1 ng/ml = 0.004 µM). After 2 hours of incubation, there was a trend in increased concentrations in ROS in the tissue exposed to BPB and BPA, which was statistically significant for 10 but not for 100 ng/ml BPB and was never significant with BPA. However, POD lipids content and the activities of antioxidant enzymes (SOD and POD) in the testis, which exhibited very high variability, did not significantly change with BPB or BPA treatment. However, since the survival of the testicular cells in these conditions is questionable, these data cannot be considered as conclusive.

Overall, although most of the spermatogenic observations were performed only qualitatively, they suggest that exposure of adults to 50 mg/kg bw/day BPB alters spermatogenesis. BPB also reduces testicular testosterone production from the lower dose (5 mg/kg bw/day). Oxidative stress was also observed. BPB exerts effects at dose levels similar or lower than BPA.

In **Ullah et al., 2019b** (ToxRTool score 3) referenced **# 5** in Table 7, Sprague-Dawley rats on PND 70-80, bred in steel cages with soy and alfalfa-free feed and tap water in polysulfone bottles, were given BPB or BPA, BPF or BPS by gavage for 28 days at doses equal to 5, 25, and 50 mg/kg bw/day (7 animals per group). Observations were performed on the 29th day.

The number of studied endpoints is limited. No indication is given about a potential general toxicity or on body weight changes. No change in the percentage of motile sperm was observed with any bisphenol. Using the comet assay, a significant increase in DNA damage was observed with the four bisphenols at 50 mg/kg bw/day. Daily sperm production was reported by the authors as decreased with the four bisphenols at 50 mg/kg bw/day, but, surprisingly for such an

important endpoint, no data are presented in the paper.

An *in vitro* experiment was also conducted in this study using sperm incubation with 0, 1, 10 and 100 ng/ml bisphenols (1 ng/ml = 0.004 µM). After 2 hours of incubation, there was a dose-dependent increase in ROS and LPO in the sperm, which became statistically significant with 100 ng/ml BPB or BPA. The activity of the antioxidant SOD was also increased with BPB and BPA. On the contrary, SOD activity was decreased after *in vivo* exposure. The authors interpreted this effect as a short term "body defence mechanism".

In conclusion, the interpretation of this study is limited by the low number of endpoints measured and the poor data reporting.

Table 7: Experimental data available on male reproduction function and BPB and comparison with BPA

Summary of the 5 studies. All reported changed are statistically significant with p<0.05 unless specified otherwise

Number and reference	Species Strain Model	Routes	Dose Exposure period	Group size	Outcomes reported			NOAEL/ LOAEL	ToxR Tool	Limits of the study
					Spermatogenesis	Endocrinology	Oxidative stress			
Gestational exposure										
- 1 - Ullah et al 2019a	Rat Sprague-Dawley	Drinking water	**BPA, BPS, BPF, BPB,** 0, 5, 25, 50 µg/L in the drinking water *i.e.* around 0.6, 3, 6 µg/kg bw/day. from GD1 to GD 21 Observation on PND16 and PND 80	8	Multiple alterations on PND 80 with the 4 BPs: - ↓ in daily sperm production at 50 µg/L, - ↓ in sperm number in caput/corpus but not in cauda epididymis from 25 µg/L, - changes in seminiferous morphometry from 50 µg/L (3 parameters) or 25 µg/L for area % of seminiferous tubules, - ↓ in different cell types in the seminiferous tubules at 50 µg/L, - ↓ of the motility of the sperm at 50 µg/L (BPA, BPB) or from 25 µg /L (BPF, BPS).	No antiandrogenic effect on PND16. With the 4 BPs, on PND 80, ↓ in plasma testosterone, LH, FSH, and ↑ in plasma œstradiol with 50 µg/L.	On PND 80: - ↓ in anti-oxidative activities from 50 µg/L with BPA and BPF and from 25 µg/L with BPB and BPS. - ↑ in oxidative stress at 50 µg/L with the 4 BPs	NOAEL : 25 µg/L ≈ 3 µg/kg bw/day LOAEL : 50 µg/L ≈ 6 µg/kg bw/day	Score 2	Moderate confidence in histological spermatogenesis analysis
Pubertal and adult exposure										
- 2 - Ullah et al 2018a	Rat Sprague-Dawley	Drinking water	**BPA, BPS, BPF, BPB,** 0, 5, 25, 50 µg/L in the drinking water from PND22 for 48 weeks. Average of the estimated absorbed doses: around 0, 0.6, 3, 6 µg/kg bw/day	7	<u>With the 4 BPs:</u> - ↓ of epididymis relative weight at 50 µg/L, - ↓ of paired testis weight at 50 µg/L (not statistically significant) - ↓ in daily sperm production at 50 µg/L, - alterations of seminiferous morphometry at 50 µg/L: diminution of epithelial height, reduction in the number of spermatogonia, spermatocytes and spermatids, - ↓ in cauda epididymis sperm number at 50 µg/L, - ↓ in caput epididymis sperm number from 25 µg/L.	With the 4 BPs: - ↓ in the seminal vesicle absolute weight from 25 µg/ and relative weight at 50 µg/L, - ↓ in plasma testosterone , LH, FSH, at 50 µg/L, - ↑ in plasma œstradiol at 50 µg/L.	With the 4 BPs: - ↓ in anti-oxidative activities from 25 µg/L. - ↑ in oxidative activities at 50 µg/L	NOAEL: 5 µg/L ≈ 0.6 µg/kg bw/day LOAEL: 25 µg/L ≈ 3 µg/kg bw/day	Score 2	Information on the purity of the test chemical, the sensitivity of hormonal assays and the number of replicates were lacking.

					With BPA and BPB, - ↓ in sperm mobility in cauda epididymis at 50 µg/L.					
- 3 - Ikhlas and Ahmad 2020	Mouse Swiss albino	I.P.	**BPB**, 12.5, 25, 37.5 mg/kg injected at PND 35/42 and then at 7-day intervals Observation on the 16th day (3 injections) or on the 28th day (5 injections)	5	Increase in sperm DNA damage from 25 mg/kg BPB at 30 day of exposure and at 37.5 mg/kg BPB at 16 day of exposure. With 3 or 5 injections: - ↑ sperms DNA damage from 25 mg/kg. - ↓ in sperm number with 37.5 mg/kg, - ↓ in sperm viability, morphology, maturity and motility from 25 mg/kg.		In sperm, with 3 injections: ↑ in ROS content at 37.5 mg/kg In sperm, with 5 injections: - ↓ in reduced glutathione at 37.5 mg/kg, - ↑ in peroxided lipids at 37.5 mg/kg, - ↑ or ↓ in anti-oxidative activities from 12.5 mg/kg, - ↑ in ROS content from 25mg/kg	Inconclusive with this experimental exposure design.	Score 2	IP route.
Adult exposure										
- 4 - Ullah et al 2018b	Rat Sprague-Dawley	Oral	**BPA, BPS, BPF, BPB,** 0, 5, 25, 50 mg/kg bw/day PND 70/80 for 28 days. Observation on the 29th day.	7	↓ in epithelial height of seminiferous tubules at 50 mg/kg bw/day without changes in the other two morphometric testicular parameters studied.	↓ in plasma and/or intratesticular testosterone, with 5 mg/kg bw/day with the 4 BPs.	↓ in anti-oxidative activities (POD) from 5 mg/kg bw/day for BPB (at 50 mg/kg bw/day for BPA) ↑ in oxidative activities at 50 mg/kg bw/day with the 4 BPs.	NOAEL: 25 mg/kg bw/day LOAEL: 50 mg/kg bw/day	Score 2-	Few sperm parameters quantitatively analysed. The modalities of the oral administration method performed are not presented and the histopathological evaluation is not sufficiently described.

- 5 - Ullah et al 2019b	Rat Sprague-Dawley	Oral (gavage)	**BPA, BPS, BPF, BPB,** 0, 5, 25, 50 mg/kg bw/day PND 70/80 for 28 days. Observation on the 29th day.	7	- DNA damage in sperms at 50 mg/kg bw/day with the 4 BPs - ↓ in daily sperm production (data not shown)			NOAEL: 25 mg/kg bw/day LOAEL: 50 mg/kg bw/day	Score 3	Values of the most important data (daily sperm production) are not presented.

General conclusion for male reproductive system

Two studies from 2 different laboratories (Ullah et al., 2018a and Ikhlas and Ahmad, 2020 # 2, 3) display a high degree of confidence. One other paper reports many endpoints which are well-studied except the testicular histological analysis (Ullah et al., 2019a # 1). A fourth study gave essentially qualitative alterations (Ullah et al., 2018b # 4) and the last one is very poor quality (Ullah et al., 2019b # 5). All these studies show that BPB alters spermatogenesis. Furthermore, a BPB-induced increase in oxidative stress in testis or in sperm was observed whenever this endpoint was evaluated (Ullah et al., 2018 a and b, Ullah et al., 2019a and Ikhlas and Ahmad, 2020 # 1, 2, 3, 4). Lastly testicular testosterone production and/or plasma testosterone level were decreased (Ullah et al., 2018 a and b, Ullah et al., 2019a # 1, 2, 4) and plasma estradiol level was increased (Ullah et al., 2019a and b # 1, 2) whenever these endpoints were measured.

Importantly, BPA causes the same effects as BPB in all the endpoints studied Ullah et al., 2018a, 2019a, 2018b, 2019b (# 1, 2, 4, 5).

It remains unknown whether the multiple differences between the experimental procedures used in the different studies (species, age at exposure to BPB, method and route of administration of BPB, duration of the exposure to BPB) can explain why the effective dose of BPB largely differs from one study to another one. In particular, differences were observed between studies performed by gavage or in drinking water. The importance of the contribution of sublingual absorption has been demonstrated with bisphenol A (Gayrard et al., 2013; Vandenberg et al., 2014). Sublingual absorption that bypasses the first pass metabolisation in the gastrointestinal tract may lead to a slower conjugation and hence higher systemic exposure to free active bisphenol when the substance is administered in drinking water compared to gavage.

A hypothetical MoA of BPB is represented in **Figure 2**.

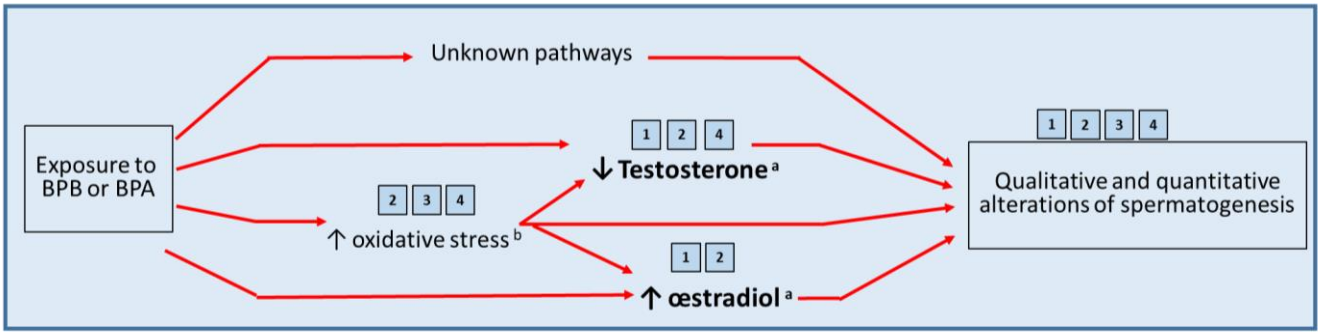


Figure 2: Hypothetical scheme of the MoA of BPB (and BPA) on spermatogenesis in rats. The numbers placed above each effect indicate the references of the studies which report this effect.

All the papers presently available provide evidence of a decrease in plasma testosterone level and an increase in plasma estradiol levels in response to BPB (or BPA) exposure. It is known that each of the observed endocrine changes (displayed in bold in figure 2) provoke alterations in spermatogenesis. It cannot be excluded that other pathways independent of endocrine changes could be implicated.

The figure 2 is drawn from 3 papers with a good or acceptable quality (Ullah et al., 2018 a and b, Ikhlas and Ahmad, 2020, # 1, 2, 3) and one qualitative study (Ullah et al., 2018b # 4). Studies (Ullah et al., 2018 a and 2019a, Ullah et al., 2018b) # 1, 2, 4 used Sprague-Dawley rats whereas study (Ikhlas and Ahmad 2020) # 3 used Swiss albino mice. There is no study investigating these endpoints that reported no effect of BPB (and BPA).

Taken together, these data provide evidence that a sub-chronic or chronic exposure to BPB alters the male reproductive system in the adult rat. These hormonal and histological effects are consistent with some of the anti-androgenic effects observed in castrated animals in the Hershberger assay. BPB also acted on the hypothalamic-

pituitary-gonadal system. BPB-induced changes in testicular hormonal production observed in animals are coherent 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 these studies.

6.4.2.2 Female reproductive system

Another recent study on rodents investigated the effect of BPB on the reproductive health in young adult female rats at early post pubertal period (**Ijaz et al., 2020**) (ToxRTool score 3). This study originated from the same group of Ullah and colleagues, and focused on the effects of several bisphenols including BPB and BPA on female rat reproductive functions using *in vivo* approaches.

Sprague-Dawley young adult female rats were housed in steel cages. Endocrine disrupting properties of the diet were not characterised in this study. Post-weaning (pubertal exposure) female rats were exposed via IP to 0, 0.05, 0.5, 5 and 50 mg/kg bw/day (5 animals per group) for 28 days. On day 29 after the beginning of the treatment, stage of the estrous cycle was noted, and animals at the estrous stage were euthanised. Ovarian weight, uterus weight and GSI (gonadosomatic index = relative ovary weight) was determined. The ovaries were sampled and cut into sections (7 µm thick) and every tenth section was observed i.e. 70 µm between two consecutively examined sections, at 10 or 20× and the different types of follicles and corpus luteum were counted. The total number of follicles from each class was defined as the mean of the counted follicles per section.

No significant increase in body weight was noted although a slight increase could be observed with BPB at 50 mg/kg bw/day (decreased from 0.500 mg/kg bw/day with BPA). A significant lower weight of the paired ovaries and of GSI was observed at the highest tested dose for BPB and BPA and also at the lowest tested dose for BPB only. There was also a significant decrease of the absolute uteri weight from the lowest tested dose level for BPB and BPA. The relative weights of uterus were significantly decreased from 500 µg/kg bw/day for BPB and 5 mg/kg bw/day for BPA. Based on the current knowledge, the apparent discrepancy behind the increased uterine blotted weight (absolute value) observed in Yamasaki et al., 2002 and the decreased absolute uteri weight in Ijaz et al., 2020 cannot be explained. However, these two studies differ in many ways, one is conducted in prepubertal immature rat (PND20 to 22) and the other one in peripubertal period (from PND28) until adulthood (PND56), the route of exposure differs (IP vs SC) as well as the dose levels. Lastly as BPB responds in the same manner as BPA in these two studies, it is judged that these uterine effects should be considered.

Hormone concentrations were determined (see **Summary of** the experimental evidence on female reproduction function and BPB

Table 8 below). It should be noted, however, that one has to be very cautious about those results. In the one hand, estradiol assay sensitivity provided by the manufacturer was much higher than the values described in the paper. In the other hand, the values given in the result were not consistent with the supposed physiological state (estrus). Nevertheless, the authors showed higher testosterone and lower FSH levels in the highest BPB dose group as compared to vehicle. Progesterone levels were significantly lower in all BPB groups and LH ones were lower with the 5 and 50 mg/kg doses. Lastly, histology of the ovaries showed changes from 50 µg/kg bw/day with BPB and from 500 µg/kg bw/day with BPA. The histological analysis of the ovaries was of poor quality with a possibility of double-counting of follicles (see for further details the table below) and insufficient details given on the number of analysed sections. With all those major limitations, the author provided results suggesting that BPB may significantly reduce antral follicles at 50 mg/kg bw/day (from 5 mg/kg bw/day for BPA) and *corpus luteum* counts at all tested doses (from 500 µg/kg bw/day for BPA) while increasing the number of

atretic as well as cystic follicles from 5 mg/kg bw/day for BPB (and BPA). Treatment with 50 and 500 µg/kg bw/day resulted in a low count of *corpus luteum* but the effect was limited as compared to the higher doses (5 and 50 mg/kg bw/day). Effects on the dimensions of the different follicle structures were also noted.

After treatments with BPB, markers of lipid peroxidation in the ovaries were significantly increased at 50 mg/kg bw/day (same effect for BPA). ROS were increased at 500 µg/kg and 5 mg/kg bw/day only for BPB while found in all animals treated with BPA, whatever the dose. The activities of some antioxidant enzymes were decreased in the tissue exposed to BPB: the decrease in CAT activity was significant from 500 µg/kg bw/day onward for BPB (and BPA but without clear dose-response relationship) and in SOD activity at 500 µg/kg bw/day and 50 mg/kg (from 500 µg/kg bw/day for BPA). No effect was observed on POD activity (BPB and BPA).

In conclusion, when looking at the most reliable results of this study i.e. the parameters without methodological limitations, it appears that a 28 day IP BPB exposure during peripubertal period is associated to several markers of female reproductive dysfunction, in particular absolute and relative reproductive organ weights -and, oxidative status of the whole ovary. The most sensitive marker, i.e. the one modified from the lowest dose, 50 µg/kg bw/day, was absolute uterus weight. A lower relative ovary weight (GSI) in the absence of significant body weight difference might suggest a delayed puberty for the highest dose. Overall, all significant and reliable effects were similar with those of BPA and, for most of them, expressed at similar doses. It can be considered that alteration of the pubertal process is a sensitive target of BPB exposure for dose as low as 50 µg/kg bw/day *via* a parenteral route. This pinpoints at BPB as a potential endocrine disruptor. However, there is not enough data from this study to firmly support this hypothesis.

Summary of the experimental evidence on female reproduction function and BPB

Table 8 : Experimental data available on female reproduction function and BPB and comparison with BPA.
All reported changed are statistically significant with p<0.05 unless specified otherwise

Reference	Species Strain Model	Dose Exposure period	Monitored parameters	Effects		Comments
				BPA	BPB	
Ijaz et al., 2020	**Rat Sprague Dawley**	Pubertal exposure (from 4-5 weeks to 8-9- weeks postnatal)	Weight gain	no	no	mistake in reported values in the publication table
		Duration: 28 days 0.05 -0.5 - 5 & 50 mg/kg bw/day Animal BW: 90-150g OECD-like 407	Absolute ovary weight	↓ at 50 mg/kg bw/day	↓ at 50 µg/kg bw/day and 50 mg/kg bw/day	
			Relative ovary weight (GSI)	↓ at 50 mg/kg bw/day	↓ at 50 mg/kg bw/day	
			Absolute uteri weight	↓ at all doses	↓ at all doses	
			Relative uteri weight	↓ from 5 mg/kg bw/day	↓ from 500 µg/kg bw/day	
			Absolute liver weight	↑ at 50 mg/kg bw/day	↑ at 50 mg/kg bw/day	
			Absolute heart weight	↑ at 50 mg/kg bw/day	↑ at 50 mg/kg bw/day	
			Absolute kidney weight	no	no	
			Ovarian antioxidant status			
			CAT activity	↓ from 500 µg/kg bw/day	↓ from 500 µg/kg bw/day	
			POD activity	no	no	
			SOD activity	↓ 500 µg and 5 and 50 mg/kg bw/day	↓ 500 µg and 5 and 50 mg/kg bw/day	non monotonic?
			ROS activity	↑ at all doses	↑ 500 µg and 5 mg/kg bw/day	non monotonic?
			TBARS concentration	↑ at 50 mg/kg bw/day	↑ at 50 mg/kg bw/day	
			Hormonal status			
			Testosterone	↑ at 5 and 50 mg/kg bw/day	↑ at 50 mg/kg bw/day	
			Estradiol	↓ 50 mg/kg bw/day	↓ 500 µg and 50mg/kg bw/day	Unreliable results since values are out of the range of the assay performance

			Progesterone LH FSH	↓from 5 mg/kg bw/day ↓ from 5 mg/kg bw/day ↓ at 50 mg/kg bw/day	↓ from 50 µg/kg bw/day ↓ from 5 mg/kg bw/day ↓ at 50 mg/kg bw/day	
			Ovarian follicle counts (absolute numbers) Corpus luteum Antral follicle Atretic follicles Preovulatory follicles	 ↓ from 500 µg/kg bw/day ↓ from 5 mg/kg bw/day ↑ from 5 mg/kg bw/day no	 ↓ at all doses ↓ at 50 mg/kg bw/day ↑ from 5 mg/kg bw/day no	-Equivocal dose dependency for BPA. -Poor quality of histology; -Possibility of same follicles counted twice (sections: 1 7µm thick section over 10 (i.e 70µm between examined sections< size of some types of follicles) -number of analyszed sections not indicated.
			Follicle and ovarian structure size Corpus luteum diameter Antral follicle diameter	 ↑ at 50 mg/kg bw/day ↑ from 5 mg/kg bw/day	 ↑ at 50 mg/kg bw/day ↑ from 500 µg/kg bw/day	
			Granulosa height	↓ at 50 and 500 µg, and 5 mg/kg bw/day	↓ at 50 and 500 µg, and 5 mg/kg bw/day ; ↑ at 50 mg/kg bw/day	Equivocal non monotonic dose-response
			Theca height	no	no	

6.4.3 Non-EATS modalities

Metabolism and obesity

One *in vivo* study that primarily investigated male reproductive function (see section 6.4.2.1) reported that prenatal exposure to BPB was as efficient as BPA at enhancing body weight of the male offspring at PND 80. No effect was observed at PND 16 (Ullah et al., 2019a # 1). In another study, adult exposure only did not lead to body weight changes (Ullah et al., 2018b # 4).

Summary of the experimental evidence on metabolism and obesity and BPB

Table 9: Experimental evidence available on metabolism and obesity and BPB

Period of exposure	Methods	Characteristics, sex, species	Outcomes surveyed		References
Pregnancy	**Sprague-Dawley rats** ; exposure to **BPA, BPB, BPF and BPS** through drinking water dosed at concentrations of 5, 25, 50 µg/L from GD1 to GD21.	**Male offspring** studied. Standard diet	The study is about the reproductive axis in males (see section 6.4.2.1). However, several outcomes can provide information regarding metabolic health including body weight gain of dams, pups and the offspring; liver weight; adrenal weight; plasma hormone levels.	No effect on maternal body weight gain. Enhanced body weight of the male offspring at PND80 (an average of 10%) at the highest dose but not at PND16. No adrenal and liver weight changes.	Ullah et al., 2019a

6.4.4 Human epidemiological data

Philips et al. (2018) evaluated the impact of BPA analogues on fecundability among 877 of the 8879 participants from the population-based Generation R pregnancy cohort (EU cohort). BP concentrations were measured in a spot urine sample obtained from each participant during the first trimester visit. They reported no association of urinary concentrations of bisphenol analogues including BPB with fecundability, but total bisphenols (including BPF, BPS, BPB, BPP[17], BPAF, BPAP[18], or BPZ[19]) could be associated with a longer time to pregnancy in women with inadequate or without folic acid supplement use before pregnancy. However, the association is not statistically significant (Philips et al., 2018). Nevertheless, the following limitations of exposure measurements should be taken into account. Few spot measurements of a substance,

[17] Bisphenol P, CAS 2167-51-3, EC No 606-820-0, EC name: 4,4'-(1,4-Phenylenediisopropylidene)bisphenol
[18] Bisphenol AP, CAS 1571-75-1, EC name: 4,4’-(1-Phenylethylidene)bisphenol
[19] Bisphenol Z, CAS 843-55-0, EC No 212-677-1, EC name: 4,4'-cyclohexylidenebisphenol

such as one of the bisphenols with a very short half-life in the body and large day-to-day within-person variability, can result in poorly estimated average levels over long periods. This results in exposure assessments that cannot be reliable for health outcomes that require a long period of latency.

6.4.5 Conclusion on the *in vivo* adverse effect data

The adverse effects of BPB have been investigated in vertebrates in two studies in fish and six studies in rodents (five experimental studies from two distinct laboratories on male reproductive function and one study on female reproductive function).

All data in rodents also evidenced the effect of BPB on the male reproductive system (altered spermatogenesis) and changes in hormones levels (decrease in T and increase in E2 levels). Ullah et al., 2018a, b, showed that a chronic exposure to low doses of BPB during the pubertal and adult periods or at adulthood alters the reproductive function in the male adult rat. BPB exerts similar effects compared to BPA sometimes the effects of BPB appear at lower doses as compared with BPA, whereas BPA is never more potent than BPB. "Spermatogenesis impairment was observed in adult rats exposed at 50 mg/kg bw/day for 28 days (Ullah et al., 2018b) or at an estimated dose of 3 µg/kg bw/day for 48 weeks (Ullah et al., 2018a). These results were reinforced by the study of Ullah et al. (2019a) that shows alterations in daily sperm production, number and motility at adulthood. This study also points out additional MoA than ED such as oxidative stress. Ikhlas and Ahmad (2020) report numerous and well-performed analyses of the sperm characteristics. They clearly show an adverse effect of BPB on sperm count and quality in mice at 25 and 37.5 mg/kg BPB *via* the IP route. Although the IP route is a non-physiological route of exposure, it provides supportive evidence to effects also observed using a physiological route of exposure by the Ullah group.

Supportive evidence of an effect on the female reproductive system in rodents is provided in one study with limitations; these effects would need to be further investigated. Increased body weight in male offspring prenatally exposed to BPB (and BPA) suggests an effect on metabolism (Ullah et al., 2019a), while adult exposure only did not lead to body weight changes (Ullah et al., 2018b). The effects on female reproductive system and metabolism are not considered to be demonstrated with a sufficient level of evidence based on the present database and are not further discussed below in the demonstration of the ED properties of BPB.

Apical effects for BPB in fish were observed through malformation and death of embryos (Catron et al., 2019) and were associated with developmental and reproductive disturbances, malformations, embryo-toxic effects at the organism level, and indications of decreased sperm count in the testis and alteration of spermatogenesis (Yang et al., 2017). In zebrafish, BPB decreased fish fecundity at the highest concentration tested, as observed with the reduced egg numbers, hatching rate and survival in a study of good quality.

In all studies in which BPA was also tested (all studies except Yang et al., 2017), the toxicity profile of BPB was largely similar to the toxicity profile of BPA considering the nature of the effects that were observed. The effects of BPB were observed at similar or at lower doses compared to BPA.

6.5 Conclusion regarding ED properties relevant for environment and human health

6.5.1 Adverse effects relevant for ED identification

In rodents, adverse effects on male reproductive function were evidenced in two species, i.e. mice and rats (see Table 10 below), with decreased sperm count in the testis and alteration of

spermatogenesis. Spermatogenesis impairment was observed in adult rats exposed at 50 mg/kg bw/day for 28 days (Ullah et al., 2018b*)* and in pubertal rats at an estimated dose of 3 µg/kg bw/day for 48 weeks (Ullah et al., 2018a). These results were reinforced by the results of Ullah et al., 2019a (reporting of histological spermatogenesis judged of bad quality) showing that exposure to BPB during fetal life provokes alterations in daily sperm production, number and motility at adulthood. Ikhlas and Ahmad, 2020 reports numerous and well-performed analyses of the sperm characteristics. They clearly show an adverse effect of BPB on sperm count and quality in mice at 25 and 37.5 mg/kg BPB *via* the IP route. Strict comparison between these studies is not possible because they either used different species, mode of administration (addition in drinking water, gavage and intraperitoneal injection) and periods of exposure (fetal exposure, pubertal, pubertal and adult exposure or adult exposure only). Nevertheless, these studies report apical effects as alterations of spermatogenesis in rat and mice with decreased daily sperm production, decreased sperm number within caput/corpus and in different cell types within the seminiferous tubule, decreased sperm motility, and decreased relative weights of male reproductive organs. Thus, all the available data show that BPB alters male reproductive function.

In fish, adverse effects included an altered hepato-somatic index and gonado-somatic index in male and female zebrafish, indication of altered testis tubules and a decrease in the amount of mature spermatids in males. BPB was demonstrated to significantly reduce fecundity of adult fish exposed for 21 days and to decrease embryos hatching and survival of F1 generation (Yang et al., 2017). Malformations and death were also noted (Catron et al., 2019).

Data (*in vivo*) providing scientific evidence of an adverse effect of BPB on the male reproductive system are summarised in Table 10 below.

Overall, the following adverse effects relevant for the ED identification of BPB have been demonstrated in reliable studies:

- **adverse effect on male reproductive system in rodents,**
- **adverse effect on male reproductive system in fish associated with reproductive disturbances.**

Supportive evidence of a developmental effect is available in fish.

Table 10: Line of evidence for BPB reproductive dysfunction in *in-vivo* studies (fish and male rat and mice).

All reported changed are statistically significant with p<0.05, unless specified otherwise.

Assay category	Biological Model	Species	Exposure duration	Parameter	Effect dose	BPA/ BPB [a]	ToxR Score	References
Histology	Testes	Male SD rats	28 days	[b]	50 mg/kg bw/day: decreased secondary spermatocytes, tubules, and elongated spermatids in the lumen (no statistical analysis conducted for these parameters).	Similar profile to BPA	2	Ullah et al., 2018b
Histology	Testes	Male SD rats	28 days	LOAEL	50 mg/kg bw/day: decreased epithelial height of seminiferous tubules. No effects on the area of seminiferous tubule, interstitium, diameter	Similar profile to BPA	2	Ullah et al., 2018b
Histology	Testes	Male SD rats	48 weeks	LOAEL	0.05 mg/L: decrease in spermatogonia, spermatocytes and spermatids number	Similar profile to BPA	2	Ullah et al., 2018a
Histology	Testes	Male SD rats	48 weeks	LOAEL	0.025 mg/L: decreased sperm number in the caput epididymis	Similar profile to BPA	2	Ullah et al., 2018a
Histology	Testes	Male SD rats	48 weeks	LOAEL	0.05 mg/L: decrease of sperm motility, daily sperm production, sperm number in the cauda epididymis (no effects on viable sperm)	Similar profile to BPA	2	Ullah et al., 2018a
Histology	Testes	Male SD rats	48 weeks	LOAEL	0.05 mg/L: decreased epithelial height. No effects on the area of seminiferous tubule, interstitium, diameter	Similar profile to BPA	2	Ullah et al., 2018a
Histology	Testes	Male zebrafish	21 days	[b]	1 mg/L: alteration of testis tubules, decrease of mature spermatids	Similar profile to BPA	1	Yang et al., 2017
Histology	Testes	Male SD rats	21 days	NOAEL	On PND80, decrease in different cell types in the seminiferous tubules at 50 µg/L (corresponding to 6 µg/kg bw/day).	Similar profile to BPA	2	Ullah et al., 2019a
Histological morphometric analysis	Testes	Male SD rats	21 days	NOAEL	On PND80, decrease of daily sperm production at 50 µg/L (corresponding to 6 µg/kg bw/day).	Similar profile to BPA	2	Ullah et al., 2019a
Histological morphometric analysis	Testes	Male SD rats	21 days	-	On PND80, no decrease of sperm number in the cauda epididymis	Similar profile to BPA	2	Ullah et al., 2019a
Histological morphometric analysis	Testes	Male SD rats	21 days	NOAEL	On PND80, decrease of sperm number in caput/corpus epididymis at 25 and 50 µg/L (corresponding to 3 and 6 µg/kg bw/day).	Similar profile to BPA	2	Ullah et al., 2019a
Histological	Testes	Male SD	21 days	LOAEL	On PND80, decrease of sperm motility at 50	Similar	2	Ullah et al., 2019a

morphometric analysis		rats			µg/L (corresponding to 6 µg/kg bw/day).	profile to BPA		
Histological morphometric analysis	Testes	Male SD rats	21 days	NOAEL	On PND80, changes in seminiferous morphometry from 50 µg/L (3 parameters) and 25 µg/L for area % of seminiferous tubules (but this data is not solid).	Similar profile to BPA	2	Ullah et al., 2019a
Histological morphometric analysis	Daily sperm production	Male SD rat	28 days	NOAEL	50 mg/kg bw/day: decreased daily sperm production (data not shown)	Similar profile to BPA	3	Ullah et al., 2019b
Histological morphometric analysis	Percentage of motile sperms	Male SD rat	28 days	NOAEL		Similar profile to BPA	3	Ullah et al 2019b
Histological morphometric analysis	Testes	Swiss albino mice	16 or 30 days	NOAEL	Decrease of the sperm viability at 25 or 37.5 mg/kg	-	2	Ikhlas and Ahmad 2020
Histological morphometric analysis	Testes	Swiss albino mice	16 or 30 days	NOAEL	Increase in the percentage of sperm with abnormal morphology at 25 or 37.5 mg/kg	-	2	Ikhlas and Ahmad 2020
Histological morphometric analysis	Testes	Swiss albino mice	16 or 30 days	NOAEL	Increase in the immaturity of the sperm evaluated by the remaining histones at 25 and 37.5 mg/kg	-	2	Ikhlas and Ahmad 2020
Histological morphometric analysis	Testes	Swiss albino mice	16 or 30 days	NOAEL	Decrease in sperm motility at 25 and 37.5 mg/kg	-	2	Ikhlas and Ahmad 2020
Histological morphometric analysis	Testes	Swiss albino mice	16 or 30 days	NOAEL	Decrease in sperm number in the cauda epididymis at 37.5 mg/kg	-	2	Ikhlas and Ahmad 2020
Genotoxic analysis	Comet assay (sperm)	Male SD rat	28 days	NOAEL	Increase in DNA damage in sperm at 50 mg/kg bw/day	Similar profile to BPA	2	Ullah et al 2019b
Genotoxic analysis	Comet assay (sperm)	Swiss albino mice	16 or 30 days	NOAEL	Increase in sperm DNA damage from 25 mg/kg BPB at 30 day of exposureand at 37.5 mg/kg BPB at 16 day of exposure.	-	2	Ikhlas and Ahmad 2020
Gonado-somatic index		Male SD rats	48 weeks	LOAEC	0.05 mg/L: decrease	Similar profile to BPA	2	Ullah et al., 2018a
Gonado-		Male	21 days	LOEC	1 mg/L: decrease	-	1	Yang et al., 2017

somatic index	zebrafish						
Gonado-somatic index	Female zebrafish	21 days	LOEC	1 mg/L: decrease	-	1	Yang et al., 2017
Hepato-somatic index	Male zebrafish	21 days	LOEC	0.1 mg/L: increase	-	1	Yang et al., 2017
Hepato-somatic index	Female zebrafish	21 days	LOEC	0.1 mg/L: increase	-	1	Yang et al., 2017
Fecundity	Adult zebrafish	21 days	LOEC	1 mg/L : decrease	-	1	Yang et al., 2017
Hatching rate (F1 generation)	Adult zebrafish	21 days	LOEC	1 mg/L : decrease	-	1	Yang et al., 2017
Survival (F1 generation)	Adult zebrafish	21 days	LOEC	1 mg/L : decrease	-	1	Yang et al., 2017
Abnormalities and death	Embryos zebrafish	10 days	AC50/ NOEC	5.8 / 5.1 µM (1.40 / 1,23 mg/L)	3.7	1	Catron et al., 2019

Note: gonado–somatic index, [gonad weight/body weight]× 100; hepatosomatic index, [liver weight/body weight] ×100
a: BPA/BPB ratio calculated with IC50 or EC50 values, when both chemicals were tested within the same study and showed activity in the same direction.
b: Qualitative assessment only, no parameter calculated.

6.5.2 Endocrine activity

Much *in vitro* and *in vivo* evidence is available regarding the estrogenic activity of BPB, as summarised in Table 11 below. The many *in vitro* results converge to indicate BPB interaction with either or both ERα and ERß signalling of humans, rodents and fish. In addition, a large body of *in vitro* data showed that ER genomic signalling pathway is activated by BPB, and BPB was also shown to activate ER extra genomic response. This estrogenic activity is consistent with the higher uterine weight of treated animals in the immature rat uterotrophic assay (Yamasaki et al. 2002 and UTDB and Kleinstreuer et al., 2016). Estrogen receptors are well preserved among vertebrates such as between fish and humans (Matthews et al. 2000). All the available *in vitro* data on steroidogenesis (Rosenmai et al. 2014, Wang et al. 2014) and *in vivo* data in fish and rodents show an increase in estrogen levels concomitantly to a decrease in testosterone (T) levels (Yang et al. 2017, Ullah et al. 2018a and 2018b, Ullah et al. 2019a, Ikhlas and Ahmad, 2020, see section 6.4.2.1). The *in vitro* estrogenic activity of BPB is also coherent with the increased levels of VTG expression in the liver of male medaka, the significant induction of hepatic VTG in the liver of male zebrafish and the increase in ER-regulated cyp19a1b expression in the brain of male zebrafish (Yang et al. 2017, Yamaguchi et al. 2015). **The estrogenic activity of BPB is therefore well established *in vitro* and *in vivo* in a coherent manner.** Regarding the anti-androgenic action of BPB, the *in vitro* data on steroidogenesis and *in vivo* data in fish and rodents demonstrate the capacity of BPB to decrease testosterone cellular levels (Rosenmai et al. 2014, Wang et al. 2014), plasmatic levels (Ullah et al. 2018a and 2018b, Yang et al. 2017), or intra-testicular levels (Ullah et al. 2018a). Based on the *in vitro* and *in vivo* mechanistic data presented above, it is however not clear whether BPB negatively acts on testosterone levels via an anti-AR mode of action. Indeed, eight out of nine *in vitro* reporter gene assays show BPB antagonist capacity (IC50 in the µM range), however the Hershberger assay provides unclear results (Yamasaki et al. 2003). Therefore, **the anti-androgenic activity of BPB is demonstrated in vertebrate cells including in human cells but has not been confirmed so far *in vivo***.

Regarding the indirect action *via* the hypothalamic-pituitary axis, the *in vivo* data showed a decrease in LH- and FSH-related gene expression in brain and gonads of male zebrafish (Yang et al. 2017) and decreased plasma LH and FSH levels in rats (Ullah et al. 2018a and 2019a).

Oxidative stress was reported in several rodent studies and may also have an impact on the testis. It is however not known whether it may be a consequence or a specific mode of action in addition to estrogenic and possible anti-androgenic effects.

Data therefore provide *in vitro* and *in vivo* evidences that BPB has estrogenic activity and suggest anti-androgenic activity as well as an effect on the hypothalamic-pituitary axis.

Table 11: Line of evidence for BPB estrogenic activity.

Assay category	Species/ Endpoint	Biological model	Exposure time	Parameter	Observed effects	BPA/BPB [a]	ToxR Score	Reference
In vitro endocrine activity								
Binding	Rat ER	Uterine cytosol	-	RBA to E2	0.086%	-	1	Blair et al., 2000
Binding	Rat ER	Uterine cytosol	-	IC50	1.05 µM	11.1	1	Blair et al., 2000
Binding	hGPER	SKRB3 breast cancer cells	<30min	IC50	3.3 µM	7.7	1	Cao et al., 2017
Binding	hERα-LBD	Radiolabelled binding	1-12h	IC50	0.215 µM	5	1	Liu, Sakai et al., 2019
Binding	hERß-LBD	Radiolabelled binding	1-12h	IC50	0.073 µM	13.7	1	Liu, Sakai et al., 2019
Binding	Mouse LBD-ERα	Recombinant	-	IC50	0.023 µM	4.8	1	Sipes et al., 2013
Binding	Bovine ER	Uterus membrane	-	IC50	0.43 µM	1.5	1	Sipes et al., 2013
Binding	hERα	Breast cancer cells	-	IC50	0.30 µM	2.7	1	Sipes et al., 2013
Binding	hERα-LBD	Fluorescence polarisation assay	-	IC50	1.45 µM	4.9	1	Zhang et al., 2018
TA - agonist activity	hERα	Yeast two-hybrid assay (ERα + TIF2)	4h	*b*	Estrogenic activity	Similar profile to BPA	3	Chen et al., 2002
TA - agonist activity	hERα	Yeast cells (YES assay)	24h	EC50	1.73 µM	19.5	3	Conroy-Ben et al., 2018
TA - agonist activity	hERα	HeLa cells (HELN ERα and ERβ cell lines)	16h	EC50	0.204 µM	2.2	2	Grimaldi et al., 2019
TA - agonist activity	hERß	HeLa cells (HELN ERα and ERβ cell lines)	16h	EC50	0.128 µM	3	2	Grimaldi et al., 2019
TA - agonist activity	hERα	Yeast two hydrid system (strain Y190)	4h	*b*	Estrogenic activity of BPB increased by S9 fraction activation	Similar profile to BPA	3	Hashimoto et al., 2001
TA - agonist activity	hERα	MCF-7 cells	24h	EC50	0.07 µM	9	3	Kitamura et al., 2005
TA - agonist activity	hERα	CHO-K1 cells	24h	EC50	0.07 µM	4.5	1	Kojima et al., 2019
TA - agonist activity	hERß	CHO-K1 cells	24h	EC50	0.05 µM	2.6	1	Kojima et al., 2019
TA - agonist activity	hERα	T47D-KBluc cells	24h	EC50	0.3 µM	1.3	1	Mesnage et al., 2017
TA - agonist activity	hERα	Yeast cells (YES assay)	48h	*b*	Estrogenic activity of BPB increased by S9 fraction activation	Similar profile to BPA	3	Okuda et al., 2011
TA - agonist activity	hERα	HepG2 cells	18h	EC50	0.32 µM	3.75	1	Pelch et al., 2019
TA - agonist activity	hERß	HepG2 cells	18h	EC50	n.a.	-	1	Pelch et al., 2019
TA - agonist activity	hERα	MCF-7 cells (MVLN cells)	24h	LOEC	1 µM	BPA not tested	3	Rivas et al., 2002
TA - agonist activity	hERα	BG1-Luc4E2 cells	22h	EC50	0.12 µM	0.7	1	Rosenmai et al., 2014

TA - agonist activity	hERα	HeLa cells (dsRED2 RNA FISH in GFP-ER:PLR-Hela assay)	30min	*b*	Agonist activity	Similar profile to BPA	1	Stossi et al., 2014
TA - agonist activity	hERβ	HeLa cells (dsRED2 RNA FISH in GFP-ER:PLR-Hela assay)	30min	*b*	No agonist activity	Similar profile to BPA	1	Stossi et al., 2014
TA - agonist activity	hERα	Yeast cells (yEGFP + hAR)	24h	EC50	5.8 µM	2.6	1	Van Leeuwen et al., 2019
TA - agonist activity	hERα	Yeast cells	24h	EC50	5 µM	4	1	Wang et al., 2014
TA - agonist activity	hERα	U2OS cells (ER-CALUX)	24h	EC50	0.12 µM	2.3	1	Wang et al., 2014
TA - agonist activity	Rat ERα	HeLa cells	24h	EC50	0.164 µM	14.7	1	Yamasaki et al., 2002
TA - agonist activity	Medaka ERα	Yeast two-hybrid assay (ERα + TIF2)	4h	EC10	0.59 µM	1.5	3	Yokota et al., 2008
TA - agonist activity	Rat ERα	Yeast two-hybrid assay (ERα + TIF2)	18h	*b*	Estrogenic activity of BPB metabolites	Some similar metabolites to BPA's	3	Yoshihara et al., 2004
TA - antagonist activity	hERα	HeLa cells HELN ERα and ERβ cell lines)	16h	IC50	n.a.	Similar profile to BPA	1	Grimaldi et al., 2019
TA - antagonist activity	hERß	HeLa cells HELN ERα and ERβ cell lines)	16h	IC50	n.a.	Similar profile to BPA	1	Grimaldi et al., 2019
TA - antagonist activity	hERα	MCF-7 cells	24h	*b*	No anti-estrogenic activity	Similar profile to BPA	3	Kitamura et al., 2005
TA - antagonist activity	hERα	CHO-K1 cells	24h	IC50	n.a.	Similar profile to BPA	1	Kojima et al., 2019
TA - antagonist activity	hERß	CHO-K1 cells	24h	IC50	n.a.	Similar profile to BPA	1	Kojima et al., 2019
TA - antagonist activity	hERα	MCF-7 cells	24h	*b*	No anti-estrogenic activity	Similar profile to BPA	3	Okazaki et al., 2017
TA - antagonist activity	hERα	HepG2 cells	18h	IC50	n.a.	-	1	Pelch et al., 2019
TA - antagonist activity	hERß	HepG2 cells	18h	IC50	n.a.	-	1	Pelch et al., 2019
TA - antagonist activity	hERα	Hela cells (dsRED2 RNA FISH in GFP-ER:PLR-Hela assay)	30min	*b*	Antagonist activity	No	1	Stossi et al., 2014
TA - antagonist activity	hERβ	Hela cells (dsRED2 RNA FISH in GFP-ER:PLR-Hela assay)	30min	*b*	Antagonist activity	Similar profile to BPA	1	Stossi et al., 2014
TA - antagonist activity	hERα	Yeast cells (yEGFP + hAR)	24h	EC50	n.a.	n.a.	1	Van Leeuwen et al., 2019

TA - antagonist activity	hERα	Yeast cells	24h	*b*	No anti-estrogenic activity	Similar profile to BPA	1	Wang et al., 2014
TA - antagonist activity	hERα	U2OS cells (ER-CALUX)	24h	*b*	No anti-estrogenic activity	Similar profile to BPA	1	Wang et al., 2014
Promoter occupancy	hERα	GFP-ERα:PLR-Hela assay	30 min	EC50	1.8 µM	2.3	1	Ashcroft et al., 2011
Promoter occupancy	hERα	GFP-ER:PLR-Hela assay	30 min	*b*	weak agonist activity	Similar profile to BPA	1	Stossi et al., 2014
Promoter occupancy	hERβ	GFP-ER:PLR-Hela assay	30 min	EC50	0.161 µM	4.5	1	Stossi et al., 2014
Cell proliferation	Human	human uterine adenocarcinoma cell line assay	72h	*b*	n.a;	-	2	Beames et al., 2019
Cell proliferation	Human	MCF-7 cells	144h	*b*	Increased cell proliferation	Similar profile to BPA	3	Hashimoto et al., 2001
Cell proliferation	Human	MCF-7 cells	144h	AC50	0.24 µM	1.5	1	Mesnage et al., 2017
Cell proliferation	Human	T47D cells	144h	*b*	Increased proliferation similar to MCF-7 cells	Similar profile to BPA	1	Mesnage et al., 2017
Cell proliferation	human	MCF-7 cells	96h	*b*	Increased cell proliferation	Similar profile to BPA	2	Pisapia et al., 2012
Cell proliferation	Human	MCF-7 BUS cells	144h	LOEC	0.1 µM: increase	BPA not tested	3	Rivas et al., 2002
Cell proliferation	Human	T47D cells	80h	RPE	128.93%	-	1	Rotroff et al., 2013
Cell proliferation	Human	T47D cells	80h	AC50	0.283 µM	1.4	1	Rotroff et al., 2013
Cell proliferation	Human	MCF-7 cells	144h	RPE	92.96%	-	1	Stossi et al., 2014
Cell proliferation	Fish	CIK cells	48h	IC50	72.44 µM	1.5	3	Zhu et al., 2020
Gene expression	Human	MCF-7 cells	48h	*b*	Genes altered are involved in the etiology of breast cancer and hormone-induced proliferative effect. Gene with the highest fold-change: progesterone receptor	Similar profile to BPA	1	Mesnage et al., 2017
Gene expression	human	MCF-7 cells	24h-48h	LOEC	No effect on ERα and cdc2 gene expression	Similar profile to BPA	3	Okazaki et al., 2017
Gene expression	human	MCF-7 cells	24h-48h	LOEC	No effects on ERβ and Erg-1 gene expression	Similar profile to BPA	3	Okazaki et al., 2017
Gene expression	human/ ps2 protein level	MCF-7 BUS cells	144h	LOEC	1 µM: increase	BPA not tested	3	Rivas et al., 2002

Gene expression	human/ pS2 mRNA	MCF-7 BUS cells	24h	LOEC	1 µM: increase	BPA not tested	3	Rivas et al., 2002
GPER signalling pathway	Human/ intracellular Ca mobilisation	SKRB3 breast cancer cells	<30min	LOEC	0.01 µM: increase	-	1	Cao et al., 2017
GPER signalling pathway	Human/ intracellular Ca mobilisation	SKRB3 breast cancer cells	<30min	EC50	1.7 µM	4.4	1	Cao et al., 2017
GPER signalling pathway	Human/ intracellular cAMP production	SKRB3 breast cancer cells	<30min	LOEC	0.010 µM: increase	-	1	Cao et al., 2017
GPER signalling pathway	Human/ intracellular cAMP production	SKRB3 breast cancer cells	<30min	EC50	0.0975 µM	> 100	1	Cao et al., 2017
GPER signalling pathway	Human/ cell migration	SKRB3 breast cancer cells	48h	LOEC	0.1 µM: increase	Similar profile to BPA	1	Cao et al., 2017
ERα signal in oocyte spindle and changes in localization pattern	Mouse ERα	Mouse oocytes	-	-	150 µM	BPA not tested	-	Zhang et al., 2020b
In vivo endocrine activity								
Organ weight	Wet uterine weight	Immature female rat	3 days	LOAEL	From 20 mg/kg bw/day : increase		2	UTDB and Kleinstreuer et al., 2016
Organ weight	Blotted uterine weight	Immature female rat	3 days	LOAEL	From 20 mg/kg bw/day : increase		2	UTDB and Kleinstreuer et al., 2016
Organ weight	Uterine blotted weight	Immature female rat	3 days	LOAEL	200 mg/kg bw/day : increase	1.3	1	Yamasaki et al., 2002
Organ weight	Ventral prostate	Castrated male Brl Han: WISTJcl (GALAS) rat	10 days	NOAEL	No effect	Similar profile to BPA	1	Yamasaki et al., 2003
Organ weight	Seminal vesicles	Castrated male Brl Han: WISTJcl (GALAS) rat	10 days	NOAEL	No effect	Similar profile to BPA	1	Yamasaki et al., 2003
Organ weight	Glans penis	Castrated male Brl Han: WISTJcl (GALAS) rat	10 days	NOAEL	No effect	Not similar profile to BPA	1	Yamasaki et al., 2003
Organ weight	BC/LA	Castrated male Brl Han: WISTJcl (GALAS) rat	10 days	NOAEL	From 200 mg/kg bw/day: decrease.	Not similar profile to BPA	1	Yamasaki et al., 2003
Organ weight	Cowper's glands	Castrated male Brl Han: WISTJcl (GALAS) rat	10 days	NOAEL	No effect	Similar profile to BPA	1	Yamasaki et al., 2003

Organ weight	Ventral prostate	Castrated male Brl Han: WISTJcl (GALAS) rat	10 days	NOAEL	From 200 mg/kg bw/day: increase when co-exposed with TP.	Not similar profile to BPA	1	Yamasaki et al., 2003
Organ weight	Seminal vesicles	Castrated male Brl Han: WISTJcl (GALAS) rat	10 days	NOAEL	At 600 mg/kg bw/day: increase when co-exposed with TP.	Not similar profile to BPA	1	Yamasaki et al., 2003
Organ weight	Glans penis	Castrated male Brl Han: WISTJcl (GALAS) rat	10 days	NOAEL	At 600 mg/kg bw/day: increase when co-exposed with TP.	Not similar profile to BPA	1	Yamasaki et al., 2003
Organ weight	BC/LA	Castrated male Brl Han: WISTJcl (GALAS) rat	10 days	NOAEL	At 600 mg/kg bw/day: increase when co-exposed with TP.	Not similar profile to BPA	1	Yamasaki et al., 2003
Organ weight	Cowper's glands	Castrated male Brl Han: WISTJcl (GALAS) rat	10 days	NOAEL	At 600 mg/kg bw/day: increase when co-exposed with TP.	Not similar profile to BPA	1	Yamasaki et al., 2003
Hormone measurement	LH level (plasma)	Male SD rats	48 weeks	LOAEC	0.003 mg/kg bw/day : decrease	-	2	Ullah et al., 2018a
Hormone measurement	FSH level (plasma)	Male SD rats	48 weeks	LOAEC	0.003 mg/kg bw/day.: decrease	-	2	Ullah et al., 2018a
Hormone measurement	Estradiol level (plasma)	Male SD rats	48 weeks	LOAEC	0.003 mg/kg bw/day: increase	-	2	Ullah et al., 2018a
Hormone measurement	Testosterone level (plasma)	Male SD rats	48 weeks	LOAEC	0.003 mg/kg bw/day: decrease	-	2	Ullah et al., 2018a
Hormone measurement	Estradiol level (plasma)	Male SD rats	21 days	NOAEL	On PND80, increase at 0.003 mg/kg bw/day).	Similar profile to BPA	2	Ullah et al., 2019a
Hormone measurement	Testosterone level (plasma)	Male SD rats	21 days	NOAEL	On PND 80, decrease at 50 µg/L. (corresponding to 3 µg/kg bw/day).	Similar profile to BPA	2	Ullah et al., 2019a
Hormone measurement	FSH level (plasma)	Male SD rats	21 days	NOAEL	On PND 80, decrease at 0.003 mg/kg bw/day).	Similar profile to BPA	2	Ullah et al., 2019a
Hormone measurement	LH level (plasma)	Male SD rats	21 days	NOAEL	On PND 80, decrease at 0.003 mg/kg bw/day).	Similar profile to BPA	2	Ullah et al., 2019a
Hormone measurement	Estradiol level (body)	Male zebrafish	21 days	LOEC	0.01 mg/L: increase	-	1	Yang et al., 2017
Hormone measurement	Estradiol level (body)	Female zebrafish	21 days	LOEC	0.01 mg/L: increase	-	1	Yang et al., 2017
Hormone measurement	Testosterone level (body)	Male zebrafish	21 days	LOEC	0.1 mg/L: decrease	-	1	Yang et al., 2017
Hormone measurement	Testosterone level (body)	Female zebrafish	21 days	LOEC	1 mg/L: decrease	-	1	Yang et al., 2017

Gene expression	vtg1 mRNA in liver	Male medaka	8h	LOEC	1.2 mg/L: increase	-	1	Yamaguchi et al., 2015
Gene expression	vtg2 mRNA in liver	Male medaka	8h	LOEC	No effect	-	1	Yamaguchi et al., 2015
Gene expression	Chg-L mRNA in liver	Male medaka	8h	LOEC	1.2 mg/L: increase	-	1	Yamaguchi et al., 2015
Gene expression	Chg-H mRNA in liver	Male medaka	8h	LOEC	1.2 mg/L: increase	-	1	Yamaguchi et al., 2015
Gene expression	ERα mRNA in brain	Male zebrafish	21 days	LOEC	0.1 mg/L: increase	-	1	Yang et al., 2017
Gene expression	ERα mRNA in brain	Female zebrafish	21 days	LOEC	0.1 mg/L: decrease	-	1	Yang et al., 2017
Gene expression	ERβ2 mRNA in brain	Adult zebrafish	21 days	LOEC	No effect	-	1	Yang et al., 2017
Protein level	VTG protein in liver	Male zebrafish	21 days	LOEC	0.1 mg/L : increase	-	1	Yang et al., 2017
Gene expression	*cyp19a1b* mRNA	Male zebrafish - Brain	21 days	LOEC	0.01 mg/L: increase	-	1	Yang et al., 2017
Gene expression	*cyp19a1b* mRNA	Female zebrafish - brain	21 days	LOEC	No effects	-	1	Yang et al., 2017
Gene expression	*lhr* mRNA	Male zebrafish - Testes	21 days	LOEC	0.01 mg/L : increase	-	1	Yang et al., 2017
Gene expression	*fshr* mRNA	Male zebrafish - Testes	21 days	LOEC	0.1 mg/L : increase	-	1	Yang et al., 2017
Gene expression	*fshβ* mRNA	Male zebrafish - Brain	21 days	LOEC	0.1 mg/L: increase	-	1	Yang et al., 2017
Gene expression	*lhβ* mRNA	Male zebrafish - Brain	21 days	LOEC	0.1 mg/L: increase	-	1	Yang et al., 2017
Gene expression	*fshβ* mRNA	Female zebrafish - brain	21 days	LOEC	1 mg/L: decrease	-	1	Yang et al., 2017
Gene expression	*lhβ* mRNA	Female zebrafish - brain	21 days	LOEC	No effects	-	1	Yang et al., 2017
Gene expression	*fshr* mRNA	Female zebrafish - ovaries	21 days	LOEC	0.1 mg/L: decrease	-	1	Yang et al., 2017
Gene expression	lhr mRNA	Female zebrafish – ovaries	21 days	LOEC	1 mg/L: decrease		1	Yang et al., 2017

Note: Only studies that tested multiple concentrations are included in the table. - , Not applicable; n.a. : not active;
a: BPA/BPB ratio calculated with IC50 or EC50 values, when both chemicals were tested within the same study and showed activity in the same direction.
b: qualitative assessment only, no parameter calculated.

6.5.3 Plausible link between adverse effects and endocrine activity

The decreased fecundity of fish and the altered spermatogenesis of rodents can result from disruption of endocrine pathways. According to the OECD Revised Guidance Document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption (OECD, 2018), vitellogenin induction in males and changes in gonadal staging such as increased proportion of early sperm stages in fish and reductions in sperm parameters in rodents, as seen with BPB, are diagnostic for the estrogen agonist and androgen antagonist mode of action.

Although many interactions are involved in the regulation of spermatogenesis, *in vitro* and *in vivo* data indeed point at least toward three main endocrine disruption pathways involved in BPB-induced gametogenesis disruption: (1) an increase of estrogenic action and/or to a lesser extent; (2) a decrease of androgenic action; and (3) an action via the hypothalamic-pituitary axis. Each of these three endocrine disruption pathways are highly inter-related.

Regarding an estrogenic MoA, a large body of *in vitro* data shows that both ER genomic and extra-genomic signalling pathways are activated by BPB. In fish, the increase in levels of VTG gene expression in the liver of male medaka (Yamaguchi et al., 2015) and male zebrafish (Yang et al. 2017), and the increase in ER-regulated cyp19a1b expression in the brain of male zebrafish (Yang et al. 2017) support the *in vitro* estrogenic activity of BPB.

Estrogens are key regulators of male physiology in vertebrates (Cooke et al., 2017) and an excess of estrogens or in 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). An increase in estradiol has been observed *in vivo* after exposure to BPB and concomitantly with alteration of spermatogenesis in male rodents (Ullah at al., 2018a; Ullah et al., 2019a) as well as in male zebrafish (Yang et al., 2017). Thus, **these data support that BPB acts via an estrogenic mode of action to alter spermatogenesis**.

Regarding an anti-androgenic MoA, effects on spermatogenesis have also been observed concomitantly with a decrease in testosterone concentrations in male rodents (Ullah at al., 2018a; Ullah et al., 2019a) as well as in male zebrafish (Yang et al., 2017). Changes in the GSI may provide additional information about the gonad maturation and spawning readiness (OECD, 2004). It describes changes in the relative weight of gonad to whole body mass and thus may be an indicator of the reproductive effort of organisms (Helfman et al., 1997). In the study by Yang et al., 2017, the gonado-somatic index of the zebrafish group exposed to 1 mg/L was significantly decreased in both male and female zebrafish. Moreover, histological analyses of the gonads, although not quantified, showed an alteration of the testis tubules and a decrease in the amount of mature spermatids after exposure to 0.1 and 1 mg/L. Reduction of GSI in male fish is regarded as a sensitive parameter in reproductive studies with estrogenic substances (OECD, 2004). In Yang et 2017, BPB exposure led to a higher expression of cyp19a in male gonads (encoding for Aromatase A) and in a higher expression of cyp19b in male brain (encoding for aromatase B). High levels of testosterone are required for spermatogenesis (review in Shiraishi and Matsuyama, 2017), thus, based on these data, **it can be hypothesised that BPB-induced spermatogenesis disruption is the consequence of a decrease of effective intra-testicular testosterone concentrations**. To validate this hypothesis, further investigations in rodents would be needed to know whether BPB acts on organs expressing aromatase (such as testis or the brain).

Regarding an action *via* the hypothalamic-pituitary axis, the *in vivo* data showed a decrease in LH- and FSH-related gene expression in brain and gonads of male zebrafish (Yang et al. 2017) and decreased plasma LH and FSH levels in rats (Ullah et al. 2018a and

2019a). LH and FSH are key regulators of spermatogenesis by acting on Sertoli (FSH) or Leydig (LH) cells (O'Donnell et al. 2017). **Thus, these results suggest that the alteration of spermatogenesis and testosterone levels might also result from an action *via* the hypothalamic-pituitary axis.**

Overall, the adverse effects on male reproductive system, the concomitant decrease in plasma testosterone levels and the increase in plasma estradiol levels and the unambiguous estrogenomimetic activity of BPB supported by the positive results in uterotrophic assays point to a highly probable causal link between BPB adverse effects on male reproduction and its estrogen agonist activity and possibly its androgen antagonist activity in rodents and fish.

Based on current understanding of endocrinology and physiology, the adverse effects observed in fish and in male rodents (mice and rat) exposed to BPB are biologically plausibly linked to its endocrine activity as an estrogen agonist. The possible activity of BPB via androgen antagonism and/or the hypothalamic-pituitary axis could also be linked to the observed adverse effects.

6.5.4 Environmental relevance

BPB causes severe adverse effects on fish, which are considered population relevant as they affect population stability and recruitment. This was observed through BPB effect *in vivo* on zebrafish where BPB 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 supportive evidence of malformation of testes and ovaries.

Data clearly provide evidence that a sub-chronic or chronic exposure to low doses of BPB alters the reproductive function in male adult rodents, with adverse effects on sperm count and quality. The effects have been observed in both mice and rats. It is plausible that these effects are also of relevance for other mammalian wildlife species. Indeed, the effects observed in experimental animals are relevant to other mammalian species on the basis of existing knowledge on male reproductive system development across species and the very high degree of conservation of hormonal regulation. There is a large degree of conservation of the primary amino acid sequences in proteins, which implies large commonalities between non-mammalian and mammalian vertebrate species in regard to hormones, enzymes and receptors involved in the EATS modalities (OECD 2018). Evidence of endocrine disruptive properties of BPB on mammalian vertebrate species therefore provides further support for similar properties in non-mammalian vertebrates, in particular with regard to disruption of estrogenic pathways.

6.5.5 Human relevance

Data demonstrates that BPB alters the reproductive function in the male adult rodent. The effects observed in experimental animals are relevant to human health on the basis of existing knowledge on male reproductive system development across species. Indeed, the main features of hormonal regulation of spermatogenesis are highly conserved in mammals.

Besides, there is no data available on BPB that contradict human relevance. Moreover, estrogen agonist and androgen antagonist activities of BPB have been reported in human cells and human receptors (see Table 11 in section 6.5.2 above) and this supports the human relevance of ED-mediated effects of BPB.

6.5.6 Comparison with BPA

The analogy of BPB and BPA effects and endocrine activities (especially estrogenic and anti-androgenic activities) bring supportive arguments for ED 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 as presented in Table 11 above (see column BPA/BPB for comparison of effects).

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 mode of action of BPA as an estrogen agonist/androgen antagonist in fish is supported by a number of *in vitro* studies, demonstrating that it is able to bind to and activate the estrogen receptor of mammals and fish and show competitive inhibition of androgenic activity at the AR in mammalian and fish cells. The SVHC dossier concluded that **BPA clearly acts as an estrogen agonist in fish**.

In medaka, VTG induction in males, changes in gonadal staging and testis ova were observed after BPA exposure. In zebrafish, VTG induction, testis-ova, sex ratio skewed towards females and reduced fertilisation success were observed. In Fathead minnow, the observed effects were VTG induction and reduced egg production. For six other fish species (*Cyprinus carpio, Gabiocypis rarus, Salmo trutta, Carassius auratus, Oncorhynchus mykiss*, *Poecillia reticulata, Xiphophorus helleri*) results show that BPA induced vitellogenin and effects on sperm quality up to disruption of spermatogenesis occurred. A similar effect was also evidenced with BPB by induction of vitellogenin gene expression in male fish (Yamaguchi et al. 2015, Yang et al. 2017). In females, the complete inhibition of ovulation (*S. trutta*) was observed with BPA. These effects are diagnostic for an estrogen agonist mode of action in fish according to OECD Revised Guidance Document 150 (OECD, 2018).

Additional endpoints that are potentially sensitive but not diagnostic with respect to an estrogen MoA in fish are growth, survival, behaviour, time to first spawn, fecundity, and fertilisation success and were observed in several fish species exposed to BPA. Zebrafish exposed to BPA had lower egg production, 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). BPA exposure also resulted in lower sperm volume and/or motility in adult zebrafish (Chen et al. 2017), brown trout (Lahnsteiner et al. 2005) and goldfish (Hatef et al. 2012a, b), and exposed japanese medaka had less spermatozoa (Metcalfe et al. 2001), supporting the likelihood of similar effects between both bisphenols in fish.

In relation to the extensive database available for BPA, additional elements are available for BPA that were not investigated with BPB.

Indicators for an estrogen-like activity of BPA were demonstrated in amphibians with induction of vitellogenin expression. Change in sex ratio and reproduction were also observed. There are also indications from various studies for possible endocrine related modes of action and related effects in molluscs (such as increase of oocytes and embryos, induction of superfemales, malformations of the genital tissues, embryotoxicity with malformed embryos and developmental disturbances).

Besides, BPA causes additional severe effects on reproduction- and development- related processes (including sexual development) in fish, that are clearly linked to its endocrine mode of action. According to OECD Revised Guidance Document 150 (OECD, 2018) a substance is almost certainly an ED causing endocrine mediated effects if the sex ratio is biased towards females and effects observed at other levels (*in vitro*, histology) fit to this observation which was observed for BPA. Indeed, BPA has been shown to cause a complete

sex reversal resulting in all-female phenotype populations.

In rodents, the estrogenic potential was confirmed for both BPA and BPB in a reliable *in vivo* mechanistic study (Yamasaki et al, 2002). In this rat immature uterotrophic assay, uterine blotted weight was significantly increased both by BPA and by BPB at 200 mg/kg and with a slightly higher magnitude of effect for BPB (+257% vs +197%).
In all four *in vivo* studies in rats by Ullah and colleagues, BPB treatment resulted in alteration of reproductive organs, including lower seminal vesicle and epididymis weights and deleterious effects on male sperm production observed using both testis histology and sperm analysis. With the same BPA treatment all these changes were similar or even slightly less pronounced (Ullah et al. 2018b, Ullah et al. 2018a, Ullah et al., 2019a, Ullah et al., 2019b) as presented in Table 7 and summarised in Table 10 above.
BPA has been identified as an ED for human health (ECHA, 2017a) but male reproductive toxicity was not included in the analysis as it was focused on the most investigated endpoints in relation to ED properties for BPA, i.e. female reproductive toxicity, alterations of mammary gland, of learning and of metabolism. However**, it has been concluded that disruption of the estrogenic pathways is the main mode of action consistently involved in these four effects.**

In addition, BPA has been classified as a reprotoxic chemical of category 1B based on reprotoxic effects in both males and females (ECHA, 2014). The classification opinion concluded that BPA induced negative effects on plasma testosterone levels, on organs of the reproductive tract and the sperm production and quality, although some divergences were noted regarding the effective BPA concentrations.

Therefore, both the estrogenic mode of action of BPA and its adverse effects on male reproductive toxicity have been previously recognised at the European level in rodents. It is also to be noted that the other ED effects of BPA (female reproductive toxicity, alterations of mammary gland, of learning and of metabolism) have been poorly or not investigated at all with BPB but the limited avalaible data are consistent with the effects identified with BPA (see 6.2.4 and 6.4.2.3 for metabolism). Regarding female reproduction, the only study available focusing on female fertility (Ijaz et al., 2019) had limitations (see also 6.4.2.2), but the effects observed on female reproductive toxicity are reinforced because they are coherent with the effects of BPA.By having a very similar structure and similar effects *in vitro* as well as in fish and rodents, particularly in males, data on BPA support the identification of both the effects and the ED mode of action of BPB. These effects were observed at similar or even lower doses as compared with BPA.

6.5.7 Conclusion

Adverse effects

Consistent adverse effects and endocrine activity are observed in rodents and fish exposed to BPB. The observed adverse effects in mammalian vertebrates are reduced sperm count and quality consistently observed in several reliable studies in two species (rats and mice). In fish, adverse effects include an altered hepato-somatic index and gonado-somatic index in male and female zebrafish. Qualitative observation of altered testis tubules and a decreased amount of mature spermatids in males also provide supportive evidence. BPB was demonstrated to significantly reduce fecundity of adult fish exposed for 21 days and to decrease embryo hatching and survival of the F1 generation in a reliable study (Yang et al., 2017). Supportive evidence is provided by the induction of malformations (no detailed information) in zebrafish in one study. **BPB therefore induces adverse effects on the male reproductive system in rodents and fish.**

Estrogenic activity

BPB exposure leads to higher estrogen and lower androgen levels in both *in vitro* and *in vivo* studies in rodents and fish. Additionally, *in vitro* data unambiguously show the estrogenic activity of BPB: competitively binding to ER of several vertebrate species, (e.g. human, bovine, rat, mouse and medaka in the µM range), activation of ER signalling pathway (e.g. ER transactivation in reporter cell lines, increased promoter occupancy and induction of ER-regulated gene expression) and physiological cell response (e.g. proliferation) with similar or higher potency than BPA. This estrogeno-mimetic activity of BPB is also supported by the results of immature rat uterotrophic assays with increases in watery uterine content and blotted uterine weight. This effect was similar to BPA, but with a slightly higher magnitude for BPB. In fish, the increase in levels of VTG gene expression in the liver of male medaka (Yamaguchi et al., 2015) and male zebrafish (Yang et al. 2017), and the increase in ER-regulated cyp19a1b expression in the brain of male zebrafish (Yang et al. 2017) also strongly support the estrogenic activity of BPB.

BPB was therefore shown to have clear estrogenic effects in rats and fish.

Other potential modes of action

BPB was shown to bind the AR and to induce an anti-androgenic response in most vertebrate cell lines including in human cells but this effect was not confirmed in the Hershberger assay. Therefore, **BPB possibly has anti-androgenic effects.**

The *in vivo* data also showed a decrease in LH- and FSH-related gene expression in brain and gonads of male zebrafish (Yang et al. 2017) and a decrease in plasma LH and FSH levels in rats (Ullah et al. 2018a and 2019a), suggesting an action of BPB via the hypothalamic-pituitary axis. It is however not known whether it may be a cause, a consequence or a specific mode of action in addition to estrogenic and possible anti-androgenic effects.

Oxidative stress was reported in several rodent studies and may also have an impact on the testis. It is however not known whether it may be a consequence or a specific mode of action in addition to estrogenic and possible anti-androgenic effects.

Plausibility of the link between effects and endocrine activity

BPB may have multiple modes of action that interact or superimpose and are difficult to distinguish from each others. The estrogenic effects of BPB is established in fish and rats and anti-androgenic effects are suggested. Estrogenic and anti-androgenic modes of action are known to be involved in the regulation of spermatogenesis and are closely inter-related. Considering the concomitant decrease in plasma testosterone levels and the increase in plasma estradiol levels, the link between these endocrine activities and the adverse effects on the male reproductive system in rodents and fish is highly plausible.

Relevance of effects and endocrine modes of action

In the present assessment, the *in* vivo available evidence on rodents shows that BPB can affect the male reproductive system. These observed adverse effects in mammalian vertebrates are considered relevant for effects on human health and on mammalian wildlife species in the environment and supportive for effects on non-mammalian vertebrate species (fish, amphibians) with respect to the underlying mode of action and adverse effects.

Supportive evidence from BPA

The link between the observed effects and these specific MoA is supported by the data on BPA, as BPB and BPA share very similar structures, adverse effects and modes of action. BPA has been identified already as SVHC due to its endocrine disrupting properties relevant for human health and the environment (ECHA, 2017a and ECHA, 2017b). It should be

noted that considering the extremely large database available for BPA, it was decided to focus the SVHC identification for BPA due to its endocrine properties for human health on the endpoints having the strongest plausible link at the time of the identification. Male reproduction was not included. However, the effects of BPA on male reproduction are acknowledged, in addition to female reproduction, in the justification to classify BPA as Repro 1B for reproduction (ECHA, 2014). In contrast, the endpoints included in the BPA SVHC identification for human health are largely not investigated for BPB. However, when data are available, they provide indications of a similar effect of BPB to BPA for female reproduction and metabolic effects. This support the consistency of effects between BPB and BPA.

These elements are summarised in Table 12 below.

Conclusion on ED properties

Overall, BPB has estrogen agonist properties and induces adverse effects on the male reproductive system in rodents and fish that are plausibly mediated by this endocrine activity.

Supportive evidence is provided by the consideration that BPB possibly has androgen-antagonist properties. This endocrine activity could also plausibly contribute to the adverse effects on the male reproductive system in rodents and fish could also be plausibly mediated by this endocrine activity.

The effects on rodents are relevant for human health and the effects in fish and rodents are relevant for the environment as an effect on the reproductive function can have consequences at a population level.

Therefore, there is scientific evidence that BPB fulfils the definition of an endocrine disruptor relevant for environment and human health.

Table 12 : Summary of evidence showing that BPB fulfils the definition of an endocrine disruptor

Adverse effects	Endocrine activity	Plausible link	Relevance
Evidence of an alteration of male reproduction • Impact on spermatogenesis and histology of testis (rodents (rat and mice) observed with BPB and BPA in the same protocol design) and fish) • Reduced egg number, hatching and	**Evidence of an estrogen agonist activity** • Demonstrated *in vitro* • Supported by findings including ED markers (VTG) and hormonal changes consistent with this MoA in experimental studies with fish and rodents • Supported by results in uterotrophic assays • Supported by analogy with BPA	Alteration of reproduction can be plausibly linked to the estrogen activity of BPB	**Environmental relevance: effects on populations and generations** • Impact on reproduction and survival of F1 generation • Supported by same MoA in rodents (several species: mice and rats are impacted) • Supported by large set of data on BPA on multiple species

survival of F1 generation (fish) • Supported by analogy with BPA	**Supportive evidence of a androgen antagonist activity** • Demonstrated *in vitro* • Supported by findings including ED markers and hormonal changes consistent with this MoA in experimental studies with fish and rodents	Alteration of reproduction can be plausibly linked to the possible anti-androgen activity of BPB	**Human relevance: effects on reproductive performance** • Hormonal regulation of spermatogenesis highly conserved in mammals • Estrogen agonist and androgen antagonist MoA in rodent (several species: mice and rats), in human cells and in human receptors.

7. Conclusions on the SVHC properties - Assessment under Article 57(f)

7.1 Summary of the data on the hazardous properties

As summarised in section 6.5.7[20]:

Adverse effects

Consistent adverse effects are observed in rodents and fish exposed to BPB. The observed adverse effects in mammalian vertebrates are reduced sperm count and quality consistently observed in several reliable studies in two species (rats and mice). In fish, adverse effects include an altered hepato-somatic index and gonado-somatic index in male and female zebrafish. Qualitative observation of altered testis tubules and a decreased amount of mature spermatids in males also provide supportive evidence. BPB was demonstrated to significantly reduce fecundity of adult fish exposed for 21 days and to decrease embryo hatching and survival of the F1 generation in a reliable study (Yang et al., 2017). Supportive evidence is provided by the induction of malformations (no detailed information) in zebrafish in one study. **BPB therefore induces adverse effects on the male reproductive system in rodents and fish.**

Estrogenic activity

BPB exposure leads to higher estrogen and lower androgen levels in both *in vitro* and *in vivo* studies in rodents and fish. Additionally, *in vitro* data unambiguously show the estrogenic activity of BPB: competitively binding to ER of several vertebrate species (e.g. human, bovine, rat, mouse and medaka in the µM range), activation of the ER signalling pathway (e.g. ER transactivation in reporter cell lines, increased promoter occupancy and induction of ER-regulated gene expression) and physiological cell response (e.g. proliferation) with similar or higher potency than BPA. This estrogeno-mimetic activity of BPB is also supported by the results of immature rat uterotrophic assays with increases in watery uterine content and blotted uterine weight. This effect was similar to BPA, but with a slightly higher magnitude for BPB. In fish, the increase in levels of VTG gene expression in the liver of male medaka (Yamaguchi et al., 2015) and male zebrafish (Yang et al. 2017), and the increase in ER-regulated cyp19a1b expression in the brain of male zebrafish (Yang et al. 2017) also strongly support the estrogenic activity of BPB.

BPB was therefore shown to have clear estrogenic effects in rats and fish.

Other potential modes of action

BPB was shown to bind the AR and to induce an anti-androgenic response in most vertebrate cell lines including in human cells but this effect was not confirmed in the Hershberger assay. Therefore, **BPB possibly has anti-androgenic effects.**

The *in vivo* data also showed a decrease in LH- and FSH-related gene expression in brain and gonads of male zebrafish (Yang et al. 2017) and a decrease in plasma LH and FSH levels in rats (Ullah et al. 2018a and 2019a), suggesting an action of BPB *via* the hypothalamic-pituitary axis. It is however not known whether it may be a cause, a consequence or a specific mode of action in addition to estrogenic and possible anti-androgenic effects.

Oxidative stress was reported in several rodent studies and may also have an impact on the testis. It is however not known whether it may be a consequence or a specific mode

[20] shaded text indicates copy and paste of corresponding section(s)

of action in addition to estrogenic and possible anti-androgenic effects.

Plausibility of the link between effects and endocrine activity

BPB may have multiple modes of action that interact or superimpose and are difficult to distinguish from each others. The estrogenic effects of BPB is established in fish and rats and anti-androgenic effects are suggested. Estrogenic and anti-androgenic modes of action are known to be involved in the regulation of spermatogenesis and are closely inter-related. Considering the concomitant decrease in plasma testosterone levels and the increase in plasma estradiol levels, the link between endocrine activities and the adverse effects on the male reproductive system in rodents and fish is highly plausible.

Relevance of effects and endocrine modes of action

In the present assessment, the *in* vivo available evidence on rodents shows that BPB can affect the male reproductive system. These observed adverse effects in mammalian vertebrates are considered relevant for effects on human health and on mammalian wildlife species in the environment and supportive for non-mammalian vertebrate species (fish, amphibians) with respect to the underlying mode of action and adverse effects.

Supportive evidence from BPA

The link between the observed effects and these specific endocrine activities is supported by the data on BPA, as BPB and BPA share very similar structures, adverse effects and modes of action. BPA has been identified already as SVHC due to its endocrine disrupting properties relevant for human health and the environment (ECHA, 2017a and ECHA, 2017b). It should be noted that considering the extremely large database available for BPA, it was decided to focus the SVHC identification for BPA due to its endocrine properties for human health on the endpoints having the strongest plausible link at the time of the identification. Male reproduction was not included. However, the effects of BPA on male reproduction are acknowledged, in addition to female reproduction, in the justification to classify BPA as Repro 1B for reproduction (ECHA, 2014). In contrast, the endpoints included in the BPA SVHC identification for human health are largely not investigated for BPB. However, when data are available, they provide indications of a similar effect of BPB to BPA for female reproduction and metabolic effects. This support the consistency of effects between BPB and BPA.

Conclusion on ED properties

Overall, BPB has estrogen agonist properties and induces adverse effects on the male reproductive system in rodents and fish that are plausibly mediated by this endocrine activity.

Supportive evidence is provided by the consideration that BPB possibly has androgen-antagonist properties. This endocrine activity could also plausibly contribute to the adverse effects on the male reproductive system in rodents and fish.

The effects on rodents are relevant for human health and the effects in rodents and fish are relevant for the environment as an effect on the reproductive function can have consequences at a population level.

Therefore, there is scientific evidence that BPB fulfils the definition of an endocrine disruptor relevant for environment and human health.

7.2 Equivalent level of concern assessment

In agreement with the REACH legal text, substances identified as SVHCs under article 57(f) shall give rise to an equivalent level of concern to those of other substances listed in points (a) to (e), on a case-by-case basis. BPB is an endocrine disruptor relevant for environment and human health as summarised above.

BPB presents similar ED properties as its structural analog Bisphenol A. The ED properties of Bisphenol A have been recognised as of equivalent level of concern to article 57 (a) to (e) in its identification as an SVHC due to its endocrine properties for human health in June 2017 and for the environment in December 2017.

A number of factors relevant to assess that an adverse health effect represents an equivalent level of concern (ELoC) is identified in a discussion paper by ECHA (2012) with a specific focus on sensitisers. The factors identified in this document to evaluate the ELoC are considered relevant for the present case and are listed below:

- Characteristics of the effects:
  - Type of possible effects
  - Irreversibility of effects
  - Delay of effects
- Other factors:
  - Quality of life affected (for human health effects)
  - Societal concern
  - Is derivation of 'safe concentration' possible?

These elements are discussed in the present analysis in a larger context that covers both environment and human health.

- **BPB causes severe effects**

For the environment, reported adverse effects in fish related to the estrogen agonist mode of action include alteration of sperm and altered testis tubules and oocyte development, decrease in egg number, hatching rate and survival indicating a decrease in fecundity. These effects of BPB are similar to what is observed for BPA and with the same mode of action. The long-term effects of these alterations in normal functioning are difficult to predict but may lead to serious fecundity problems. The severity of these effects and significance for the population level have already been demonstrated for BPA and different alkylphenols (i.e. 4-tert-butylphenol, 4-pentylphenol, 4-heptylphenol, 4-tert-octylphenol, 4-nonylphenol) with an estrogenic mode of action and similar apical effects. Further data are available for BPA and provide information on the adverse effects on reproduction and development-related processes. BPA alters sex ratio leading to a feminisation of male fish in different species, reaching even a complete sex reversal at high concentration (>1.3 mg/L) definitely impairing population development in the long-term.

Apical effects of BPB are associated with developmental and reproductive disturbances, malformations, or embryo-toxic effects in fish at the organism level. These effects can severely affect population stability and recruitment and they are considered as serious effects for the environment.

The spectrum of effects on the male reproductive system observed in rats and mice include decreased sperm count in the testis and alteration of spermatogenesis (see Table 10). The available data do not include a measurement of fertility in rodents. However, the fact that no sperm were observed in the seminiferous tubules of rats treated with high doses of BPB informs on the probable effect on fertility (Ullah et al. 2018a). In addition, these effects were similar to what was observed for BPA, which was included in the experimental protocol covering the same range of concentration (order of magnitude of µg/kg/day and

even higher) and are likely to result from the same mode of action. The significance for human health of effects on male reproductive function in terms of severity has already been demonstrated for BPA. 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 (ECHA, 2014). The CLP opinion 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 noted regarding the effective BPA concentrations.

BPB exposure impairs plasma testosterone levels, organs of the reproductive tract, and sperm production and quality in rodents. These effects relevant for human health are considered severe as similar effects in humans could cause sub- and infertility. Moreover, BPA, a very close structural analog of BPB, is classified as a reprotoxic chemical of category 1B based on reprotoxic effects in both males and females (ECHA, 2014) based on the same effects as those described with BPB in all the endpoints studied.

- **BPB causes irreversible and delayed effects that may have consequences in the long term**

The reduced sperm count and quality observed in rodent studies are irreversible and are shown to occur later in life after exposure to BPB during gestation (Ullah et al., 2019a). In this study, BPB alters male reproductive function in adult rats after fetal exposure only. In addition, these effects were similar to what was observed for BPA. There is therefore a long latency period between early exposure and occurrence of the adverse effects. Impacts during early development, which adversely affect reproductive ability, such as reduced sperm and, spermatogonia, spermatocyte and spermatid production, as well as testicular changes, will not manifest themselves fully until reproductive age. Due to its MoA, a short time exposure may be sufficient to provoke long-term effects even if exposure ceases. This is in particular the case when exposure occurs during critical time windows, life stages during development or during specific seasons. This is in line with our knowledge about the endocrine system. Endocrine modulation is a very complex feedback process that is set up during critical life stages. As summarised in WHO/IPCS (2002) disturbance of this set up may result in effects during the entire life-time. The disturbances of a transient exposure during sensitive life stages are irreversible and result in effects during the entire life and even in the next generation with long-term consequences at the (sub)-population level.

Effects on the following generations or population development are usually not covered with standard test protocols and data availability for BPB is scarce. In fish species, BPA exposure, even during a short period of time, resulted in reproductive abnormalities (reduced fertilisation rates, hatching success and survival of embryos) in the future generation without any further exposure to BPA. Moreover, different studies showed that the sensitivity of fish increases from one generation to the other when continuously exposed to BPA, reflected by the sensitivity of future generations for reproductive endpoints such as egg production, VTG induction, fertilisation rate and embryo survival. Strong similarities between BPB and BPA structures, sameness in MoA and in estrogenic agonist effects allow to estimate that BPB can produce the same adverse effects in the long term even after short term exposure by impairing both the actual generation of organisms living in the environment and their descendants.

- **BPB probably affects a large variety of species in different ecosystems**

BPB may adversely affect a high variety of different ecologically important species in different ecosystems. As the substance is not registered in Europe, data are still scarce. However, effects are demonstrated in fish species for the aquatic compartment and in rodent for terrestrial organisms. Its structural analog BPA that shares a similar MoA was

demonstrated to impact a high variety of different ecologically important species in different ecosystems (nine fish species, six amphibian species, as well as a high number of invertebrate species) in the SVHC support document for BPA (ECHA, 2017b). BPB can therefore presumably affect a high number of organisms. As data on only a small proportion of the existing species are available, potential effects on further organisms remain unknown and there is a potential that a number of further species may be sensitive to exposure to BPB. Adverse effects are thus not expected to be restricted to certain taxonomic groups or species, in agreement with the large conservation of the main endocrine systems among vertebrate species in various environments.

Moreover, BPB has an ubiquitous occurrence and may enter the environment via emissions from various sources, which is supported by occurrence and monitoring studies. Occurrence data reports increasing concentrations over recent years indicating that BPB is used more and more as an alternative to BPA due to the similarities in structure. Besides, BPB is not restricted to certain environmental compartments, local sites or specific time points.

- **Concern related to co-exposure and combined effects**

Moreover, BPB can act jointly with other chemicals occurring in the environment having the same bisphenol structure and displaying the same effect. BPB is part of the group of bisphenols, some of which share common MoA and may have additive effects. Common MoA and effects are demonstrated for BPA but some evidence also exists for other bisphenols i.e. bisphenol S, bisphenol F (Rosenmai et al., 2014, Rochester & Bolden, 2015; Le Fol et al., 2017; Pelch et al., 2019; Faheem & Bhandari 2021). Besides, environmental occurrence and human biomonitoring data (see section 3.2) show that BPB is detected in the environment, in environmental species as well as in human fluids together with BPA and other bisphenols. Typical examples are sewage plant effluents where BPB occurs jointly with other bisphenols.

- **Quality of life (element relevant for human health)**

Sub- and infertility is not only detrimental for species survival, but also has a major impact on quality of life. A reduced ability to reproduce considerably affects the quality of life for the individuals affected as well as for their partners and families. Overall, the strong similarity between BPB and BPA structures, sameness in MoA and estrogenic agonist activities and impacts on male reproductive function, and the recognised ED properties of BPA, allow to estimate that BPB will produce similar adverse effects on male reproductive function and will have a similar adverse impact on quality of life.

- **Societal concern**

Reduced fertility in humans is of general concern in the EU countries. Infertility rates have remained stable (Kortenkamp et al., 2012) in recent decades ranging from 1.7 to 3.5% in developed countries (Boivin et al., 2007). However, the demand for assisted reproductive technologies (ART) treatment in Europe – as expressed in treatment cycles performed in European countries – has increased by 59% in the five years from 1997 to 2002 (HEAL 2014). Futhermore, it is now generally admitted that, despite geographic variations in semen quality, a global decrease in sperm count has occurred over the past five decades (Le Moal et al 2014). Analysis of ejaculates from more than 26,000 men representative of the general population showed that sperm concentration in France has been declining by 1.9% per year from 1996 to 2005 (Rolland et al., 2013). A potential role of EDCs is generally considered as plausible (Marques-Pinto et al., 2013). A reduced ability to reproduce negatively affects society as it contributes to an increased financial burden e.g. on the health care sector, providing counselling, clinical treatment and assisted fertilisation treatments. In humans, fertility treatment and counselling carries high societal costs. Any substance that has the capacity to contribute to these effects raises a concern.

In relation to the environment, the impairment of fertility can be an issue regarding species survival. There is an increasing concern related to the preservation of biodiversity and increasing evidence that it is threatened due to various causes including global warming and excessive pressure due to human activities (Jenssen, 2006). EDCs may also contribute to the challenge of survival of endangered species (Tubbs and McDonough, 2018). It was well demonstrated that BPA impaired fertility of various species potentially threatening biodiversity (ECHA, 2017b). As a very close structural analog expressing the same MoA and adverse effects, BPB could potentially be of equivalent concern. Preservation of biodiversity is a part of a number of governmental and non-governmental initiatives. Preserving and restoring ecosystems and biodiversity is one of the key aims of the European Green Deal (EC, 2019) that is an integral part of the European Commission's strategy to implement the United Nation's 2030 Agenda and the sustainable development goals[21].

- **Is derivation of a 'safe concentration' possible?**

With regard to alteration of the male reproductive function when considering oral rodent studies, adverse health effects such as reduced sperm count and quality are observed (see section 6.4.2.1) at relatively high doses in some studies (50 mg/kg bw/day by gavage in Ullah et al 2018b and Ullah et al., 2019b) and at relatively low doses in other studies (1.5 or 3 µg/kg bw/day via drinking water in Ullah et al., 2018a and in Ullah et al., 2019a). All the papers presently available consistently report a decrease in plasma testosterone level and an increase in plasma oestradiol level in response to BPB (or BPA) exposure. It is known that each of these endocrine changes provoke alterations in spermatogenesis, also observed in several studies. It remains unknown whether the multiple differences between the experimental procedures used in the different studies (species, age at exposure to BPB, method and route of administration of BPB, duration of the exposure to BPB) can contribute to the dose-response uncertainty. In particular, a role of the mode of administration (gavage vs drinking water) may partly explain the differences but lack of toxicokinetic data and the magnitude of the difference raise uncertainty in the dose-response. Importantly, BPA provokes the same effects as BPB in all the endpoints studied and the same apparent uncertainty in the dose response was also observed. Uncertainty in the dose response was also acknowledged by the Risk Assessment Committee (RAC) in its restriction opinion on bisphenol A (ECHA, 2015). Therefore it is difficult to establish a concentration which could be regarded as safe for human health.

In addition, the effects of BPA have been investigated in a much larger number of studies and additional ED-related adverse effects established (ECHA 2017a): in relation to its capacity to disrupt estrogenic pathways, BPA has been recognised to alter female reproductive function, development of the mammary gland, memory and learning as well as metabolism. Despite the large database available for BPA, our knowledge of the ED-related effects may still not be complete (ECHA, 2017a). In particular, there is some emerging evidence of an effect of BPA on the immune function that has been recently investigated (Menard et al., 2014a and 2014b). The database of BPB is scarce compared to BPA, but shows strong similarity with the MoA and effects of BPA. Considering the wide range of functions influenced by hormones, it is also highly challenging to fully characterise the effects related to ED properties of BPB. The scope of the effects of BPB may therefore be underestimated with consequences on the knowledge of levels that can be considered as safe.

Regarding the endocrine properties of BPB in the environment, on the basis of the available data it appears difficult to derive a safe level in the environment, although it might exist. One reason is that it is difficult to determine definite low effect concentrations as effects may only be observed in certain life stages or time windows. Moreover, as observed for

[21] https://sustainabledevelopment.un.org/post2015/transformingourworld

BPA, there might be seasonal effects leading to difficulty predicting its impact on the development of different groups of organisms or individuals from the same group.

Besides, concentration response-relationships are often not monotonic. For BPB, this could be observed in fish where hepatic estrogen-responsive gene regulation was upregulated at low and high concentration of BPB, but not in between (Yamagushi et al., 2015). The same type of effects can be observed on sperm quality in fish exposed to BPA. In addition, with exposure of invertebrates to BPA, egg production was sometimes stimulated at lower but reduced at higher concentrations. An explanation for these contradicting effects is that the hormonal receptors are sensitive to certain trigger concentrations, or that different modes of action are triggered. It was shown that BPB, similarly to BPA, elicits effects via estrogenic agonism but other MoA are concomitant such as anti-androgenicity for BPB and thyroid effect and anti-ecdysteroid action in arthropods for BPA (not investigated for BPB). These multiple effects on various receptors and endpoints explain why a great variety of organisms may possibly be affected.

Moreover, it has been demonstrated for BPA that certain species (e.g. fish, amphibians and snails) are sensitive at low concentrations (even below or around 1 µg/L), levels which are indeed measured in the environment. Thus, as endangered species such as amphibian species are affected by BPA it may be the case for BPB. A limitation to take into account is that literature always provide data on species used in standard tests and commonly found in specific environments. But it was demonstrated for a large set of substances, that non-standard test species and non-traditional endpoints may be much more sensitive than endpoints usually considered in OECD standard test protocols (ECHA, 2017b). A great variety of taxonomic groups essential for the well-functioning of ecosystems were shown to be affected by BPA, making it probable that BPB will also affect the environment and other species living in it.

Although the endocrine system with its hormones and functioning is conservative among vertebrate species, the specific hormones affected, binding affinities and modes of action differ between taxa. Owing to the lack of in-depth knowledge of their endocrine system and the lack of test systems it is difficult to estimate which species are most sensitive and therefore difficult to establish a concentration which could be regarded as safe for the entire environment.

- **Avoid regrettable substitution as soon as possible**

The substance is not yet registered under REACH. However, it has been shown that BPB is used outside the EU and products containing it can be imported into the EU. Additionally, industry might invest in BPB as a substite for BPA. In this context, regulating BPB for its endocrine properties in the same way as BPA is necessary to avoid regrettable substitution and to protect human health and wildlife.

Conclusion on the ELoC

The effects of BPB due to its endocrine disrupting properties are considered to be of equivalent level of concern to substances listed in Article 57 points (a) to (e). The concern is substantiated by the severity and irreversibility of the effects on organisms and populations that may have long term consequences, the large variety of species that may be adversely affected and the difficulties to quantify a safe level of exposure with regard to the endocrine mediated effects. An equivalent level of concern is also supported by the potential for combined exposure with other bisphenols that share similar modes of action. The assessment shares similar lines of argumentation as for previous SVHC identifications of BPA for its ED properties, for which a considerable amount of data is available. Due to the very close structural similarity between BPB and

BPA, commonalities of effects and of modes of action, the main arguments justifying the equivalent level of concern of BPA are also relevant to BPB.

In conclusion, there is scientific evidence that BPB causes probable serious effects to the environment and human health due to its endocrine disrupting properties which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of the REACH Regulation.

7.3 Conclusion on the hazard properties and equivalent level of concern assessment

As summarised in section 6.5.7 and in the conclusion of section 7.2[22]:

Adverse effects

Consistent adverse effects are observed in rodents and fish exposed to BPB. The observed adverse effects in mammalian vertebrates are reduced sperm count and quality consistently observed in several reliable studies in two species (rats and mice). In fish, adverse effects include an altered hepato-somatic index and gonado-somatic index in male and female zebrafish. Qualitative observation of altered testis tubules and a decreased amount of mature spermatids in males also provide supportive evidence. BPB was demonstrated to significantly reduce fecundity of adult fish exposed for 21 days and to decrease embryo hatching and survival of F1 generation in a reliable study (Yang et al., 2017). Supportive evidence is provided by the induction of malformations (no detailed information) in zebrafish in one study. **BPB therefore induces adverse effects on the male reproductive system in rodents and fish.**

Estrogenic activity

BPB exposure leads to higher estrogen and lower androgen levels in both *in vitro* and *in vivo* studies in rodents and fish. Additionally, *in vitro* data unambiguously show the estrogenic activity of BPB: competitively binding to ER of several vertebrate species (e.g. human, bovine, rat, mouse and medaka in the µM range), activation of ER signalling pathway (e.g. ER transactivation in reporter cell lines, increased promoter occupancy and induction of ER-regulated gene expression) and physiological cell response (e.g. proliferation) with similar or higher potency than BPA. This estrogenomimetic activity of BPB is also supported by the results of immature rat uterotrophic assays with increases in watery uterine content and blotted uterine weight. This effect was similar to BPA, but with a slightly higher magnitude for BPB. In fish, the increase in levels of VTG gene expression in the liver of male medaka (Yamaguchi et al., 2015) and male zebrafish (Yang et al. 2017), and the increase in ER-regulated cyp19a1b expression in the brain of male zebrafish (Yang et al. 2017) also strongly support the estrogenic activity of BPB.

BPB was therefore shown to have clear estrogenic effects in rats and fish.

Other potential modes of action

BPB was shown to bind the AR and to induce an anti-androgenic response in most vertebrate cell lines including in human cells but this effect was not confirmed in the Hershberger assay. **Therefore, BPB possibly has anti-androgenic effects.**

The *in vivo* data also showed a decrease in LH- and FSH-related gene expression in brain and gonads of male zebrafish (Yang et al. 2017) and a decrease in plasma LH and FSH levels in rats (Ullah et al. 2018a and 2019a), suggesting an action of BPB *via* the hypothalamic-pituitary axis. It is however not known whether it may be a cause, a

[22] shaded text indicates copy and paste of corresponding section(s)

consequence or a specific mode of action in addition to estrogenic and possible anti-androgenic effects.

Oxidative stress was reported in several rodent studies and may also have an impact on the testis. It is however not known whether it may be a consequence or a specific mode of action in addition to estrogenic and possible anti-androgenic effects.

Plausibility of the link between effects and endocrine activity

BPB may have multiple modes of action that interact or superimpose and are difficult to distinguish from each others. The estrogenic effects of BPB is established in fish and rats and anti-androgenic effects are suggested. Estrogenic and anti-androgenic modes of action are known to be involved in the regulation of spermatogenesis and are closely inter-related. Considering the concomitant decrease in plasma testosterone levels and the increase in plasma estradiol levels, the link between these endocrine activities and the adverse effects on the male reproductive system in rodents and fish is highly plausible.

Relevance of effects and endocrine modes of action

In the present assessment, the *in* vivo available evidence on rodents shows that BPB can affect the male reproductive system. These observed adverse effects in mammalian vertebrates are considered relevant for effects on human health and on mammalian wildlife species in the environment (such as mice, rats) and supportive for non-mammalian vertebrate species (fish, amphibians) with respect to the underlying mode of action and adverse effects.

Supportive evidence from BPA

The link between the observed effects and these specific endocrine activities is supported by the data on BPA, as BPB and BPA share very similar structures, adverse effects and modes of action. BPA has been identified already as SVHC for HH and ENV due to its endocrine disrupting properties relevant for human health and the environment (ECHA, 2017a and ECHA, 2017b). It should be noted that considering the extremely large database available for BPA, it was decided to focus the SVHC identification for BPA due to its endocrine properties for human health on the endpoints having the strongest plausible link at the time of the identification. Male reproduction was not included. However, the effects of BPA on male reproduction are acknowledged, in addition to female reproduction, in the justification to classify BPA as Repro 1B for reproduction (ECHA, 2014). In contrast, the endpoints included in the BPA SVHC identification for human health are largely not investigated for BPB. However, when data are available, they provide indications of a similar effect of BPB to BPA for female reproduction and metabolic effects. This support the consistency of effects between BPB and BPA.

Conclusion on ED properties

Overall, BPB has estrogen agonist properties and induces adverse effects on the male reproductive system in rodents and fish that are plausibly mediated by this endocrine activity.

Supportive evidence is provided by the consideration that BPB has possible androgen-antagonist properties. This endocrine activity could also plausibly contribute to the adverse effects on the male reproductive system in rodents and fish.

The effects on rodents are relevant for human health and the effects in fish and

rodents are relevant for the environment as an effect on the reproductive function can have consequences at a population level.

Therefore, there is scientific evidence that BPB fulfils the definition of an endocrine disruptor relevant for environment and human health.

The effects of BPB due to its endocrine disrupting properties are considered to be of equivalent level of concern to substances listed in Article 57 points (a) to (e). The concern is substantiated by the severity and irreversibility of the effects on organisms and populations that may have long-term consequences, the large variety of species that may be adversely affected and the difficulties to quantify a safe level of exposure with regard to the endocrine mediated effects. An equivalent level of concern is also supported by the potential for combined exposure with other bisphenols that share similar modes of action. The assessment shares similar lines of argumentation as for previous SVHC identifications of BPA for its ED properties, for which a considerable amount of data is available. Due to the very close structural similarity between BPB and BPA, commonalities of effects and of modes of action, the main arguments justifying the equivalent level of concern of BPA are also relevant to BPB.

In conclusion, there is scientific evidence that BPB causes probable serious effects to the environment and human health due to its endocrine disrupting properties which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of the REACH Regulation.

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