CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification:

4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol; bisphenol AF

EC Number: 216-036-7

CAS Number: 1478-61-1

Index Number: -

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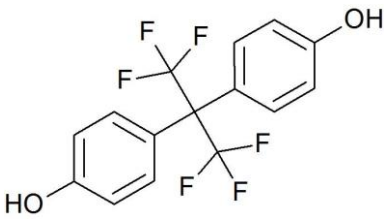
Version number: 2 **Date: 16/12/2019**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol 2,2-Bis(4-hydroxyphenyl)hexafluoropropane 4,4'-(1,1,1,3,3,3-hexafluoropropane-2,2-diyl)diphenol 4,4'-(Hexafluoroisopropylidene)diphenol 4,4'-[2,2,2-Trifluoro-1-(trifluoromethyl)ethylidene]bisphenol 4,4'-[2,2,2-Trifluoro-1-(trifluoromethyl)ethylidene]diphenol 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenol Bisphenol AF
Other names (usual name, trade name, abbreviation)	BIS-AF BISPHENOL AF BPAF
ISO common name (if available and appropriate)	n.a.
EC number (if available and appropriate)	216-036-7
EC name (if available and appropriate)	4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol
CAS number (if available)	1478-61-1
Other identity code (if available)	n.a.
Molecular formula	C15H10F6O2
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	336.233 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	n.a
Description of the manufacturing process and identity of the source (for UVCB substances only)	n.a

Degree of purity (%) (if relevant for the entry in Annex VI)	n.a

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol EC no. 216-036-7	99.5-100%	None	Eye Dam. 1, H318 Eye Irrit. 2, H319 Repr. 1B, H360 Skin Irrit. 2, H315 STOT RE 2, H373 STOT SE 3, H335 Aquatic Acute 1, H400 Aquatic Chronic 1, H410 Not classified

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
-				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
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2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed harmonised classification and labelling according to the CLP critieria.

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal		4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol; bisphenol AF	216-036-7	1478-61-1	Repr. 1B	H360F	GHS08 Dgr	H360F			
Resulting Annex VI entry if agreed by RAC and COM		4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol; bisphenol AF	216-036-7	1478-61-1	Repr. 1B	H360F	GHS08 Dgr	H360F			

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	harmonised classification proposed	**Yes**
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

No previous harmonised classification and labelling.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level. 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol; bisphenol AF, hereafter referenced as BPAF, has CMR properties (reproductive toxicity). Harmonised classification and labelling for CMR and respiratory sensitisation is a community-wide action under Article 36 of the CLP regulation.

5 IDENTIFIED USES

The substance is used as a reactive process regulator in polymer materials and in rubber production and processing. BPAF is used as a crosslinking agent for certain fluoroelastomers and as a monomer for polyimides, polyamides, polyesters, polycarbonate copolymers and other specialty polymers.

6 DATA SOURCES

- Data on BPAF in the publically disseminated REACH registration dossier (ECHA dissemination, 2019)
- Search in PubMed open literature using search terms like “bisphenol AF”, “BPAF”, “Bisphenol A analogues” and similar.
- Access to the full study report of the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422; Study report, 2011) was given from the registrant.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20°C and 101,3 kPa	Solid	REACH registration (ECHA dissemination, 2019)	Measured
Melting/freezing point	161.7 °C	REACH registration (ECHA dissemination, 2019)	Measured
Boiling point	The test item does not have a measurable boiling point when subject to atmospheric pressure, decomposition occurs at ≥ 350 °C, prior to the onset of boiling.	REACH registration (ECHA dissemination, 2019)	Measured
Relative density	1.573 Dimensionless quantity	REACH registration (ECHA dissemination, 2019)	Measured
Vapour pressure	5 x 10-6 Pa at 20.0 °C	REACH registration (ECHA dissemination, 2019)	Measured
Surface tension	Based on structural assessment the substance	REACH registration (ECHA	

Property	Value	Reference	Comment (e.g. measured or estimated)
	is not expected to be surface active.	dissemination, 2019)	
Water solubility	222.4 mg/L	REACH registration (ECHA dissemination, 2019)	Measured
Partition coefficient n-octanol/water	2.79 at 20 °C	REACH registration (ECHA dissemination, 2019)	Measured
Flash point	Flash point is only relevant to liquids and low melting point solids		
Flammability	Not flammable.	REACH registration (ECHA dissemination, 2019)	Measured
Explosive properties	Not explosive.	REACH registration (ECHA dissemination, 2019)	Measured
Self-ignition temperature	No self-ignition observed under the test conditions	REACH registration (ECHA dissemination, 2019)	Measured
Oxidising properties	No oxidising properties.	REACH registration (ECHA dissemination, 2019)	Measured
Granulometry	Particle Size Distribution L10 D (v, 0.1) = 4.40 µm, the L50 D (v, 0.5) = 13.96 µm and the L90 D (v, 0.9) = 36.33 µm	REACH registration (ECHA dissemination, 2019)	Measured
Stability in organic solvents and identity of relevant degradation products	Not relevant		
Dissociation constant	Not relevant		
Viscosity	Not relevant		

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this CLH proposal.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Non-test guideline, in vivo experimental study. Disposition of BPAF was studied in male and female Harlan Sprague-Dawley rats and B6C3F1/N mice following oral administration to C14-labelled BPAF (97% purity) at doses of 3.4, 34 or 340 mg/kg (4 animals per group). A dose of 34 mg/kg was also given intravenously (to 4 animals per group except for male mice: N=3). Urine and feces were collected at 8 (urine only) and 24 h, and 24-h intervals throughout the study. Blood, bile and tissue samples (skin, muscle, adipose, bladder, spleen, heart, pancreas, liver, kidney, brain, lung, thyroid, ovaries, uterus, testes, small intestine, large intestine, cecum and stomach) were collected.	Rats: BPAF was well absorbed and excreted mainly in the feces after oral administration. Female rats excreted more BPAF in urine compared to male rats. Total residual radioacitivity in tissues was low (72 h after substance administration). The concentration in liver was higher than in other organs (except for GI tract). In male rats, 52% of a 340 mg/kg oral dose was excreted in bile (24 h), which mostly included BPAF glucuronide. Mice: As in rats, BPAF was excreted mainly in the feces after oral administration. Female mice excreted more BPAF in urine compared to males. Highest tissue BPAF levels were found in gall bladder.		Waidyanatha et al. 2015.
Non-test guideline, in vivo experimental study. Toxicokinetics and bioavailability of BPAF (>99% purity) was studied in Harlan Sprague Dawley rats and B6C3F1/N mice of both sexes. No information was found on the number of animals used. Single oral doses at 34, 110 and 340 mg/kg were given to rats and a dose of 34 mg/kg was given to mice. In addition, a dose of 34 mg/kg was given intravenously (IV) to both rats and mice. Blood samples were collected at target times 0, 5, 15, 30 min, 1, 2, 4, 8, 12, 24, 32 and 48 h.	Rats: BPAF was detected at all time points in plasma of both males and females after oral administration. BPAF was rapidy absorbed. The bioavailability was low. Total BPAF was higher than free BPAF, indicating rapid and extensive conjugation. Total BPAF was rapidly eliminated with half-lives ranging from 2.60 to 4.61 h (depending on dose) after oral administration, except for high dose females for which the elimination half-life was 20.2 h. Free BPAF was cleared more rapidly than total BPAF. Mice: BPAF was absorbed rapidly after oral administration. The substance was cleared rapidly, with elimination half-lives of 4.22 h for males and 1.33 h for females (free BPAF). Conjugation was rapid and extensive. Total BPAF was cleared from plasma with an elimination half-life of 0.753 h		Waidyanatha et al. 2019.

Method	Results	Remarks	Reference
	and 0.804 h for males and females, respectively. No sex differences were seen. In both rats and mice BPAF was rapidly and extensively conjugated following oral administration. The bioavailability was low in both species, though slightly higher in mice than in rats (3-6% vs. 1%).		
Non-test guideline, in vivo experimental study. BPAF (98% purity) 10 mg/kg was given orally to 4 male Sprague Dawley rats, for 2 consecutive weeks. Urine and feces samples were collected, as well as serum and tissues, including kidneys, liver, testis, adipose and muscle.	High levels of BPAF were detected in liver, kidney and serum. BPAF was also detected in other organs, such as testis. The liver seemed to be the main organ responsible for metabolism. The highest level of BPAF was found in feces (in unconjugated form).		Yang et al. 2012.
Non-test guideline, in vivo experimental study. For urine analysis: BPAF (98% purity) was given orally (200 mg/kg) to 8 weeks-old male Sprague-Dawley rats, daily, for 2 consecutive weeks. No information was found on the number of animals used. For serum analysis: 6 animals per treatment group were given a single oral dose of 20 mg/kg or 100 mg/kg.	Four metabolites of BPAF were identified in urine: BPAF diglucuronide, BPAF glucuronide, BPAF glucuronide dehydrated and BPAF sulfate. BPAF glucoronide was the major metabolite formed in vivo. Glucuronidation was a rapid and efficient pathway for biotransformation of BPAF. The peak of BPAF glucuronide in plasma was observed at 30 minutes after treatment, followed by a rapid decline in the next 3 hours, indicating a fast clearance in rats. The peak of BPAF was observed at 1 hour, and was completely eliminated after 48 hours. The three other metabolites were not found in plasma.		Li et al. 2013

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

No test guideline studies on toxicokinetics of BPAF are available. The section on toxicokinetics is based on data available in the open literature (experimental studies).

Absorption

Oral

In rats and mice, BPAF was rapidly absorbed after oral administration. The bioavailability was low (Waidyanatha et al. 2015).

Dermal

No dermal absorption study is available with BPAF.

Inhalation

No inhalation absorption study is available with BPAF.

Distribution

High levels of BPAF has been detected in gall bladder (Waidyanatha et al. 2015) and in liver, kidney and serum in rats after oral administration (Yang et al 2012). BPAF has also been detected in other organs, such as testis, after oral exposure. BPAF given orally to female rats during gestation and lacation showed that BPAF could transfer via cord blood and breast milk and distribute to offspring testes (Li et al. 2016).

Metabolism

The liver seems to be the major organ responsible for metabolism (Yang et al 2012). Four metabolites of BPAF has been identified in urine: BPAF diglucuronide, BPAF glucuronide, BPAF glucuronide dehydrated and BPAF sulfate. BPAF glucoronide was the major metabolite formed in vivo. Glucuronidation was rapid and efficient pathway for biotransformation of BPAF (Li et al. 2013). The peak of BPAF glucuronide in plasma was observed at 30 minutes, followed by a rapid decline in the next 3 hours. The peak of BPAF was observed at 1 hour, and BPAF was completely eliminated after 48 hours (Li et al. 2013).

Elimination

Waidyanatha et al. (2015) showed that BPAF was exreted primarily via bile. BPAF was found mainly in feces by 72 h in both rats and mice after oral administration (Waidyanatha 2015). The majority of fecal radioactivity (> 94%) was in the form of BPAF. BPAF metabolites are excreted via urine (Li et al. 2013). Female mice excreted a larger proportion of the dose in urine compared to male mice, and male mice excreted more in feces, compared to females (Waidyanatha et al. 2015).

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity oral route

Not evaluated in this CLH proposal.

10.2 Acute toxicity dermal route

Not evaluated in this CLH proposal.

10.3 Acute toxicity inhalation route

Not evaluated in this CLH proposal.

10.4 Skin corrosion/irritation

Not evaluated in this CLH proposal.

10.5 Serious eye damage/eye irritation

Not evaluated in this CLH proposal.

10.6 Respiratory sensitisation

Not evaluated in this CLH proposal.

10.7 Skin sensitisation

Not evaluated in this CLH proposal.

10.8 Germ cell mutagenicity

Not evaluated in this CLH proposal.

10.9 Carcinogenicity

Not evaluated in this CLH proposal.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 9: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Combined repeated dose toxicity study with reproduction/developmental toxicity screening test (oral:gavage) according to GLP. OECD Test Guideline 422 Rat (Sprague-Dawley) male/female Treatment groups: 12 males + 12 females per treatment group. Recovery groups: 5 males + 5 females per treatment group. Relability Score 1 according to registrant	2,2-Bis (4-hydroxyphenyl)-hexafluoropropane (Bisphenol AF). Purity 99.69%. Dose levels: 0, 30, 100, 300 mg/kg bw/day. Duration of exposure: Test groups and controls: 55 consecutive days (including a 2 week maturation phase, pairing, gestation and early lactation for females), Recovery groups: treated for 42 consecutive days and then maintained without treatment for 14 days. Recovery animals were not mated.	Pregnancy rates were reduced at all doses tested. None of the rats exposed to the highest dose (300 mg/kg bw/day) did achieve pregnancy. Fertility index was 100%, 83%, 64% and 0% for control, low, mid and high dose, respectively. Reproductive organs, including epididymides and testes, were significantly smaller (20 and 11%, respectively) in male rats treated at the highest dose (300 mg/kg bw/day). Female rats treated with BPAF (30 and 100 mg/kg bw/day) demonstrated significantly lower mean body weight (7-10%) during gestation and lactation periods.	Study report, 2011.
OECD Test Guidelines 407 Repeated Dose 28-day Oral Toxicity Study in Rodents, GLP compliant Rats Crj:CD(SD) rats Reliability Score 1 according to registrant	4,4´-(Hexafluoroisopropylidene)diphenol (Bisphenol AF) Dose levels: 0, 10, 30, 100 mg/kg/day Duration of exposure: 28 days, beginning at 8 weeks of age.	Significantly lower body weight gains (12%) were seen in high dose male rats. The absolute weights of reproductive organs, such as prostate and seminal vesicles were significantly lower in high dose males (-23 and -28%, respectively) compared to controls. Furthermore, significant atrophy of Leydig	Umano et al. 2012.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		cells was seen in high dose treated rats.	

Table 10: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 11: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Uterotrophic assay GLP compliant Reliability Score 3 according to registrant	BPAF: Purity 98.8%.	Crj:CD rats, 19-days-old at the start of the experiment, 6 animals per dose group. Dose levels: 0, 8, 40, 100 mg/kg/day Duration of exposure: subcutanous injection for 3 consecutive days.	BPAF exposure caused significantly increased uterine blotted weight at all doses tested. In addition, watery uterine contents were grossly detected in the high dose group (100 mg/kg). The body weights of treated animals were not significantly different compared to control animals.	Yamasaki et al. 2003.
Hershberger assay GLP compliant Reliability Score 3 according to registrant	BPAF: Purity 98.8%.	Male BRL Han:WIST Jcl (GALAS) rats, 6 animals per dose group. Dose levels: 0, 50, 200, 600 (400*) mg/kg/day Duration of exposure: oral gavage for 10 consecutive days, beginning at postnatal days 56. *Dose was reduced because toxic signs were observed during the study.	BPAF exposure at 600 mg/kg caused a significant increase in relative glans penis weight. However, there were signs of general toxicity at the two highest doses which included significantly decreased body weight gain and decreased spontaneous locomotion. In addition, the control values varied considerably and an androgen agonistic property could not be determined in this study.	Yamasaki et al. 2003.
Non-guideline study, in vivo experimental study Species, strain and sex: Adult rats (Sprague	BPAF	Doses: 0, 2, 10, 50, 200 mg/kg bw/day Duration of exposure: 14 days.	The concentrations of BPAF in the testes increased with increasing dose. Cholesterol levels in serum decreased significantly in animals in the two highest dose groups. Testosterone in serum decreased significantly in the high dose group, whereas luteinizing hormone and follicle-stimulating homone increased. Testicular mRNA levels of Inhibin B,	Feng et al. 2012.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Dawley) males Treatment groups: 8 animals per group.			estrogen receptor and lutenising hormone receptor decreased in high-dose animals.	
Zebrafish exposed to BPAF from 4 hour post-fertilization to 120 day-post-fertilization OECD TG 236	BPAF	Long-term effects of BPAF on hormonal balance and genes of hypothalamus-pituitary-gonad axis and liver of zebrafish, and the impact on offspring were studied. Dose levels: 0, 5, 25, 125 µg/L	The fertilization rate of spawn eggs was significantly decreased at the highest concentration tested. In the F1 generation, the malformation rate was significantly increased in the high dose group, and survival rate was lower compared to controls. Male zebrafish (F0) exposed to BPAF had significantly increased concentrations of plasma 17β-estradiol (E2) compared to controls, and significantly decreased levels of plasma testosterone. In females (F0), E2 levels were significantly increased in the high dose group. Vitellogenin gene expression was significantly increased in the liver of treated male zebrafish (F0). In the testis, expressions of fshr, cyp19a and cyp11a1 were significantly increased in the high dose group, and star and cyp17 were significantly decreased. In ovaries, fshr was significantly increased and star was decreased, compared to control group.	Shi et al. 2015
Zebrafish exposed to BPAF for 28 days.	BPAF	Dose levels: 0, 0.05, 0.25, 1.0 mg/L	Exposure to 1 mg/L BPAF caused liver damaged in male fish, but no effects were seen in females. The highest dose affected the testis and caused a retardation of oocyte development in the ovaries. In male fish, the testosterone levels decreased dose-dependently, while estradiol levels increased (at 1.0 mg/L in males and at 0.05 and 0.025 mg/L in females). Upregulated vitellogenin was seen in both sexes.	Yang et al. 2016
Zebrafish exposed to BPAF for 21 days.	BPAF	Transcription of genes related to thyroid endocrine disruption in male zebrafish following exposure to	BPAF affected genes related to thyroid hormones production and receptor activation, thyroid gland development and deiodinase activity.	Kwon et al. 2016

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		BPAF was studied. Dose: 24.7 µg/L		
Zebrafish larvae exposed to BPAF short-term. OECD TG 236	BPAF	Whole-body total 3,3′,5-triiodothyronine, total 3,5,3′,5′-tetraiodothyronine, free 3,3′,5-triiodothyronine and free 3,5,3′,5′-tetraiodothyronine levels were examined following 168 h post-fertilization exposure to BPAF. Dose levels: 0, 5, 50, 500 µg/L	The results showed that thyroid hormones decreased significantly after BPAF treatment, indicating endocrine disruption of the thyroid. The expression of genes involved in the hypothalamic-pituitary-thyroid axis was also affected by BPAF exposure.	Tang et al. 2015
In vitro study in human breast cancer cells	BPAF		Binding of BPAF to G protein-coupled estrogen receptor (GPER). BPAF had a 9-fold stronger binding affinity than BPA. BPAF exhibited stronger agonistic activity to GPER compared to BPA.	Cao et al. 2017
In vitro study in human cervical cancer cell line (HeLa)	BPAF		BPAF bound to estrogen receptor with a receptor-binding activity three times stronger for ERβ than for ERα. BPAF fully activated ERα in a dose-dependent manner, but was almost completely inactive for Erβ.	Matsushima et al. 2010
In vitro study in human breast cancer cell lines	BPAF		BPAF functioned as an agonist of ERα at lower concentrations (nanomolar order), and as an anti-estrogenic compound via the induction of ERβ at higher concentrations.	Okazaki et al. 2017
In vitro study in human breast cancer cell lines	BPAF		Estrogenicity of BPA analogues was studied. BPAF exposure stimulated cell growth in an ER-mediated cell proliferation assay and induced estrogen response element-mediated transcription in a luciferase assay. BPAF was the most potent BPA analogue, followed by BPB, BPZ, BPA, BPAP and BPS.	Mesnage et al. 2017
In vitro study in human breast cancer cell line and two-hybrid yeast screen systems	BPAF		BPAF was more potent than BPA for estrogenic and thyroidal effects in yeast (ERa and thyroidal hormone receptor a agonistic activity).	Lei et al. 2017

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In vitro study in human adrenocortical cell line	BPAF		Exposure to BPAF altered steroidogenesis in H295R cells. BPAF induced progesterone and reduced testosterone levels. BPAF was more potent in inducing cell toxicity compared to BPA, BPS and BPF.	Feng et al. 2016
In vitro study in Yeast (XenoScreen XL YES/YAS assay)	BPAF		BPAF demonstrated agonistic estrogenic activity in a yeast assay. BPAF was more potent than BPA. BPAF also showed anti-androgenic activity with a higher potency compared to BPA.	Fic et al. 2014
In vitro study: NIH3T3 Luciferase Reporter Assay	BPAF		BPAF demonstrated clear agonistic estrogenic activity in the MCF-7 Estrogen Luciferase Reporter Assay. BPAF showed inhibitory effects on the androgenic activity of 5a-dihydrotestosterone in mouse fibroblast cell line NIH3T3.	Kitamura et al. 2005
In vitro study in African green monkey kidney CV1 cells	BPAF		BPAF demonstrated agonistic estrogenic activity and and acted as an AR antagonist in this luciferase reporter assay.	Teng et al. 2013

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) of BPAF in rat (Study report, 2011)

In an OECD TG 422 guideline study, BPAF was administered by gavage to 3 dose groups each of 12 male and 12 female Sprague-Dawley Crl:CD (SD) IGS BR Strain rats, for 55 days (including a 2 week maturation phase, pairing, gestation and early lactation for females), at dose levels of 30, 100 and 300 mg/kg/day. A control group of 12 males and 12 females was dosed with vehicle alone (Arachis oil BP).
Two recovery groups (5 males and 5 females), were treated with the high dose (300 mg/kg/day) or the vehicle alone for 42 consecutive days and then maintained without treatment for 14 days. Recovery animals were not mated.

Fertility, parturition and sexual function

The number of pairing days until mating did not vary significantly between controls and treated animals (in average 3.9 days for controls and 4.0 days for high dose animals). The number of estrous stages without mating was slightly higher in mid- and high dose females compared to control animals (0.9 ±1.3 and 0.6 ± 1.2, vs. 0 in controls). Irregular estrous cycles were observed in 2 of 11 high dose animals (18%) and in 1 of the mid dose females (8%).

Sperm reading scores (based on number of sperm detected) indicate sperm effects from treatment, since high dose males had scores of 0 (n=1), +1 (n=3), +2 (n=1) and +3 (n=5) compared to control males which all had scores of +3 (n=11). (Score 1+: few spermatozoa present, score 2+: continuous few spermatozoa in all fields, and score 3+: many spermatozoa in all fields)

The mating index did not differ signficantly between controls and treated animals (91% in high dose animals vs 92% in controls) (Table 12). However, exposure to BPAF did have a clear impact on the pregnancy outcomes, since no pregnancy was induced in any of the high dose females that mated (10 of 11 animals, since 1 animal pair did not mate). The incidence of non-pregnant females increased with increasing dose and the fertility index was 100%, 83%, 64% and 0 for controls, low-, mid- and high dose groups, respectively.

Pre-implantation losses were higher in mid dose animals compared to controls (19% vs. 12%), however not significantly higher. There were large individual variations within the dose groups, which resulted in large standard deviations for this parameter. The number of corpora lutea and implantations were lower in treated females compared to controls (14.0 vs. 16.7 and 10.4 vs. 14.1, respectively), however, not significantly lower (Table 12). Implantation index was lower, 81% in mid dose females vs. 88% in controls. Total litter loss was observed for one female each of the low- and mid dose group, compared to none among control females.

Table 12: Fertility parameters, OECD TG 422 study, 2011

Dose levels (mg/kg bw/day)	**0**	**30**	**100**	**300**
No. of pairs examined	12	12	12	11
Estrous cycle (days)	4.0 ± 0.0	3.9 ± 0.2	4.3 ± 0.4	4.2 ± 0.2
Irregular estrous cycle	0/12	0/12	1/12 (8%)	2/11 (18%)
No. of pairs with successful mating	11	12	11	10
Mating index (%) = (No. of pairs with successful mating/ No. of pairs examined) X 100	91.7	100.0	91.7	90.9
No. of pregnant females	11	10	8	0
Fertility index (%) = (No. of pregnant animals/ No. of pairs with successful mating) x 100	100.0	83.3	63.6	0
Pairing days until mating	3.9 ± 3.5	2.3 ± 1.3	2.4 ± 1.4	4.0 ± 4.1
No. of estrous stages without mating	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 1.2	0.9 ±1.3
Total litter loss in utero	0	1 of 10 (10%)	1 of 8 (13%)	-
Gestation length (days)	22.9 ± 0.6	23.0 ± 0.6	22.9 ± 0.2	-
No. of corpora lutea	16.7 ± 3.6	14.7 ± 5.8	14.0 ± 7.8	-
No. of implantations	14.1 ± 1.9	12.1 ± 3.8	10.4 ± 4.8	-
Implantation index (%) = (No. of implantation sites/ No. of corpora lutea) x 100	88.1 ± 13.2	89.4 ± 18.2	81.3 ± 16.8	-
Pre-implanation loss (No. of corpora lutea - No. of implantation sites/No. of corpora lutea x 100) (mean %)	11.9 ± 13.2	10.6 ± 18.2	18.7 ± 16.8	-

Reproductive organ weights and histopathology

Males

The absolute weights of the epididymides and testes were significantly lower in the high dose males compared to control animals (20 and 11%, respectively) (Annex I, Table 3). Similarly, the relative weight of epididymides in the high dose group was significantly lower (13%) compared to control animals.

Significantly reduced secretory content in the prostate was seen in treated animals and the incidence increased with increasing dose (Table 13). This effect was present in 67% of high dose animals and in 80% of high dose recovery males, compared to none in control animals. In addition, significantly reduced secretory content of seminal vesicles was observed in all high dose males (in 100% vs. 8% of control animals) and the incidence increased with increasing dose.

Leydig cell atrophy was present in 11 of 12 high dose males (92%) compared to 1 of 12 (8%) of control animals (Table 13). In the mid dose group, 3 of 12 males showed this effect. There was also moderate to severe atrophy of testes in 2 of 5 high dose recovery males.

Fifty percent of high dose males showed tubuloalveolar differentiation of the mammary glands (slight and moderate effects) (Table 13). In high dose recovery males, 4 of 5 animals demonstrated this effect. No effects on mammary glands were seen in control animals.

Table 13: Number of male animals with histopathological findings in reproductive-related organs. Incidence in percent in parenthesis. Study report, 2011

Dose levels (mg/kg/day)	**0**	**30**	**100**	**300**	**Recovery 0**	**Recovery 300**
No. of animals	n = 12	n = 12	n = 12	n = 12	n= 5	n= 5
Mammary gland - Tubuloalveolar differentiation						
No section	2	2	2	3	0	0
Absent	7	4	3	1	5	1
Minimal	3 (25%)	4 (33%)	6 (50%)	2 (17%)	0	0
Slight	0	2 (17%)	1 (8%)	4 (33%)	0	1 (20%)
Moderate	0	0	0	2 (17%)	0	3 (60%)
Prostate - Reduced secretory content						
No section	0	0	1	0	0	0
Absent	23	11	7	4	5	1
Present	0	1 (8%)	4 (33%)	8 (67%)	0	4 (80%)
Prostate - Chronic inflammatory cell foci						
Absent	12	12	11	11	5	5
Slight	0	0	0	1 (8%)	0	0
Seminal vesicles – Reduced secretory content						
Vesicle 1						
No section	0	0	1	0	0	0
Absent	11	10	5	0	5	2

Dose levels (mg/kg/day)	**0**	**30**	**100**	**300**	**Recovery 0**	**Recovery 300**
Present	1 (8%)	2 (16%)	6 (50%)	12 (100%)	0	3 (60%)
Vesicle 2						
No section	0	0	1	0	5	5
Absent	11	10	6	0	0	0
Present	1 (8%)	2 (16%)	5 (42%)	12 (100%)	0	0
Testes - Atrophy						
Testis 1						
Absent	12	12	12	12	5	3
Moderate	0	0	0	0	0	1 (20%)
Severe	0	0	0	0	0	1 (20%)
Testis 2						
Absent	12	12	12	12	5	4
Severe	0	0	0	0	0	1 (20%)
Leydig cell						
Absent	11	12	9	1	5	4
Present	1 (8%)	0	3 (25%)	11 (92%)	0	1 (20%)

Pregnant females

In mid dose females that got pregnant, a higher incidence of follicular/fluid-filled cysts in the ovaries was observed compared to controls (43% vs. 18%) (Annex I, Table 6).

Cystic corpora lutea were found in the ovaries among both pregnant controls and treated animals (in 45% of control animals, vs. 14% in mid dose females).

Non-pregnant females

Minimal glandular hyperplasia of the mammary gland was seen in 4 of 11 (36%) of the high dose group among non-pregnant females (Table 14). Ovarian cysts were found in several of the non-pregnant females of each treatment group. Nine of the non-pregnant high dose females (82%) had follicular/fluid-filled cysts on the ovaries, which was absent in the control female that did not mate. In addition, a higher incidence of follicular/fluid-filled cysts in the ovaries was found in 4 of 5 (80%) of the high dose recovery females (Annex I, Table 6).

Five of 11 (45%) of the high dose (non-pregnant) females had minimal follicular cell hypertrophy of the thyroid, an effect also noted in 1 animal in the low dose group. However, since the number of non-pregnant females was much lower in the lower dose groups and controls, the incidence results should be interpreted with caution.

Effects of uterus/cervix and vagina (dilatation horn, endometrial gland proliferation and keratinisation cervix and epithelial hyperplasia, epithelial keratinisation and keratin cysts, respectively) were observed in a few non-pregnant female animals of all dose groups, but not in the single non-pregnant control female. Epithelial hyperplasia of the vagina was seen in 4 (36%) of the non-pregnant high dose females compared to none in the other dose groups.

Table 14: Number of female animals with histopathological findings in reproductive-related organs, only females that failed to mate/non-pregnant. Incidence in percent in parenthesis. Study report, 2011

Dose levels (mg/kg/day)	**0**	**30**	**100**	**300**
No. of animals	n=1	n=2	n= 4	n= 11
No. animals that failed to mate	1 of 12 (8%)	0	1 of 12 (8%)	1 of 11 (9%)
No. of animals not pregnant	0	2 of 12 (16%)	3 of 12 (25%)	10 of 11 (90%)
Mammary gland				
Glandular hyperplasia (minimal)	0	0	0	4 of 11 (36%)
Ovaries				
Cystic corpora lutea	0	1 of 2 (50%)	1 of 4 (25%)	3 of 11 (27%)
Follicular/fluid-filled cyst	0	0	2 of 4 (50%)	9 of 11 (82%)
Haemorrhagic cyst	0	0	0	1 of 11 (9%)
Vacuolation stroma	0	0	0	2 of 11 (18%)
Thyroid				
Follicular cell hypertrophy (minimal)	0	1 of 2 (50%)	0	5 of 11 (45%)
Uterus/Cervix				
Dilatation horn 1				
Minimal	0	0	1 of 4 (25%)	1 of 11 (9%)
Slight	0	1 of 2 (50%)	1 of 4 (25%)	1 of 11 (9%)
Dilatation horn 2				
Minimal	0	0	2 of 4 (50%)	1 of 11 (9%)
Slight	0	1 of 2 (50%)	0	1 of 11 (9%)
Endometrial gland proliferation	0	0	0	1 of 11 (9%)
Keratinisation cervix	0	2 of 2 (100%)	3 of 4 (75%)	1 of 11 (9%)
Vagina				
Epithelial hyperplasia				
Minimal	0	0	0	4 of 11 (36%)
Epithelial keratinisation	0	2 of 2 (100%)	1 of 4 (25%)	2 of 11 (18%)
Keratin cyst	0	0	0	1 of 11 (9%)

General toxicity

Clinical signs included increased salivation and staining around the mouth post-dosing for animals in all treatment groups (in a dose-response manner). Dehydration and staining around the ano-genital region was observed for two high dose females. One high-dose female, which was killed on Day 6, demonstrated severe clinical signs that were considered caused by incorrect administration of the test substance. There were no

effects observed related to behaviour, functional performance or sensory reactivity in any of the treated groups.

No significant changes in mean body weights for the treated males were seen, although there was a tendency of lower weights among animals in mid- and high dose groups compared to controls (Annex I, Table 3). In recovery males, however, significant lower mean weights were demonstrated in treated animals (16% and 22% compared to controls, on days 22 and 43, respectively) (data not shown). It is noted that recovery control males had a higher mean body weight than non-recovery control males. Two weeks off treatment the weights of high dose treated animals (recovery group) were still significantly lower compared to controls (17%).

Table 15: Mean female body weights (g), Study report, 2011

Dose levels (mg/kg/day)	**0**	**30**	**100**	**300**
Maturation, No. of females	n = 12	n = 12	n = 12	n = 11
Day 1	241 ± 15	238 ± 14	233 ± 10	242 ± 11
Day 15	256 ± 15	246 ± 17	239 ± 14	250 ± 16
Gestation, No. of females	n = 11	n = 9	n = 6	No pregnant animals
Day 0	271 ± 20	253 ± 22	248 ± 17	
Day 20	421 ± 31	**377* ± 32** (-10%)	382 ± 39	
Lactation, No. of females	n = 11	n = 9	n = 7	
Day 0	324 ± 20	**301* ± 27** (-7%)	**292* ± 13** (-10%)	
Day 4	332 ± 24	**300** ± 25** (-10%)	**301* ± 15** (-9%)	

*p<0.05, **p<0.01

The mean body weight *change* during week 1 of maturation was significantly lower in the mid- and high dose groups compared to control (0 and 1%, respectively, compared to 4% for control animals). However, there were no significant differences on mean body weights among females in the different dose groups during maturation weeks 1 and 2. A significantly lower mean body weight (10%) was observed for females in mid dose group compared to controls at day 20 of gestation (Table 15). During lactation (day 0 and 4) both the low- and mid dose group females demonstrated significantly lower mean body weights compared to controls (range: 7 to 10%). Among recovery females there were no significant differences in body weights between control and exposed animals (measured at days 1, 8, 15, 22, 29, 36, 43, 50 and 57).

Among treated males, the mean food consumption was significantly lower in the two highest dose groups (9% and 22%, respectively) during the first week of treatment. Treated females (mid- and high dose groups) also demonstrated significantly lower food consumption during maturation week 1 (19% and 25% compared to controls, respectively). During gestation days 7-14, females in the low- and mid dose groups had a lower food intake (13%) and during days 14-20, the mid dose group demonstrated lower food intake (11%) compared to controls. No high dose females (non-pregnant) were included in comparative evaluations after maturation and mating weeks.

Water consumption in males was significantly higher among animals in all treatment groups compared to controls at all assessments points (week 1-2 and weeks 5-6). In the high dose group, water consumption ranged between +19% and +39% compared to controls. Water consumption increased significantly also in

treated females during pre-mating days 1-7 (mid- and high dose groups), with an increase of 30% and 39% compared to controls, respectively, and during days 8-14 (high dose group; 11%).

Few changes of hematology parameters or blood chemistry of toxicological relevance/significance were reported. Measurements were conducted at day 14 and day 42/day 4 post partum. Significant reductions in haemoglobin and erythrocyte counts in high dose males were demonstrated prior to termination on day 42 (8 and 9%, respectively). Furthermore, ALAT values were significant higher (35-37%) in mid- and high dose males compared to control animals at day 42. Among high dose female rats, the ALAT value was significantly higher (74%) compared to control animals during the maturation phase (day 14).

The mean relative weights of adrenals and liver were significantly higher in males of the high dose group (25 and 10%, respectively) compared to controls (Annex I, Table 3). In recovery animals, the mean absolute liver weight of the high dose males was significantly lower compared to control animals (18%) and relative organs weights for adrenal, brain, spleen and thymus were significantly higher compared to controls (data not shown).

The mean relative brain weights of females in the low- and mid dose groups were significant higher compared to controls (7% and 9%, respectively) (Annex I, Table 3). Likewise, the mean absolute heart weights were significantly lower in the low- (17%) and mid dose (15%) females compared to control animals. No significant effects on organ weights were seen in treated recovery females (data not shown). Non-pregnant females in the high dose group were not included in comparative evaluations after maturation and mating.

Conclusion

A clear effect on fertility was evident in high dose animals (300 mg/kg bw/day) in the Study report, 2011. Among the females treated at this dose level, which mated, no pregnancies were achieved. The incidence of animals without induced pregnancy increased with increasing dose starting from the lowest dose (30 mg/kg bw/day). No marked general toxicity was noted.

28-day repeated dose toxicity test (OECD TG 407) of BPAF in rats (Umano et al. 2012)

A repeated-dose toxicity study conducted according to the OECD test guideline 407 (in vivo screening tests to detect endocrine-mediated effects) was performed using Crj:CD rats. Rats were orally gavaged for 28 days with 0, 10, 30 and 100 mg/kg bw/day BPAF, each dose group comprised 10 males and 10 females.

Reproductive organs and histopathology

Absolute weights of prostate, ventral prostate, and seminal vesicle were significantly lower in the high dose group (23%, 25%, and 28%, respectively) (Table 16).

Histopathological findings demonstrated significant atrophy of testicular Leydig cells in high dose males (Table 17). Atrophy of the mammary glands was also seen in 3 of 10 males in the high dose group, compared to none in the other groups, however this effect was not significantly altered compared to controls.

A sperm analysis did not reveal any abnormalities in treated males.

In females, there were no signs of histopathological effects. However, irregular estrous cycles were observed in females in mid- and high dose groups.

General toxicity

In the high dose group of male rats, mean body weights were significantly lower (12%) compared to controls (Table 16), an effect also seen in females rats in the mid- (7%) and high dose (8%) groups. In high dose males white blood cell counts, total cholesterol and albumin values were significantly lower. Among female rats cholinesterase and total cholesterol values were lower than for controls, and total bilirubin values were

higher among high dose animals. Serum T4 levels were significantly higher in the high dose group of both sexes, compared to controls.

Organ weight measurements revealed significantly higher relative weights in high dose males of kidney (9%), adrenals (23%) and brain (15%), whereas liver (18%), heart (12%) and spleen (17%) were significantly lower in this dose group. Histopathological examination revealed significant hypertrophy of the adrenal zona fasciculate, and decreased hepatocytic glycogen in the high dose males, compared to controls (Table 17). Among high dose females the absolute heart weight was significantly lower (10%) and the relative brain weight was higher (8%) (Table 16).

Conclusion

Significantly lower absolute weights of reproductive organs were observed among BPAF treated males. The effects observed on mammary glands in males, testis and estrous cycle indicate endocrine-mediated (estrogenic) mechanisms underlying the toxicity of BPAF.

Table 16: Body weights and organ weights (mean ± SD) (Umano et al. 2012)

Dose levels (mg/kg/day)	**0**	**10**	**30**	**100**
Males	n = 9	n = 10	n = 10	n = 10
Initial body weight (g)	324 ± 14	324 ± 13	325 ± 15	326 ± 13
Terminal body weight (g)	449 ± 27	451 ± 26	450 ± 33	**396** ± 29**
Prostate (mg)	1068 ± 188	1134 ± 179	1061 ±189	**827* ± 183**
Prostate (g/100 g)	0.237± 0.045	0.251 ± 0.031	0.236 ± 0.041	0.208 ± 0.039
Ventral prostate (mg)	631 ± 166	741 ± 110	676 ± 150	**474* ± 112**
Ventral prostate (g/100 g)	0.139 ± 0.034	0.164 ± 0.021	0.150 ± 0.030	0.120 ± 0.027
Seminal vesicle (g)	1.41 ± 0.19	1.43 ± 0.30	1.38 ± 0.24	**1.02* ± 0.36**
Seminal vesicle (g/100 g)	0.312 ± 0.045	0.317 ± 0.069	0.307 ± 0.045	0.254 ± 0.079
Liver (g)	17.13 ± 2.18	16.90 ± 1.37	16.38 ± 2.85	**14.10** ± 1.31**
Liver (g/100 g)	3.778 ± 0.322	3.747 ± 0.179	3.623 ± 0.399	3.564 ± 0.271
Kidney (g)	3.02 ± 0.23	3.01 ± 0.18	3.00 ± 0.37	2.89 ± 0.30
Kidney (g/100 g)	0.667 ± 0.021	0.671 ± 0.058	0.666 ± 0.064	**0.729* ± 0.043**
Heart (g)	1.28 ± 0.09	1.32 ± 0.08	1.28 ± 0.09	**1.13** ± 0.13**
Heart (g/100 g)	0.283 ± 0.013	0.293 ± 0.018	0.285 ± 0.011	0.286 ± 0.017
Spleen (g)	0.71 ± 0.08	0.70 ± 0.08	0.71 ± 0.10	**0.59* ± 0.09**
Spleen (g/100 g)	0.158 ± 0.018	0.155 ± 0.015	0.158 ± 0.020	0.149 ± 0.016
Adrenals (mg)	58 ± 8	58 ± 11	56 ± 4	63 ± 9
Adrenals (g/100 g)	0.013 ± 0.002	0.013 ± 0.002	0.012 ± 0.001	**0.016** ± 0.003**
Brain (g)	2.17 ± 0.05	2.21 ± 0.09	2.23 ± 0.06	2.19 ± 0.07
Brain (g/100 g)	0.482 ± 0.034	0.491 ± 0.032	0.498 ± 0.036	**0.554** ± 0.035**
Females	n = 10	n = 10	n = 10	n = 10
Initial body weight (g)	211 ± 7	215 ± 11	213 ± 10	214 ± 11
Terminal body weight (g)	274 ± 18	277 ± 18	**255* ± 18**	**253* ± 15**

Dose levels (mg/kg/day)	0	10	30	100
Heart (g)	0.87 ± 0.08	0.88 ± 0.05	0.82 ± 0.11	**0.78* ± 0.06**
Heart (g/100 g)	0.316 ± 0.015	0.319 ± 0.021	0.321 ± 0.024	0.308 ± 0.020
Brain (g)	2.03 ± 0.05	2.00 ± 0.06	2.05 ± 0.10	2.02 ± 0.08
Brain (g/100 g)	0.742 ± 0.042	0.725 ± 0.044	**0.809* ± 0.070**	**0.799* ± 0.039**

*Significantly different from control, p<0.05 ** Significantly different from control, p<0.01

Table 17: Significant histopathological findings in male rats (Umano et al. 2012)

Dose levels (mg/kg/day)	0	10	30	100
Testis: atrophy of Leydig cells	0	0	0	**5***
Adrenal gland: hyperthrophy of zona fasciculata	1	1	0	**8****
Liver: decreased hepatocytic glycogen	1	0	1	**8****

*Significantly different from control, p<0.05 ** Significantly different from control, p<0.01

Uterotrophic assay and Hershberger assay, Yamasaki et al. 2003

Yamasaki et al. studied estrogenic and androgenic effects of BPAF given on 3 consecutive days to 19-day-old rats in the uterotrophic assay, at doses 0, 8, 40 and 100 mg/kg per day, and for 10 consecutive days in the Hershberger assay at doses 0, 50, 200 and 600 mg/kg per day. BPAF tested positive in the uterotrophic assay (dose-response), with significantly increased uterine blotted weight at all doses tested, suggesting estrogenic agonistic properties of BPAF. In addition, watery uterine contents were detected in the high dose group (100 mg/kg) (Table 18). No significant differences on body weights were seen among treated animals, compared to controls.

In the Hershberger assay (0, 50, 200 and 600 mg/kg) the relative glans penis weight increased significantly in rats given 600 mg/kg BPAF per day (Table 19). However, there were signs of general toxicity at the two highest doses which included significantly decreased body weight gain and decreased spontaneous locomotion. In addition, the control values for this organ varied considerable, and according to the authors an androgen agonistic property could not be determined in this study.

Table 18: Results from an uterothropic assay, Yamasaki et al. 2003

Dose levels (mg/kg/day)	0	8	40	100
Body weight (g)	56.1 ± 4.3	55.0 ± 4.5	56.6 ± 4.0	54.7 ± 4.2
Uterus blotted weight, absolute (mg)	28.6 ± 4.9	**47.2** ± 9.9**	**65.9** ± 9.8**	**96.4** ± 9.0**
Uterus blotted weight, relative (mg/100 g)	50.9 ± 7.4	**85.1** ± 11.9**	**116.0** ± 11.7**	**177.2** ± 22.2**

** Significantly different from control at p<0.01.

Table 19: Results from the Hershberger assay, Yamasaki et al. 2003

Dose levels (mg/kg/day)	0	50	200	600 (400)
Body weight (g)	275.1 ± 9.7	257.09 ± 18.2	**259.99* ± 12.8**	219.69 ± 30.9
Ventral prosate (mg/100 g bw)	5.89 ± 0.9	5.59 ± 1.5	5.79 ± 1.4	6.29 ±1.6
Seminal vesicle (mg/100 g bw)	11.39 ± 2.1	12.09 ± 1.8	13.29 ± 2.3	13.69 ± 1.2
BC/LA (mg/100 g bw)	53.29 ± 6.3	55.99 ± 8.4	**44.29* ± 4.7**	48.99 ± 3.5

Dose levels (mg/kg/day)	0	50	200	600 (400)
Glans penis (mg/100 g bw)	12.69 ± 1.0	12.89 ± 1.1	12.29 ± 2.0	**15.19* ± 1.8**
Cowper's gland (mg/100 g bw)	1.59 ± 0.5	1.79 ± 0.5	1.39 ± 0.4	1.79 ± 0.3

* Significantly different from control at p<0.05.

Mode of action – mechanistic information supporting adverse effects seen in vivo

Several mechanistic studies on the effects of BPAF in zebrafish, and in vitro, are available. Short summaries of these are found in Table 11. Results consistently indicate estrogenic and anti-androgenic effects of BPAF, mechanisms of relevance for the effects seen on fertility in vivo. Several in vitro studies also show that BPAF can cause DNA damage, oxidative stress and induce apoptosis (Lee et al. 2013, Mokra et al. 2017, Lei et al. 2017, Ding et al. 2017).

Summary of available studies

In an OECD TG 422 study (GLP compliant) BPAF was administered to 12 male and 12 female Sprague-Dawley rats, for 55 days (including a 2 week maturation phase, pairing, gestation and early lactation for females), at dose levels of 0, 30, 100 and 300 mg/kg/day.

A clear effect on fertility was evident in high dose animals. Among the females treated at this dose level, which mated, no pregnancies were achieved. The incidence of animals without induced pregnancy increased with increasing dose. Pre-implantation and post-implantation losses were higher in treated animals compared to controls, but not significantly different. Total litter loss was observed for one female each from the low- and mid dose groups, compared to none among control females.

Among high dose treated females (which all were non-pregnant), the incidence of follicular cysts of the ovaries was high (82%). The same effect was seen in 80% of high dose females (recovery group) compared to none of the controls. In females that got pregnant (control, low- and mid-dose groups), the incidence of ovarian cysts increased with increasing dose. Effects on uterus/cervix and vagina were also observed in a few non-pregnant female animals of all dose groups, but not in the single control female that was non-pregnant.

In some of the treated females exposed to BPAF at 300 mg/kg bw/day in the OECD TG 422 study (and at 100 mg/kg bw/day in the OECD TG 407 study) irregular estrous cycles were seen. However, no differences were detected in the mating performance between controls and treated animals in the TG 422 study. In high dose males, absolute and relative weights of several reproductive organs were significantly smaller compared to controls, including epididymides and testes. In the 28-days repeated dose toxicity study (OECD TG 407), similar effects on males reproductive organs were seen in animals treated with BPAF at 100 mg/kg bw/day, including lower absolute weights of prostate and seminal vesicles. Correspondingly, histopathological effects were found in these organs, including Leydig cell atrophy in testes and reduced secretory contant in prostate and seminal vesicles. In addition, tubuloalveolar differentiation and atrophy of mammary glands were demonstrated among males of all dose groups, in both studies.

Although a few signs of systemic toxicity were observed in the Study report (2011) (reduced body weight, decreased food consumption, increased water consumption) the general toxicity of BPAF at the dose levels tested appears not to be marked. Thus, the reduced fertility seen at all doses (dose response) is considered a direct effect of the substance. Since treatment-related effects were seen in both treated males and females, both sexes may be affected by the substance, leading to a reduced fertility.

Results from the updated OECD TG 407 (28 days study with possibility to detect endocrine-mediated effects) demonstrated effects on mammary glands, testis and estrous cycle, indicating endocrine-mediated mechanisms involved (estrogenic effects). This result is in line with data from an uterotrophic assay that revealed significantly increased uterine blotted weight at all doses tested, suggesting estrogenic agonistic properties of BPAF. Several mechanistic studies on zebrafish and in vitro further support the estrogenic properties of BPAF. In addition, several comparative studies with BPA indicate a stronger estrogenic potency for BPAF.

BPAF is a structural analogue to BPA, which has a harmonised classification as Repr. 1B (H360F). Effects on sexual function and fertility of BPA mentioned as evidence in RAC's opinion[1] from 2014 include decreased ovarian weights and increased number of follicular cysts on ovaries in females, decreased serum testosterone and sperm production, and effects on reproductive organs in males.

Furthermore, adverse effects by BPA on fertility included decreased number of litters, litter size and number of live pups per litter, reported at 600 and 1200 mg/kg bw/day (mice) and at 500 mg/kg bw/day (rats)[1]. Based on the findings summarised in the current proposal, BPAF seems to be more potent than BPA with a lower fertility index observed already at the lowest dose (30 mg/kg bw/day). However, it is not clear how the mechanisms underlying these effects differ from BPA.

10.10.3 Comparison with the CLP criteria

The criteria for classification in Repr. 1B for adverse effects on sexual function and fertility are considered fulfilled since: A clear effect on fertility was evident in high dose animals in the Study report, 2011. Of the females treated at this dose level, which mated, no pregnancies were achieved. The incidence of animals without induced pregnancy increased with increasing dose.

In a total weight of evidence the available data provide clear evidence of an adverse effect on both male and female sexual function and fertility and there is no mechanistic evidence to indicate that the observed effects are not relevant for humans. Classification in Repr. 1B, H360F is therefore warranted.

Classification in Repr 1A is not appropriate as it should be based on human data and no human data are available.

Classification in Repr. 2 is not appropriate as the evidence for adverse effects on sexual function and fertility from existing experimental data on BPAF is considered as clear evidence and not some evidence.

10.10.4 Adverse effects on development

Table 20: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (oral:gavage) according to GLP. OECD Test Guideline 422 Species, strain and sex:Rat (Sprague-Dawley) males and females Treatment groups: 12 males + 12 females per treatment group. Reliability Score 1	BPAF. Purity: 99.69% Doses: 0, 30, 100, 300 mg/kg bw/day.	Pregnancy rates were reduced at all doses tested. None of the rats exposed to the highest dose (300 mg/kg bw/day) did achieve pregnancy. Female rats treated with BPAF (30 and 100 mg/kg bw/day) demonstrated lower mean body weight (7-10%) during gestation and lactation periods, compared to controls. (High dose non-pregnant females were not included in comparative evaluations after maturation and mating). No adverse effects were seen on offspring exposed to BPAF in utero (30 and 100 mg/kg bw/day). However, no offspring was produced in the highest dose group (300 mg/kg	Study report, 2011.

[1] RAC Opinion proposing harmonised classification and labelling at EU level of Bisphenol A; 4,4'-isopropylidenediphenol: https://www.echa.europa.eu/documents/10162/51436fe4-c531-e3aa-dbe8-42f9c75c83db

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
according to registrant		bw/day).	

Table 21: Summary table of human data on adverse effects on development

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data.				

Table 22: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Non-guideline study, in vivo experimental study Effects of BPAF on development and long-term health of the mammary gland in female offspring was investigated.	BPAF. Purity 98%	Doses: 0, 0.05, 0.5, 5 mg/kg bw given twice per day Species, strain and sex: Mice: CD-1 (Pregnant females) Treatment groups: 10-12 animals per group. Duration of exposure: GD 10.5 to GD 17.5. Offspring was followed for up to 16 months.	BPAF exposure caused accelerated pubertal mammary development. By 14 months of age, a significant dose-related increase in non-neoplastic lesions was found in BPAF-exposed groups, including cysts, inflammation, lobuloalveolar hyperplasia and squamous metaplasia.	Tucker et al. 2018.
Non-guideline study, In vivo experimental study Effects of gestational and lactational exposure to BPAF on male offspring were studied. Reliability Score 4 according to registrant	BPAF. Purity 97%	Species, strain and sex: Rats: Sprague Dawley females Treatment groups: 30 females per dose group (GD 3-19), 15 females per dose group (PND 3-19). Doses: 0, 100 mg/kg bw/day Duration of exposure: GD 3 to GD 19 and PND 3 to PND 19.	Lactational exposure caused significantly increased levels of BPAF in serum and in testis, showing that BPAF was transferred via breast milk. Gestational and lactational exposure lead to increased testosterone and decreased Inhibin B levels in male offspring. Androgen receptor levels in testes increased following BPAF exposure.	Li et al. 2016.
Non-guideline in vivo study of the effects on maternal BPAF exposure during	BPAF	Pregnant mice were orally exposed daily from GD 1 to GD 19. On PND 35, 10 pups per litter were randomly selected to undergo	Fetal exposure to BPAF induced anxiety- and depressive-like behaviours in male adolescent offspring. In addition, BPAF exposure	Gong et al 2017.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
pregnancy on neurobehaviours in adolescent mice offspring.		behavioural tests. Doses: 0, 0.4, 4 mg/kg bw/day	impaired memory formation in both sexes.	
OECD TG 236 Effects of BPAF exposure on zebrafish embryo-larvae	BPAF	Acute toxicity, teratogenic and estrogenic effects of BPAF were studied in zebrafish embryo-larvae. Doses: 0, 0.5, 0.75, 1.0 , 2.0 mg/L	BPAF was the most acute toxic substance among several bisphenols tested, and the most potent bisphenol of those tested for developmental effects. The substance caused cardiac edema. BPAF was the most potent studied estrogen in an estrogen-responsive transgenic fish Tg(ERE:Gal4ff)(UAS:GFP) among the chemicals tested in the study (e.g. BPA).	Moreman et al. 2017
Non-guideline short-term in vivo study to detect disruption of fetal testosterone synthesis in rats exposed in utero.	BPAF	BPAF was given to pregnant rats from GD 14 to GD 18, the testes of fetal male rats were removed and incubated in media for ex vivo testis hormone production for 3 hours. Doses: 0, 200, 300, 400, 500 mg/kg bw	BPAF exposure had no effect on testosterone levels at doses 200-500 mg/kg/day.	Furr et al 2014.

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) in rat, Study report, 2011

In the OECD TG 422 study described in more detail above (section 10.10.2), no significant effects were seen on offspring treated in utero. There were no differences in sex ratio and body weights of offspring between treated animals and controls. Table 13 in Annex I shows necropsy findings in offspring. No evident effects from BPAF treatment is noted. The pups were examined until PND 5 (examination and termination at PND 13 is indicated in current OECD test guideline, adopted in 2016). Importantly, there were no pups at all produced by animals in the high dose group treated with 300 mg/kg bw/day.

Tucker et al. 2018 study

The earliest stages of mammary gland development in late gestation and just prior and after birth have been reported as susceptible time windows for effects of endocrine disrupters (Tucker et al. 2018 and references therein). Tucker et al. investigated the effects of BPAF on development and long-term health of the mammary gland in female CD-1 mice. Pregnant dams were gavaged twice daily with BPAF 0.05 mg/kg bw (n=10), 0.5 mg/kg bw (n=11) or 5 mg/kg bw (n=11) from GD 10.5 (prior to formation of the rudimental mammary epithelial bud) until GD 17.5. Female offspring were followed for up to 16 months.

Table 23: Pubertal mammary gland development score[a] for female offspring exposed to BPAF in utero, Tucker et al. 2018

Dose levels (mg/kg bw twice/day)	**0**	**0.05**	**0.5**	**5**
PND 20	2.03 ± 0.07	**2.77 ± 0.27****	**2.79 ± 0.19*****	**2.80 ± 0.17*****
PND 28	2.33 ± 0.18	2.79 ± 0.33	2.57 ± 0.18	**3.25 ± 0.23***
PND 35	1.92 ± 0.21	**3.12 ± 0.35 ***	1.96 ± 0.18[b]	**3.05 ± 0.46***
PND 56	2.39 ± 0.15	2.95 ± 0.17	2.78 ± 0.28	2.93 ± 0.37

[a]Pubertal mammary gland development scores were calculated for female offspring exposed in utero. Scoring:1=poor development and 4=best development. Scores were based on lateral and longitudinal epithelial growth, presence or absence of terminal end buds (TEB), branching density, budding and appearance of ductal ends. Significantly different from control group: *p<0.05, **p<0.01, ***p<0.001.[b]Mean body weight was significantly smaller compared to controls.

Mammary glands of females treated in utero with BPAF exhibited greater longitudinal growth (mm) and branching density, higher terminal end buds (TEB) counts and more TEBs/mm2. These findings indicate accelerated mammary gland development during puberty in offspring exposed in utero. Table 23 shows significantly higher mammary gland development scores for female offspring at PND 20-35, compared to control offspring. Measurements done at PND 56 demonstrated no statistical differences between the groups, and the control group seemed to have caught up the accelaterated growth and development.

Table 24: Histopathological findings - mammary gland lesion incidences, Tucker et al. 2018

Dose levels (mg/kg bw twice/ day)	**0**	**0.05**	**0.5**	**5**
3 months, no. of animals	n=8	n=9	n=8	n=7
Inflammation, mixed cell	1	1	1	2
8 months, no. of animals	n=5	n=7	n=5	n=6
Lubuloalveolar hyperplasia	0	0	0	0
Inflammation, mixed cell	0	0	0	1
Squamous metaplasia, ductal	0	0	0	1
14 months, no. of animals	n=13	n=14	n=18	n=22
Carcinoma	0	0	0	0
Fibroadenoma	0	0	0	0
Histiocytic sarcoma	0	0	0	0
Lipoma	0	0	0	1
Cyst	0	0	0	**3* (14%)**
Duct dilation	0	0	0	0
Hemorrhage, focally extensive	0	0	0	0
Inflammation, lymphoplasmacytic perivascular	2	7	7	5
Inflammation, mixed cell	1	1	2	**8** (36%)**
Inflammation, neutrophilic	0	0	0	1
Inflammation, not specified	0	0	0	1

Keratin	0	1	0	1
Lobuloalveolar hyperplasia	0	0	1	**5** (23%)**
Lymph node: inflammation neutrophilic	0	0	0	0
Lymph node: Inflammation, mixed with eosinophilic crystals	0	0	0	0
Lymph node:Squamous cell carcinoma or met from Zymbal's gland	0	0	0	1
Lymph node: vascular angiectasis	0	0	0	0
Lymph node: increased cellularity, plasma cells	0	0	0	1
Papillary hyperplasia, multifocal	0	0	0	0
Squamous metaplasia, ductal	0	1	2	**7** (32%)**

*Significant trend P<0.05 ** Significant trend P<0.01

By 14 months of age, there was a significant dose-related increase in diagnoses of non-neoplastic lesions development in BPAF-exposed groups. This included cysts, inflammation, lobuloalveolar hyperplasia and squamous metaplasia (Table 24). In one BPAF-treated high dose animal (5 mg/kg), a squamous cell carcinoma was found in the mammary gland.

Prior to vaginal opening at PND 20, the mean serum estradiol concentration of female offspring of all BPAF-treated animals were significantly higher than the levels of control animals. In the high dose group the progesterone levels were also higher compared to controls. Testosterone levels were lower compared to control animals in offspring treated in utero with BPAF 0.05 mg/kg (at PND 28 and PND 35). Later in life hormone levels in serum normalised.

This study indicates accelerated mammary gland development during early puberty after BPAF exposure in utero, an effect that persisted into adulthood. Other indicators of puberty, such as timing of vaginal opening, age at first estrus or estrous cyclicity were not affected.

This study is considered as supporting, since findings in this study points to effects on development of the offspring but are not sufficient for classification. Moreover, the robustness of this study is limited due to poor reporting.

Li et al. study 2016

Li et al. studied effects on gestational and lactational exposure to BPAF on male offspring (daily exposure of maternal Sprague Dawley rats of 0 or 100 mg/kg on GD 3-19 and PND 3-19). The study was a cross-fostering study (GD 3-19: 2 control groups and 2 treatment groups, PND 3-19: unexposed controls (CC) pups exposed prenatally (TC), pups exposed postnatally (CT) and pups exposed both pre- and postnatally (TT)).

Gestational and lactational exposure of BPAF resulted in significantly increased levels of free and total BPAF in both serum and in testis, compared to the control group. These results show that BPAF was transferred via cord blood and breast milk, although BPAF concentrations were not measured directly in these body fluids. Gestational and lactational exposure lead to significantly decreased Inhibin B levels in serum and in testis of male offspring, compared to controls. Testosterone levels in serum and testis were increased in all treatment groups compared to controls, but only significantly increased in the TT group (no data available, results are shown in bar charts). Estradiol, lutenizing hormone and follicle-stimulating hormone levels in serum did not differ between the four groups, except that estradiol levels in CT and TC

groups were significantly lower than in the TT group. Furthermore, androgen receptor levels in testes increased following BPAF exposure.

Maternal weights of treated animals were significantly lower compared to controls, when measured at GD 12, GD 15 and GD 19 (no data available, results are shown in bar chart). However, no significant differences related to litter size, birth weight, sex ratio and survival rate of pups were found between control and treated groups. Offspring exposed to BPAF during lactation (CT) had significantly lower body weight compared to controls, at PND 12, PND 18 and PND 23 (no data available, results are shown in bar charts). The body weight of offspring exposed during both gestation and lactation was also significantly lower at PND 6, PND 12 and PND 16, compared to controls. No differences between the groups were seen for absolute and relative weights of the testis and epidydimes.

This study is considered as supporting, since findings in this study points to effects on development of the offspring but are not sufficient for classification. Moreover, the robustness of this study is limited due to poor reporting since results were mainly shown in bar charts.

Gong et al. 2017

In a non-guideline in vivo study, male and female adolescent offspring exposed in utero to BPAF was examined using several behaviour tests. Pregnant female mice were treated with 0, 0.4 and 4 mg/kg BPAF per day from GD 1 to GD 19. Different behavioural tests were performed from PND 35 to PND 42 including an Open field test, a Novelty-suppressed feeding test, a Sucrose preference test, a Tail suspension test, a Forced swimming test, a Novel object recognition task and a Contextual fear conditioning test. Test results indicate that maternal BPAF exposure could induce anxiety- and depressive-like behaviors in male mice. Furthermore, this study indicates that BPAF exposure in utero could have adverse effects on memory formation in both male and female mice offspring.

This study is considered as supporting, since findings in this study points to effects on development of the offspring but are not sufficient for classification. Moreover, the robustness of this study is limited due to poor reporting.

Summary of available studies

The results from the OECD TG 422 study (GLP compliant) do not indicate adverse effects on offspring. However, no offspring were produced in the highest dose group. The pups were followed until PND 5 and no histopathological investigations were conducted.

There are a few non-guideline studies that show effects on offspring treated during the fetal period. These effects include e.g. abnormal mammary gland development and mammary gland lesions (dose response trends), transfer of BPAF in breast milk during lactation and impact on hormone levels in serum and testes in offspring, and indications of behavioural changes such as anxiety in male mice. These parameters were not assessed in the Study report, 2011. The available data on developmental effects is mainly generated by non-guideline studies, which makes the quality and relevance of results difficult to assess. The studies are considered as supporting, since the findings point to effects on development of the offspring. However, the studies are considered not sufficient for classification, based on methodological deficiencies and due to poor reporting.

In addition to the in vivo experimental studies mentioned above, there are several mechanistic studies on the effects of BPAF in zebrafish, and in vitro available and listed in Table 11. Results consistently indicate estrogenic and anti-androgenic effects of BPAF, mechanisms of potential relevance for the effects seen on development in vivo.

There are currently ongoing studies on BPAF within the National Toxicology Program (NIEHS), which includes a Modified One-Generation (MOG) study. No results are publically available at the time of writing this proposal. However, results generated from the MOG study may be used to clarify this endpoint in the future.

10.10.6 Comparison with the CLP criteria

In three non-guideline experimental studies in rat and mouse, BPAF was shown to cause effects on development, including effects on development of mammary glands (0.05-5 mg/kg bw, twice/day), impact on hormone levels in serum and testes in offspring (100 mg/kg bw/day), and behavioural changes such as anxiety in male mice (0.4-4 mg/kg bw/day). The studies are non-guideline studies, which makes the quality and relevance of results difficult to assess. The studies are considered not robust enough as basis for classification, because of methodological deficiencies and due to poor reporting. The weight of evidence for developmental toxicity is considered weak.

Classification in Repr. 1A is not justified since there is no human data available on BPAF.

Classification in Repr. 1B is not justified since the evidence for developmental toxicity from existing experimental data on BPAF is not considered to be clear evidence.

Classification in Repr. 2 is not justified since the evidence for developmental toxicity based on existing experimental data on BPAF is not conclusive and cannot at present be considered as some evidence.

10.10.7 Adverse effects on or via lactation

Table 25: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (oral:gavage) according to GLP. OECD Test Guideline 422 Species, strain and sex:Rat (Sprague-Dawley) males and females Treatment groups: 12 males + 12 females per treatment group. Reliability Score 1 according to registrant	Test substance: 2,2-Bis (4-hydroxyphenyl)-hexafluoropropane (Bisphenol AF) Purity: 99.69% Dose levels: 0, 30, 100, 300 mg/kg bw/day.	No adverse effects were seen on offspring exposed to BPAF in utero (30 and 100 mg/kg bw/day). However, no offspring was produced in the highest dose group (300 mg/kg bw/day).	Study report, 2011.
Non-guideline study, Cross-fostering study Effects of gestational and lactational exposure to BPAF on male offspring were studied. Reliability Score 4 according to registrant	Test substance: BPAF (purity 97%) Species, strain and sex: Rats: Sprague Dawley females Treatment groups: 30 females per dose group (GD 3-19), 15 females per dose group	Lactational exposure caused significantly increased levels of BPAF in serum and in testis, showing that BPAF was transferred via breast milk. Lactational exposure lead to significantly decreased Inhibin B levels in male offspring. In addition, androgen receptor levels in testes increased following lactational BPAF exposure. Statistically significant decreased body weights in pups exposed via lactation. Maternal body weights of treated females were significantly decreased at GD 12 - GD 19, compared to controls.	Li et al. 2016.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	(PND 3-19). Dose level: 0, 100 mg/kg/day Duration of exposure: GD 3 to GD 19 and PND 3 to PND 19.		

10.10.7 Short summary and overall relevance of the provided information on effects on or via lactation

Results from the Study report, 2011, do not indicate any effects of BPAF on or via lactation. Pups were followed until PND 5.

In a non-guideline cross-fostering study (Li et al. 2016) BPAF was given to female rats during the lactation, which resulted in transfer of BPAF via breast milk to the pups. Lactational exposure of BPAF resulted in significantly increased levels of BPAF in both serum and in testis. Lactational exposure also lead to significantly decreased Inhibin B levels in serum and in testis of male offspring, compared to controls. Furthermore, androgen receptor levels in testes increased following BPAF exposure. Maternal weights of treated animals were significantly lower compared to controls (estimated by Dossier submitter to be less than 10% based on bar chart), when measured at GD 12, GD 15 and GD 19. Offspring exposed to BPAF during lactation also demonstrated significantly lower body weight compared to controls.

This non-guideline study is not considered robust enough as basis for classification due to poor reporting. The registrant has assigned the study a reliability score of 4. It is not possible to draw conclusions on adverse effects via lactation based on these findings.

10.10.8 Comparison with the CLP criteria

Since no conclusive data are available, comparison with the CLP criteria is inapplicable.

According to CLP Annex I classification of substances for effects on or via lactation can be assigned on the:

(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

10.10.9 Conclusion on classification and labelling for reproductive toxicity

Classification of BPAF for adverse effects on sexual function and fertility is warranted: Repr. 1B H360F. A specific concentration limit for adverse effects on sexual function and fertility is not considered justified

since the estimated ED10 value is within the medium potency group (4 mg/kg bw/day < ED10 value < 400 mg/kg bw/day).

10.11 Specific target organ toxicity-single exposure

Not evaluated in this CLH-proposal.

10.12 Specific target organ toxicity-repeated exposure

Not evaluated in this CLH-proposal.

10.13 Aspiration hazard

Not evaluated in this CLH-proposal.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this CLH-proposal.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this CLH-proposal.

13 ADDITIONAL LABELLING

Not relevant.

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