Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Adopted

18 March 2021

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 4,4'-[2,2,2-TRIFLUORO-1-(TRIFLUOROMETHYL)ETHYLIDENE]DIPHENOL; BISPHENOL AF

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]diphenol; bisphenol AF
EC number: 216-036-7
CAS number: 1478-61-1
Dossier submitter: Sweden

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
05.05.2020	Germany		MemberState	1
Comment received				
Toxicokinetics: Bioavailability It is stated on page 8 of the CLH report that the bioavailability after oral exposure was low in rats (only 1 %) citing the study from Waidyanatha et al. (2019). Some systemic availability is the prerequisite to induce the observed reproductive effects. It appears from this study that this low value refers only to free BPAF in plasma as reference and did not consider the conjugated metabolites. It is furthermore stated in this study that after oral exposure an extensive first pass conjugation in the intestine and liver occurs. Thus, the bioavailability (and systemic availability) of BPAF including metabolites should be clearly higher than 1 %.				
Dossier Submitter's Response				
Thank you for your comment. We have cited the results as stated in the paper, but we do agree with your reasoning. In this case, however, this TK data may be of less importance since there are clear toxicological effects demonstrated in several studies, including the Repro screening study (OECD 422).				
RAC's response				
RAC agrees with the view of the Dossier Submitter. It is noted that the 1% bioavailability in rats refers to free BPAF only. Unlike for BPA it is currently not known whether free BPAF alone or free and conjugated BPAF are the active forms.				

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2020	Belgium		MemberState	2
Comment received				
BE CA would thank Swedish Chemicals Agency for this CLH dossier proposal. BECA strongly supports the proposal to classify Bisphenol AF as Repr. 1B H360.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2020	Belgium		MemberState	3
Comment received				
BECA strongly supports the proposal to classify Bisphenol AF as Repr. 1B H360F based on the severe observed fertility effects. In the Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (study report, 2011), performed in rats (SD), the fertility index was clearly dose dependently decreased. And at the highest dose, no pregnancy was induced (11, 10, 8 and 0 females respectively at 0, 30, 100 and 300 mg/kg bw/d). Furthermore, the number of corpora lutea and the number of implantations were also dose-dependently reduced. In this combined study, males exhibited also effects as reproductive organs weight were affected. Moreover, Leydig cell atrophy was noted in 3 males out of 12 of the mid dose and in 11 males out of 12 of the highest dose level (compared to only in 1 male in control group). This effect was confirmed by the 28-day repeated dose toxicity study (Umamo et al., 2012) in which Leydig cell atrophy was observed in 5 male rats out 10 exposed to 100 mg/kg bw/d (highest dose tested). Clear and severe fertility effects were demonstrated in absence of general toxicity. BECA supports the proposal to classification Bisphenol AF as Repr. 1B H360F. BE CA is of the opinion that a prenatal developmental toxicity study is needed to correctly assess the developmental toxicity endpoint. Although the results of the available combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) were negative, indications of developmental toxicity shown in literature mentioned in the CLH point however to a concern. Furthermore, one of the standard information requirements of Annex IX of the REACH Regulation (No 1907/2006) for substances manufactured or imported in quantities of 100T or more is the performance of a prenatal developmental toxicity study with one species using the most appropriate route of administration.				
Dossier Submitter's Response				
Thank you for your support and for your comment about a prenatal developmental toxicity study. A modified one-generation study to generate information on BPAF's effect				

on prenatal development, postnatal development, and reproduction is ongoing at the National Toxicology Program (NIH, US). Data is currently under review and when these results are available they may help to further clarify this endpoint.
RAC's response
Both aspects were considered in the opinion document.

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2020	France		MemberState	4
Comment received				
We agree with the proposal Repr. 1B –H360F. This classification is fully justified, in particular, since there is no pregnant females in the OECD 422 study at the highest tested dose. Regarding effect on development and on or via lactation, we agree that the available data do not allow proposing a classification.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
05.05.2020	Germany		MemberState	5
Comment received				
Fertility The evaluation of the reproductive toxicity of BPAF was mainly based on a screening test according to OECD TG 422 and a 28-day study according to OECD TG 407 in rats with oral administration of the test substance. Supporting information from an uterotrophic assay and a Hershberger assay as well as several mechanistic studies are given. The screening study was performed in rats using dose levels of 0, 30, 100 and 300 mg/kg bw/day. BPAF caused a dose-dependent decrease in the fertility index down to 83 %, 64 %, and 0 % for 30, 100, and 300 mg/kg bw/day, respectively, compared to 100 % for the control group. In the highest dose group no pregnancy was induced in any of the mated females. Pre- and post-implantation loss was increased in the low and mid dose compared to the con-trol, this increase was, however, not significant. The number of corpora lutea and implanta-tions was lower in treated animals as compared to the control; this effect again was not sig-nificant. A high incidence of follicular ovarian cysts was noted in the non-pregnant high dose females (including the recovery group). Moreover, in pregnant females of the other dose groups, ovarian follicles increased with dose. Absolute and relative weights of several repro-ductive organs (e.g. testes) were significantly decreased in the high dose males as compared to the control. In the 28-day study according to OECD TG 407 similar significant effects on male reproduc-tive organs were detected at 100 mg/kg bw/day (e.g. prostate, seminal vesicles) including histopathological effects (e.g. Leydig cell atrophy in testes). A NOAEL of 30 mg/kg bw/day can be derived.				

In both tests irregular oestrus cycle was noted in some of the high dose females and atrophy of mammary glands in high dose males. The results indicate endocrine-mediated oestrogenic effects (effects on mammary glands, testes, oestrus cycle) that were underlined by the uterotrophic assay where BPAF significant-ly increased the uterine blotted weight in all dose groups (8, 40, 100 mg/kg/day). Several other studies support the oestrogenic and anti-androgenic activity of BPAF (in vivo studies in zebrafish, in vitro studies). Comparative studies indicate a stronger oestrogenicity for BPAF as compared to BPA. With the dose-dependent significant decrease of the fertility index in the OECD TG 422 from the lowest dose (30 mg/kg bw/day), the significantly lower reproductive organ weights in males in the screening and the 28-day studies and the signs of oestrogenic/anti-androgenic activity clearly indicate an impairment of sexual function and fertility in both sexes. No marked general (parental) toxicity was seen in any study. Therefore, classification of BPAF as Repr. 1B, H360F is supported. Developmental toxicity: For the evaluation of developmental toxicity, a guideline study according to OECD TG 422 and few non-guideline studies are available. In the screening study, no adverse effects on offspring were seen up to PND 5. However, it has to be noted that in the high dose group no offspring were produced, so developmental effects cannot be excluded at 300 mg/kg bw/day. In non-guideline studies, effects of BPAF on offspring were shown, such as accelerated pubertal mammary gland development in female mice, transfer of BPAF in breast milk and alteration of hormone levels in serum and testes of male rats, and impacts on behaviour (e.g. anxiety in males, impaired memory formation in both sexes) of mice. However, due to poor reporting of these studies and lacking GLP compliance, these studies cannot be used for classification. Due to the lack of guideline-conform developmental toxicity data, the DE CA agrees that with the available information a classification of BPAF as developmental toxicant is not indicated. Adverse effects via lactation In the screening study according to OECD TG 422, no effects of BPAF via lactation until PND 5 were found. A cross-fostering study in rats showed transfer of BPAF via breast milk and subsequent ef-fects on Inhibin B and androgen receptor levels as well as effects on body weight of off-spring. However, the results of the study are not robust for classification due to poor reporting and lack of GLP-compliance.
Dossier Submitter's Response
Thank you for your support.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
07.05.2020	Netherlands		MemberState	6
Comment received				

We agree with the proposed classification in Repr. 1B for adverse effects on sexual function and fertility. Clear effects on fertility were observed in the OECD 422 study, starting at the lowest dose, without marked systemic toxicity. The clear effects on fertility observed in this study alone is considered sufficient for classification as Repr. 1B H360F. The mechanistic studies indicate an endocrine-mediated mechanism is involved, further supporting the proposed classification,.

Regarding developmental toxicity, the following was noted:

- OECD 422 study, oral, 0-30-100-300 mg/kg bw/day, rats
o No significant effects on offspring treated in utero.
o No differences in sex ratio and body weights of offspring between treated animals and controls.
o Necropsy findings in offspring: no evident effects from BPAF treatment
o Note: no pups at all produced by animals in the high dose group treated with 300 mg/kg bw/day.

- In vivo study mammary gland, exposure GD 10.5-17.5, follow-up offspring until 16 months, CD-1 mice, 0, 0.05, 0.5, 5 mg/kg bw twice per day:
o BPAF exposure caused accelerated pubertal mammary development.
o 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.

- In vivo study on effects on offspring, SD rats, exposure GD 3-19 and PND 3-19, 0 and 100 mg/kg/bw/d:
o Lactational exposure caused significantly increased levels of BPAF in serum and in testis, showing that BPAF was transferred via breast milk.
o 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.

- In vivo study on neurobehaviours in adolescent mice offspring, exposure GD 1-19, 0-0.4- 4 mg/kg bw/day.
o Fetal exposure to BPAF induced anxiety- and depressive-like behaviours in male adolescent offspring. In addition, BPAF exposure impaired memory formation in both sexes.
o Note: no exact numbers given in the research article, no information on parental toxicity.

Perhaps a discussion for classification as category 2 developmental toxicant would be possible, but it seems there is insufficient robust reporting to draw conclusions on possible developmental toxicity.
Overall, there are indications of treatment-related developmental effects, but the evidence is inconclusive for classification and we agree that the available information is

insufficient for classification for developmental toxicity and for classification for effect on or via lactation.
Dossier Submitter's Response
Thank you for your support. A modified one-generation study to generate information on BPAF's effect on prenatal development, postnatal development, and reproduction is ongoing at the National Toxicology Program (NIH, US). Data is currently under review and when these results are available they may help to further clarify this endpoint.
RAC's response
The feasibility of your proposal to consider classification for developmental toxicity has been checked. Finally RAC agreed with the DS on the lack of robust data and noting that a new 1-generation study is soon awaited.