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

EVALUATION OF ENDOCRINE DISRUPTING PROPERTIES FOR THE ENVIRONMENT

Substance Name:	4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol ("BPAF") and its salts
EC Number:	216-036-7
CAS Number:	1478-61-1
Submitted by:	Germany
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ABBREVIATIONS

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)
CLH	Harmonised Classification and Labelling
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
E(R)R	Estrogen (related) receptor
FSH	Follicle-stimulating hormone
hpf	hours post-fertilisation
IC	Inhibitory concentration
JRC	Joint Research Center
LC	Lethal concentration
LH	Luteinising hormone
LC-MS	Liquid chromatography/mass spectroscopy
LOEC	Lowest Observed Effect Concentration
MAPK	mitogen-activated protein kinase
MoA	Mode of action
MSC	Member State Committee
(MS)CA	(Member State) Competent Authority
NO(A)EC	no observed (adverse) effect concentration
OECD	Organisation for Economic Co-operation and Development
4-OHT	4-Hydroxytamoxifen
PCR	Polymerase chain reaction
RAC	Committee for Risk Assessment
SEv	Substance Evaluation
SVHC	Substance of very high concern
VTG	Vitellogenin
WHO	World Health Organisation
WoE	Weight of Evidence

Background

The German Competent Authority (DE CA) has indicated through an entry on 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'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol ("BPAF") and its salts**
EC number(s):	216-036-7
CAS number(s):	1478-61-1

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'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol (Bisphenol AF, BPAF) 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 BPAF show clear adverse and population relevant effects on reproduction in zebrafish. The observed effects fit to an estrogenic and/or anti-androgenic mode of action (MoA) and no indications of further non-ED mediated pathways were found in the key long-term fish study

Endocrine activity

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

Plausible link between adverse effects and endocrine activity

The estrogenic and/or anti-androgenic effects of BPAF are established in zebrafish by the available data showing significant adverse effects on the fertilization rate of spawned eggs. 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 (E2) levels as well as the increase in vitellogenin (VTG) levels and gene expression in male fish demonstrate an estrogenic and/or anti-androgenic MoA. Thus, the link between these endocrine activities and the observed adverse effects on egg fertilization is highly plausible.

Other supporting evidence

The link between the observed effects and the specific estrogenic and/or anti-androgenic activity of BPAF is further supported by the analogy of BPAF to BPA and BPB. The data available for BPA and BPB, both of which share very similar chemicals structures compared to BPAF, 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.

Read across to BPAF salts

The conclusion on the ED properties for BPAF is read across to the eight salts of BPAF due to the high ratio of BPAF counter ion and the fact that under environmental conditions the substances can be expected to dissociate to BPAF.

Conclusion on ED properties

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

Furthermore, *in vivo* and *in vitro* evidence is provided that BPAF has androgen antagonistic properties. This endocrine activity could also plausibly contribute to the observed adverse effects on reproduction in zebra fish.

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

Furthermore, RAC concluded in the opinion on a CLH dossier that the available data provide clear evidence of adverse effects of BPAF on sexual function and fertility in mammals. Changes in male reproductive organ weight, size and histopathology are indicative of an (anti-androgenic) endocrine mechanism. Based on the available data, estrogenic or anti-androgenic mechanism are thought to play a dominant role *in vivo*. This conclusion fits to the observed endocrine activity and adverse effects of BPAF in fish. Adverse effects on sexual function and fertility have to be considered as population relevant effects. Thus, beside the fish data discussed herein, also mammalian data support the conclusion that BPAF exerts endocrine mediated adverse and population-relevant effects in the environment.

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

Registration dossiers submitted for the substance: Yes

1. Identity of the substance and physical and chemical properties

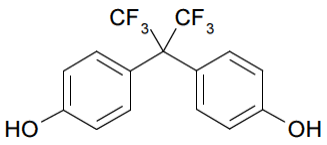
1.1. Name and other identifiers of the substance

Table 1: Substance identity

EC number:	216-036-7
EC name:	4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol
SMILES:	Oc2ccc(C(c1ccc(O)cc1)(C(F)(F)F)C(F)(F)F)cc2
CAS number:	1478-61-1
IUPAC name:	4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol
Index number in Annex VI of the CLP Regulation	Not available
Molecular formula:	$C_{15}H_{10}F_6O_2$
Molecular weight range:	336.23 g/mol
Synonyms:	BIS-AF BISPHENOL AF BPAF

Substance type: mono-constituent

Structural formula:



Besides BPAF itself, eight salts of the substance are registered under REACH, identifiers and data of which are given in the table below. The term "BPAF and its salts" used within this report refers to these substances.

Table 2 Substance identity of registered salts of BPAF

Substance name	EC No.	CAS No.	Remarks
benzyltriphenylphosphonium, salt with 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]bis[phenol] (1:1)	278-305-5	75768-65-9	
'T-6627'	425-060-9		Disodium salt
'COMPOUND 7518'	443-330-4		Methyltributyl-ammonium salt
Tributyl-2-methoxypropylphosphonium salt with 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]bis[phenol]	468-740-0		
Not (publicly) available	469-080-6		UVCB
benzyl(diethylamino)diphenylphosphonium 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenolate	479-100-5	577705-90-9	
Reaction mass of 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol and benzyl(diethylamino)diphenylphosphonium 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenolate (1:1)	943-265-6		
Reaction mass of 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol and benzyltriphenylphosphonium, salt with 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]bis[phenol] (1:1)	947-368-7		

1.2. Physicochemical properties

Table 3: Overview of physicochemical properties of BPAF[1]

Property	Value [Unit]	Reference/source of information
Physical state at 20°C and 101.3 kPa	*Purple grey powder @ 20 °C & 1013 hPA*	*Visual assessment*
Melting/freezing point	*161.7 °C @ 101.3 kPa*	*OECD 102*
Boiling point	*Not determined (decomposition at ≥350 °C)*	*OECD 103*
Particle size distribution (granulometry)	*L10D (v, 0.1) = 4.40 µm,* *L50D (v, 0.5) = 13.96 µm,* *L90D (v, 0.9) = 36.33 µm.*	*CIPAC MT 18*
Vapour pressure	*0 Pa @ 20-50 °C*	*OECD 104*
Density	*1.573 g/cm^3 @ 20 °C*	*OECD 109*
Water solubility	*222.4 mg/L @ 20 °C @ pH 7.32*	*OECD 105*

[1] Values taken from ECHA dissemination page on BPAF: https://echa.europa.eu/de/registration-dossier/-/registered-dossier/23236/

2. Harmonised classification and labelling

BPAF and its salts are currently not covered by Index numbers in part 3 of Annex VI to the CLP Regulation.

However, a CLH proposal to classify BPAF as Repr. 1B, H360F has been submitted by the Swedish CA in December 2019.[2]

In addition, for the following four of the eight registered salts of BPAF, CLH dossiers have also been submitted by the SE CA in parallel to the dossier on BPAF itself, also proposing classification as Repr. 1B, H360F: EC 278-305-5,[3] EC 479-100-5,[4] EC 943-265-6,[5] and EC 947-368-7.[6]

The RAC opinions on the CLH dossiers supporting the classification proposal of the SE CA were adopted on 18 March 2021.[7]

3. Environmental hazard assessment – endocrine disruption

3.1. General approach

The available data for BPAF 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, 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 (WoE) 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

[2] https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e1830f8b24
[3] CLH section for EC 278-305-5: https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e18402a830
[4] CLH section for EC 479-100-5: https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e184029b1d
[5] CLH section for EC 943-265-6: https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e18402b2bf
[6] CLH section for EC 947-368-7: https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e1840283d4
[7] RAC opinion on BPAF: https://echa.europa.eu/documents/10162/d4b588a0-d1e2-9978-a65b-3ebe209d16fa ; EC 278-305-5: https://echa.europa.eu/documents/10162/0f521dfe-58a2-b45a-fcb4-39de06a98643 ; EC 479-100-5: https://echa.europa.eu/documents/10162/bf51d78c-bbad-a64b-f448-64d73ab3efae ; EC 943-265-6: https://echa.europa.eu/documents/10162/22996dd5-4055-c372-18f2-2f39c80c96ce ; EC 947-368-7: https://echa.europa.eu/documents/10162/cffc6225-06bc-9533-8345-c2ca455a53b8 ; all adopted 18 March 2021.

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 to answer questions regarding an endocrine mode of action (MoA) 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 BPAF

In their opinion on the CLH proposal on BPAF, RAC concluded that the available data provide clear evidence of adverse effects of BPAF on sexual function and fertility in mammals. Changes in male reproductive organ weight, size and histopathology are indicative of an (anti-androgenic) endocrine mechanism. Based on the available data, estrogenic or anti-androgenic mechanism are thought to play a dominant role in vivo. RAC further states that the observed effects on male and female sexual function and fertility are not considered to be a secondary non-specific consequence of parental systemic toxicity.

3.3. Assessment of information on ED properties of BPAF

In the following sections the available data relevant for the environment from the registration dossier as well as from literature studies are presented and discussed to conclude on the endocrine activity and possibly related adverse and population relevant effects of BPAF in the environment.

3.3.1. In vitro information indicative of endocrine activity

There are three studies available investigating estrogenic and anti-estrogenic activity of BPAF. The studies are all rated as Klimisch 2.

The following table provides an overview of the available *in vitro* data, which are further discussed below.

Table 4: Available *in vitro* data on BPAF

Method	Results	Reference
In vitro models for evaluation of effects on ER α and β using three human cell lines (Ishikawa, HeLa, and HepG2) 10 – 100 – 1000 nM	Estrogenic activity as agonist for ERα in a dose-dependent manner At lower concentrations BPAF acted as antagonist for ERβ in HeLa cells ERE-mediated activation was via AF-2 function of ERα Endogenous ERα target genes and rapid signaling vie the p44/42 MAPK pathway were activated by BPAF	Li et al., 2012 (Klimisch 2)
HepG2 and HeLa cells used to determine the agonistic activity of BPAF on ERα and ERβ via the luciferase reporter assay. Ishikawa cells stably expressing ERα used	Activation of ERα3xERE-mediated responses in HepG2 cells. Significantly induction of 3xERE and pS2ERE mediated activity. Activation of ERβ3xERE and pS2ERE mediated activity. effects on ERα target genes (PR, pS2, GREB1, SPUVE, WISP2, and SDF-1) using real-time PCR	Li et al., 2013 (Klimisch 2)

to determine changes in endogenous ER target gene expression by bisphenol-AF.	in Ishikawa/ERα stable cells: significant induction of endogenous ERα target genes PR,pS2, GREB1, SPUVE, WISP2 and SDF-1. In contrast, expression of target genes in the Ishikawa/vector stable cells did not change with bisphenol-AF treatments.	
Receptor binding activity to ER α and β + competitive binding (BPA, 17β-estradiol, 4-OHT)	Binding to ERs over ERRgamma Receptor binding activity 3-times stronger for ERβ (IC_{50} = 18.9 nM) than for ERα Almost completely inactive in stimulating the basal constitutive activity of ERβ Antagonist against activity of 17β-estradiol	Matsushima et al., 2010 (Klimisch 2)

3.3.1.1. Estrogen pathway

Two cellular assays (Li et al., 2012 and Li et al., 2013) analysed the ability of BPAF to interact with the ERα and ERβ proteins using luciferase reporter gene assays. The study performed by Li et al. (2012) found estrogen agonistic activity of BPAF for ERα in a dose-dependent manner. At lower concentrations BPAF acted as antagonist for ERβ in the HeLa cell assay. Gene expression analysis showed that endogenous ERα target genes and rapid signaling vie the p44/42 MAPK pathway were activated by BPAF exposure of the cells.

Li et al. (2013) observed activation of ERα3xERE-mediated responses in HepG2 cells as well as a significant induction of 3xERE and pS2ERE mediated activity after BPAF exposure. Gene expression studies using real-time PCR revealed effects on ERα target genes (PR, pS2, GREB1, SPUVE, WISP2, and SDF-1) in BPAF treated Ishikawa/ERα stable cells.

The third study performed by Matsushima et al. (2010) investigated the receptor binding activity of BPAF to ERα and ERβ proteins in a competitive binding assay. The authors found that receptor binding activity of BPAF was three times stronger for ERβ (IC_{50} = 18.9 nM) compared to ERα. Furthermore, BPAF almost completely inactivated the basal constitutive activity of ERβ and acted as an antagonist against the activity of 17β-estradiol. Hence, the authors concluded that BPAF is a full agonist for the ERα but a highly specific antagonist for ERβ.

3.3.1.2. Conclusion

BPAF shows significant endocrine activity in the nanomolar range in the available in vitro studies in a dose-dependent manner. Most prominent are estrogen agonistic effects, but also specific antagonistic activity to the ERβ receptor protein subtype was observed. These results are consistent across the different cell lines used in the available studies and the receptor binding assay.

3.3.2. In vivo information indicative of endocrine activity

In the following table, the available *in vivo* fish data for BPAF is summarised. The studies performed by Shi et al. (2015) and Yang et al. (2016) are rated as Klimisch 1 since they represent high quality studies that were performed according to OECD TG 234 and 215, respectively. The study by Song et al. (2014) is rated as Klimisch 2 since it is an exploratory study of high quality.

Table 5: Available *in vivo* data on BPAF

Method	Results	Remarks	Reference
Fish Sexual Development Test	120d-$NOEC_{F0\text{-}mortality}$ > 125 µg/ (nominal) 120d-$NOEC_{F1\text{-}time\_to\_hatch}$ < 5 µg/L	VTG concentrations	Shi et al., 2015

equivalent to OECD TG 234 *Danio rerio* AB strain No analytical monitoring Vehicle: ethanol 100 µL/L Semi-static 3 replicates with 120 embryos – 50 larvae – 4 males and 2 females SC – 5 – 25 – 125 µg/L (nominal)	(nominal) F0: Fecundity of female decreased but not statistically significant in 125 µg/L-group; F0: significant reduction in fertilisation success of spawn eggs in 125 µg/L-group; F1: malformation rates in 125 µg/L-group significantly higher than SC after 3 dpf (most malformed larvae died in first few days); survival rates sign lower after 6 dpf in 125 µg/L-group F0 males: sign increased plasma E2 in 25 and 125 µg/L-groups; plasma T sign reduced in 25 and 125 µg/L-groups F0 females: sign increased E2 levels in 125 µg/L-group; T levels unchanged F0 gene expression: liver of male fish vtg1 sign increased in 25 and 125 µg/L-groups; vtg1 unchanged in female fish; brain expression of gnrh2, fshb, lhb, cyp19b in male sign increased in 125 µg/L-group; no changes in female brain; gonads expression of fshr, cyp19a, cyp11a1 sign increased; testis star with cyp17 sign decreased in 125 µg/L-group; ovaries increase of fshr + decrease of star in 125 µg/L-group	not determined; VTG gene expression via measurements of mRNA as indicator for ER stimulation	(Klimisch 1)
Danio rerio Analytical monitoring (LC/MS) Vehicle: DMSO Embryo test: n.a. + reproduction test: semi-static, 2 mo old fish Embryo test: 144 dpf; reproduction test: 21d 3 replicates with 30 embryos OR with 10 males randomly selected Control - SC - 0.5 – 1.0 – 1.5 – 2.0 mg/L (nominal)	144h-$LC_{50,embryos}$ = 1.75 mg/L 144h-$NOEC_{larval\_development}$ = 1 mg/L 144h-$NOEC_{embryo\_mortality}$ = 1.5 mg/L 21d-$NOEC_{adult\_mortality}$ ≥ 1.5 mg/L Male adults: VTG induction in all concentrations of BPAF (21d) Embryo test: 100% mortality at 2.0 mg/L after 144 hpf; statistically sign increase of pericardial oedema at 1.5 mg/L slightly delayed hatching in 1.0 and 1.5 mg/L-group (not sign) Not reported: body length, number fish in swim-up stage at one or more time periods, behavioural abnormalities	Combination of embryo development test (144 hpf) with reproduction test (21 d)	Song et al., 2014 (Klimisch 2)
Fish juvenile growth test equivalent to OECD TG 215 *Danio rerio* (2 mo old) No analytical monitoring Vehicle: DMSO Semi-static 3 replicates with 9 fish SC - 0.05 – 0.25 – 1 mg/L (nominal)	28d-$NOEC_{mortality}$ ≥ 1 mg/L (n) 28d-$NOEC_{length\_increased}$ = 0.05 mg/L (n); 28d-$NOEC_{weight\_increased}$ = 0.05 mg/L (n) Male fish hepatocytes swollen and irregularly shaped in 1 mg/L-group Vacuolization in liver in 1 mg/L-group but no hepatic damage in any female fish No obvious alterations in the gills and intestines of both sexes Males: germ cells in all stages of spermatogenesis females: significantly higher proportion of stage I cells in 0.25 and 1 mg/L-groups	Dissolved O_2-concentration not reported	Yang et al., 2016 (Klimisch 1)

	Male fish: T-levels in whole-body homogenates reduced in dose-dependent manner + E2 levels increased with increase of BPAF concentration Female fish: T levels increased in 0.05 and 0.25 mg/L-groups + decreased in 1 mg/L group; increase in E2 levels in 0.05 and 1 mg/L-groups but slightly decrease in 0.25 mg/L-group VTG gene in liver sign upregulated in males of 1 mg/L-group		

3.3.2.1. Fish data

The OECD 234 study performed by Shi et al. (2015) showed that after 120 d of exposure of zebrafish to BPAF, E2 and E2/testosterone ratio was significantly increased in both males and females in a dose-dependent manner. In male fish, several HPG axis related genes were either significantly suppressed or induced in all tissues. In the liver of male fish vtg1 expression significantly increased in the highest two concentrations tested, whereas no changes were observed in female fish. Genes involved in the regulation of gonadotropin releasing hormone, LH and FSH were also affected, and thus the regulation of testosterone and estrogen may be affected.

Song et al. (2014) observed VTG induction in male fish in all concentrations of BPAF tested in a 21-d fish study using zebrafish. Additionally, the authors reported a slight but not statistically significant delayed hatching rate in the 1.0 and 1.5 mg/L treatment group.
The fish juvenile growth test performed by Yang et al. (2014) according to OECD TG 215 showed germ cells in all stages of spermatogenesis in male fish and significantly higher proportion of stage I cells in 0.25 and 1 mg/L treatment groups of female fish. Furthermore, testosterone levels in whole-body homogenates of male fish were reduced in a dose-dependent manner, and E2 levels significantly increased with increase of BPAF concentration. In female fish, testosterone levels increased in 0.05 and 0.25 mg/L groups and decreased in 1 mg/L group. Additionally, an increase in E2 levels in the 0.05 and 1 mg/L groups but a slight decrease in the 0.25 mg/L treatment group was observed in females. VTG gene expression in the liver was significantly upregulated in males of the 1 mg/L treatment group.

3.3.2.2. Conclusion

The available fish data clearly and consistently show an estrogenic and/or anti-androgenic MoA of BPAF. The effect concentrations are in the low mg/L range (0.05-1 mg/L) and hence comparable to those observed for BPA and 4-nonylphenol,[8] which are already identified ED substances based on their estrogenic activity.

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

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 Shi et al. (2015). In this study, *Danio rerio* was exposed in a semi static set up to 0.5, 1.0, 1.5 and 2.0 mg/L BPAF according to the test design and parameters described in OECD guideline

[8] 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

234. For the F0 generation there was a significant reduction in fertilisation success of spawned eggs in the 125 µg/L treatment group. Additionally, the malformation rate of offspring was also significantly increased at the highest concentration. Fecundity of female fish decreased but not statistically significant in the 125 µg/L treatment group. Signs of systemic toxicity were not reported in this study up to the highest concentration of BPAF tested. Thus, there are no indications for other toxic modes of action that could explain the observed adverse effects on fertility in the fish.

3.3.3.2. Conclusion

The long-term fish studies performed by Shi et al. (2015) with *Danio rerio* clearly show an adverse effect on the reproductive capacity of zebra fish after exposure to BPAF. The study performed according to an OECD TG 234 set up demonstrated significant adverse effects on the fertilization rate of spawned eggs at 125 µg/L. The same study shows effects for an estrogenic and/or anti-androgenic MoA 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. BPAF shows adverse effects on reproduction (decrease in fertility) that fit to the observed estrogenic and anti-androgenic activity of the substance in further *in vivo* and *in vitro* studies.

Read-across to the salts of BPAF

The conclusion on the ED properties for BPAF is read across to the eight salts of BPAF due to the occurrence of BPAF as the counter anion in these substances.

Under environmental conditions the substances can be expected to dissociate to the cation and the anion (BPAF). In many of the registration dossiers of the BPAF salts data for physicochemical properties and aquatic toxicity have been given separately for the cation and the anion; in many cases aquatic toxicity data from BPAF has hence been submitted.

Under physiological conditions, dissociation of the salts is also expected. Based on the nature of the substances, it is concluded that the ED properties for the environment relevant for BPAF apply to the salts as well.

3.4. Conclusion regarding ED properties relevant for environment

3.4.1. Adverse effects relevant for ED identification

The available data for BPAF show a clear adverse and population relevant effects on reproduction in zebrafish. The observed effects fit to an estrogenic and/or anti-androgenic MoA and no indications of further non-ED mediated pathways were found in the key long-term fish study.

3.4.2. Endocrine activity

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

3.4.3. Plausible link between adverse effects and endocrine activity

The estrogenic and/or anti-androgenic effects of BPAF are established in zebra fish by the available data showing significant adverse effects on the fertilization rate of spawned eggs. 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 VTG levels and gene expression in male fish demonstrate an estrogenic and/or anti-androgenic MoA. Thus, the link between these endocrine activities and the observed adverse effects on egg fertilization is highly plausible.

3.4.3.1. Other supporting evidence

The link between the observed effects and the specific estrogenic and/or anti-androgenic activity of BPAF is further supported by the analogy of BPAF to BPA and BPB. The data available for BPA and BPB, both of which share very similar chemicals structures compared to BPAF, 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, BPAF has estrogen agonistic properties and induces adverse effects in zebra fish that are plausibly mediated by this endocrine activity.

Furthermore, *in vivo* and *in vitro* evidence is provided that BPAF has androgen antagonistic properties. This endocrine activity could also plausibly contribute to the observed adverse effects on reproduction in zebra fish.

The effects observed in 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 to conclude that BPAF fulfils the definition of an endocrine disruptor in the environment.**

The conclusion on the ED properties for BPAF is read across to the eight salts of BPAF due to the high ratio of BPAF counter ion and the fact that under environmental conditions the substances can be expected to dissociate to BPAF.

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