Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

benzyl(diethylamino)diphenylphosphonium 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenolate

EC Number: 479-100-5
CAS Number: 577705-90-9

CLH-O-0000006967-56-01/F

Adopted

18 March 2021

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18 March 2021

CLH-O-0000006967-56-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **benzyl(diethylamino)diphenylphosphonium 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenolate**

EC Number: **479-100-5**

CAS Number: **577705-90-9**

The proposal was submitted by **Sweden** and received by RAC on **16 December 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Sweden has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **9 March 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **8 May 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Agnes Schulte**

Co-Rapporteur, appointed by RAC: **Ruth Moeller**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **18 March 2021 by consensus**

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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 submitters proposal	TBD	benzyl(diethylamino)diphenylphosphonium 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenolate	479-100-5	577705-90-9	Repr. 1B	H360F	GHS08 Dgr	H360F			
RAC opinion	TBD	benzyl(diethylamino)diphenylphosphonium 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenolate	479-100-5	577705-90-9	Repr. 1B	H360F	GHS08 Dgr	H360F			
Resulting Annex VI entry if agreed by COM	TBD	benzyl(diethylamino)diphenylphosphonium 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenolate	479-100-5	577705-90-9	Repr. 1B	H360F	GHS08 Dgr	H360F			

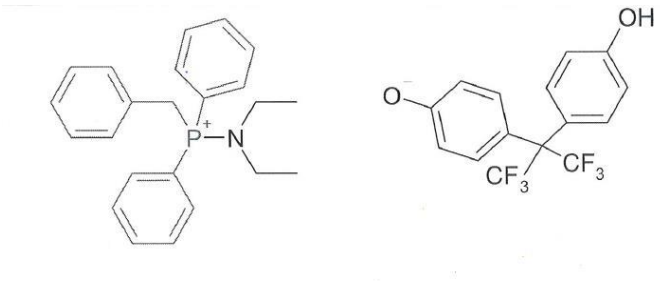
GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Benzyl(diethylamino)diphenylphosphonium 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenolate (from here on called BDDP-BPAF) is registered under REACH with a tonnage band of 1–10 t/a. Tonnage data from another individual registrant of a NONS substance is claimed as confidential and according to the ECHA website manufacture was ceased in 2019.
The substance BDDP-BPAF is a salt of the bisphenol AF (BPAF; EC No. 216-036-7)-based anion moiety, and a quaternary phosphonium cation, i.e. the Benzyl(diethyl-amino)diphenylphosphonium (BDDP) cation. The substance thus contains two components, each at ca. 50%.

The substance is used in the fluoropolymers manufacturing. The sector of end use is manufacturing of rubber and plastic products.

The substance has the following chemical structure:



BDDP-BPAF has currently no harmonised classification. It is self-classified as:

Eye Irrit. 2; H319
Acute Tox. 4; H302
Aquatic Chronic 2; H411

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.

The DS proposed read across from BPAF to this substance. Bisphenol AF is an organofluorine compound and derivative of bisphenol A (BPA) having the methyl hydrogens replaced by fluorines. RAC agrees with the DS that the CLH proposal under consideration can be assessed based on data for BPAF.

BDDP-BPAF is an ionic organic compound expected to quickly dissociate in water and biological fluids to BPAF and BDDP. Due to the dissociation behaviour, the substance toxicity can be assessed based on its two dissociation products. Bisphenol AF can be used as the source substance to read across to BDDP-BPAF, because the CLH proposal under consideration, Repr. 1B; H360F, is based on the data on the BPAF component. The organism will be exposed to the common compound BPAF, and the properties of the target substance are predicted to be

quantitatively equal to that of the source substance BPAF. No data has been made available in the CLH report on the BDDP component of the substance and the prediction of the toxic properties is based on data on BPAF only.

Bisphenol AF, which is contained in the salt BDDP-BPAF at a concentration of approximately 50%, is a structural analogue and functionally similar to BPA. In 2014, BPA was classified as Repr. 1B; H360F by RAC (ECHA, 2014). In December 2020, RAC classified 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 BPAF, and thus BDDP-BPAF, 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".

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS used data on the constituent BPAF as the basis for this CLH proposal, as BDDP-BPAF is a salt of a BPAF-based anion moiety and contains approximately 50% BPAF.

Adverse effects on sexual function and fertility

The DS evaluated adverse effects on sexual function and fertility of BDDP-BPAF mainly based on a screening study with BPAF in rats performed according to OECD TG 422 and a 28-day study in rats with BPAF 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 BPAF, 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 BPAF 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 BPAF 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 BPAF.

BPAF (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 BPAF.
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 BPAF in zebrafish and mammalian cells *in vitro* were available and results consistently indicated oestrogenic and anti-androgenic effects of BPAF, 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 BPAF.

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 BPAF, and thus BDDP-BPAF, as Repr. 1B; H360F is warranted.

Adverse effects on the development of the offspring

The DS evaluated the developmental toxicity of BDDP-BPAF mainly based on the OECD TG 422 screening study in rats with BPAF, as well as on a few non-guideline studies with BPAF, 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) currently performs a Modified One-Generation (MOG) study with BPAF.

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 BPAF 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 BPAF 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 BPAF 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 BPAF 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 BPAF 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 BDDP-BPAF via lactation mainly based on the OECD TG 422 screening study in rats with BPAF, as well as on a non-guideline cross-fostering study with BPAF 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 BPAF 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), BPAF was given to female rats during lactation. BPAF was transferred via breast milk to the pups. The lactational exposure resulted in significantly increased levels (free and total) of BPAF 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 BPAF-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 standard consultation

Three 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 BDDP-BPAF as Repr. 1B; H360F based on read across from BPAF, as the substance contains ca. 50% of BPAF as an anion.

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 with BPAF and 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 BPAF on male and female sexual function and fertility.

OECD TG 422

In an OECD TG 422 study, BPAF 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 BPAF 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 BPAF 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 BPAF 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 implantation sites) x 100 (mean %)	9.5 ± 10.3 (n=10)	7.8 ± 7.6 (n=7)[###]	22.9 ± 35.5 (n=7)[####]	-
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 Keratinisation cervix	0 0	0 2/2 (100%)	0 3/4 (75%)	1/11 (9%) 1/11 (9%)
Vagina				
Epithelial hyperplasia Minimal	0	0	0	4/11 (36%)
Epithelial keratinisation Keratin cyst	0 0	2/2 (100%) 0	1/4 (25%) 0	2/11 (18%) 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 BPAF 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 BPAF 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 BPAF on fertility (no. of pregnant females) are considered as unequivocal substance-related effects justifying 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 BPAF 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 BPAF-treated males. Effects observed on testis and oestrous cycle further indicate endocrine-mediated mechanisms underlying the toxicity of BPAF. 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 BPAF.

Uterotrophic assay and a Hershberger assay

The assays were conducted according to GLP with BPAF (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 BPAF 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. BPAF was tested **positive in the Uterotrophic assay** (dose-response), with significantly increased uterine blotted weight at all doses tested, suggesting oestrogenic agonistic properties of BPAF. 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 BPAF. 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 BPAF 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 BPAF 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 BPAF for male SD rats was <10 mg/kg bw/d.

Other mechanistic studies

Several mechanistic studies on the effects of BPAF in zebrafish, and *in vitro*, are available. **Results consistently indicate oestrogenic and anti-androgenic effects of BPAF** (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 BPAF 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). BPAF 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). BPAF 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, BPAF 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 BPAF 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 BPAF, 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 BPAF has been demonstrated in human derived cell lines. Data on BPA and BPS are considered as supportive for the classification proposal for BPAF 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 BPAF 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 BPAF (only data on oral route and on effects in young adult/parental animals available), BPAF-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 BPAF), 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 BDDP-BPAF mainly based on a screening test with BPAF in rats performed according to OECD TG 422, as well as on a few non-guideline studies with BPAF, which were identified during a literature search.

RAC takes note of the upcoming Modified One-Generation (MOG) study of the National Toxicology Program (NIEHS) with BPAF from which tabled summary results have recently been published[1], but for which a study report is not yet published[2].

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

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

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 BPAF 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 BPAF 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 BPAF, 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 BPAF via breast milk during lactation and impact of BPAF treatment on testosterone levels in serum and androgen 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 BPAF 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.

(A more detailed review may be considered in a later CLH dossier on the developmental toxicity, if justified by data of the new NTP study or other data.)

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 BPAF. RAC, however, also notes that the (raw) data of the modified one generation study highlighted by the DS are already available, while a summary report is still pending. This additional data might provide sufficient information for deciding on whether classification of BPAF, and thus BDDP-BPAF for developmental toxicity is warranted.

Adverse effects on or via lactation

The DS evaluated effects of BDDP-BPAF on or via lactation based on the OECD TG 422 study and a non-guideline cross-fostering study with BPAF (Li *et al*., 2016). Results from the above described screening study are not indicative of any effects of BPAF 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 BPAF, and thus BDDP-BPAF, 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 BPAF 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 the main constituent of BDDP-BPAF, i.e. BPAF.

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 BPAF via an oestrogenic main mode of action.

RAC concludes that classification for adverse effect on sexual function and fertility of BDDP-BPAF 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 BDDP-BPAF 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 insufficient data.**

Additional references

Mandrup, K. *et al*. (2016): Low-dose effects of bisphenol A on mammary gland development in rats. Andrology. 2016 Mar 1; 673-683.

Waidyanatha, S. *et al*. (2020): Comparative toxicokinetics of bisphenol S and bisphenol AF in male rats and mice following repeated exposure via feed. Xenobiotica. 2020 Oct 6;1-12.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).