Article 77(3)(c) Request
Supporting Information Report

EVALUATION OF ENDOCRINE DISRUPTING PROPERTIES FOR THE ENVIRONMENT

Substance Name:	4,4'-methylenediphenol ("Bisphenol F")
EC Number:	210-658-2
CAS Number:	620-92-8
Submitted by:	Germany
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TABLES

ABBREVIATIONS

AhR	Aryl-hydrocarbon receptor
AR	Androgen receptor
ARN	Assessment of Regulatory Needs
BPA	Bisphenol A (EC 201-245-8, CAS 80-05-7)
BPAF	Bisphenol AF (EC 216-036-7, CAS 1478-61-1)
BPB	Bisphenol B (EC 201-025-1, CAS 77-40-7)
BPF	Bisphenol F (EC 210-658-2, CAS 620-92-8)
BPS	Bisphenol S (EC 201-250-5, CAS 80-09-1)
DHT	Eihydrotestosterone
dpf	days post-fertilisation
E2	Estradiol
EAG	Expert Advisory Group
EC	Effective concentration
ECHA	European Chemicals Agency
ED	Endocrine Disruptor
EDC	Endocrine Disrupting Compound
EFSA	European Food Safety Authority
ER	Estrogen receptor
hpf	hours post-fertilisation
GSI	Gonadosomatic index
HSI	Hepatosomatic index
HPT	Hypothalamic-pituitary-thyroid
IC	Inhibitory concentration
JRC	Joint Research Center
LC	Lethal concentration
LC-MS	Liquid chromatography/mass spectroscopy
LDH	Lactate dehydrogenase
LOEC	Lowest Observed Effect Concentration
MoA	Mode of action
MSC	Member State Committee
(MS)CA	(Member State) Competent Authority
NF	Nieuwkoop and Faber (developmental staging for xenopus laevis)
NO(A)EL	no observed (adverse) effect level
OECD	Organisation for Economic Co-operation and Development
(q)PCR	(quantitative) Polymerase chain reaction
RAC	Committee for Risk Assessment
RMOA	Risk Management Option Analysis
SEv	Substance Evaluation
SVHC	Substance of very high concern
T3	Triiodothyronine
T4	Thyroxine
TH	Thyroid hormone
TR	Thyroid hormone receptor
TTR	Transthyretin
VTG	Vitellogenin
WHO	World Health Organisation
WoE	Weight of Evidence

Background

The German Competent Authority (DE CA) has indicated through an entry in the Registry of Intentions that they plan to submit a proposal to restrict 4,4'-isopropylidenediphenol (EC 201-245-8, CAS 80-05-7, Bisphenol A) and bisphenols exhibiting a similar concern for the environment from uses which lead to emissions to the environment.
Following submission of the restriction dossier and a positive conformity check, the hazards and risks of the proposal would be evaluated by ECHA's Risk Assessment Committee (RAC).

In addition to bisphenols with confirmed endocrine disrupting properties for the environment, the restriction proposal is planned to also include the following two bisphenols for which no EU-wide consensus has so far been reached on their endocrine disrupting properties for the environment:

- 4,4'-methylenediphenol (EC 210-658-2, CAS 620-92-8, "Bisphenol F")
- 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol (EC 216-036-7, CAS 1478-61-1, "Bisphenol AF") and its salts

Generally, EU-wide opinion forming on whether a substance has endocrine disrupting properties for the environment is achieved through its identification as a Substance of Very High Concern (SVHC), which involves opinion making by the ECHA Member State Committee (MSC). MSC has extensive previous experience of such evaluations. To avoid a situation where RAC is required to conclude on whether a substance fulfils the criteria for endocrine disruption for the environment, rather than the MSC, it is anticipated that ECHA will facilitate an ad-hoc process under Article 77(3)(c) of REACH to enable the MSC to give an opinion on the endocrine disrupting properties of the substances identified above, which can then be used by RAC as the basis of their evaluation. This assessment report is submitted to ECHA by the DE CA to be used as the basis for the opinion-making of the MSC.

RESULTS OF EVALUATION OF ENDOCRINE DISRUPTING PROPERTIES FOR THE ENVIRONMENT

Substance name(s):	**4,4'-methylenediphenol ("BPF")**
EC number(s):	210-658-2
CAS number(s):	620-92-8

The substance acts as an endocrine disruptor (ED) in the environment according to the ED criteria of the World Health Organisation (WHO).

Summary of how the substance meets the criteria for an environmental ED:

4,4'-methylenediphenol (Bisphenol F, BPF) acts as an ED in the environment based on available information from *in vitro* and *in vivo* data in fish:

Adverse effects relevant for ED identification

The available data for BPF show clear and consistent adverse and population relevant effects on reproduction and sexual development in two zebrafish studies. The observed effects fit to an estrogenic and/or anti-androgenic mode of action and no indications of further non-ED mediated pathways were found in the two studies.

Endocrine activity

The available *in vitro* as well as *in vivo* mechanistic data clearly and consistently demonstrate an estrogenic and anti-androgenic activity of BPF.

Additionally, two *in vitro* studies also show an interference with testosterone production in cellular assays.

Plausible link between adverse effects and endocrine activity

BPF may have multiple modes of endocrine action (estrogenic, anti-androgenic, thyroidal activity and interference with steroidogenesis) that might interact and are difficult to distinguish from each other. However, the estrogenic and/or anti-androgenic effects of BPF in fish are consistently observed in the available studies showing significant effects on egg production, hatching and survival of F1 larvae, sex ratio and gonadal development. Estrogenic and anti-androgenic modes of action are well known to be involved in the regulation of sexual development and reproduction. Considering the observed concomitant decrease in plasma testosterone levels and the increase in plasma estradiol levels as well as the increase in vitellogenin (VTG) levels and gene expression in male fish, the link between these endocrine activities and the observed adverse effects on fish is highly plausible.

Other supporting evidence

In vitro and in vivo data from fish and amphibians point to an interference of BPF with the hypothalamic-pituitary-thyroid (HPT) axis.

Additionally, the available human health data support the conclusion for an estrogenic and/or antiandrogenic activity of BPF, even though the reliability of these studies have not

been assessed here. Studies on rats show consistently a decrease in serum testosterone levels and a decrease in sperm motility in the offspring of treated female rats. An increase in uterus weight was observed in juvenile rats.

The link between the observed effects and the specific estrogenic and/or anti-androgenic activity of BPF is further supported by the analogy of BPF to BPA and BPA. The data available for BPA and BPB, both of which share very similar chemicals structures compared to BPF, show well defined adverse effects and modes of action that fit to an estrogenic mode of action in fish. Based on these data BPA and BPB have been identified already as SVHC due to its endocrine disrupting properties in the environment.

Conclusion on ED properties

Overall, BPF has estrogen agonistic properties and induces adverse effects in fish that are plausibly mediated by this endocrine activity.

Furthermore, *in vivo* and *in vitro* evidence shows that BPF has androgen antagonistic properties. This endocrine activity could also plausibly contribute to the observed adverse effects on reproduction and sexual development in fish.

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

Therefore, there is scientific evidence to conclude that BPF fulfils the definition of an endocrine disruptor in the environment.

Registration dossiers submitted for the substance: No

1. Identity of the substance and physical and chemical properties

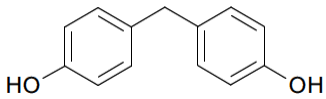
1.1. Name and other identifiers of the substance

Table 1: Substance identity

EC number:	210-658-2
EC name:	4,4'-methylenediphenol
SMILES:	Oc2ccc(Cc1ccc(O)cc1)cc2
CAS number:	620-92-8
IUPAC name:	4,4'-Dihydroxydiphenylmethane
Index number in Annex VI of the CLP Regulation	Not available
Molecular formula:	$C_{13}H_{12}O_2$
Molecular weight range:	200.20 g/mol
Synonyms:	Bisphenol F BPF

Substance type: mono-constituent

Structural formula:



1.2. Identity and composition of structurally related substances

In lieu of disseminated data from a registration dossier on BPF itself, the following data is reported from the ECHA dissemination database on EC 908-921-9, the multi-constituent substance comprising BPF and its two stereoisomers, section "Physical & chemical properties".[1]

[1] Via https://echa.europa.eu/de/registration-dossier/-/registered-dossier/26309 accessed 25 April 2022

Table 2: Structurally related substance(s) identity

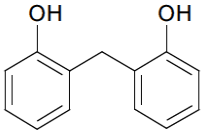
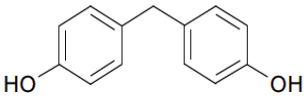
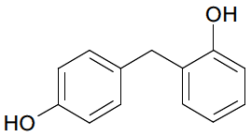
EC number:	908-912-9
EC name:	2-[(2-hydroxyphenyl)methyl]phenol; 2-[(4-hydroxyphenyl)methyl]phenol; 4-[(4-hydroxyphenyl)methyl]phenol
SMILES:	See below
CAS number (in the EC inventory):	1333-16-0
CAS number:	1333-16-0
IUPAC name:	Reaction mass of 2,2'-methylenediphenol and 4,4'-methylenediphenol and o-[(4-hydroxyphenyl)methyl]phenol
Index number in Annex VI of the CLP Regulation	N/A
Molecular formula:	$C_{13}H_{12}O_2$
Molecular weight range:	200.20 g/mol
Synonyms (trade names):	GX-460 KD-9005 KD-9007 KD-9009 KDF-214M KDF-438 KDN-253 SP-2000 SP-2000P YDPN-638A80 YDPN-638N

Substance type: multi-constituent

Structurally related substance(s) formula:

The registered substance is an isomeric mixture of (from left to right) 4,4’-BPF, 2,4’-BPF and 2,2’-BPF (see below).

Table 3: Constituents of structurally related substance EC 908-912-9

Constituents	EC Number	CAS number	SMILES / structural formula
2,2'-methylenediphenol	219-578-2	2467-02-9	Oc1ccccc1Cc2ccccc2O 
4,4'-methylenediphenol	210-658-2	620-92-8	Oc2ccc(Cc1ccc(O)cc1)cc2 
o-[(4-hydroxyphenyl)methyl]phenol	219-579-8	2467-03-0	Oc2ccc(Cc1ccccc1O)cc2 

1.3. Physicochemical properties

Table 4: Overview of physicochemical properties

Property	Value [Unit]	Reference/source of information
Physical state at 20°C and 101.3 kPa	*Crystalline, light red*	*Safety data sheet (Sigma-Aldrich 2022)*[2]
Melting/freezing point	*162-164 °C*	*Safety data sheet (Sigma-Aldrich 2022)*[2]
Boiling point	*291 °C @ 101.3 kPa*	*OECD 103 (Test substance: EC 908-912-9)*[1]
Vapour pressure	*0 Pa @ 20-25 °C*	*OECD 104 (Test substance: EC 908-912-9)*[1]
Density	*1.279 g/cm^3 @ 20 °C*	*OECD 109 (Test substance: EC 908-912-9)*[1]
Water solubility	*2.97 g/L @ 20 °C @ pH 6.2-6.3*	*OECD 105 (Test substance: EC 908-912-9)*[1]
Partition coefficient n-octanol/water (log value)	*log P_{ow} = 2.91 @ 20 °C*	*OECD 107 (Test substance: EC 908-912-9)*[1]

2. Harmonised classification and labelling

BPF is not covered by an Index number in part 3 of Annex VI to the CLP Regulation.

[2] https://www.sigmaaldrich.com/DE/en/sds/ALDRICH/B47006

3. Environmental hazard assessment – endocrine disruption

3.1. General approach

The available data for BPF is presented with the focus on tests and endpoints that can be conclusive for their endocrine disrupting properties in the environment. Similarity of concern to BPA and BPB with respect to intrinsic hazardous properties in this case is considered to be met if based on the available data the substance fulfils all of the following criteria in accordance with the WHO/IPCS definition of an endocrine disruptor in the environment as interpreted by the EC ED EAG (JRC, 2013):

- Show an adverse and population relevant effect in organisms. With regard to this, all effects that impact the survival, the growth or the reproduction of an organism are considered to be adverse and of population relevance.
- Show an endocrine activity; and
- An endocrine mode of action (MoA), i.e. there is a biologically plausible link between the endocrine activity and the adverse effects observed.

To conclude if these criteria are fulfilled, a weight of evidence approach is used. All relevant and reliable data available when compiling this dossier are considered. This comprises *in silico*, in vitro and in vivo data from standard as well as from non-standard exploratory studies. 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): I) *in vitro* and *ex vivo* mechanistic parameters, II) in vivo mechanistic parameters and III) parameters providing 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 relevance of a given study is assumed if the study design allows answering questions regarding an endocrine mode of action and/or regarding adverse effects that are or can be mediated via an endocrine MoA. To judge on the reliability of data, all studies were assessed according to the Klimisch scoring system (Klimisch et al., 1997).

3.2. Previous assessments of the endocrine properties of BPF

In March 2021 the SE CA provided an RMOA for BPF also discussing the ED concern with a focus on ED properties for human health. The SE CA concluded that the current evidence might not be strong enough to be used as basis for SVHC identification (ED HH) but might be used as the basis for a read-across to BPA to propose a classification of BPF as Repr. 1B. Evaluation of ED properties for the environment was not the main focus of the SE CA's RMOA. [3]

3.3. Assessment of information on ED properties of BPF

A literature search was performed to identify all studies available in open literature relevant for the assessment of endocrine disrupting properties of Bisphenol F with respect to the environment. As the substance itself is not registered under REACH, an assessment

[3] Risk Management Option Analysis Conclusion Document on 4,4'-methylidenediphenol (Bisphenol F) dated 24 March 2021: https://echa.europa.eu/documents/10162/785a51c1-3391-1b81-e532-cc83b665ea60

of the results presented in study reports from the registration dossier could not be included in the assessment here.
The last PubMed search was performed on September 10th 2021. This resulted in 498 hits. The respective search strings and number of hits are presented in the table below.

Date of search	Database	Search string	Number of articles	Comment
Again on 10.09.2021	PubMed	(Bisphenol F) OR (4,4'-methylenediphenyl) OR (620-92-8 OR (210-658-2) AND ((fish OR human OR toxicity) OR (endocrine* OR hormone* OR androgen* OR estrogen* OR thyroid* OR steroid*))	498 publications Manual screening narrowed the result to 36 potentially relevant publications	The title and abstracts of the identified articles were screened manually. Expert judgement was also used to determine whether a study was likely to provide information of relevance for the ED assessment.

The quality and reliability of all in vitro and in vivo studies were assessed, and each study was assigned a reliability score based on the Klimisch categories 1, 2, 3 or 4 (Klimisch et al., 1997) combined with an expert judgement statement.

The following sections report and discuss the relevant and reliable data to conclude on the environmental ED properties of BPF according to the approach described above.

3.3.1. In vitro information indicative of endocrine activity

There are eleven studies available investigating estrogenic, anti-estrogenic, androgenic, anti-androgenic, thyroidal and steroidal activity of BPF. Additionally, growth hormone production, metabolism and cytotoxicity were investigated in some studies. All studies are rated as Klimisch 2, except the study performed by Park et al. (2020), which is rated Klimisch 1 as it follows OECD TG 455.

The following table provides an overview of the available in vitro data which are further discussed in the respective subsections.

Table 5: Available *in vitro* data on BPF

Method	Results	Reference
Culture system (foetal testis assay) tested with BPS, BPF	BPA: 10 nM reduces basal testosterone secretion of human foetal testis explants Addition of LH in FeTA system enhances BPA min effective conc in mouse and human but not in rat foetal testis BPS / BPF: 10 nM decreased basal testosterone secretion by human foetal testis BPA and BPF: 1000 nM min effective concentration in foetal mouse testes BPA / BPS / BPF: 10000 nM reduced *Insl3* expression in cultured mouse foetal testes	Eladak, et al., 2015 (Klimisch 2)
Luciferase reporter gene assay with HepG2 cells transiently transfected with ERα or ERβ	ERα-agonism: 63.1% max efficacy, rel. EC_{50} = $1.6 \cdot 10^{-6}$M (BPA: 110.3%, $1.2 \cdot 10^{-6}$M) ERβ-agonism: 97.9 %max Efficacy, rel. EC_{50} = $1.3 \cdot 10^{-6}$M (BPA: 97.4%, $3.5 \cdot 10^{-7}$M) ERα-antagonism: 37.6% max. inhibition, rel. EC_{50} = - (BPA: 11.9%, -) ERβ-antagonism: 12.5 % max. inhibition, rel. EC_{50} = -	Pelch et al., 2019 (Klimisch 2)

	(BPA: 35.2%, -) AR antagonism: 66.2% max. inhibition, rel. IC_{50} = $5.9 \cdot 10^{-6}$M (BPA: 98.8%, $5.1 \cdot 10^{-7}$M)	
Mixture effects: Stably transfected transcriptional activation (STTA) assay according to OECD TG 455: Human ERα-expressing HeLa9903 cells + MCF-7 cells	**ER transactivation activity in Hela9903 cells**. HeLa9903 cells treated with E2 (1 nM) or: BPA: 1.0 x 10^{-10} to 1.0 x 10^{-4}M → 1.51 µM compared to 100% activity induced by E2 BPF: 1.0 x 10^{-10} to 1.0 x 10^{-4}M → 3.96 µM **ERα protein expression in MCF-7 cells** MCF-7 cells were treated with E2 (10 nM) or: BPA: 1 µM decreased the Erα-expression 0.69-fold BPF: 1 µM no change observed, 10 µM 0.53-fold reduced ERα protein levels (for BPA, BPF, BPS) **androgenic activities in AR-EcoScreen cells** AR-Ecoscreen cells were treated with DHT (10 nM) and: BPA: $1.0 \cdot 10^{-10}$ to $1.0 \cdot 10^{-5}$ M; BPF: $1.0 \cdot 10^{-9}$ to $1.0 \cdot 10^{-5}$ M → no AR agonist activity; **anti-androgenic activities in AR-EcoScreen cells** AR-Ecoscreen cells were treated with DHT (500 pM) or/and HF (100 nM) and BPA $1.0 \cdot 10^{-10}$ to $1.0 \cdot 10^{-5}$ M, BPF $1.0 \cdot 10^{-9}$ to $1.0 \cdot 10^{-4}$ M BPA and BPF had antagonistic activity at 10 µM Compared with the 100% activity induced by 500 pM DHT (positive control), AR activity was decreased 50% by 8.93 µM BPA, 10.8 µM BPF. **AhR activities in DR-EcoScreen cells**. DR-Ecoscreen cells were treated with TCDD (100pM) and BPA $1.0 \cdot 10^{-10}$ to $1.0 \cdot 10^{-5}$M, BPF $1.0 \cdot 10^{-9}$ to $1.0 \cdot 10^{-4}$ M → 10 µM BPA, 100 µM BPF, and 100 µM BPS had very low, but significant, AhR-mediated activity	Park et al., 2020 (Klimisch 1)
Comparative study *in vitro* (and *in vivo*) sperms and testicular tissue of rats (BPA, BPB, BPF, BPS) doses (0, 1, 10, and 100 ng/ml) stock solutions with ethanol tissue from 7 Sprague–Dawley male adult rats	Antioxidant enzyme activities and oxidative stress markers were induced in the testes, whereas testosterone production was reduced	Ullah et al., 2018 (Klimisch 2)
In vitro (and *in vivo*) zebrafish	Efficient transactivation of all zebrafish estrogen receptor (zfER) subtypes in zebrafish hepatic reporter cell lines (ZELH-zfERs). BPA was selective for zfERα while BPS and BPF were slightly more potent on zfERβ subtypes	Le Fol et al., 2017 (Klimisch 2)
HPLC-APCI/APPI-HRMS was coupled with CALUX *in vitro* reporter gene bioassays	TTR binding potency: BPAF = BPF > BPA = BPE anti-androgenic activities: BPAF > BPE > BPA > BPTMC > BPF > BPS	Šauer et al., 2021 (Klimisch 2)
Hepatocytes isolated from liver of *O.mykiss* 24 h expo to BPF 0, 15.63, 31.25, 62.50, 125, 250, and 500 µM	LDH assay: increasing cytotoxicity in cells (dose-dependent) Increased malondialdehyde content (indicative of lipid peroxidation) sign between 15.63 and 250 µM.	Aykut and Kaptaner, 2021 (Klimisch 2)

LDH (lactate dehydrogenase assay) and antioxidant defence system indicators	Toxic mechanism mainly based on cell membrane damage and oxidative stress	
2,2'-BPF (CAS 2467-02-9); 4,4'-BPF (CAS 620-92-8) 1) Caco-2 cells: in vitro intestinal transport study 2) HepaRG: in vitro liver metabolism study 3) QIVIVE for EC_{50}/IC_{50} extrapolation	2) 4,4'-BPF slightly slower metabolization than BPA and 2,2'-BPF Yeast estrogen bioassay EC_{50} (4,4'-BPF) = 20 µM EC_{50} (2,2'-BPF) = NA but anti-ER IC_{50}= 70 µM EC_{50} (BPA) = 20 µM Yeast anti-androgen bioassay: IC_{50} (4,4'-BPF) = 20 µM IC_{50} (2,2'-BPF) = NA IC_{50} (BPA) = 30 µM	Punt et al., 2019 (Klimisch 2)
fluorescence competitive binding assay Coactivator recruitment assay TR-mediated luciferase reporter gene system using GH3 cells T-screen assay	Fluorescence competitive binding assay: BPS and BPF bound to TH receptors (TRα and TRβ) (binding potencies an order of magnitude lower than BPA) Coactivator recruitment assay: BPS and BPF recruited coactivator to TRβ but not TRα, with weaker potencies than BPA TR-mediated reporter gene transcription assay: agonistic actions in the absence or presence of T3 BPA, BPF, BPS induced TH-dependent GH3 cell proliferation BPA and BPF inhibited T3 induction in presence of T3	Zhang et al., 2018 (Klimisch 2)
In vitro ERE-luciferase reporter assay using MCF-7 cells + ARE-luciferase reporter assay + induction of growth hormone production in GH3 cells	MCF-7 estrogen luciferase reporter assay: BPF-EC_{50} = 1.0 µM BPA-EC_{50} = 0.63 µM BPS-EC_{50} = 1.1 µM Luciferase Reporter Assay (anti-androgenic act. against DHT): BPF-IC_{50} = 12 µM BPA-IC_{50} = 4.3 µM BPS-IC_{50} = 17 µM Growth hormone production assay of GH3 cells: BPA, BPF showed no activity	Kitamura et al., 2005 (Klimisch 2)

3.1.1.1. Estrogen pathway

Several in vitro reporter gene and transactivation assays show a clear agonistic activity of BPF on both ERα and ERβ subtypes of the receptor proteins (Kitamura et al., 2005, Le Fol et al., 2017, Pelch et al., 2019, Park et al., 2020). In these studies, BPF (i.e. 4,4´-BPF) showed similar EC_{50} values in the low µM range as BPA. Additionally, there is a yeast estrogen bioassay performed by Leuwen et al. (2019) which also shows estrogen agonistic activity of BPF and BPA, both at EC_{50} values of 20 µM.
Anti-estrogenic activities of BPF were observed in the studies by Pelch et al. (2019) and Park et al. (2020) in the transactivation assay set up and in an MCF-7 cell-based ERα protein expression level.

3.1.1.2. Androgen pathway

No androgen agonistic activity is reported in the available studies. Anti-androgenic activity was observed in several assays (Kitamura et al., 2005, Punt et al., 2019, Pelch et al., 2019, Park et al., 2020, Šauer et al., 2021). Reported IC_{50} values and effect concentrations

of BPF and BPA were similar or at least in the same order of magnitude.

3.1.1.3. Thyroid pathway

BPF showed binding to both subtypes of the thyroid receptor protein in a competitive binding assay (Zhang et al., 2018). The same study found that BPS and BPF recruited coactivator to TRβ but not to TRα in a coactivator recruitment assay but with weaker potencies compared to BPA. Additionally, Zhang et al. (2018) performed a TR-mediated reporter gene transcription assay and found agonistic actions of BPF in the absence or presence of T3. The study further showed that BPA, BPF, BPS induced TH-dependent GH3 cell proliferation and that BPA and BPF inhibited T3 induction in the cells in presence of T3. Šauer et al. (2021) showed that BPF can bind to the TTR transport protein. TTR binding potency of BPF was comparable to that of BPAF and higher compared to BPA in this assay.

3.1.1.4. Steroid pathway

Eladak et al. (2015) found that BPF and BPA comparably decreased basal testosterone secretion by human and mouse foetal testis in a culture assay set up. Growth hormone production was analysed in GH3 cells by Kitamura et al. (2005) without any effects of BPF and BPA.
Cytotoxicity of BPF in hepatocytes isolated from liver of *Oncorhynchus mykiss* was investigated by Aykut and Kaptaner (2021) using a LDH assay. This study observed significant cytotoxic effects based on oxidative stress (lipid peroxidation and membrane damage) between 15.63 and 250 µM.

3.1.1.5. Conclusion

BPF shows significant endocrine activity in the available in vitro studies. Most prominent are estrogen agonistic effects and anti-androgenic effects with effect concentrations (in the low µM range) in the same order of magnitude as or even equal to the concentrations observed for BPA, which is already identified as an ED in the environment based on estrogenic and or anti-androgenic effects in fish. These results are consistent across different cell lines and studies. BPF also shows thyroidal activity based on binding to the thyroid receptor protein as well as to the transport protein TTR. One study demonstrated an inhibitory effect of BPF on testosterone production in a testis cell culture assay.
Significant cytotoxicity of BPF was observed in one study using isolated hepatocytes from rainbow trout, however severe effects were seen at higher concentrations compared to effect concentrations triggering ED specific endpoints in the other available in vitro studies.

3.3.2. In vivo information indicative of endocrine activity

In the following table the available *in vivo* fish and amphibian data is summarised. All studies are rated as Klimisch 2 since they represent high quality exploratory studies.

Table 6: Available in vivo data with fish on BPF

Method	Results	Reference
Danio rerio (AB strain) 28 ± 0.5 °C 6 male and 6 female fish in 10-L glass aquaria control, 0.001, 0.01, 0.1, and 1 mg/L BPF for 21 days following OECD guidelines 229 and 230 0.01% DMSO Two replicates	Geometric mean conc.: 0.00071, 0.0072, 0.079, 0.75 mg/L No mortalities in any treatment HSI at 1 mg/L sign higher in males + GSI sign decreased in females and males at 1 mg/L Egg production, hatching rate, survival rate sign reduced at 1 mg/L Exposing parental fish resulted in	Yang et al., 2017 (Klimisch 2)

Semi-static Histological analysis under microscope Hormone measurements using ELISA RNA extraction and gene expression analysis	malformed embryos and larvae (sign at 0.1 and 1 mg/L) Histological examination: decrease in number of early sperm stages, enlargement of interstitial space in male fish, females: lower proportion of later follicular stages; no intersex individuals Sign differences in concentrations of steroid hormones: decrease in T conc. In homogenate of 0.001, 0.1 and 1 mg/L + sign increase of E2 conc. In 0.1 and 1 mg/L Liver: sign increased vtg1 expression in males (dose-dependent), but not in females.	
Zebrafish (*Danio rerio,* AB strain) Acute tox test: 20 eggs (2 hpf) in 6-well cell culture plates with 10 mL test solution in triplicate (0, 1, 10, 100, 1000, 2000, 4000, 6000, or 8000 µg/L, 0.01% DMSO) expo: 2 hpf to 120 hpf 16 h light per day 28 ± 0.5 °C Long-term expo: 500 eggs in 500 mL glass beakers in triplicate (0, 1, 10, 100, or 1000 µg/L) Semi-static At 10 dpf larvae transferred to 10 L glass aquaria Expo: 1 to 60 dpf Gonadal histology (microscope) ELISA Rea-time PCR Analytical verification: LC-MS Statistics: Levene's test on homogeneity of variances + Kolmogorov-Smirnov test on normality; one-way analysis of variance Tukey's post -hoc test ($p < 0.05$)	Mean measured conc: 0.93, 11.51, 98.56, and 1047.93 µg/L 96h-LC_{50} = 10,030 µg/L 120h-LC_{50} = 9391 µg/L 100 and 1000 µg/L BPF: - led to trend for a female sex ratio bias - Gonad histology: 10 and 22 % intersex fish + all male fish developed ovo-testes - Abnormal testicular development Steroid hormone conc.: - Conc. T decreased sign in ≥ 10 µg/L - E2 increased sign in ≥ 10 µg/L VTG expression increased sign from 20 dpf in ≥ 10 µg/L expo groups	Yang et al., 2018 (Klimisch 2)
Zebrafish embryos (wild-type WIK strain) BPA, BPF, BPS, BPAF Ethanol 0.01% LC-MS analysis Co-exposure with ICI 182780 (CAS 129453-61-8) (ER antagonist) 20 embryos per group in 100 mL water in triplicate 28 ± 1 °C Semi-static Toxic effects + morphological abnormalities: OECD TG 236 (96h) Estrogenic response by GFP induction 0 to 120 hpf	96h-LC_{50}: BPA 12 mg/L BPF 32 mg/L BPS 199 mg/L Hatching success 72hpf EC50: BPA 5.7 mg/L BPF 14 mg/L BPS 155 mg/L Similar morphological abnormalities, including acriac edema, spinal malformation and craniofacial abnormalities (96 hpf) + lack of pigmentation at 10 mg BPF/L and above	Moreman et al., 2017 (Klimisch 2)
Zebrafish (wild-type AB) Control – solvent control - 0.0005 – 0.5 – 5.0 mg/L triplicates 2 hpf in 24-well plates (RNA-Seq Test)	All treatments with BPF: Reduction of swimming distance and locomotive activity at 6 dpf; Activity range and swim speed of BPF-treated larvae generally lower	Yuan et al., 2019 (Klimisch 2)

OR QPCR 2 hpf in 1 L beaker (150 mL exposure solution) with 120 embryos; at 48 hpe 85 embryos from each replicate OR Chip-seq test	than control 0.5 and 5.0 mg BPF/L: sign affect motor neuron development at 72 hpf with an inhibition of axon growth → comparative analysis: BPF stronger influence on zebrafish motor neuron development than BPA No transcriptional changes observed for estrogenic genes in BPF treatment	
Danio rerio (AB strain) 28 ± 0.5 °C 14 h light per day 7.08 – 72.43 – 700.43 µg/L (mean measured conc.) TUNEL staining	OECD TG 212: 96h-LC_{50} = 7.40 mg/L Free-swimming total distance: tended to decline in a dose-dependent manner (sign at 70 and 700 µg/L) 300 µg/L CPF (positive control): same effect Oxidative stress in 3 dpf and 6 dpf larvae: decrease of activities of SOD, increase of activity of MDA (dose-dependent) Apoptosis in larvae brain: increased number of death cell in larvae brain with increasing BPF conc. (sign at 70 and 700 µg/L) Decreased expressions of genes regulating neurodevelopment (dose-dependent) Histopathological abnormalities in brain (sign at 700 µg/L)	Gu et al., 2020 (Klimisch 2)
Danio rerio Embryo (< 4 hpf) Until 120 hpf DMSO < 0.1% Carbon-filtered tap water 25 ± 1 °C 14 h light/day 96-well plates Observation of survival, hatching, malformation: 1 larva per well, 6 wells per replicate, 4 replicates per treatment at 0.4, 2, and 10 mg/L for both BPA and BPF; Measurement of THs in larval fish: 160 larvae per replicate, three replicates per treatment in a glass beaker (200 mL volume) at 0.08, 0.4, and 2 mg/L for both BPA and BPF at 0.4 and 2.0 mg/L for both BPA and BPF; Transcriptional analysis: mass chamber (50 mL of media/batch) with 25 per replicates and three replicates per treatment at 0.08, 0.4, and 2 mg/L for both BPA and BPF	Survival: NOEC = 2.0 (BPA) or 10 (BPF) mg/L Time-to-hatch: LOEC = 2.0 (BPA) or 0.08 (BPF) mg/L Hatchability: No sign. effect for BPF; NOEC = 2.0 mg/L for BPA Sign. increase of T3: At 0.4 mg/L BPA Sign increase of T4: At 2.0 mg/L for BPF Transcriptional changes in TH regulating genes: Up-regulated hematopoietically expressed homeobox (hhex), transthyretin (ttr) and ridine diphosphate glucuronosyltransferase 1 ab (ugt1ab) genes by BPA, BPF, and BPS.	Lee et al., 2019 (Klimisch 2)
Danio rerio (wild-type AB strain and transgenic lines Tg(cyp19a1b:GFP)) eggs collected 2hpf 28.5 °C	Hatching success (measured at 55 hpf): BPA sign. at both conc. BPF no sign. effect	Coumailleau et al., 2020 (Klimisch 2)

80 embryos per treatment Glass flask (100 mL medium) 28.5°C ± 0.5°C 14 hour light per day DMSO, EE2 (1nM = positive control) BPA, BPS, BPF, BPAF, BPAP: 1 and 0.1 µM	BPAF sign. at 1 µM BPS sign. at both conc. Locomotor activity (using ZEBRALAB): No sign effect on swimming distance, but reduction of swimming speed over time for all substances tested. BPAF showed as ignificantly reduced locomotor activity following 6 days of exposure.	
Danio rerio (AB strain) 40 fish (20 males + 20 females) for one group 2 replicates per conc. 14 days exposure 1, 10, 100, 1000 µg/L ≤ 0.01% DMSO Semi-static (24h renewal) ELISA analysis RNA-seq analysis qRT-PCR analysis DNA extraction	Sign increased 1L-1ß and TNF-α levels (indicating intestinal inflammation) ELISA analysis showed induced oxidative damage and inflammatory response Different changes in microbial community of zebrafish intestine	Wang et al., 2021 (Klimisch 2)
(*In vitro* and) *in vivo* zebrafish quantifying the expression of brain aromatase using a transgenic *cyp19a1b*-GFP zebrafish embryo assay	BPA, BPF, BPS: induced GFP in a concentration-dependent manner. BPS only partially induced brain aromatase at the highest tested concentrations (>30 µM) while BPA and BPF strongly induced GFP, in an ER-dependent manner, at 1–10 µM. Additionally, BPF strongly induced vitellogenin synthesis in adult male zebrafish.	Le Fol et al., 2017 (Klimisch 2)
Danio rerio 21 d exposure to BPA, BPF, or BPS 30 µg/L – 0.005% v/Vv ethanol Acclimatisation or acute test (18°C and 28 °C) 14 h light per day 9-15 fish per treatment	BPF, BPS, not BPA: reduced swimming performance – no interactions between bisphenol exposure and acclimatisation	Wu and Seebacher, 2021 (Klimisch 2)
Acute toxicity testing to algae, daphnia, fish + chronic	Daphnia 48h-EC50= 7.3 mg BPA/L or 8.7 mg BPF/L Zebrafish 72h-EC50 (hatching inh)= 4.0 mg BPA/L or 6.8 mg BPF/L Zebrafish 48h LC50= 15.9 mg BPA/L or BPF not determined as not as toxic	Tišler et al., 2016 (Klimisch 2)
Zebrafish embryos and larvae	reduced amount of eggs and hatching rate	Ren et al., 2017 (Klimisch 2)
Danio rerio 96h- acute toxicity + gene expression 1, 2, 4, 6, 8, 10, 12, 14 $mg \cdot L^{-1}$	96h-LC_{50} = $1.6 \cdot 10^6$µM	Han et al., 2021 (Klimisch 2)
Zebrafish (wild type AB) Juvenile 10 – 15 g 5 L glass tanks 5 fish per tank, 4 replicates 0.005% DMSO, blank, 0.1, 1, 10, 100, and 1000 µg/L Analytical monitoring	60d: no sign difference in fish body weight, survival rates, body length compared to control Increased expression of reproductive neuroendocrine-related genes and increased levels of hormones in the zebrafish brain as	Qiu et al., 2019 (Klimisch 2)

Semi-static 100 µg/L BPA or BPF for 60 d – survival rate, body weight, body length, HSI	well as increased levels of vitellogenin in liver	
Cyprinus carpio Juvenile 10 – 15 g 5 L glass tanks 5 fish per tank, 4 replicates 0.005% DMSO, blank, 0.1, 1, 10, 100, and 1000 µg/L Analytical monitoring Semi-static 100 µg/L BPA or BPF for 60 d – survival rate, body weight, body length, HSI,…	60d: no sign difference in fish body weight, survival rates, body length compared to control HSI sign increased at 1000 µg/L BPF Oxidative stress at 100 and 1000 µg/L BPF BPF similar effects as BPA on immune modulation	Qiu et al., 2018 (Klimisch 2)
Zebrafish (wild-type AB) embryos (2 hpf) Developmental tox test: 24-well plates Blank -solvent control (Acetone) – 0.005 – 0.5 – 5 mg/L; 3 replicates; 96h expo Transcriptomic test: 1-L beakers; 3 replicates; Blank -solvent control (Acetone) – 0.005 – 0.5 mg/L; 50 embryos per beaker; at 48 hpf: 25 hatched larvae per replicate evaluated qPCR validation: 0.0005, 0.5 and 5.0 mg/L in 1L-beakers; 100 embryos; 3 replicates; at 48 hpf: 25 hatched larvae per replicate evaluated analytical verification of conc. (measured less than 20% different from nominal)	Pigmentation reduction (sign less melanin in eyes, yolk sac, notochord) Size and number of melanocytes sign reduced in embryos of 5 mg/L BPF treatment Developmental defects: abnormal spontaneous movements at 0.5 and 5.0 mg/L; decreased heart rate hatch inhibition sign at 5.0 mg/L Sign. effect on motor neuron development at 0.5 or 5.0 mg/L	Mu et al., 2019 (Klimisch 2)
Zebrafish (*Danio rerio*, AB strain) <u>Acute tox test:</u> embryos 1.55-1.7 hpf in 24-well plates with 2 mL exposure solution in triplicate Expo: 4 days Semi-static (renewal every 24h) 14 hours light per day 26°C <u>Developmental Toxicity Test:</u> embryos 1.55-1.7 hpf in 24-well plates with 2 mL exposure solution in triplicate (control, 0.1, 0.5, 1.0 mg/L) <u>Estrogenic Activity Test:</u> about 100 embryos in 1-L-beakers with 500 mL exposure solution in triplicate (0.2, 2, 10 mg/L) Expo: 2 hpf to 96 hpf 60 embryos per replicate collected <u>ELISA tests:</u> 30 embryos per sample homogenized with saline on ice <u>Gene Expression Analysis:</u> Total RNA extracted from 30 embryos per sample + qPCR validation <u>Chemical confirmation of substances:</u> UPLC-MS	No solvent effect 96h-LC_{50} = 19.59 mg/L Sign inhibition of embryo hatching ratio: 15% at 5 mg/L and 65% at 10 mg/L at 72 hpf Pigmentation of eyes, yolk sac, notochord of embryos sign reduced (35% at 1 mg/L; 57% at 5 mg/L; 82% at 10 mg/L)	Mu et al., 2018
Danio rerio (Zebrafish, AB strain)	Increase of T3 and decrease of T4	Huang et al.,

larvae 0.01% DMSO, 0.2, 2, 20, and 200 µg/L 2 hpf to 144 hpf 500 embryos; 6 replicates LC-MS/MS	contents, increased ratios of T3/T4 TSH content sign induced in concentration-dependent manner Increased gene transcription of *dio2*, *crh* and *nis* and *tg*	2016 (Klimisch 2)

Table 7: Available *in vivo* data with amphibia on BPF

Method	Results	Reference
Xenopus laevis T3-induced metamorphosis assay Tadpoles stage 52 in glass tanks (9 per tank); 3 replicates 0.001% DMSO 10, 100, 1000, 10000 nM in absence or presence of 1 nM T3 22 ± 1 °C 24h expo In vitro tail assay Spontaneous metamorphosis assay Histological examination	In the presence of T3, higher concentrations of BPF (100–10000 nM) antagonized T3-induced TH-response gene transcription and morphological changes in a concentration-dependent manner, whereas 10 nM BPF exerted stimulatory effects on T3-induced integral metamorphosis, displaying TH signaling disrupting effects with complicated concentration–response relationships In the absence of T3, BPF inhibited development at metamorphic climax, but promoted pre- and pro-metamorphic development, displaying a developmental stage-dependent manner agonistic actions of BPF on Notch signaling in the intestine	Zhu et al., 2018 (Klimisch 2)
Pelophylax nigromaculatus tadpoles (Gosner stage 27) TH-response gene transcription assay 0.01–10 µM BPA, BPS, or BPF in the presence or absence of 0.2 nM T3 for 48 h Three replicate test beakers	BPA, BPF, BPS induced TH-response gene transcription in the tadpoles In presence of T3 altered T3-induced gene transcription in biphasic concentration-response manner	Zhang et al., 2018 (Klimisch 2)
Xenopus laevis NF 52 stage tadpoles Semi-static test system T3-induced (1nM) *Xenopus* metamorphosis assay 10, 100, and 1000 nM BPA or BPF in absence or presence of 1 nM T3 Glass tanks (4L water) 9 tadpoles per tank with three replicates 0.001% 8v/v) DMSO 96h test duration	TH-response gene expression: BPA increases like *st3, dio3* and *thibz* expression in brain – BPF upregulated *dio3* but not the other; co-exposure: BPA antagonised T2-induced upregulation of *tgm2, thibz, st3*, and *dio3* expression - In the presence of T3, BPF exhibited more remarkable biphasic effects on T3-induced expression of all the test TH-response genes except for *tgm2.* Brain morphology: BPA and BPF antagonised T3-induced brain remodelling	Niu et al., 2021 (Klimisch 2)

3.3.2.1. Fish data

There is a clear indication of an estrogenic endocrine MoA in the zebrafish studies. One study observed a significant dose-dependent increase in VTG levels in male zebrafish after 7 days of exposure to 0.1 µM BPF in the water (Le Fol et al., 2017). Another study also observed a significant increase in VTG in both male and female zebrafish, after 60 days exposure to 0.0001 mg/L water (Yang et al., 2018). In addition, the endocrine MoA is

supported by an observation of increased estradiol levels in both juvenile and adult male and female zebrafish, at doses of 0.01 mg/L water (juvenile) and 0.1 mg/L water (adult) (Yang et al., 2017 and 2018). Furthermore, Qui et al. (2019) showed that long-term exposure to low and environmentally relevant levels of BPF in vivo leads to increased expression of reproductive neuroendocrine-related genes and increased levels of hormones in the zebrafish brain as well as increased levels of VTG in liver. The authors suggest that chronic BPF exposure affects regulation of the reproductive neuroendocrine system through activation of the estrogen receptor.

3.3.2.2. Amphibian data

In vivo mechanistic data from *Xenopus laevis* and *Pelophylax nigromaculatus* tadpoles show that BPF exerts some activity on the HPT axis. Zhou et al. (2018) found that in the absence of T3, BPF inhibited development of the tadpoles at metamorphic climax, but promoted pre- and pro-metamorphic development, displaying a developmental stage-dependence. The study performed by Zhang et al. (2018) on *Pelophylax nigromaculatus* tadpoles showed that BPA, BPF, BPS induced TH-response gene transcription in the tadpoles. In presence of T3 altered T3-induced gene transcription was observed in a biphasic concentration-response manner. Niu et al. (2021) found effects of BPF on thyroid specific gene expression as well as antagonised T3-induced brain remodelling in *X. laevis*.

3.3.2.3. Conclusion

The available fish data clearly and consistently show an estrogenic and/or anti-androgenic mode of action of BPF. The effect concentrations are in the low mg/L range (0.01-1 mg/L) and hence comparable to those observed for BPA and 4-nonylphenol[4] which are already identified ED substances based on their estrogenic activity.

This observed *in vivo* endocrine activity fits to the observed endocrine effects of BPF in the available *in vitro* data.

Furthermore, BPF shows activity on the HPT axis, which also fits to the effects observed in the available *in vitro* data and effects reported for BPA.

3.3.3. In vivo adverse effect data

3.3.3.1. Fish data

Adverse and population relevant effects in fish were observed in the study of Yang et al. (2017). In this study *Danio rerio* (AB strain, 6 males and 6 females in two replicates) was exposed in a semi static set up to 0.001, 0.01, 0.1, and 1 mg/L BPF for 21 days following OECD guidelines 229 and 230. Histological analysis was performed via microscopy, hormone measurements (testosterone and E2) via ELISA were included and a gene expression analysis (vtg1) was done. Egg production in F0, hatching rate and survival rate of F1 were significantly reduced at 1 mg/L of BPF. Hence, clear adverse effects on reproduction could be shown.

HSI at 1 mg/L was significantly higher in males and GSI was found to be significantly decreased in females and males at 1 mg/L. The histological examination revealed a decrease in the number of early sperm stages and an enlargement of interstitial space in

[4] Annex XV dossier containing the proposal for identification of 4-nonylphenol, branched and linear […] as a Substance of Very High Concern based on its environmental ED properties: https://echa.europa.eu/de/registry-of-svhc-intentions/-/dislist/details/0b0236e180e4ba35

male fish. In female fish a lower proportion of later follicular stages was observed and no intersex individuals could be detected. Significant differences in concentrations of steroid hormones were observed with a decrease in testosterone concentration in the full body homogenate of the 0.001, 0.1 and 1 mg/L treatment. A significant increase of E2 was observed at concentrations of 0.1 and 1 mg/L BPF. The gene expression analysis performed in liver homogenate revealed a significant increased vtg1 expression in males (dose-dependent), but not in females.
Furthermore, the long-term fish study performed by Yang et al. (2018) on zebrafish showed adverse and population relevant effects. In this study, 500 eggs were exposed in triplicate to 0, 1, 10, 100, or 1000 µg/L of BPF under semi-static conditions. At 10 dpf the larvae were transferred to 10 L glass aquaria, and exposure to BPF was continued to 60 dpf. Histology of the gonads was performed at the end of the test, and E2 and testosterone concentrations in full body homogenate were measured via ELISA. Real-time PCR was performed for gene expression analysis (vtg1 in liver). Adverse effects were seen at 100 and 1000 µg/L BPF since histology of the gonads revealed that there is a significant increase in intersex fish of 10 and 22%, respectively. Simultaneously, all males developed ovo-testes and showed an abnormal development. Additionally, there was a trend towards a female-biased sex ratio with a statistically significant shift of the sex ratio towards females at 100 µg/L.
Hormone measurements showed a decrease in testosterone concentration significant from 10 µg/L onwards and an increase of E2 also significant from ≥ 10 µg/L. Liver VTG expression increased significantly from 20 dpf in the ≥ 10 µg/L exposure groups.

3.3.3.2. Conclusion

Two fish studies performed with *Danio rerio* clearly show consistent adverse effects on the reproductive capacity of the animals after exposure to BPF. The study performed by Yang et al. followed an OECD TG 229 (fish short-term reproduction assay) and demonstrated adverse effects on egg production in F0 females as well as on hatching and survival of the larvae of the F1 generation. The same study shows effects for an estrogenic and/or anti-androgenic mode of action in absence of indications for unspecific systemic toxicity. Hence, according to OECD GD 150 this study can be used to conclude on ED mediated adversity.
The long-term fish study of Yang et al. (2018) investigated the sexual development of zebrafish. Here, clear adverse effects could be shown on gonadal development and on the sex ratio. The further endpoints investigated in this study clearly point to an underlying estrogenic and/or anti-androgenic mode of action of these adverse effects. Indications for systemic toxicity that could have triggered the observed effects on sexual development were not seen in this study. According to OECD GD 150, the effects observed in this study are also conclusive for ED mediated adverse and population relevant effects.

3.4. Conclusion regarding ED properties relevant for environment

3.4.1. Adverse effects relevant for ED identification

As described above, the available data for BPF show clear and consistent adverse and population relevant effects on reproduction and sexual development in two zebrafish studies. The observed effects fit to an estrogenic and/or anti-androgenic mode of action and no indications of further non-ED mediated pathways were found in the two studies.

3.4.2. Endocrine activity

The available *in vitro* as well as *in vivo* mechanistic data clearly and consistently demonstrate an estrogenic and anti-androgenic activity of BPF.

Additionally, *in vitro* and *in vivo* data from fish and amphibians point to an interference of

BPF with the HPT axis.

Two *in vitro* studies also show an interference with testosterone production in cellular assays.

3.4.3. Plausible link between adverse effects and endocrine activity

BPF may have multiple modes of endocrine action (estrogenic, anti-androgenic, thyroidal activity and interference with steroidogenesis) that might interact and are difficult to distinguish from each other. However, the estrogenic and/or anti-androgenic effects of BPF in fish are consistently observed in the available studies showing significant effects on egg production, hatching and survival of F1 larvae, sex ratio and gonadal development. Estrogenic and anti-androgenic modes of action are well known to be involved in the regulation of sexual development and reproduction (AOP Wiki including examples therein[5], e.g. AOP 345). Adverse effect such as feminization of fish, fertilization success, ability to produce viable offspring and gonadal development and their link to an estrogenic and/or anti-androgenic mode of action have been reviewed i.a. by Jobling et al. (2002 and 1998), Miller et al. (2012).

Considering the observed concomitant decrease in plasma testosterone levels and the increase in plasma estradiol levels as well as the increase in VTG levels and gene expression in male fish, the link between these endocrine activities and the observed adverse effects on fish is highly plausible.

3.4.3.1. Other supporting evidence

In vitro and *in vivo* data from fish and amphibians point to an interference of BPF with the hypothalamic-pituitary-thyroid (HPT) axis.

Additionally, the available human health data support the conclusion for an estrogenic and/or antiandrogenic activity of BPF, even though the reliability of these studies have not been assessed here. Studies on rats show consistently a decrease in serum testosterone levels and a decrease in sperm motility in the offspring of treated female rats. An increase in uterus weight was observed in juvenile rats.

The link between the observed effects and the specific estrogenic and/or anti-androgenic activity of BPF is further supported by the analogy of BPF to BPA and BPA. The data available for BPA and BPB, both of which share very similar chemicals structures compared to BPF, show well defined adverse effects and modes of action that fit to an estrogenic mode of action in fish. Based on these data BPA and BPB have been identified already as SVHC due to its endocrine disrupting properties in the environment.

3.4.4. Conclusion on ED properties

Overall, BPF has estrogen agonistic properties and induces adverse effects in fish that are plausibly mediated by this endocrine activity.

Furthermore, *in vivo* and *in vitro* evidence shows that BPF has androgen antagonistic properties. This endocrine activity could also plausibly contribute to the observed adverse

[5] https://aopwiki.org/aops

effects on reproduction and sexual development in fish.

The effects observed in fish are relevant for the environment as an effect on the reproductive function and the sexual development can have consequences at a population level. **Therefore, there is scientific evidence to conclude that BPF fulfils the definition of an endocrine disruptor in the environment.**

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