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'-sulphonyldiphenol; bisphenol S

EC Number: 201-250-5

CAS Number: 80-09-1

CLH-O-0000006929-56-01/F

Adopted

10 December 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 4,4'-SULPHONYLDIPHENOL; BISPHENOL S

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'-sulphonyldiphenol; bisphenol S
EC number: 201-250-5
CAS number: 80-09-1
Dossier submitter: Belgium

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
07.02.2020	Germany	CHEM Trust Europe	International NGO	1
Comment received				
- CHEM Trust would like to thank the dossier submitter for their important work on the CLH report to classify BPS as a substance toxic to reproduction. - The similar substance Bisphenol A is already classified as Rep 1B and has been identified and included in the REACH Candidate List as a SVHC due to its endocrine disrupting properties. - An identification as endocrine disruptor may also be warranted for BPS as many studies have shown estrogenic activity like BPA (see e.g. https://academic.oup.com/toxsci/article/139/1/35/2338266). - Already in 2015 RAC highlighted that BPS has similar properties to BPA and still the substances was marketed without any information on its potential hazards despite indications for the damage on fertility (see insufficient self-classification by companies in the classification and labelling inventory, as summarized in the CHEM Trust report `From BPA to BPZ. https://www.chemtrust.org/wp-content/uploads/chemtrust-toxicsoup-mar-18.pdf) - At the same time BPS was increasingly used as a replacement for BPA in thermal paper, as has been found in an ECHA survey (https://echa.europa.eu/-/bpa-being-replaced-by-bps-in-thermal-paper-echa-survey-finds). - Use of BPS as a replacement for BPA may potentially lead to higher internal exposure to endocrine active substances as there may be a higher systemic bioavailability after oral ingestion of BPS compared to BPA as recently reported when studied in pigs (https://doi.org/10.1289/EHP4599). - In order to avoid replacing a harmful substance with one with similar properties we urge taking a group approach to bisphenols in the necessary subsequent risk management measures.				

- BPS has been included in ChemSec`s SIN list in 2014 as an endocrine disrupter based on its estrogenic properties. It has shown to be estrogenic in in vitro studies. In vivo studies have shown impaired reproduction in zebrafish and uterine growth in rat. (https://sinsearch.chemsec.org/chemical/80-09-1). - The need for classification due to other hazards e.g. acute toxicity should be scrutinised as a recent study in isolated mouse hearts may indicate a possibility for instant heart effects after exposure to BPS in amounts that mimicked typical human levels (https://www.news-medical.net/news/20200109/Study-Bisphenol-S-can-hinder-heart-function-within-minutes-of-exposure.aspx). - It is very concerning that there is widespread exposure of the general population to BPS. The substance has also been included as a priority in the ongoing Human Biomonitoring Initiative https://www.hbm4eu.eu/mdocs-posts/hbm4eu-ici-equas-report-bisphenols-in-urine-round-2/ - BPS is meanwhile already ubiquitous in the environment, and has been shown to affect the development of Zebrafish larvae (Wu et al. 2018). Wu L-H et al, 2018 Occurrence of bisphenol S in the environment and implications for human exposure: A short review. Sci Total Environ.615, 87-98; https://doi.org/10.1016/j.scitotenv.2017.09.194
Dossier Submitter's Response
Thank you for your comment.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
07.02.2020	Germany	BASF SE	Company-Manufacturer	2
Comment received				
Comments provided by the EU-REACH registrants ECHA note – An attachment was submitted with the comment above. Refer to public attachment 80-09-1_DHDPS_comments on CLH dossier_public consultation_clean.pdf				
Dossier Submitter's Response				
Thank you for your comment. See the response to comment number 9.				
RAC's response				
Noted. Please see the response to comment number 9.				

Date	Country	Organisation	Type of Organisation	Comment number
06.02.2020	Denmark	DTU National Food Institute	Academic institution	3
Comment received				
We support that BPS is classified with Repr. 1B H360FD				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
07.02.2020	Sweden		MemberState	4
Comment received				
The Swedish CA supports classification of bisphenol S (CAS No. 80-09-1) as Repr 1B H360 FD. SE agrees with the rationale for classification into the proposed hazard class and differentiations.				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.02.2020	Germany	CHEM Trust Europe	International NGO	5
Comment received				
- CHEM Trust supports the classification of BPS as a reprotoxic substance, category 1B (labelling GHS08 Dgr, H360FD) because: - A new EORGTS study from 2019 (OECD 443) supported by a reproduction/developmental toxicity screening test and further one combined with a repeated dose toxicity study (OECD 421 and 422) clearly show adverse effects on sexual function and fertility which cannot be related to a general toxicity. - A new EORGTS study from 2019 (OECD 443) supported by a prenatal developmental toxicity study (OECD 414) and a combined repeated dose toxicity study/reproduction/developmental toxicity screening test (OECD 422) clearly show adverse effects on development which cannot be related to a general toxicity.				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.01.2020	Germany		MemberState	6
Comment received				
For bisphenol S the classification Repr. 1B, H360FD is proposed. The proposal is based on effects seen in rats after administration of the test substance by gavage. Fertility In a reproductive toxicity study according to OECD 421 significant effects on fertility parameters were detected (Anonymous 12, 2000). In the highest dose group the mean oestrus cycle was significantly longer as compared to the control group. Also the fertility index was markedly lower in the high dose group (58 % as compared 92 % in the control group). A decrease in implantation sites was noted and the implantation index was significantly lower as compared to the control. In males a decrease of the relative pituitary weight and an increase of the weight of the seminal vesicles were observed in				

the highest dose group.
In an extended one generation reproductive toxicity study in rats similar effects were found (Anonymous 13, 2019). A significant longer oestrus cycle and a slight decrease of implantation sites were observed in F0 females at the highest dose level (180 mg/kg bw/d), as well as significant increased mean number and percentage of post implantation loss (also in Cohort 1B). A slight but significant decrease of sperm motility was noted in in all dose groups in F0 males; however, fertility was not affected. Additionally a significant lower number of liveborn and a higher number of stillborn pups were noted F0 females of the highest dose group.
Similarly a study according to OECD TG 422 in rats (Anonymous 14, 2017) observed a significantly increased oestrus cycle at the highest dose level (300 mg/kg bw/day) as well as a significantly lower mean number of implantation sites. Post-implantation loss (%) was significantly higher and the mean number of delivered pups was significantly lower at 300 mg/kg bw/d.
Maternal toxicity was not very pronounced, comprising slight effects on body weight, liver and kidney. No mortality was noted in any of the dose groups.
Because of absence of significant maternal toxicity and the increased length of oestrus cycle and marked reduction of implantation sites in three reproductive toxicity studies, the DE CA agrees that a classification as Repr. 1B, H360F is warranted.

Developmental toxicity

The classification of bisphenol S as Repr. 1B H360D is based on a significant increase of post-implantation losses in two studies in the absence of significant maternal toxicity. Indeed, post-implantation loss was significantly higher at the highest dose levels (300 mg/kg bw/d) in a combined repeated dose toxicity study with reproduction/developmental toxicity screening test (300 mg/kg bw/d; Anonymous 14, 2017) and an EOGRTS (60 + 180 mg/kg bw/d; Anonymous 13, 2019). In a prenatal developmental toxicity study according to OECD TG 414 (Anonymus 19, 2014) mean post-implantation loss is also noticeably increased at 300 mg/kg bw/d).
Maternal toxicity was minimal and thus, the classification of bisphenol S as Repr. 1B H360D is supported.

Dossier Submitter's Response
Thank you for your comment and support.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
06.02.2020	Denmark	DTU National Food Institute	Academic institution	7
Comment received				
Arguments are provided below for support for a classification of Repr. 1B H360FD with reference to the CLP report and open literature. Severe decreased number of implantation sites and severe higher estrus duration were observed in three different studies. These effects were more pronounced in the reproductive toxicity test (Anonymous 12, 2000) and in the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (Anonymous 14, 2017). In the EOGRTS (Anonymous 13, 2019), the highest tested dose was only 180				

mg/kb bw/d (compared to 300 mg/kg bw/d in Anonymous 12 (2000) and Anonymous 14 (2017)). At this top dose, nearly absent general toxicity was observed. The DS wants to highlight that females exposed to 180 mg/kg bw/d exhibited already significant fertility effects, which would be more pronounced if the study would have been dosed higher as it is the case in the Anonymous 12 (2000) and Anonymous 14 (2017).
The setting of the top dose in Reproductive studies and Cancer studies is currently discussed in EU (incl. ECHA) and globally in OECD. In this EOGRTS study (Anonymous 13, 2019), the top dose might have been set too low, but still adverse reproductive toxicity effects (fertility) were seen. This supports a classification as with this low top dose no maternal toxicity or general toxicity is seen.
I strongly support that this also according to the CLP criteria leads to a classification as Repr. 1B for adverse effects on sexual function and fertility. This classification is warranted based on the above mentioned severe effects observed in the available studies, which cannot be related to a general toxicity (see also comment on top dose).

According to the CLP criteria a classification as Repr. 1B for adverse effects on development is warranted based clear evidence of an adverse effect on development in the absence of toxic effect. Severe higher incidence of post-implantation loss were observed in two different studies, which cannot be related to a general toxicity. I support that BPS is classified with Repr. 1B H360FD
Moreover, in the open literature, also several papers show similar effects on reproduction as BPA (Ahsan et al. 2018). The results in this study suggest that neonatal exposure to higher concentrations of BPS can lead to BPA like structural and endocrine alterations in female rats.
ANSES have made a report in 2013 entitled Substitution of bisphenol A: review of alternatives to BPA, identification of the hazards of potential substitutes for bisphenol A" (ANSES, 2013) Both the ANSES report and other previous studies have shown that most BPA analogous including BPAF share common mechanistic properties such as estrogenic activity.

Ref. Ahsan N, Ullah H, Ullah W, Jahan S. Comparative effects of Bisphenol S and Bisphenol A on the development of female reproductive system in rats; a neonatal exposure study. Chemosphere. 2018;197:336–343.
doi:10.1016/j.chemosphere.2017.12.118

Dossier Submitter's Response

Thank you for your comment, your support and the new references.
As mentioned in your comment, the Ashan *et al.* article (2018) demonstrates that female pup rats, exposed to BPS by subcutaneous injection from PND 1 to 10 (0, 0.5, 5 or 50 mg/kg), revealed alterations in different reproductive parameters such as onset of puberty, estrous cyclicity, gonadal maturity, number of ovarian follicles and plasma reproductive hormones.

- Day of puberty onset was dose related changed: 37.00, 38.80, 40.37 and 41.66** respectively at 0, 0.5, 5 and 50 mg/kg (same trend for BPA : 42.40** at 50 mg/kg)
- GSI (gonadosomatic index) was dose related reduced: 0.079, 0.075; 0.073 and 0.066*** respectively at 0, 0.5, 5 and 50 mg/kg (same trend for BPA : 0.059*** at 50 mg/kg)
- Corpus luteum was dose related changed : 13.38, 12.63, 11.50* and 9.65** respectively at 0, 0.5, 5 and 50 mg/kg (same trend for BPA : 8.38*** at 50 mg/kg)

- LH was dose related decreased : 2.65, 2.34, 2.30 and 2.21*** ng/ml respectively at 0, 0.5, 5 and 50 mg/kg (BPA : 2.05*** ng/ml at 50 mg/kg)
- FSH was dose related reduce : 4.69, 5.54, 4.47 and 4.19* mlU/ml respectively at 0, 0.5, 5 and 50 mg/kg (BPA : 3.89** at 50 mg/kg)

Furthermore, the percentage of females that conceived was decreased (100 % in control, low and mid dose groups, while 60 % in the highest dose). The mean number of pups born per female was dose related and significantly lower at the highest dose (8.80, 8.80, 8.60 and 5.33** respectively at 0, 0.5, 5 and 50 mg/kg).

RAC's response

Noted. Please see the response to comment number 8.

Date	Country	Organisation	Type of Organisation	Comment number
06.02.2020	France		MemberState	8

Comment received

In relation to fertility, based on the data provided in the C&L dossier, an effect on female cyclicity is demonstrated with consistent findings in the 3 studies that investigate this parameter: increased cycle duration and prolonged dioestrus. Proper cyclicity is essential for a functional reproductive function in mammals and humans in particular. An effect on implantation is also consistently observed. An impact on the fertility index is observed at the highest doses and the effect cannot be attributed to the modest general toxicity. A classification Repr 1B for fertility is fully supported on this basis.

In males, effects on the weight of reproductive organs are noted in several studies (effect on seminal glands in OECD 421 at 300 mg/kg, on prostate in the EOGRTS at 180 mg/kg, on prostate and seminal gland in the 28-day study, on testis and epididymis in the 90-day study). Atrophy of mammary gland is also consistently observed in males. Although it has no impact on the reproductive function, this effect may indicate sex hormone disturbance, together with increased pituitary weight in the OECD 421 study. No histological findings are reported in male reproductive organs. However, sperm parameters are very poorly investigated in the dataset. A decrease of sperm motility is reported in F0 in the EOGRTS and to our understanding this parameter has not been investigated in any other studies. An effect on male reproductive function is therefore suspected but is insufficiently characterised.

In relation to developmental toxicity, a significant effect in post-implantation loss in the OECD 422 study as well as in the EOGRTS is observed. The number of stillborn F1 pups was also increased in the EOGRTS and modifications of fetal weight are noted. The effects cannot be attributed to the modest maternal toxicity and a classification Repr 1B for development is fully supported on this basis.

In the EOGRTS, the DNT cohort gave a negative outcome and the DIT cohort was inconclusive. The results of the DNT cohort should have been reported with more details in Annex I to allow adequate analysis. In particular, data supporting the reliability and sensitivity of the test method (i.e. positive and historical control data) should be specified as well as statistical treatment of results, including statistical models used to analyze the data, and the results, regardless of whether they were significant or not (OECD TG 443). Lastly only means are reported in Cohort 2 without any indication on the standard deviation (SD) or the standard error of the mean (SEM). Moreover some published

studies (see literature reference list below) show effects on the expression of maternal behavior and anxiety-related behaviour. Lastly the highest dose in this study is considered insufficiently high and prevent from any conclusion.

In conclusion, the proposed classification is supported for BPS.

It is also noted that the effects observed with BPS are similar to effects of its close structural analogue BPA, that is classified Repr 1B for fertility and identified as an SVHC for its endocrine disrupting properties relevant for health and environment.

In addition, it is noted that a number of studies have been published, in particular in the very recent years and relates to investigation of effects of BPS on reproductive function as well as developmental effects of BPS. A non-exhaustive list of publications is provided below. These data seem to :
- Provides further evidence that BPS affect female reproductive function as well as male reproductive function, in rats and mice
- Provides some indications that developmental exposure to BPS may alter metabolic function, behaviour and mammary gland development
- Provide indication about human impregnation including in pregnant woman
- Give some warnings about potential human health effects linked to BPS exposure but further investigations on its health effects in humans are warranted.

The data presented in the C&L dossier fully justify a classification Repr 1B for fertility and development and an in-depth assessment of published studies may not be necessary. But these publications emphasise that the scope of the reproductive and developmental effects of BPS is not fully characterised yet. None of the published studies is in contradiction with the proposed classification.

We note that BPS is evaluated by Belgium (within the CORAP list). In this context, we recommends that these data should also be included in the registration dossier by registrants to provide an exhaustive evaluation of the effects of the substance

Editorial comment:
The OECD 407 study seems to be wrongly described on page 26 as a dietary study whereas BPS was given by gavage in this study.

Additional bibliographic references:
1: Jing J, Pu Y, Gingrich J, Veiga-Lopez A. Gestational Exposure to Bisphenol A and Bisphenol S Leads to Fetal Skeletal Muscle Hypertrophy Independent of Sex. Toxicol Sci. 2019 Dec 1;172(2):292-302. doi: 10.1093/toxsci/kfz198. PubMed PMID: 31501865; PubMed Central PMCID: PMC6876539.

2: Ullah A, Pirzada M, Jahan S, Ullah H, Razak S, Rauf N, Khan MJ, Mahboob SZ. Prenatal BPA and its analogs BPB, BPF, and BPS exposure and reproductive axis function in the male offspring of Sprague Dawley rats. Hum Exp Toxicol. 2019 Dec;38(12):1344-1365. doi: 10.1177/0960327119862335. PubMed PMID: 31514588.

3: Ijaz S, Ullah A, Shaheen G, Jahan S. Exposure of BPA and its alternatives like BPB, BPF, and BPS impair subsequent reproductive potentials in adult female Sprague Dawley rats. Toxicol Mech Methods. 2020 Jan;30(1):60-72. doi: 10.1080/15376516.2019.1652873. Epub 2019 Sep 13. PubMed PMID: 31424294.

4: Shi M, Whorton AE, Sekulovski N, MacLean JA, Hayashi K. Prenatal Exposure to Bisphenol A, E, and S Induces Transgenerational Effects on Male Reproductive Functions in Mice. Toxicol Sci. 2019 Dec 1;172(2):303-315. doi: 10.1093/toxsci/kfz207. PubMed PMID: 31532523.

5: Prokešová Š, Ghaibour K, Liška F, Klein P, Fenclová T, Štiavnická M, Hošek P, Žalmanová T, Hošková K, Řimnáčová H, Petr J, Králíčková M, Nevoral J. Acute low-dose bisphenol S exposure affects mouse oocyte quality. Reprod Toxicol. 2019 Dec 24. pii: S0890-6238(19)30692-6. doi: 10.1016/j.reprotox.2019.12.005. [Epub ahead of print] PubMed PMID: 31881267.

6: da Silva BS, Pietrobon CB, Bertasso IM, Lopes BP, Carvalho JC, Peixoto-Silva N, Santos TR, Claudio-Neto S, Manhães AC, Oliveira E, de Moura EG, Lisboa PC. Short and long-term effects of bisphenol S (BPS) exposure during pregnancy and lactation on plasma lipids, hormones, and behavior in rats. Environ Pollut. 2019 Jul;250:312-322. doi: 10.1016/j.envpol.2019.03.100. Epub 2019 Apr 9. PubMed PMID: 31003143.

7: Yin N, Liang X, Liang S, Liang S, Yang R, Hu B, Cheng Z, Liu S, Dong H, Liu S, Faiola F. Embryonic stem cell- and transcriptomics-based in vitro analyses reveal that bisphenols A, F and S have similar and very complex potential developmental toxicities. Ecotoxicol Environ Saf. 2019 Jul 30;176:330-338. doi: 10.1016/j.ecoenv.2019.03.115. Epub 2019 Apr 2. PubMed PMID: 30951980.

8: Meng Z, Wang D, Liu W, Li R, Yan S, Jia M, Zhang L, Zhou Z, Zhu W. Perinatal exposure to Bisphenol S (BPS) promotes obesity development by interfering with lipid and glucose metabolism in male mouse offspring. Environ Res. 2019 Jun;173:189-198. doi: 10.1016/j.envres.2019.03.038. Epub 2019 Mar 21. PubMed PMID: 30921577.

9: Ullah A, Pirzada M, Jahan S, Ullah H, Khan MJ. Bisphenol A analogues bisphenol B, bisphenol F, and bisphenol S induce oxidative stress, disrupt daily sperm production, and damage DNA in rat spermatozoa: a comparative in vitro and in vivo study. Toxicol Ind Health. 2019 Apr;35(4):294-303. doi: 10.1177/0748233719831528. Epub 2019 Mar 14. PubMed PMID: 30871434.

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10;126(8):087003. doi: 10.1289/EHP3189. eCollection 2018 Aug. PubMed PMID: 30102602; PubMed Central PMCID: PMC6108869.

13: Nevoral J, Kolinko Y, Moravec J, Žalmanová T, Hošková K, Prokešová Š, Klein P, Ghaibour K, Hošek P, Štiavnická M, Řimnáčová H, Tonar Z, Petr J, Králíčková M. Long-term exposure to very low doses of bisphenol S affects female reproduction. Reproduction. 2018 Jul;156(1):47-57. doi: 10.1530/REP-18-0092. Epub 2018 May 10. PubMed PMID: 29748175.

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PMC5847463.

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19: Žalmanová T, Hošková K, Nevoral J, Adámková K, Kott T, Šulc M, Kotíková Z, Prokešová Š, Jílek F, Králíčková M, Petr J. Bisphenol S negatively affects the meotic maturation of pig oocytes. Sci Rep. 2017 Mar 28;7(1):485. doi: 10.1038/s41598-017-00570-5. PubMed PMID: 28352085; PubMed Central PMCID: PMC5428703.

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30. Machtinger, R., et al. (2018). "Urinary concentrations of phthalate metabolites, bisphenols and personal care product chemical biomarkers in pregnant women in Israel." Environment International 116: 319-325.

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32. Philips, E. M., et al. (2018). "First Trimester Urinary Bisphenol and Phthalate Concentrations and Time to Pregnancy: A Population-Based Cohort Analysis." J Clin Endocrinol Metab 103(9): 3540-3547.

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Dossier Submitter's Response

Thank you for your comment and your support.

Thank you also for the new references which will be added in the evaluation performed by Belgium.

The full study report, received by the DS, only mentioned HCD regarding the % of sperm motility. The period was comprised between April 2013 and September 2017, the mean % of sperm motility was of 86% (with a minimum of 79% and a maximum of 93%, Q25 of 84 and Q75 of 88%). No other HCD was made available to the DS.

Regarding DNT and DIT cohorts, you can find below a summary of the investigations performed with more details in bold.

For cohort 2A :

- *number of animals at the start of the test :* 10/sex/dose
- *time of death during the study and whether animals survived to termination :* no mortality observed during the study period.
- *clinical observations:* excessive salivation was observed immediately after exposure in 1 female and in 3 males exposed to 180 mg/kg bw/d
- *body weight data :*

Table 39 : body weight data

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
D 0	102.5	101.9	104.2	101.0	89.0	87.3	93.4	95.3
D 21	288.0	281.6	297.4	294.1	187.3	185.3	193.6	199.1
D 42	402.5	399.6	413.3	408.2	235.8	235.2	237.8	253.0

- *startle response examination at PND 24 :*
  - *Mean max. ampl.(block 1-5) :* 265.9, 188.4*, 229.8 and 272.1 in males and 218.0, 214.2, 221.6 and 225.4 in females, respectively at 0, 20, 60 and 180 mg/kg bw/d

Additional table

	Males				**Females**			
Dose level (in mg/kg bw/d)	**0**	**20**	**60**	**180**	**0**	**20**	**60**	**180**
Mean Max. Ampl.	**265.9**	**188.4***	**229.8**	**272.1**	**218.0**	**214.2**	**221.6**	**225.4**
SD	**76.4**	**46.9**	**47.7**	**65.0**	**59.0**	**54.4**	**75.1**	**45.3**

(Stat test :Kruskal-Wallis + Wilcoxon tests)

  - *Mean latency (block 1-5) :* 19.4, 20.3, 19.6 and 19.6 msec in males and 20.7, 19.8, 21.1 and 19.2 msec in females, respectively at 0, 20, 60 and 180 mg/kg bw/d

Additional table

	Males				**Females**			
Dose level (in mg/kg bw/d)	**0**	**20**	**60**	**180**	**0**	**20**	**60**	**180**
Mean latency (msec)	**19.4**	**20.3**	**19.6**	**19.6**	**20.7**	**19.8**	**21.1**	**19.2**
SD	**1.1**	**2.0**	**0.9**	**1.7**	**2.9**	**1.5**	**2.8**	**1.6**

(Stat test :Kruskal-Wallis + Wilcoxon tests)

- *FOB examination at D 75 :*
  - Home cage observations : animals did not exhibit tremors, convulsions, abnormal movements. 1 females of the control group, 2 males and 3 females of the mid dose and 2 males of high dose were sitting or laying and not walking during the observation.
  - Open field observations : animals did not exhibit resistance against handling, salivation, nasal discharge, lacrimation, abnormal eyes/pupil size, abnormal posture, abnormal respiration, tremors, convulsions, abnormal movements/stereotypy. 1 males of the control group and 1 males of the highest dose were not walking during the observation.

- Sensorimotor tests/reflexes : animals did show reactions during the examination the approach and touch responses. Moreover, no abnormal reactions were detected during the examination of audition, pinna reflex, coordination of movements, behaviour during handling and pain perception.

Table 40 :

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
Rearing (N)	8	8	6	6	12	13	12	13
SD	**3**	**3**	**2**	**3**	**3**	**4**	**5**	**3**
GS F (Newton)	9.7	10.2	10.5	10.3	7.8	8.0	7.4	8.6
SD	**0.8**	**1.5**	**1.1**	**1.5**	**1.2**	**1.4**	**1.1**	**0.9**
GS H (Newton)	5.7	5.4	6.1	6.3	4.4	4.3	4.6	4.7
SD	**0.8**	**1.3**	**1.0**	**1.2**	**0.7**	**1.0**	**0.7**	**0.7**
FST (cm)	12.6	11.6	12.8	12.0	10.1	11.4	10.4	11.1
SD	**1.4**	**2.0**	**1.0**	**1.7**	**1.8**	**1.6**	**1.5**	**2.0**

(Stat test : Kruskal-Wallis + Wilcoxon test (two-sided))

- *Motor activity at D 75 :* Sum of the interr. 1-12 was of 2811.8, 2951.3, 2495.9 and 2487.7 in males and of 3731.3, 3685.1, 3389.9 and 3227.5 in females, respectively at 0, 20, 60 and 180 mg/kg bw/d

Additional table

	Males				**Females**			
Dose level (in mg/kg bw/d)	**0**	**20**	**60**	**180**	**0**	**20**	**60**	**180**
Sum of the interr 1-12	**2811.8**	**2951.3**	**2495.9**	**2487.7**	**3731.3**	**3685.1**	**3389.9**	**3227.5**
SD	**729.9**	**875.2**	**562.1**	**1045.2**	**1344.3**	**1491.0**	**995.2**	**1061.7**

Stat test : Kruskal-Wallis + Wilcoxon test (two-sided)

- *Rearing at D 75 :* Sum of the interr. 1-12 was of 528.4, 573.6, 477.3 and 482.2 and of 609.5, 584.6, 535.3 and 461.2 in females, respectively at 0, 20, 60 and 180 mg/kg bw/d

Additional table

	Males				**Females**			
Dose level (in mg/kg bw/d)	**0**	**20**	**60**	**180**	**0**	**20**	**60**	**180**
Sum of the interr 1-12	**528.4**	**573.6**	**477.3**	**484.2**	**609.5**	**584.6**	**535.3**	**461.2**
SD	**182.5**	**228.6**	**147.2**	**180.3**	**163.5**	**217.3**	**197.2**	**137.3**

Stat test : Kruskal-Wallis + Wilcoxon test (two-sided)

- *Morris water maze :* no difference observed in the distance to and the time spent in the target quadrant between control and treated groups. **(Test stat : Wilcoxon test (one-side+)**

Table 41 : morris water maze data : learning on PND 60

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
Mean cumul. Distance (in cm)								
D 1	109939.4	120616.2	113809.1	119463.9	93981.0	140207.3	123301.7	154551.5*
D 2	44195.3	36789.1	44854.6	46090.5	48406.2	43069.4	36504.2	41593.1
D 3	26804.5	23618.1	16086.4	24773.5	36996.6	55256.4	35006.3	34114.6
D 4	23282.4	30615.9	30908.4	41351.6	47550.5	32484.5	26717.0	31356.4
Median latency time (in ms)								
D 1	41232.0	37162.8	36791.3	45872.0	33985.3	42142.3*****	39123.8	69668.3******
D 2	11431.0	11992.3	10572.0	12863.8	19182.0	17311.3	15481.8	15442.8

D 3	12551.3	10222.3	7784.3	8281.0	10582.8	11709.8	11652.0	13753.3
D 4	9971.5	9332.0	8992.3	8651.3	16452.5	8160.8	10001.5	14222.0

Table 42 : morris water maze data : relearning on PND 67

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
Mean cumul. Distance (in cm)								
D 6	39305.2	67564.2	33109.5	42331.4	41815.2	41719.3	43567.1	33506.2
D 7	25605.5	26582.4	35749.7	31374.5	24911.5	20785.0	26228.4	29318.7
D 8	18368.6	22044.8	23446.0	19910.2	33169.2	28938.8	21488.0	32905.8
D 9	17520.7	24455.7	16109.0	25269.0	26111.0	23675.0	17311.8	28368.1
Median latency time (in ms)								
D 6	13411.3	14649.0	10961.8	12582.8	11321.8	10982.5	14851.8	12472.5
D 7	8241.8	9132.8	12343.0	8802.0	11372.8	8384.0	9132.8	9992.8
D 8	7662.0	6284.3	6271.5	8811.5	12952.0	12582.8	10461.8	11802.5
D 9	6172.3	10982.5	7150.8	5191.0	11011.3	7872.5	6854.3	10361.3

- *necropsy findings :* no treatment related effects were noted
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals :*

Table 43 : brain weight data

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
FBW (in g)		394.72	394.66	408.31	402.59	236.19	227.32	233.66	248.19
Brain	Abs (g)	2.262	2.166	2.223	2.242	2.047	2.02	2.033	2.077
	Rel	0.579	0.55	0.546	0.557	0.871	0.897	0.872	0.842

- *Length and width of brain :*
  - *Length :* 2.20, 2.17, 2.21 and 2.22 cm in males and 2.12, 2.12, 2.13 and 2.13 cm in females, respectively at 0, 20, 60 and 180 mg/kg bw/d **(corresponding to 100, 99, 100 and 101% in males, at 0, 20, 60 and 180 mg/kg bw/d, and to 100% in all doses in females).**
    (Stat test : Wilcoxon + Bonferroni-Holm adjustement)
  - *Width :* 1.62, 1.61, 1.63 and 1.60 cm in males and 1.58, 1.58, 1.57 and 1.59 cm in females, respectively at 0, 20, 60 and 180 mg/kg bw/d **(corresponding to 100, 100, 100 and 99% in males and 100, 100, 100 and 101% in females, respectively at 0, 20, 60 and 180 mg/kg bw/d).**
    (Stat test : Wilcoxon + Bonferroni-Holm adjustement)
- *histopathological findings: nature and severity :* no treatment related effects were observed
- ***Morphometry :***

Additional table *: Mean measurements of brain sections (in mm) (Stat test : Wilcoxon test)*

	Males		**Females**	
Dose level (in mg/kg bw/d)	**0**	**180**	**0**	**180**
Frontal cortex left	**1.97**	**1.89 (96%)**	**1.85**	**1.88 (102%)**

Frontal cortex right	**1.96**	**1.85 (94%)**	**1.84**	**1.86 (101%)**
Nucleus caudatus width left	**4.28**	**3.84** (90%)**	**3.90**	**4.25** (109%)**
Nucleus caudatus width	**4.26**	**3.93 (92%)**	**4.04**	**4.21 (104%)**
Parietal cortex left	**2.00**	**1.96 (98%)**	**1.85**	**1.88 (102%)**
Parietal cortex right	**2.03**	**1.99 (98%)**	**1.91**	**1.95 (102%)**
Corpus callosum width	**0.88**	**0.73* (83%)**	**0.79**	**0.69 (87%)**
Hippocampus left	**1.45**	**1.59 (110%)**	**1.52**	**1.47 (97%)**
Hippocampus right	**1.49**	**1.59 (107%)**	**1.55**	**1.48 (95%)**
Base of lobus vermis cerebelli No 8	**0.99**	**1.01 (102%)**	**0.98**	**0.99 (101%)**

*** : p<0.05 ; ** : p<0.01 ; () : comparison between high and control groups**

For cohort 2B :

- *necropsy findings :* no abnormalities observed
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals :*

Table 44 : brain weight data

		Males				Females			
Dose level (in mg/kg bw/d)		0	20	60	180	0	20	60	180
FBW (in g)		59.88	57.64	60.29	60.71	56.1	57.39	58.25	59.61
Brain	Abs (g)	1.828	1.783	1.855	1.819	1.757	1.74	1.751	1.801
	Rel	3.063	3.109	3.087	3.004	3.146	3.041	3.016	3.023

- *Length and width of brain :*
  - *Length :* 1.95, 1.91, 1.94 and 1.95 cm in males and 1.91, 1.91, 1.92 and 1.92 cm in females respectively at 0, 20, 60 and 180 mg/kg bw/d **(corresponding to 100, 98, 99 and 100% in males, at 0, 20, 60 and 180 mg/kg bw/d, and to 100% in all doses in females).**
    (Stat test : Wilcoxon + Bonferroni-Holm adjustement)
  - *Width :* 1.53, 1.53, 1.53 and 1.55 cm in males and 1.51, 1.52, 1.52 and 1.51 cm in females, respectively at 0, 20, 60 and 180 mg/kg bw/d **(corresponding to 100, 100, 100 and 101% in males, at 0, 20, 60 and 180 mg/kg bw/d, and to 100, 101, 101 and 100% in females, at 0, 20, 60 and 180 mg/kg bw/d).**
    (Stat test : Wilcoxon + Bonferroni-Holm adjustement)
- *histopathological findings: nature and severity :* no abnormalities observed

For cohort 3 :

- *number of animals at the start of the test :* 10/sex/dose
- *clinical observations:* no effects were observed
- *time of death during the study and whether animals survived to termination :* one female of the lowest dose was found dead on study day 18
- *body weight data :*

Table 45 : body weight data (in g)

	Males				Females			
Dose level (in mg/kg bw/d)	0	20	60	180	0	20	60	180
D 0	100.2	100.6	105.9	98.7	91.2	93.1	88.8	92.9
D 14	214.5	219.2	228.4	219.3	160.8	160.8	161.1	173.2[A]
D 28	328.8	339.9	344.1	344.6	203.7	204.8	202.4	217.4[B]

[A] : S.d : 15.7, 12.9, 21.5 and 14.2, respectively at 0, 20, 60 and 180 mg/kg bw/d
[B] : S.d : 23.3, 13.7, 25.1 and 15.3, respectively at 0, 20, 60 and 180 mg/kg bw/d

- *T-cell dependent antibody response (SRBC) at D 63 :*
  - *Males :* 3738, 3727, 4414 and 3599 U/ml respectively at 0, 20, 60 and 180 mg/kg bw/d (positive control : 927 U/ml)
  - *Females :* 13647, 8239, 9598 and 14555 U/ml respectively at 0, 20, 60 and 180 mg/kg bw/d (positive control : 1546 U/ml)

	Males					Females				
Dose level (in mg/kg bw/d)	0	20	60	180	PC	0	20	60	180	PC
T-cell dependent antibody response (SRBC) in U/ml at D63	3738	3727	4414	3599	927	13647	8239	9598	14555	1546
SD	**2918**	**2408**	**1710**	**2808**	**564**	**12787**	**5678**	**8936**	**11711**	**889**

(test stat : Kruskal-Wallis + Wilcoxon test (two-sided); PC : positive control

- ***Lymphocytes subpopulations in spleen (at D90):***

	Males				**Females**			
Dose level (in mg/kg bw/d)	**0**	**20**	**60**	**180**	**0**	**20**	**60**	**180**
B_SPL (%)	**39.98**	**40.64**	**40.88**	**37.97**	**35.77**	**37.00**	**38.15**	**36.32**
SD	**4.73**	**7.11**	**4.22**	**3.43**	**5.01**	**5.60**	**6.48**	**4.21**
T_SPL (%)	**43.26**	**45.25**	**44.21**	**46.90**	**54.16**	**51.07**	**51.82**	**51.48**
SD	**5.54**	**8.12**	**6.74**	**7.36**	**6.61**	**5.67**	**7.96**	**4.27**
CD4_SPL (%)	**40.93**	**42.60**	**42.08**	**43.88**	**43.52**	**43.29**	**43.28**	**39.26**
SD	**7.95**	**4.76**	**6.25**	**6.69**	**7.82**	**7.67**	**5.47**	**3.83**
CD8_SPL (%)	**48.79**	**48.52**	**48.09**	**47.20**	**48.51**	**48.70**	**49.56**	**52.67**
SD	**7.46**	**5.42**	**5.89**	**6.58**	**9.03**	**7.46**	**5.76**	**4.51**
NK_SPL (%)	**6.12**	**5.46**	**5.64**	**6.18**	**4.35**	**4.99**	**5.09**	**5.73**
SD	**1.85**	**2.29**	**1.89**	**2.04**	**1.17**	**1.38**	**2.06**	**1.59**

(test stat : Kruskal-Wallis + Wilcoxon test (two sided). No info for positive control

- *necropsy findings :* no treatment related effects were observed.
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals :*

Table 46 : organ weight data

		Males					Females				
Dose level (in mg/kg bw/d)		0	20	60	180	PC	0	20	60	180	PC
FBW (g)		332.29	345.04	345.59	349.43	323.71	198.92	200.711	198.8	211.29	197.48
Spleen	Abs (g)	0.717	0.705	0.668	0.677	0.467**	0.465	0.479	0.416	0.478	0.324**
	Rel	0.217	0.205	0.193	0.193	0.144**	0.233	0.239	0.211	0.227	0.164**
Thymus	Abs (mg)	620.4	602.6	645.7	530.1	529.6	478.1	467.222	488.1	486.6	399.8
	Rel	0.187	0.176	0.187	0.152*	0.166	0.239	0.231	0.247	0.231	0.204

* : p<0.05

- *histopathological findings: nature and severity :* examination not performed

RAC's response

Noted.

A selection of the studies was included in the assessment. These were the studies by Ghaya *et al*. (2019), Wan *et al*. (2018), Shi *et al*. (2017), Ashan *et al*. (2018), Ullah *et al*. (2018), Ullah *et al*. (2019), and Ijaz *et al*. (2019). Although several of these studies do use uncommon routes of exposure such as subcutaneous or intra peritoneal, the effects observed are in line with findings observed in the oral reproductive screening studies and EOGRTS. It is acknowledged that the data presented in the CLH dossier fully justify a classification as Repr. 1B for fertility and development and an in-depth assessment of published studies may not be necessary.

Although marginal, some effects on specific neuro- and immunodevelopmental effects were noted in the OECD TG 443 study. As provided by the DS above, in cohort 2A the OECD TG 443, there were some effects observed regarding brain morphometry:

- statistically significant alteration in left nucleus caudatus width in males (10% reduced) and females (9% increased) at 180 mg/kg bw/day;
- reduction in the corpus callosum width in males (17% reduced) at 180 mg/kg bw/day

RAC considers these effects insufficient for classification on their own, but they contribute to the overall concern for effects on the developing organism.

Date	Country	Organisation	Type of Organisation	Comment number
07.02.2020	Germany	BASF SE	Company-Manufacturer	9

Comment received

Please see the attached document

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 80-09-1_DHDPS_comments on CLH dossier_public consultation_clean.pdf

Dossier Submitter's Response

Thank you for your comment.

See below for the response to the 23 points of the attached document :

1. In table 8 of the CLH report (page 11), the DS reported that "Organ weight : in male : sign. increase of relative (rel.) pituitary and rel. liver weights and sign. decrease of seminal vesicle weight (see table 12)". The extract, below, from Table 12 of the CLH report confirms that the absolute weight is significantly reduced at the highest dose as mentioned in the CLH report. The relative weight was of 0.552, 0.531, 0.546 and 0.498, respectively at 0, 10, 60 and 300 mg/kg bw/d. A reduction of the relative seminale weight was observed at the highest dose although this change was not significant.

Table 12 : organ weights

	Males				Females			
Dose level (mg/kg bw/d)	0	10	60	300	0	10	60	300
Sem. ves. (g)	2.825	2.718	2.860	2.428**	-	-	-	-

* : p<0.05; ** : p<0.01

Regarding the historical control data, it is mentioned in the attached document that the HCD were taken from Charles River Ashland Crl:CD (SD) rats in a time period from 12/2000 to 08/2018. The guidance on the application of the CLP criteria mentions that "In a general sense, the historical control data set should be matched as closely as possible to the study being evaluated. The historical data must be from the same animal strain/species, and ideally, be from the same laboratory to minimise any potential confounding due to variations in laboratory conditions, study conditions, animal suppliers, husbandry etc.". However the reproductive toxicity study (Anonymous 12, 2000) was performed on animals which were obtained from Tsukuba breeding center, Charles River Laboratories Japan. Furthermore, the guidance explained that "the historical data should be contemporary to the study being evaluated (e.g. within a period of up to around 5 years of the study).". The period of the HCD provided in the comment covers more than 10 years. This could explain the discrepancy between the HCD range provided (1.82 g to 2.44 g) and the seminal vesicle weight observed in the study (2.825 g in control).

2. Regarding sperm motility, DS confirms, as mentioned in the CLH report, that the sperm motility was significantly affected in all doses of the F0 generation and not affected in the cohort 1A. The data of sperm motility in the cohort 1A were available in the Annex 1 to the CLH report page 18 : "% of motile sperm : 84, 83, 84 and 83 % respectively at 0, 20, 60 and 180 mg/kg bw/d".
   Sperm motility parameter was not considered in the section 10.10.3 Comparison with the CLP criteria. Even if, as mentioned in the comment number 8 by France, the sperm parameters were not investigated in the other available studies, uncertainties remain regarding the male reproductive parameters as literature studies demonstrate some effects :

   - Ghayda et al. (2019), Urinary Bisphenol S concentrations : potential predictors of and associations with semen quality parameters among men attending a fertility center (Env. Inter., 131): tested men at the Massachusetts General Hospital Fertility center : associations of urinary BPS concentrations with lower ejaculate volume, sperm concentration, total sperm count and motility were demonstrated. Some of these associations were only observed among overweight and obese men.
   - Shi et al, 2017, Effects of bisphenol A analogues on reproductive functions in mice *(Reprod Toxicol. 73:280-291)*: Sperm count and motility sign. decreased in CD-1 mice at PND60

Dose BPS	Sperm count at PND 60	Sperm motility at PND 60
0	6.4 ± 0.2 x 10^6/ml	76.8 ± 1.2 %
50 µg/kg bw	2.5 ± 0.2 x 10^6/ml**	67.2 ± 1.7 %*
10 mg/kg bw	3.8 ± 0.3 x 10^6/ml**	63.1 ± 2.1 %**

- Ullah et al, 2018, Impact of low-dose chronic exposure to bisphenol A and its analogue bisphenol B, bisphenol F and bisphenol S on hypothalamo-pituitary-testicular activities in adult rats: A focus on the possible hormonal mode of action *(Food Chem Toxicol. 121:24-36) :* Sperm motility and daily sperm production sign. decreased in rats

Dose (µg/kg)	Motile sperms (%)	Daily sperm production (x 10^6)
0	79.56 ± 0.54	53.34 ± 0.6
5	78.12 ± 0.51	52.24 ± 0.5
25	75.27 ± 1.10*	50.32 ± 0.8
50	74.28 ± 0.74***	48.22 ± 0.5**

Furthermore, regarding the HCD, same as for the response to your point 1, the HCD must be from the same animal strain/species, and ideally, from the same laboratory. The rat used in the Anonymous 13 (2019) was male and female Sprague-Dawley rats, strain Crl:CD(SD), supplied by Charles River Laboratories, Research Models and Services, Germany GmbH. No information on the time period was available in your comment, if it is the same as the point 1, the time period was not appropriate as "the historical data should be contemporary to the study being evaluated (e.g. within a period of up to around 5 years of the study).".

Finally, DS agrees that the lowest value observed in F0 generation corresponds to the control value of cohort 1A. However, DS noted the presence of an outlier showing <50% of sperm motility in the control group of cohort 1A. The registrant did not consider the value of this animal as an outlier according to predefined criteria. But these criteria were developed for Wistar rats, and not for Sprague-Dawley that were used in this study. In absence of acceptable HCD, this value is considered as an uncertainty.

3. As mentioned in table 43 of the CLH report, DS agrees that the mean number of post-implantation loss was significantly lower at the mid and high dose groups in F0 females. Furthermore, the mean number of post-implantation loss was significantly reduced at the highest dose in F1 females (see table 45 of the CLH report).

Table 43 : post implantation data

Dose level (in mg/kg bw/d)	0	20	60	180
Mean number of post-implantation loss	0.5	0.8	1.3*	1.5**
Mean % of post-implantation loss	3.1	5.9	9.4*	10.5**

* : p<0.05 ; ** : p<0.01

Table 45 : female reproduction data

Dose level (in mg/kg bw/d)	0	20	60	180

Nb of females with liveborn pups	24	24	21	21
Nb of females with stillborn pups	6	2	2	6
Mean nb of implantation sites	15.2	14.6	15.2	13.7
Tot. nb of post-implantation loss	22	18	25	76
Mean nb of post-implantation loss	0.9	0.8	1.1	3.3**
% of post-implantation loss	6.4	5.3	11.1	24.6**
Duration of gestation (in day)	22.0	21.9	22.0	22.0
Mean nb of pups delivered	14.3	13.8	14.9	11.4**

** : $p<0.01$

Regarding the HCD, same comment as for the point 2.

4. DS never mentioned that the number of stillborn pups was dose-dependent. In table 8 page 12 of the CLH report, it is noted "Sign. lower tot. nb. of liveborn pups (285* at 180 mg/kg bw/d vs 340 in control) and sign. higher nb of stillborn pups (8* at 180 mg/kg bw/d vs 2 in control). In section 10.10.5 page 42, it is mentioned that "Furthermore, the number of liveborn pups was significantly reduced at the highest dose (340, 289, 322 and 285* pups respectively at 0, 20, 60 and 180 mg/kg bw/d) and the number of stillborn pups was also significantly increased at the highest dose (2, 5, 3 and 8* pups respectively at 0, 20, 60 and 180 mg/kg bw/d).". Furthermore, table 45 reported the number of stillborn pups of the second generation. As mentioned in your comment, no historical control data were available to dismiss this modification.

5. Thank you for your summary table 5.1, DS agrees that no dose-dependent effect was observed on the thymus weight. And regarding T-cell dependent antibody response, it was never mentioned that the change was dose dependent or significant (page 43 of the CLH report : "In this cohort, T-cell dependent antibody response (SRBC) was examined and revealed slight changes in the low and mid dose groups in females").
   DS only mentioned the results of the DIT and DNT cohorts to have an overview of the EOGRTS.

6. DS agrees that the units of the food consumption is g/animal/d.

7. It was never mentioned in the CLH report that the number of implantation sites was altered in a dose-dependent manner or was statistically significantly changed. Extract of the CLH report : "Furthermore, a declining tendency in the number of implantation sites and a significant decrease of implantation index were observed at the highest dose level."

   Furthermore, DS want to point out that the number of implantation sites is affected in 3 different studies :
   - In Anonymous 12 (2000) : 10.7 at 300 mg/kg bw/d vs 15.9 in control group
   - In Anonymous 13 (2019) :
     14.3 at 180 mg/kg bw/d vs 15.5 in control group in F0
     13.7 at 180 mg/kg bw/d vs 15.2 in control group in cohort 1B
   - In Anonymous 14 (2017) 10.4** at 300 mg/kg bw/d vs 15.8 in control group

Regarding the HCD, see the response to your point 1. The HCD must be from the same animal strain/species, and ideally, from the same laboratory.

8. DS recognize that 3.43** is the correct value and not 4.43**.

9. DS recognize that 11/12*** is correct and not 11/12**

10. In the CLH report it was not mentioned that death of female of the low dose group was treatment related. DS only described the mortality observed in the study.

11. In the CLH report it was not mentioned that decrease of the mean number of F1 pups observed in the mid and high doses were significant.
Regarding the HCD, see the response to your point 1. The HCD must be from the same animal strain/species, and ideally, from the same laboratory and on an appropriate time period.

12. In the CLH report it was not mentioned that the number of females with liveborn pups was significantly and/or dose-dependency affected. Moreover, the data are included in the table 21.

    Regarding oestrous cycle data, DS does not agree that the mean oestrous cycle duration was comparable, as it was of 3.9, 4.0, 4.0 and 4.5, respectively at 0, 20, 60 and 180 mg/kg bw/d (approximately 102.6, 102.6 and 115.4% compared to control).
    Furthermore, DS want to point out that the mean oestrus cycle duration is affected in 3 different studies :
    - Anonymous 12 (2000) : 5.57**d at 300 mg/kg bw/d vs 4.08d in control group
    - Anonymous 13 (2019) : 4.1*d at 180 mg/kg bw/d vs 3.9d in control group in F0
      4.1d at 180 mg/kg bw/d vs 3.9d in control group in cohort 1B (no statistical analysis was performed on this parameter)
    - Anonymous 14 (2017) : 5.16**d at 300 mg/kg bw/d vs 4.02d in control group

    In the registration dossier, regarding the Anonymous 14 (2017) study, it is stated that "High-dose F0 parental females (300 mg/kg bw/d) had a distinctly prolonged estrous cycle". (https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/14986/7/9/2/?documentUUID=af270686-4d9e-4b06-80d0-daa330a1f5de)

    Regarding the HCD, see the response to your point 1. The HCD must be from the same animal strain/species, and ideally, from the same laboratory and on an appropriate time period.

13. The registration dossier is the source of the data reported in table 23. (https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/14986/7/9/2/?documentUUID=af270686-4d9e-4b06-80d0-daa330a1f5de)

Table 4: Reproductive performance parameters

mg/kg bw	0	30	100	300
dams				
pregnant	10/10	9/10	10/10	6/10
fertility index [%]	100	90	100	60
without implantation sites	0/10	0/10	0/10	2/10
Mean implantation sites	15.8	15	15.5	10.4**

Same data were directly provided to us by the registrant. DS did not receive the full study report but only an extract of the IUCLID data.
Therefore we are unable to modify the actual observed data.

14. In the CLH report it was never mentioned that relative uterus weight was significantly higher at the highest dose. However, a severe increase was observed at the highest dose (approximately 155.83% higher compared to the control group).

    In your attached document, it is mentioned that the relative weights were of 0.157/0.244/0.224/0.307. However, for the low dose group, it is mentioned in the registration dossier that the relative uterus weight was of 0.197%.

    Furthermore, DS did not receive the full study report for this study but only an extract of the IUCLID data. In this document, the absolute weight was not mentioned. With the new data received in the attached document, an uterus weight's change was exhibited as the asbsolute weight was increased of approximately 141.1% compared to the control group.

15. DS agrees that the data were the relative organ weight and expressed in %.

16. In the CLH report it was only mentioned that "A significantly lower bwg value was noted in males of the highest dose level (no further information available)", as it is the only data that DS has in his possession. DS never received the full study report.
    Exract of the registration dossier :

Details on results (P0)

Clinical examinations:
- Salivation
- Dose dependent, and at 600 mg/kg bw significantly decreased body weight gain in males

    The data mentioned in your attached document confirm that a strong (but not significant) body weight decrease was observed at the highest dose compared to the control group (430.1 vs 487.4, corresponding approximately to 88.2%), and confirm that a significantly (at the highest dose) and dose dependent lower bwg was noted in males.

17. DS agrees with the modification.

18. Regarding the statement in the CLH report page 34 : "In the EOGRTS (Anonymous 13, 2019), the number of implantation sites was moderately modified only in the cohort 1B (13.7 vs 15.2 at 180 and 0 mg/kg bw/d, respectively). However, the DS wants to highlight that this effect appeared at a much lower dose than in Anonymous 12 (2000) and in Anonymous 14 (2017)."

    The DS agrees that in the P0 generation, the number of implantation sites was 15.3, 14.8, 14.9, and 14.3 respectively at 0, 20, 60, and 180 mg/kg bw/d, and in the F1 generation 15.2, 14.6, 15.4, and 13.7, respectively at 0, 20, 60, and 180 mg/kg bw/d. it was never mentioned that the modification was significant and/or dose-dependent.

    - In the EOGRTS, the reduction, observed at the highest dose (180 mg/kg bw/d), were approximetaly of -7 % in the P0 generation and -9.9 % in the F1 generation compared to control group.
    - In Anonymous 12 (2000), the number of implantation sites at the highest dose (300 mg/kg bw/d) was lower of approximetaly – 32.7% compared to control group.
    - In Anonymous 14 (2017), the number of implantation sites at the highest dose (300 mg/kg bw/d) was reduced of approximetaly - 34.2% compared to control group.

    The number of implantation sites was lower in 3 different studies. The reduction was higher in the Anonymous 12 and 14 than in the EOGRTS. This difference could be explained by the fact that the highest tested dose was lower in the EOGRTS (180 mg/kg bw/d in the EOGRTS compared to 300 mg/kg bw/d in the Anonymous 12 and 14).

    As mentioned before, the HCD should be taken with precaution. The guidance on the application of the CLP criteria mentiones that "In a general sense, the historical control data set should be matched as closely as possible to the study being evaluated. The historical data must be from the same animal strain/species, and ideally, be from the same laboratory to minimise any potential confounding due to variations in laboratory conditions, study conditions, animal suppliers, husbandry etc."

19. Regarding the oestrous cycle length, DS agrees that the changes were more important in the OECD 421 and the OECD 422 studies. In these 2 studies, tested doses were higher than the EOGRTS. However, in those 3 studies, maternal toxicity cannot explain the reproductive effects.

    As mentioned above, DS is of the opinion that the HCD should be taken with precaution and cannot dismiss the observed modification.
    For the OECD 421 study, the rats were provided by Tsukuba Breeding Center, Charles River Laboratories Japan.
    For the OECD 422 study, the rats were provided by Charles River Laboratories, Research Models and Services, Germany GmbH/ Charles River Laboratories, UK.

For the EOGRTS, the rats were provided by Charles River Laboratories, Research Models and Services, Germany GmbH / Charles River Laboratories, Italy.
The HCD provided in your attached document were for Charles River Ashland, Crl:CD(SD) or for France RjHan:SD (rats CD®) (from Janvier or Charles River).

20. DS agrees that maternal care could be replaced by "however, the general condition was not affected".

21. DS recognizes that the litter size has an impact on the pups body weight (the bigger litter size, the lower pups body weight). However it's difficult to say that the significant increase is only a secondary consequence of the litter size. Accordingly the statement could be moderated as following: "Moreover the mean pup body weight was significantly higher at the 2 highest dose levels (see table 44). This could be partially linked to the smaller litter size in these two groups."

22. The origin of the sentence in the CLH report page 44 "At PND 21, a higher body weight value was noted in male pups of the low dose group (+ 6.6 % compared to the control group)." is from the registration dossier, the only information available by the DS. It was, however, never mentioned that the change was dose-dependent and/or significant as DS never received the full study report.

Results: F1 generation

General toxicity (F1)

Clinical signs:	no effects observed
Mortality / viability:	no mortality observed
Body weight and weight changes:	no effects observed
Description (incidence and severity):	A mild statistically significant increase in bw was observed in male pups of the low dose at PND21 (+6.6%).
Gross pathological findings:	no effects observed

23. The post-implantation loss was affected in 3 different studies. Even if, the modification was not significant and within the HCD for the developmental toxicity study, modification was observed and was significantly affected in the EOGRTS and in the screening test (Anonymous 14).

DS doesn't agree that the most relevant and conclusive study for regulatory purposes is the EOGRTS. DS is of the opinion that all available and relevant studies in the CLH report must be taken into account and be considered in a WoE approach. Moreover, the reproductive toxicity test (Anonymous 12, 2000), OECD 421, is included in the registration dossier as a key study by the registrant.

Regarding the NOAEL, DS wants to highlight that effects were already observed at the mid dose group, then the NOAEL was of 20 mg/kg bw/d.

In conclusion, DS is still of the opinion that a classification as Repr. 1B H360DF is warranted.

RAC's response
The information you provided on the fertility index, oestrus cycle length, and mean body weights of male pups has been incorporated in the assessment. Furthermore, the historical control data (HCD) you provided are used for comparison in the assessment where deemed appropriate (post-implantation loss; mean number of implantation sites per dam; mean number of pups delivered; oestrus cycle duration). The animals in the studies included in the CLH dossier were provided by the following laboratories: • For the OECD TG 421 study (2000), the SD rats were provided by Tsukuba Breeding Center, Charles River Laboratories Japan. • For the OECD TG 422 study (2017), the SD rats were provided by Charles River Laboratories, Research Models and Services, Germany GmbH/ Charles River Laboratories, UK. • For the OECD TG 443 study (2019), the SD rats were provided by Charles River Laboratories, Research Models and Services, Germany GmbH / Charles River Laboratories, Italy. • For the OECD TG 414 study (2014), the Wistar rats were provided by BASF Laboratories The HCD provided were from the US (Charles River Ashland, Crl:CD(SD)), from France (RjHan:SD; rats CD®) (from Janvier or Charles River), or from BASF test lab (Wistar). The HCD from the US summarise data from 89/91 OECD 412/422/443 studies in a time period from 12/2000 to 08/2018, and those from France summarise data from the F0 and F1 of an unknown number of OECD 443 studies from 02/2016 to 04/2020. The guidance on the application of the CLP criteria mentions that *"In a general sense, the historical control data set should be matched as closely as possible to the study being evaluated. The historical data must be from the same animal strain/species, and ideally, be from the same laboratory to minimise any potential confounding due to variations in laboratory conditions, study conditions, animal suppliers, husbandry etc.". Furthermore, the guidance explained that "the historical data should be contemporary to the study being evaluated (e.g. within a period of up to around 5 years of the study)".* RAC observes the following: • The strains of rats for which IND provided HCD are Crl:CD(SD), RjHan:SD, and Wistar which are the same type of strains as used in the studies included in the CLH dossier; • The laboratories of which IND provided HCD are located in the US and France, which are not the same locations as the laboratories where the rats in the studies stem from (Japan, Germany, Italy); • The period of the HCD from Charles River Ashland covers a period of over more than 10 years; • The study year of one study (2000) is on the skewed end of the HCD range provided (2000-2018).

Hence, the HCD may provide an indication of the normal ranges, but its use may be limited due to the uncertainties mentioned above. The within-study controls are therefore used as most important reference to compare with treatment.

RAC concludes that the adverse effects of bisphenol S on the mean number of implantation sites, the decrease in fertility index, and the effect on the oestrus cycle warrant classification as **Repr. 1B; H360F.**

Furthermore, RAC concludes that the adverse effect of bisphenol S on the post-implantation loss and the mean number of pups delivered per dam are sufficient for classification as **Repr. 1B; H360D.** The effects observed are severe and are not resulting from maternal toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
07.02.2020	Sweden	ChemSec	International NGO	10

Comment received

ChemSec strongly supports the classification of BPS as reprotoxic 1B. It is noted that aside from the references mentioned in the dossier, a very high number of supportive studies in different species are available, especially from the recent years. As is stated, there is a lack of studies in humans (for obvious reasons), but the following two studies can be of interest as they also support the classification on our opinion.

Relationship between maternal exposure to bisphenol S and pregnancy duration, 2018
https://doi.org/10.1016/j.envpol.2018.03.057
Urinary bisphenol S concentrations: Potential predictors of and associations with semen quality parameters among men attending a fertility center, 2019
https://www.sciencedirect.com/science/article/pii/S0160412019313856

Dossier Submitter's Response

Thank you for your comment and your support.
As you mentioned in your comment, many literature studies are available regarding BPS. Even if limitations were described, Wan *et al.* (2018)'s article revealed that higher maternal urinary BPS concentration in pregnant Chinese women was associated with longer pregnancy duration.
Your second reference, Ghayda *et al.* (2019), tested men at the Massachusetts General Hospital Fertility center and demonstrated associations of urinary BPS concentrations with lower ejaculate volume, sperm concentration, total sperm count and motility. Some of these associations were only observed among overweight and obese men.

RAC's response

Noted. Please see the response to comment number 8.

PUBLIC ATTACHMENTS
1. 80-09-1_DHDPS_comments on CLH dossier_public consultation_clean.pdf [Please refer to comment No. 2, 9]