Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

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

EC Number: 216-036-7
CAS Number: 1478-61-1

CLH-O-0000006961-68-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

18 March 2021

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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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 4,4'-[2,2,2-TRIFLUORO-1-(TRIFLUOROMETHYL)ETHYLIDENE]DIPHENOL; BISPHENOL AF

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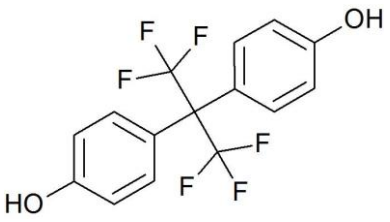
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 4,4'-[2,2,2-TRIFLUORO-1-(TRIFLUOROMETHYL)ETHYLIDENE]DIPHENOL; BISPHENOL AF

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
-					

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

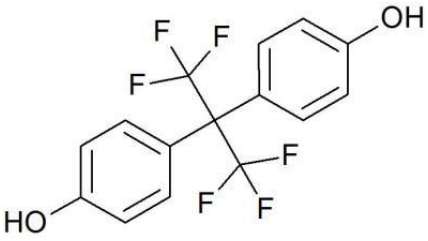
3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

No previous harmonised classification and labelling.

RAC general comment

Bisphenol AF is registered under REACH with an annual tonnage of 100 – 1000 t/a. The substance is used as a reactive process regulator in polymer materials and in rubber production and processing. Bisphenol AF is further used as a crosslinking agent for certain fluoroelastomers and as a monomer for polyimides, polyamides, polyesters, polycarbonate copolymers and other specialty polymers.

The substance has the following chemical structure:



Bisphenol AF currently has no harmonised classification.

The dossier submitter (DS) restricted the current CLH proposal to adverse effects on sexual function and fertility, for which classification in Category 1B was proposed, as well as adverse effects on development of the offspring and adverse effects on or via lactation, for which no classification was proposed.

Bisphenol AF is a structural analogue and functional replacement of bisphenol A (BPA). In 2014, BPA was classified as Repr. 1B; H360F by RAC (ECHA, 2014). In December 2020, RAC agreed on the harmonised classification of another analogue, bisphenol S (BPS), as Repr. 1B (H360FD) based on similar toxicological properties. Despite the structural and functional similarities between the substances, the classification proposal for bisphenol AF is based on data for the substance itself. The similarity of the hazard profile to BPA and BPS has been acknowledged in this opinion under "Further considerations".

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 is not expected to be surface active.	REACH registration (ECHA 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		

Property	Value	Reference	Comment (e.g. measured or estimated)
	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	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		Waidyanatha et al. 2015.

Method	Results	Remarks	Reference
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.	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.		
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 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%).		Waidyanatha et al. 2019.
Non-test guideline, in vivo	High levels of BPAF were		Yang et al. 2012.

Method	Results	Remarks	Reference
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.	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).		
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 cells was seen in high dose treated rats.	Umano et al. 2012.

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 Dawley) males Treatment groups: 8 animals per group.	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, estrogen receptor and lutenising hormone receptor decreased in high-dose animals.	Feng et al. 2012.
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	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	Shi et al. 2015

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		µg/L	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.	
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 was studied. Dose: 24.7 µg/L	BPAF affected genes related to thyroid hormones production and receptor activation, thyroid gland development and deiodinase activity.	Kwon et al. 2016
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	BPAF		Binding of BPAF to G protein-coupled estrogen receptor (GPER).	Cao et al. 2017

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
human breast cancer cells			BPAF had a 9-fold stronger binding affinity than BPA. BPAF exhibited stronger agonistic activity to GPER compared to BPA.	
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
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	BPAF		BPAF demonstrated clear agonistic estrogenic activity in the MCF-7 Estrogen Luciferase Reporter Assay. BPAF showed	Kitamura et al. 2005

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Reporter Assay			inhibitory effects on the androgenic activity of 5a-dihydrotestosterone in mouse fibroblast cell line NIH3T3.	
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
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%)

Dose levels (mg/kg/day)	**0**	**30**	**100**	**300**	**Recovery 0**	**Recovery 300**
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**
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***

Dose levels (mg/kg/day)	0	10	30	100
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
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

[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

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 according to registrant	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 bw/day).	Study report, 2011.

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 pregnancy on neurobehaviours in adolescent mice offspring.	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 behavioural tests. Doses: 0, 0.4, 4 mg/kg bw/day	Fetal exposure to BPAF induced anxiety- and depressive-like behaviours in male adolescent offspring. In addition, BPAF exposure impaired memory formation in both sexes.	Gong et al 2017.
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)	Moreman et al. 2017

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			among the chemicals tested in the study (e.g. BPA).	
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 (PND 3-19). Dose level: 0, 100 mg/kg/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. 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.

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).

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

The DS evaluated adverse effects on sexual function and fertility of bisphenol AF mainly based on a screening study in rats performed according to OECD TG 422 and a 28-day study in rats performed according to OECD TG 407, both using the oral route of

exposure. Supporting information in the form of a Uterotrophic assay and a Hershberger assay, as well as several mechanistic studies, were also included in the proposal. The DS proposed classification for adverse effects on sexual function and fertility in category 1B (Repr. 1B; H360F).

In the OECD TG 422 screening study (0, 30, 100, 300 mg/kg bw/d bisphenol AF, purity 99.69%), treatment-related effects on sexual function and fertility in females included irregular oestrus cycles, dose-dependent increases in the incidence of non-pregnant females with a fertility index down to 0% for the high dose females (300 mg/kg bw/d). The pre-implantation losses were non-significantly higher in mid dose animals compared to controls; however, there were large individual variations. The implantation index was lower in treated mid dose females, and total litter loss was observed in one female each of the low (30 mg/kg bw/d) and mid dose (100 mg/kg bw/d) groups. A higher incidence of follicular/fluid-filled cysts in the ovaries, minimal glandular hyperplasia of the mammary gland, and epithelial hyperplasia of the vagina was seen in high dose females (300 mg/kg bw/d). A direct comparison to the control group however was compromised due to 0% pregnancy in the high dose. In males, adverse effects on number of spermatozoa, significant reductions in absolute and relative epididymis and absolute testes weights in the high dose males, as well as a significantly and dose-dependently reduced secretory content in the prostate and of the seminal vesicles, were seen in treated animals. Leydig cell atrophy was noted in mid and high dose males alongside tubule-alveolar differentiation of the mammary glands at the high dose. General toxicity was not marked according to the DS, concluding that a clear effect of bisphenol AF on sexual function and fertility was evident as pregnancy incidences were reduced at all doses.

In the OECD TG 407, 28-day study (0, 10, 30, 100 mg/kg bw/d), significantly lower absolute weights of reproductive organs were observed in bisphenol AF treated males, including absolute weights of prostate, ventral prostate, and seminal vesicles. Furthermore, atrophy of testicular Leydig cells, and of the mammary glands noted in males, as well as irregularities observed in the females' oestrous cycles indicated endocrine-mediated (oestrogenic) mechanisms underlying the toxicity of bisphenol AF.

Bisphenol AF (0, 50, 200, 600 mg/kg bw/d) tested positive in the Uterotrophic assay with a clear dose-response and significantly increased uterine blotted weight at all doses tested, suggesting oestrogen agonistic properties of bisphenol AF.
In the Hershberger assay the relative glans penis weight increased significantly in rats at the high dose. However, due to general toxicity and considerable variability in the controls, an androgen agonistic property could not be verified according to the study authors.

Several mechanistic studies on the effects of bisphenol AF in zebrafish and mammalian cells *in vitro* were available and results consistently indicated oestrogenic and anti-androgenic effects of bisphenol AF, mechanisms were considered relevant for the effects seen on fertility *in vivo*.

The DS further highlighted that the structurally similar BPA has a harmonised classification as Repr. 1B (H360F) affecting the reproductive system similarly but not as potently as bisphenol AF.

In a weight of evidence approach, the DS concluded that the available data provided clear evidence of adverse effects on both male and female sexual function and fertility, that there was no mechanistic information indicating that the observed effects were not relevant for humans, and that classification of bisphenol AF as Repr. 1B; H360F is thus warranted.

Adverse effects on the development of the offspring

The DS evaluated the developmental toxicity of bisphenol AF mainly based on the OECD TG 422 screening study in rats, as well as on a few non-guideline studies, which were identified during a literature search. The DS proposed no classification for effects on development. The DS highlighted that the National Toxicology Program (NIEHS) is currently performing a Modified One-Generation study with bisphenol AF.

In the OECD TG 422 study, no pups at all were produced by the parental animals treated with 300 mg/kg bw/d. Otherwise, no significant effects of bisphenol AF on *in utero* treated offspring were observed. Post-implantation loss was higher in the mid dose group, but this effect was statistically not significant. Viability index and percentages of live births were not affected. No differences in sex ratio and body weights of offspring of treated and control animals were noted. No evident effects from bisphenol AF treatment were noted during necropsy. The DS reported that pups were examined only until PND 5, although examination and termination at PND 13 was indicated in the current OECD TG 422 (adopted in 2016).

There were a few recent non-guideline studies that reported effects on offspring following treatment of dams with bisphenol AF during the foetal period. These effects included, among others, accelerated mammary gland development and mammary gland lesions (trends in dose-response) in female offspring, transfer of bisphenol AF via breast milk during lactation, and an impact on testosterone serum levels and androgen receptor levels in testes of male offspring. Increased anxiety- and depressive-like behaviours in male adolescent offspring after foetal bisphenol AF treatment were reported in another study. The DS noted that these parameters were not assessed in the OECD TG 422 study.

The DS concluded that there is a concern for developmental toxicity, but the available studies from the scientific literature (non-guideline, non-GLP) were considered not robust enough as a basis for classification due to methodological deficiencies and poor reporting. The weight of evidence for developmental toxicity was thus considered weak and no classification for developmental toxicity was proposed.

Adverse effects on or via lactation

The DS evaluated the effects of bisphenol AF on or via lactation mainly based on the OECD TG 422 screening study in rats, as well as on a non-guideline cross-fostering study identified during the literature search. Toxicokinetic information was also included in the CLH report.

Results of the OECD TG 422 study did not indicate any effect of bisphenol AF on or via lactation. However, in this study pups were only observed until PND 5. In a non-guideline cross-fostering study (assigned Klimisch 4), bisphenol AF was given to female rats during

lactation. Bisphenol AF was transferred via breast milk to the pups. The lactational exposure resulted in significantly increased levels (free and total) of bisphenol AF and significantly decreased Inhibin B levels in both serum and testes of male offspring, and in increased androgen receptor levels in testes. Maternal weights of bisphenol AF-treated dams were significantly lower at several gestational days (GDs) and the offspring that was exposed during lactation also had significantly lower bw compared to controls.

The DS considered the available data not sufficiently robust for classification due to poor reporting, and that no conclusions could be drawn regarding classification for adverse effects on or via lactation.

Comments received during consultation

Four Member State Competent Authorities (MSCAs) submitted comments on the CLH proposal during the consultation. No comments from stakeholders were received. All commenting MSCAs supported the proposed classification of bisphenol AF (BPAF) as Repr. 1B; H360F.

Three MSCAs further agreed that the data on developmental toxicity was not sufficient for classification of bisphenol AF. One additional MSCA proposed to discuss classification of bisphenol AF as a Category 2 developmental toxicant but agreed that the available data seemed insufficiently robust for classification. One MSCA explicitly highlighted that a prenatal developmental toxicity study was needed to properly assess the developmental toxicity endpoint, as there were indications of developmental toxicity of bisphenol AF reported in the literature mentioned in the CLH report. Furthermore, this MSCA pointed out that "one of the standard information requirements of Annex IX of the REACH Regulation (No 1907/2006) for substances manufactured or imported in quantities of 100 t or more is the performance of a prenatal developmental toxicity study with one species using the most appropriate route of administration". In their response, the DS mentioned that a modified one-generation study was ongoing at the National Toxicology Program (NIH, US), generating information on bisphenol AF's effects on prenatal development, postnatal development, and reproduction. During the process of the consultation, data was still under review. However, it was later noted that while the raw data of this study had become available, a summary report is still pending[2].

With respect to lactation, one MSCA did not comment on this hazard class, whereas three MSCAs supported no classification of bisphenol AF as the available data did not allow a firm conclusion on classification. One MSCA explicitly considered the available data on lactation as insufficiently robust for classification, due to poor reporting and no GLP-compliance.

One MSCA highlighted that the reported toxicokinetic results should be handled with caution, as it was assumed that the reason for the low bioavailability after oral exposure (i.e. 1%) reported in rats may have been that only free bisphenol AF in plasma was measured in this study, while the conjugated metabolites were not considered. As it was further stated in this study that after oral exposure an extensive first pass conjugation in the intestine and liver occurred, the commenting MSCA was of the opinion that bioavailability (and systemic availability) of bisphenol AF including metabolites should

[2] https://tools.niehs.nih.gov/cebs3/views/?action=main.dataReview&bin_id=14942

have been clearly higher than 1%. The DS agreed with this reasoning but stated that the results had been cited as reported in the publication. The DS concluded that overall, the toxicokinetic data "may be of less importance since there are clear toxicological effects demonstrated in several studies, including the Repro screening study (OECD TG 422)".

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

The DS included a screening study in rats performed according to OECD TG 422 and a 28-day study in rats performed according to OECD TG 407 (both GLP-compliant and rated Klimisch 2 and 1, respectively, by the registrant(s)), both using the oral administration route for the test substance, for the assessment of sexual function and fertility. Supporting information from a Uterotrophic assay and a Hershberger assay as well as several mechanistic studies were available. Most of the provided studies indicated adverse effects of bisphenol AF on male and female sexual function and fertility.

OECD TG 422

In an OECD TG 422 study, bisphenol AF was administered by gavage to SD rats (males for 42 days, females for 55 days, including a 2-week maturation phase, pairing, gestation and early lactation for females), at 0, 30, 100 and 300 mg/kg bw/d for control, low, mid, and high dose, respectively. Two recovery groups (5/sex/group, high dose and control) were treated for 42 days with a subsequent post-exposure observation period of 14 days. Recovery animals were not mated. Regarding study reliability, it was indicated in the REACH registration dossier that the exposure duration in males was not consistent with the guideline requirements for repeated dose toxicity, which restricted the reliability of the endpoint for males only. Furthermore, it was noted by the DS that pups were examined only until PND 5, although examination and termination at PND 13 was indicated in the current OECD TG 422.

Mating performance and pregnancy outcomes

The number of pairing days until mating was not affected by treatment with bisphenol AF and the mating index did not differ significantly between controls and treated animals (91% in high dose animals vs. 92% in controls). Irregular oestrous cycles were observed in 2/11 (18%) high dose animals. At this dose, one animal with continuous anoestrus interval was reported to fail to mate, and another female that showed extended oestrus, was reported to not become pregnant. One female of the 100 mg/kg bw/d treatment group (1/12) and one control female (1/12) did not mate either.

Notably, exposure to bisphenol AF had a clear impact on the pregnancy outcomes, as no pregnancy was induced in any of the high dose females that were mated (10/11 mated animals, 1/11 animal pair did not mate). The incidence of females that mated successfully but did not become pregnant increased with increasing dose (0/11, 2/12, 3/11 and 10/10 for controls, low, mid and high dose, respectively). The fertility index was 100%, 83%, 64% and 0% for the controls, low, mid, and high dose, respectively. Pre-implantation losses were slightly higher in the mid dose animals compared to the controls (19%±17 vs. 12%±13), but this effect was not statistically significant. The number of corpora lutea and implantations were reported to be lower in the treated females compared to the controls (corpora lutea: 16.7±3.6, 14.7±5.8, 14.0±7.8 for

control, low, mid dose; implantations: 14.1±1.9, 12.1±3.8, 10.4±4.8 for control, low, mid dose); however, these effects were not statistically significant either. In line with these findings, the implantation index was lower for the mid dose with 81% vs. 88% in control females. RAC notes that there are discrepancies within the treatment groups between the number of pregnant females and the number of females investigated for corpora lutea, implantation sites, pre- and post-implantation loss, as well as for implantation index. In the original study report, for some treated females the individual data for these parameters are missing, although these females were reported to have given birth to offspring. No justification was provided for the missing values. Before the RAC plenary, the REACH registrants were asked to clarify the issue regarding the missing values for the abovementioned parameters. In response, the registrants stated that they were not the original study monitors and that the study sponsor was the Japanese Ministry of Economy, Trade and Industry (METI), which was why they could not give any specific explanation or reasoning but could only speculate. Accordingly, RAC considers that the comparison of these parameters to the controls is compromised, which is why analysis of the impact of bisphenol AF on these parameters is essentially hampered. The study author concluded that for these parameters, no statistically significant effect was observed.

Total litter loss was observed for one female each of the low dose (1/10 = 10%) and mid dose (1/8 = 13%) group, compared to none among control females. Gestation index was dose-dependently affected by treatment (100%, 90% and 88% for controls, low and mid dose females, respectively); however, these effects were reported to be not statistically significant.

Table: *Fertility parameters*

Dose levels (mg/kg bw/d)	**0**	**30**	**100**	**300**
No. of pairs examined	12	12	12	11[#]
Oestrous cycle (days)	4.0 ± 0.0	3.9 ± 0.2	4.3 ± 0.4	4.2 ± 0.2
Irregular oestrous cycle	0/12	0/12	0/12[##]	**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**	**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 oestrous 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 Index (%) = (No. of females with live born pups/No. of pregnant females) x 100	100	90	87.5	-
Gestation length (days)	22.9 ± 0.6	23.0 ± 0.6	22.9 ± 0.2	-
No. of corpora lutea	16.7 ± 3.6 (n=11)	14.7 ± 5.8 (n=9)	14.0 ± 7.8 (n=7)[####]	-
No. of implantation sites	14.1 ± 1.9 (n=10)	12.1 ± 3.8 (n=7)[###]	10.4 ± 4.8 (n=7)[####]	-
Pre-implantation loss ((No. of corpora lutea - No. of implantation sites)/No. of corpora lutea) x 100 (mean %)	11.9 ± 13.2 (n=10)	10.6 ± 18.2 (n=7)[###]	18.7 ± 16.8 (n=7)[####]	-
Implantation index = (No. of implantation sites/No. of corpora lutea) x 100 (mean %)	88.1 ± 13.2 (n=10)	89.4 ± 18.2 (n=7)[###]	81.3 ± 16.8 (n=7)[####]	-
Post-implantation loss ((No. of implantation sites – Total no. of offspring born)/No. of	9.5 ± 10.3 (n=10)	7.8 ± 7.6 (n=7)[###]	22.9 ± 35.5 (n=7)[####]	-

implantation sites) x 100 (mean %)				
Females with live offspring (no.)	11	9	7	-
Delivery index = (No. of pups delivered/No. of implantation sites) x 100	90.5 ± 10.3	92.2 ± 7.6	77.1 ± 35.5	-
No. of females rearing young to day 5 of age	11	9	7	0

One high dose female failed to mate.
in CLH report: 1/12; corrected value after study report access
###No justification is provided in the study report as to why the number of females for the parameter number of implantation sites, pre-/post-implantation loss and implantation index is n=7 instead of n=9. The numbers are missing in the individual tabled data. Data on number of corpora lutea are also missing for one of these 2 females. Individual tabled data further indicates that the very same 2 females, for which this information is missing, gave birth to offspring.
No justification is provided in the study report as to why the number of females for the parameters corpora lutea, number of implantation sites, pre-/post-implantation loss and implantation index is n=7 instead of n=8. The numbers are missing in the individual tabled data. Individual tabled data further indicates that the very same female, for which this information is missing, gave birth to offspring.

Only limited information on historical control data (HCD) was given in the study report (see the table below). Data as to July 2007 on values for the group mean (plus 2 standard deviations) were reported for a limited number of animals. As the TG 422 study was conducted in 2009/10, the quality of the HCD is very limited and no information was given on the source of it. Before the RAC plenary, the registrants were asked to clarify the issue regarding the relevance of the historical control data. In response, the registrants stated that they were not the original study monitors and that the study sponsor was the Japanese Ministry of Economy, Trade and Industry (METI), which is why they could not provide any further relevant information.

Table: *Historical control data (range = mean ± 2 standard deviations; values in brackets indicate group mean and standard deviation, respectively)*

	Range	**No. of animals**
Gestation length (days)	21.0 (22.2) – 23.4 (0.6)	85
No. of corpora lutea	14 (18) – 22 (2)	72
No. of implantation sites	12 (16) – 19 (2)	73
Pre-implantation loss (%)	2 (12) – 22 (5)	55
Post-implantation loss (%)	3 (7) – 11 (2)	31

Female reproductive organs

Minimal glandular hyperplasia of the mammary gland was seen in 4/11 (36%) non-pregnant females of the high dose group, which might suggest a treatment-related (endocrine) effect, but was not seen in the recovery control or 300 mg/kg bw/d (non-mated) females at study observation end. Also, a direct control for non-pregnant females was missing as only one individual did not mate in the control group. Historical control data was not provided in the dossier. Ovarian cysts were found in several of the non-pregnant females of each treatment group. Follicular/fluid-filled cysts appear to be dose-dependently increased (although there is no directly comparable control), 9/11 high dose females (82%) had follicular/fluid-filled cysts on the ovaries, an effect that was absent in the one non-pregnant control female (0/1, 0/2 (0%), 2/4 (50%), 9/1 (82%) for control, low, mid and high dose, respectively). This could be a treatment-related effect, the interpretation is supported by the observation of follicular cysts seen in the treated recovery females (4/5 or 80%) versus recovery controls (0/5) suggesting that the effect is treatment related and did not regress during recovery. 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. Even though only one individual did not mate in the control group, no dose-response can be inferred for the treatment groups. The study author rated this finding as normal cyclical changes in the female rat and that there was no convincing effect of the treatment in this study. Epithelial hyperplasia of the vagina was seen in 4/11 (36%) of the non-pregnant high dose females compared to none in the other dose groups. However, this effect was described as minimal. Again, the study author, allowing for normal cyclical changes, considered there was insufficient evidence to suggest an effect of treatment.

Table: *Number of female animals with histopathological findings in reproductive-related organs, only females that failed to mate/non-pregnant**

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

*No HCD available

Male reproductive organs

Treatment-related effects on sexual function and fertility in males included adverse effects on no. of spermatozoa. Sperm reading scores (based on number of sperm detected) in high dose males were 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). Moreover, significant reductions in absolute epididymis and testes weights in the high dose males compared to controls (-20% and -11%, respectively) were recorded. Similarly, relative epididymis weight was significantly lower (-13%) in high dose males, whereas relative testis weight was not affected. A significant and clearly dose-dependent reduction in secretory content of the prostate, indicated by smaller organ size, was seen in treated animals (up to 8/12 (67%) in high dose males; 4/5 (80%) in high dose

recovery males; none in respective controls). In addition, significantly and dose-dependently reduced secretory content of seminal vesicles was reported for all bisphenol AF treatment groups with 100% males affected in the high dose (main study: 12/12 (100%) vs. 1/11 (8%) in controls; recovery group: 3/5 (60%) at high dose vs. 0/5 (0%) in controls); thus, the recovery groups did indicate a trend but no convincing regression of these changes. Leydig cell atrophy was present in 1/12 (8%), 0/12 (0%), 3/12 (25%), 11/12 (92%) control, low, mid and high dose males, respectively, thus dose-dependently increased. This is considered a treatment-related effect. This effect was visible in 1/5 treated recovery males vs. 0 males in recovery controls. The lower rate of 20% after recovery (instead of 92% seen in high dose males) may indicate a trend for regression. However, the treated recovery group was small. Overall, an impact on endocrine status in males is suggested. Decreased weight of seminal vesicles and ventral prostate in rats is a relatively sensitive indicator of reduced androgen levels, also supported by the observed Leydig cell atrophy. The moderate to severe atrophy of testes reported for 2/5 high dose recovery males cannot be excluded as being a treatment-related effect by RAC. However, as no other treatment or control group displayed such change a relation to treatment appears uncertain. Again, HCD was not presented.

Tubuloalveolar differentiation of mammary glands was observed dose-dependently in males with increasing severity and incidence. 50% high dose males (6/12) showed this effect (slight to moderate in severity), while still 4/5 high dose recovery males were affected at termination. Thus, a regression of this effect was not evident. No controls had a slight to moderate severity of this effect, but 3/12 (25%) control males exhibited minimal tubuloalveolar differentiation of mammary glands.

Table: *Number of male animals with histopathological findings in reproductive-related organs. Incidence in percent in parenthesis**

Dose levels (mg/kg bw/d)	**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%)
Seminal vesicles – Reduced secretory content						
Vesicle 1						
No section	0	0	1	0	0	0
Absent	11	10	5	0	5	2
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 (atrophy**)						
Absent	11	12	9	1	5	4
Present	1 (8%)	0	3 (25%)	11 (92%)	0	1 (20%)

*No HCD data available

**Leydig cell atrophy is commonly a result of Leydig cell necrosis or apoptosis and subsequent loss of Leydig cells. No further information was given in the report.

General toxicity

One female (1/12) treated with 300 mg/kg bw/d had to be killed *in extremis* on Day 6 following severe clinical signs, which were attributed to an error in the administration of the test material formulation.

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 anogenital 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 to be 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. Mean body weights showed no significant and treatment-related changes in males. The DS reported a tendency towards lower bw among animals of the mid and high dose compared to controls; however, due to the lack of reporting of standard deviations, median values and/or confidence intervals for bw, it is premature to interpret the differences in the given mean values as potential treatment effect. In high dose recovery males, however, significantly lower mean bw were reported from day 15 until the end of the experiment (range: -13% at day 15 up to -22% at day 43). Two weeks post-exposure, bw of high dose males (recovery group) was still significantly lower compared to controls (-17%). It is noted that recovery control males generally had a higher mean bw than non-recovery control males. Despite slight body weight gain changes, there were no significant differences on mean body weights among females in the different dose groups during maturation. A significantly lower mean body weight (-10%) was observed for low dose females at GD 20 (however, no dose-response was evident during gestation), during lactation (day 0 and 4) for both the low and mid dose females (range: 7 to 10%).

Mean food consumption for males was significantly lower in the two highest dose groups (-9% and -22%, respectively) during the first week of treatment. Water consumption was significantly higher among all groups of treated males at all assessment points (change for high dose males +19% to +39% compared to controls). Treated high dose females also demonstrated significantly lower food consumption during maturation week 1 (-19% mid dose and -25% high dose). At GD 7-14 and GD 14-20, females had a lower food intake (low and mid dose (-13%) on GD 7-14 and the mid dose (-11%) on GD 14-20,). No high dose females (non-pregnant) were included in comparative evaluations after maturation and mating weeks. As for males, water consumption increased significantly during pre-mating days 1-7 (mid dose +30% and high dose +39%), and during days 8-14 (high dose group +11%).

Some changes in haematology and blood chemistry were reported, including significant reductions in Hb and RBC (-8% and -9%, respectively, prior termination day 42) in high dose males, significant higher ALAT values in mid and high dose males (+ 35-37%, day 42), and significantly higher ALAT value (+ 74%) for high dose females during the maturation phase (day 14).

Regarding organ weights, mean relative weights of adrenals and liver were significantly higher in high dose males (+25% and +10%, respectively), while in recovery high dose males the mean absolute liver weight was significantly lower compared to control animals (-18%). In treated recovery males, relative organ weights for adrenal, brain, spleen and thymus were significantly higher as well when compared to controls. For females, relative brain weights in the low and mid dose groups were significantly higher compared to controls (+7% and +9%, respectively). The mean absolute heart weights were significantly lower in the low (-17%) and mid dose (-15%) females compared to controls. Non-pregnant females in the high dose group were not included in comparative evaluations after maturation and mating.

RAC conclusion

RAC concludes that clear treatment-related dose-dependent effects of bisphenol AF on fertility were observed with no pregnancies achieved at the top dose of 300 mg/kg bw/d (0% fertility index) and fewer pregnancies at the low and mid dose of 30 and 100 mg/kg bw/d, respectively. Some indications of general toxicity were noted in the mid and high dose animals; however, the effects where rather of mild to moderate nature and RAC considers that the general toxicity was not marked. Some effects on food consumption and bw development were seen in male high dose animals, but no consistent effect was noted in high dose pregnant females or non-pregnant females of the recovery group. Therefore, 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. Uncertainties with respect to effects on corpora lutea, number of implantation sites, pre-/post-implantation loss and implantation index, were noted by RAC, not allowing a firm conclusion on these parameters. However, effects of bisphenol AF on fertility (no. of pregnant females) are considered as unequivocally substance-related and justify classification.

OECD TG 407

Supporting evidence comes from a 28-day repeated-dose toxicity study conducted according to OECD TG 407 (*in vivo* screening tests to detect endocrine-mediated effects) using Crj:CD rats. Rats were given 0, 10, 30 and 100 mg/kg bw/d bisphenol AF by oral gavage, for 28 days, and each dose group comprised 10 males and 10 females.

Reproductive organs and histopathology

For high dose males, absolute weights of prostate, ventral prostate, and seminal vesicle were significantly lower (-23%, -25%, and -28%, respectively), and histopathological findings demonstrated significant atrophy of testicular Leydig cells in treated males. RAC notes that this is well in line with the OECD TG 422 study results. Atrophy of the mammary glands was also seen in 3/10 high dose males, compared to none in the other groups. Although, this effect was not statistically significant, it might be indicative of an endocrine-mediated effect. No adverse effects on sperm were reported. In females, no

histopathological effects on reproductive organs were observed, while irregular oestrous cycles were reported. No details were included in the CLH report, whereas in the REACH registration dossier, irregular oestrous in the 30 and 100 mg/kg bw/d groups, and the dioestrous stage continued in some animals were reported. The mean duration of oestrous cycles was prolonged in the study at high dose, however without statistical significance (control: 4.2±0.4 days, 100 mg/kg bw/d: 4.9±0.9 days). Oestrous cycling days could not be measured in 1/10 and 3/10 rats of the mid (30 mg/kg bw/d) and high dose (100 mg/kg bw/d), respectively, due to irregularity of their oestrous cycles.

General toxicity

Terminal body weights in high dose males were significantly lower (-12%) compared to controls. In mid and high dose females, mean bw was significantly lower compared to controls (-7% and -8%, respectively). The effect was reported to be accompanied by decreased food consumption.

Regarding blood chemistry and haematology, in high dose males white blood cell counts (WBC), total cholesterol levels, as well as albumin values were significantly lower, and serum T4 levels were significantly higher compared to controls (+28%). In high dose females, cholinesterase and total cholesterol values were lower when compared to controls, whereas total bilirubin was higher. As with males, serum T4 levels were significantly higher in those animals than in controls (+53%).

Organ weight measurements revealed significantly higher relative kidney (+9%), adrenals (+23%) and brain (+15%) weights in high dose males, whereas absolute weights of liver (-18%), heart (-12%) and spleen (-17%) were significantly lower in these animals. Histopathological examination revealed significant hypertrophy of the adrenal zona fasciculate (8/10 vs. 1/10), and decreased hepatocytic glycogen (8/10 vs. 1/10) when compared to controls. Among high dose females, the absolute heart weight was significantly lower (-10%) and the relative brain weight was higher (+8%). It seems, however, that in females only these two organs were weighed, while no values were presented for any other organ.

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

Dose levels (mg/kg bw/d)	0	10	30	100
Animals per group	10	10	10	**10**
Testis: atrophy of Leydig cells	0	0	0	**5***
Adrenal gland: hypertrophy 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, $p<0.01$

RAC conclusion

RAC concludes that significantly lower absolute weights of reproductive organs were observed among bisphenol AF-treated males. Effects observed on testis and oestrous cycle further indicate endocrine-mediated mechanisms underlying the toxicity of bisphenol AF. Decreased weight of seminal vesicles and ventral prostate in rats is a relatively sensitive indicator of reduced androgen status, which is also supported by the

observed Leydig cell atrophy in treated males. No change in female reproductive organs was detected, despite irregularities in oestrous cycle (in line with the OECD TG 422 results), suggesting a potentially weak effect on the female reproductive tract. It is, however, noted that dose levels were rather moderate in this study (under the conditions of the OECD TG 422 described above, animals tolerated longer and higher dosing), and it is unclear whether the effects would have been more pronounced, if the top dose tested had been higher.

Mechanistic and non-guideline studies

The DS briefly summarised a range of mechanistic studies in the CLH report. These comprised Level 3 Endocrine Disruptor (ED) assays (Uterotrophic and Hershberger assay) and a range of non-guideline studies (*in vivo* and *in vitro*) all performed with bisphenol AF.

Uterotrophic assay and a Hershberger assay

The assays were conducted according to GLP with bisphenol AF (98.8% purity). The DS reported Klimisch score 3 with reference to the registration dossier, however the registrant assigned them Klimisch 1 as the studies were conducted according to OECD TG 440 and 441, without deviations. Yamasaki *et al*. (2003) studied oestrogenic and androgenic effects of bisphenol AF orally given on 3 consecutive days to 19-day-old rats in the Uterotrophic assay, at doses of 0, 8, 40 and 100 mg/kg bw/d, and for 10 consecutive days in the Hershberger assay at doses of 0, 50, 200 and 600 mg/kg bw/d via oral gavage. Bisphenol AF was tested **positive in the Uterotrophic assay** (dose-response), with significantly increased uterine blotted weight at all doses tested, suggesting oestrogenic agonistic properties of bisphenol AF. In addition, watery uterine contents were detected in the high dose group (100 mg/kg bw/d). No significant differences in body weights were seen among treated animals, compared to controls.

Table: *Results from the Uterotrophic assay, Yamasaki et al., 2003*

Dose levels (mg/kg bw/d)	**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.

In the Hershberger assay, the relative glans penis weight increased significantly in rats given 600 mg/kg bw/ d of bisphenol AF. However, there were signs of general toxicity at the mid and high dose, which included significantly decreased body weight gain and decreased spontaneous locomotion (no further details available). In addition, the control values for this organ varied considerably, and according to the authors, an androgen agonistic property could not reliably be determined in this study.

Subacute in vivo study

In a non-guideline 14-day *in vivo* study (Feng *et al*., 2012), adult SD rats (8

males/group) were dosed with bisphenol AF at 0, 2, 10, 50 and 200 mg/kg bw/d. Key finding included decreased total serum cholesterol at doses of 50 and 200 mg/kg bw/d. Moreover, concentrations of bisphenol AF increased dose-dependently in the testes, while **testosterone in serum decreased** significantly in the high dose group. Levels of luteinising hormone and follicle-stimulating hormone increased. Testicular mRNA levels of Inhibin B, oestrogen receptor and luteinising hormone receptor decreased in high dose animals. The NOAEL for bisphenol AF for male SD rats was <10 mg/kg bw/d.

Other mechanistic studies

Several mechanistic studies on the effects of bisphenol AF in zebrafish, and *in vitro*, are available. The results consistently indicate oestrogenic and anti-androgenic effects of bisphenol AF (decreased testosterone in male fish, increased oestradiol levels and upregulated vitellogenin in males and females), mechanisms of relevance for the effects seen on fertility *in vivo* (Shi *et al*., 2015, Yang *et al*., 2016). In addition, some studies are indicative of endocrine disruption of the thyroid (Kwon *et al*., 2016, Tang *et al*., 2015).

In vitro studies

Several *in vitro* studies show **oestrogen receptor binding activity,** including binding of bisphenol AF to G protein-coupled oestrogen receptor in human breast cancer cells (Cao *et al*., 2017), binding and activation of oestrogen receptors in HeLa cells (3-fold stronger binding to ERβ than ERα; fully activated ERα, but being almost completely inactive for ERβ) (Matsushima *et al*., 2010), ERα-agonistic behaviour at lower concentrations (nanomolar) and anti-oestrogenic action via the induction of ERβ at higher concentrations in human breast cancer cells (Okazaki *et al*., 2017). Bisphenol AF was the most potent BPA analogue, followed by BPB, BPZ, BPA, BPAP and BPS in stimulating cell growth in an ER-mediated cell proliferation assay and inducing oestrogen response element-mediated transcription in a luciferase assay (Mesnage *et al*., 2017). Bisphenol AF altered steroidogenesis in H295R cells inducing progesterone levels and reducing testosterone levels (Feng *et al*., 2014), exhibited agonistic oestrogenic activity in the MCF-7 Oestrogen Luciferase Reporter Assay and inhibitory effects on the androgenic activity of 5a-dihydrotestosterone in the mouse fibroblast cell line NIH3T3 (Kitamura *et al*., 2005). Moreover, bisphenol AF elicited oestrogenic and thyroidal effects in two-hybrid yeast bioassay (Lei *et al*., 2017) and agonistic oestrogenic and AR-antagonist activity in a luciferase reporter assay using African green monkey kidney cells (Teng *et al*., 2013).

Further considerations: structural similarity to BPA and BPS

The structural similarity of bisphenol AF to BPA was highlighted by the DS. Bisphenol A has a harmonised classification as Repr. 1B (H360F) and affects the reproductive system similarly. Fertility assessment concluded significantly decreased number in litters/pair in two- and multigeneration studies. Although data are mainly from animals exposed *in utero* and/or postnatally, irregularities in the oestrus cycle, ovarian cysts and decreased numbers of implantation sites were also observed for BPA. In males, exposure to BPA decreased the levels of testosterone, sperm production and weights of reproductive organs.

Disruption of oestrogenic signalling was considered to be the main mode of action for the effects of BPA on fertility. The hormonal systems are well conserved between mammalian species, and the effects that have been observed in rodents were therefore also considered relevant for humans (ECHA RAC, 2014).

RAC recently assessed another structural analogue of bisphenol AF, BPS. Bisphenol S

consistently and severely disturbed reproductive parameters and RAC classified BPS as Repr. 1B; H360F, based on adverse effects on fertility, reproduction and pregnancy outcome, including a decreased number of implantation sites, reduction of fertility index down to 60%, and prolongation and irregular oestrus cycle at comparable dosing with 300 mg/kg bw/d (ECHA RAC, 2020).

RAC conclusion

In line with the DS, and in a weight of evidence approach, RAC concludes that the available data provide clear evidence of adverse effects on sexual function and fertility, especially with regards to the fertility index from doses of 30 mg/kg bw/d and above. Changes in male reproductive organ weight, size and histopathology are indicative of an (anti-androgenic) endocrine mechanism. Based on the available data oestrogenic or anti-androgenic mechanism are thought to play a dominant role *in vivo*. As there is no mechanistic information indicating that the observed effects are not relevant for humans, adverse effects on sexual function and fertility are relevant for classification. In fact, oestrogen receptor binding activity of bisphenol AF has been demonstrated in human derived cell lines. Data on BPA and BPS are considered as supportive for the classification proposal for bisphenol AF on this endpoint.

Effects on male mammary glands (transformation to tubuloalveolar pattern observed with higher incidence and severity in male rats at 300 mg/kg bw/d compared to controls in an OECD TG 422 study) may indicate the presence of additional endocrine mechanisms/targets. While this sign of increased cellular growth could be interpreted as 'feminisation', the atrophy of the mammary gland observed in male rats at 100 mg/kg bw/d bisphenol AF in the 28-day study indicates a suppressive effect on the mammary gland. Although the BPA data on the mammary gland effects in offspring following *in utero*/perinatal exposures (which indicate that the mammary gland in female offspring is a target organ) are not directly comparable to the data for bisphenol AF (only data on oral route and on effects in young adult/parental animals available), bisphenol AF-related effects on the mammary gland were generally only seen in male (young adult) rats, but not in female (young adult) rats.

In male rats, mammary gland effects were of depressive nature in treated young adults (as a result of the 28-day treatment with 100 mg/kg bw/d of bisphenol AF), while increased proliferation was seen at 300 mg/kg bw/d after a longer treatment period (42 days, with and without recovery, in the TG 422 study).

Due to the uncertainty, as the database is limited (based on the TG 407 and TG 422 studies only) and the inconsistency of the nature of effects (atrophy versus increased tubuloalveolar differentiation), no robust conclusion on the potential for 'feminisation' of the mammary glands of male animals can be drawn at this time for the endpoint fertility.

Adverse effects on development of the offspring

The DS evaluated adverse effects on development of bisphenol AF mainly based on a screening test in rats performed according to OECD TG 422, as well as on a few non-guideline studies, which were identified during a literature search.

RAC takes note of the upcoming Modified One-Generation (MOG) study of the National Toxicology Program (NIEHS) from which tabled summary results have recently been

published[3], but for which a study report is not yet published[4].

OECD TG 422

In the OECD TG 422 study (described in more detail under Adverse effects on sexual function and fertility), no pregnant females and, thus, no pups were produced at the high dose of 300 mg/kg bw/d. Therefore, developmental effects at this dose could not be assessed in this study.

At the lower doses, no significant effects on *in utero* treated offspring were observed. Post-implantation loss was higher in the mid dose group, but differences were not statistically significant (10%, 8% and 23% for controls, low and mid dose groups, respectively).

Viability index and percentages of live births were not affected. There were further no differences in sex ratio and body weights of offspring between treated animals and controls. No evident effects from bisphenol AF treatment were noted during necropsy. The DS noted that pups were examined only until PND 5, although examination and termination at PND 13 is indicated in the current OECD TG 422 (adopted in 2016).

Further data

There are a few recent non-guideline studies that report effects on offspring treated during the foetal period.

Tucker *et al*. (2018) investigated the effects of bisphenol AF on development and long-term health of the mammary gland in female CD-1 mice. Pregnant dams were given 0.05 mg/kg bw/d (n=10), 0.5 mg/kg bw/d (n=11) or 5 mg/kg bw/d (n=11) of bisphenol AF, via oral gavage, twice daily 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. The reported effects included, among others, accelerated mammary gland development of female offspring treated *in utero* and mammary gland lesions in the female offspring of treated dams. The DS indicated that these effects were dose-dependent. On closer inspection, a clear dose-dependency may not be inferred for the histopathological lesions, but significant effects are reported for the high dose, including mammary gland cysts (3/22 [14%], 0% for other groups), and mixed cell inflammation found at low incidences in all treatment groups (1/12 [8%], 1/14 [7%] and 2/18 [11%] in controls, low dose and mid dose groups, respectively) in contrast to the high dose group where 8/22 (36%) females exhibited this effect. Furthermore, lobuloalveolar hyperplasia in mammary glands was observed at mid and high dose (1/18 [6%] and 5/22 [23%], respectively, significant trend $p<0.01$), and squamous metaplasia was reported with increasing incidences (0/13 [0%], 1/14 [7%], 2/18 [11%], 7/22 [32%], for control, low, mid and high dose, respectively, significant trend $p<0.01$). The assessment of mammary gland development showed significant results between PND 20 and 35, including greater longitudinal growth and branching density, higher terminal endbuds (TEB) counts and more TEB/mm^2, indicating a potential treatment-related accelerated growth.

In another study (Li *et al*., 2006), transfer of bisphenol AF via breast milk during lactation and impact of bisphenol AF treatment on testosterone levels in serum and androgen

[3] https://tools.niehs.nih.gov/cebs3/views/?action=main.dataReview&bin_id=14942

[4] https://ntp.niehs.nih.gov/whatwestudy/testpgm/status/ts-08002.html?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=ts-08002

receptor levels in testes of male offspring of treated dams were reported. Furthermore, increased anxiety and depressive-like behaviours in male adolescent offspring due to foetal bisphenol AF treatment were reported in a further study. The DS noted that these parameters were not assessed in the OECD TG 422 study.

Further considerations: structural similarity to BPA

There are a number of studies that reported increased cellular growth in the mammary gland of female animals (rats and mice), at several sites (ductal, alveolar buds and/or terminal buds, not all consistent), following *in utero*, perinatal and/or postnatal exposure to BPA. A number of them showed effects only at low doses without effects at higher doses and some inconsistencies in the effect patterns (for review see Table in Mandrup *et al*., 2016). Few studies observed mammary gland effects in male offspring.

The observation of low dose effects of BPA and the transient nature of findings was confirmed by the more recent study of Mandrup *et al*. (2016). Perinatal exposure (GD 7-21) of BPA to rats induced mammary gland longitudinal growth in male offspring (only) on post-natal day (PND) 22 at oral doses of 0.025 mg/kg bw/d of BPA, and ductal hyperplasia in adult females at 0.25 mg/kg bw/d at PND 400, but not at PND 100 (Mandrup *et al*., 2016). These effects on male and female rats were not seen at higher doses, and effects in male rats were not seen at PND 100 or 400. Although a tubuloalveolar pattern with lumens was not present in male rats, authors considered the changes as an early shift toward a female-like morphology.

RAC conclusion

RAC concludes that a robust *in vivo* developmental toxicity study is lacking in the CLH report, thus, hampering full assessment of developmental toxicity of bisphenol AF. RAC, however, also notes that the (raw) data of the by the DS highlighted modified one generation study study are already available, while a summary report is still pending. This additional data might provide sufficient information for deciding on whether classification of bisphenol AF for developmental toxicity is warranted.

Adverse effects on or via lactation

The DS evaluated effects on or via lactation based on the OECD TG 422 study and a non-guideline cross-fostering study (Li *et al*., 2016). Results from the above described screening study are not indicative of any effects of bisphenol AF on or via lactation. However, pups were followed until PND 5 only. The cross-fostering study was not considered robust enough by the DS to be used as basis for classification due to poor reporting (the registrant(s) assigned Klimisch 4).

RAC conclusion

RAC agrees that it is not possible to draw conclusions on adverse effects of bisphenol AF on or via lactation based on the available information.

Comparison with the CLP criteria

Repr. 1A; H360 (known human reproductive toxicant): The classification of a substance in Category 1A is largely based on evidence from humans.

Repr. 1B; H360 (presumed human reproductive toxicant): The classification of a

substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.

Repr. 2; H361 (suspected human reproductive toxicant): Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

With respect to adverse effects on sexual function and fertility, RAC concludes that bisphenol AF has a clear and adverse impact on pregnancy outcomes in rats with a dose-dependent decrease in fertility index and no pregnancies achieved at the top dose of 300 mg/kg bw/d. RAC highlights the steep dose-response curve, seen at doses at which general toxicity was not marked and not severe enough to explain the observed effects on fertility. Thus, these effects are considered a direct toxic effect and not of secondary non-specific nature. Further experimental observations support this conclusion, as disturbance of oestrus cycle, adverse effects on testis, a positive outcome of the Uterotrophic assay, as well as various mechanistic *in vitro* studies on oestrogenicity suggest an endocrine mediated mechanism of action for bisphenol AF.

Classification in Category 1A is not appropriate as no relevant human data is available supporting harmonised classification in this category. Classification in Category 2 is not appropriate as the evidence for adverse effects on sexual function and fertility is considered clear evidence. This conclusion is line with the previous RAC assessments of the two structurally very similar chemicals, BPA and BPS, which both were concluded to elicit similar but not as potent effects on pregnancy outcomes and fertility index as bisphenol AF via an oestrogenic main mode of action.

RAC concludes that **classification for adverse effect on sexual function and fertility of bisphenol AF as Repr. 1B; H360F, is justified.**

With regards to developmental toxicity, the assessment of adverse effects on offspring development is essentially hampered, as no robust *in vivo* prenatal developmental toxicity study is available in the CLH dossier. In the OECD TG 422 study no effects on *in utero* treated offspring were noted that would warrant classification, and at the high dose no pregnancy was achieved at all. However, RAC notes that an OECD TG 422 does not provide adequate information on developmental toxicity, e.g. compared to an OECD TG 414.
The effects on female mice mammary gland development and male offspring testosterone levels and adolescent behaviours reported in the supplementary studies retrieved from the public literature are considered insufficiently robust for classification, although indicating a concern. RAC notes that the (raw) data from the Modified One Generation study conducted by the U.S. NTP are publicly available already, while a summary report

is still pending. This data was therefore not considered in the current opinion. Hence, **RAC concludes that classification of bisphenol AF for adverse effects on development of the offspring is not warranted due to inconclusive data.**

In line with the DS, RAC considers that is not possible to draw conclusions on adverse effects on or via lactation based on the limited information available. **No classification is proposed for adverse effects on or via lactation due to lack of data.**

Supplemental information - In depth analyses by RAC

Toxicokinetics and bioavailability of bisphenol AF was investigated in rodents after oral and i.v. dosing with single and repeated doses (non-guideline *in vivo* experimental studies). No guideline-conform toxicokinetics studies with bisphenol AF are available.

Single dosing

Single dosing studies were conducted rather recently by the U.S. NTP.

Waidyanatha (2015) assessed clearance and metabolism of bisphenol AF (purity: 97%) in rodents following oral administration of 3.4, 34 or 340 mg/kg [(14)C]bisphenol AF. [(14)C]bisphenol AF-associated radioactivity was excreted primarily in faeces by 72 h after oral administration to rats (65-80%) and mice (63-72%). Females excreted [(14)C]bisphenol AF to a higher extent in urine than males. Metabolites identified in bile were bisphenol AF-glucuronide, -diglucuronide, -glucuronide sulfate and –sulfate, while >94% of faecal radioactivity was present as bisphenol AF, suggesting extensive deconjugation in the intestine.

Waidyanatha (2019) assessed toxicokinetics and bioavailability of bisphenol AF (purity: 99%) in male and female Harlan Sprague Dawley rats and B6C3F1/N mice following a single gavage administration of 34, 110, or 340 mg/kg bw/d. Plasma concentrations were measured 0, 5, 15, 30 min, 1, 2, 4, 8, 12, 24, 32 and 48 h post-exposure. Bisphenol AF was rapidly absorbed in rats with the maximum plasma concentration (C_{max}) of free bisphenol AF reached at ≤2.20 h. Bisphenol AF was cleared rapidly with a plasma elimination half-life of ≤3.35 h. C_{max} of the sum of free and conjugated bisphenol AF was reached ≤1.07 h in rats with both Cmax (≥27-fold) and AUC0-∞ (≥52-fold) being much higher than corresponding free values, demonstrating rapid and extensive conjugation of bisphenol AF following oral administration. In mice after oral administration of 34 mg/kg, C_{max} of free bisphenol AF was reached more rapidly (C_{max}: ≤0.455 h) than in rats and bisphenol AF was cleared more rapidly, with an elimination half-life of ≤4.22 h. The authors used $AUC_{0-\infty}$ of free bisphenol AF following gavage and IV administration (Bioavailability (%F) as AUC/Dose (oral) ÷ AUC/Dose (i.v.) x 100) to estimate oral bioavailability and concluded that the bioavailability of free bisphenol AF in rats was ~1% with no apparent dose-related effect. Bioavailability of free bisphenol AF in mice was slightly higher than in rats (males ~6%, females ~3%). Overall, bisphenol AF was rapidly and extensively conjugated likely due to extensive first pass conjugation in the intestine and the liver, with rapid elimination from plasma or serum.

Repeated dosing

Yang *et al*. (2012) analysed traces in various tissues and excreta of bisphenol AF (purity: 98%) given orally at doses of 10 mg/kg bw to 4 male Sprague Dawley rats, daily for 2 consecutive weeks. Urine and faeces samples were collected, as well as serum and

tissues, including kidneys, liver, testis, adipose and muscle. High levels of bisphenol AF were detected in liver, kidney and serum. Bisphenol AF was also detected in other organs, such as testes. The liver seemed to be the main organ responsible for metabolism. The highest level of bisphenol AF was found in faeces (in unconjugated form).

In a study by Li *et al*. (2013), bisphenol AF (purity: 98%) was given orally (200 mg/kg bw/d) to male Sprague-Dawley rats, daily for 2 consecutive weeks (no. of animals not reported). For serum analysis animals were given a single oral dose of 20 mg/kg or 100 mg/kg (n=3). The study identified four metabolites including bisphenol AF-diglucuronide, -glucuronide (bisphenol AF-G), -glucuronide dehydrated and -sulphate in the urine of the rats.
Bisphenol AF-glucuronide was the major metabolite formed *in vivo*. Glucuronidation was a rapid and efficient pathway for biotransformation of bisphenol AF. The peak of bisphenol AF-glucuronide in plasma was observed 30 minutes after treatment, followed by a rapid decline during the next 3 hours, indicating a fast clearance in rats. The peak of bisphenol AF was observed at 1 hour, and bisphenol AF was completely eliminated after 48 hours. The three other metabolites were not found in plasma.

RAC takes note of a recent publication by NTP, not part of the CLH report (Waidyanatha, 2020), assessing comparative toxicokinetic of bisphenol AF (338, 1125, and 3750 ppm) and BPS following exposure via feed for 7 days in male rats and mice. The study confirms glucuronidation to be a major metabolic pathway *in vivo*. Elimination half-lives for free substance (4.41-10.4 h) were comparable between species and analogues. In rats, the exposure concentration-normalised maximum concentration [C_{max}/D (ng/mL)/(ppm)] and area under the concentration time curve [AUC/D (h × ng/mL)/(ppm)] was higher for free BPS (C_{max}/D: 0.476-1.02; AUC/D: 3.58-8.26) than for free bisphenol AF (C_{max}/D: 0.017-0.037; AUC/D:0.196-0.436). BPS and bisphenol AF were highly conjugated; AUC values for total BPS and total bisphenol AF were higher than corresponding free values (BPS: rats ≥18-fold, mice ≥17-fold; bisphenol AF: rats ≥127-fold, mice ≥16-fold). Data demonstrated that there are analogue and species differences in the kinetics of BPS and bisphenol AF.

The authors estimated the actual average daily intake of bisphenol AF based on feed consumption data was 23.4, 70.5 and 193.0 mg/kg bw/d for male rats and 69.4, 236.0 and 1590 mg/kg bw/d for male mice. For BPS, the estimated daily doses were 23.0, 76.8 and 298.0 mg/kg bw/d for male rats and 131.4, 428.0 and 1176.0 mg/kg bw/d for male mice. Thus, the doses administered to rats were similar to the low and mid dose treatment group in the OECD TG 422 study with bisphenol AF (30, 100, 300 mg/kg bw/d for low, mid and high dose, respectively).

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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