Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

Perfluoroheptanoic acid; tridecafluoroheptanoic acid

EC Number: 206-798-9
CAS Number: 375-85-9

CLH-O-0000006908-60-01/F

Adopted

10 December 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON PERFLUOROHEPTANOIC ACID; TRIDECAFLUOROHEPTANOIC ACID

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.

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Substance name: perfluoroheptanoic acid; tridecafluoroheptanoic acid
CAS number: 375-85-9
EC number: 206-798-9
Dossier submitter: Belgium

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Belgium	Chemours Netherlands B.V.	Company-Importer	1
Comment received				
We believe that only substances that are placed on the market in the EU or substances in scope of Article 4(2) of the CLP regulation can be subject to the CLH procedure, and hence the proposal is not applicable for this substance which is only a degradant and not placed on the market. Further details on this argument can be found in the attachment "Public comments to the CLH proposal Perfluoroheptanoid acid". Furthermore, we object to some of the information in the CLH proposal, see "Confidential comments to CLH proposal" attached. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public comments to CLH proposal Perfluoheptanoic acid_final 24Jan2020.pdf ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential comments to CLH proposal Perfluoheptanoic acid_final24Jan2020.pdf				
Dossier Submitter's Response				
Thank you for your comment. The substance perfluroheptanoic acid (EC 206-798-9) is notified in the C&L inventory implying that the substance is placed on the market. Indeed, any manufacturer or importer placing a substance on the market which meets the criteria for classification and labelling shall notify to the Agency (art. 40 (1) of the CLP regulation), whom includes then the received information into the C&L inventory (art. 42(1) of the CLP regulation). This means that instead of article art. 4(2), art. 4(1) of the CLP regulation [" Manufacturers, importers and downstream users shall classify substances or mixtures in accordance with Title II before placing them on the market] is therefore of				

application. Substances that fulfill the classification and labelling criteria shall normally be subject to harmonized classification (art. 36 of CLP).
RAC's response
RAC agrees with the response provided by the BE CA.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Germany		MemberState	2
Comment received				
P/C-Properties, Table 7: • Please check unit of water solubility. Is it correct with 4.238 mg/L or is it g/L? • The Pow seems to be a log value.				
Dossier Submitter's Response				
Thank you for your remark. Regarding water solubility, we confirm the unit mg/L. The partition coefficient n-octanol/water is indeed expressed as a log value.				
RAC's response				
RAC agrees.				

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Sweden		MemberState	3
Comment received				
We agree that read-across from sodium perfluoroheptanoate, for the purpose of classification of perfluoroheptanoic acid in reproductive toxicity and specific organ toxicity – repeated exposure, is justified based on the formation of the common heptanoate anion under physiological conditions. Are toxicokinetic studies available for perfluoroheptanoic acid or sodium perfluoroheptanoate? This information would have been helpful in the assessment, and potential comparison (e.g. as supportive read-across) with other perfluorinated carboxylic acids . The close longer homologues, perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), with 8 and 9 carbon atoms in the alkyl chain, respectively, as compared to the 7 carbons in PFHpA, are both classified as e.g. Repr. 1B for development and STOT RE 1 for liver toxicity.				
Dossier Submitter's Response				
Thank you for your comment. To our knowledge, there are still no toxicokinetic study available with PFHpA. Also, in contrast to PFOA, experimental data on PFHpA are very scarce. Please refer to our response to your CA below (Comment No. 9) for more details about the read-across.				
RAC's response				
RAC agrees.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	France		MemberState	4

Comment received

FR agrees with the classification proposal.

However, regarding the few toxic effects on F0 generation, it could be considered that the authors did not use high enough concentrations when considering OECD guideline.

Moreover, as far as we understand, cleft palates are only seen on dead animals. Is there an explanation?

Dossier Submitter's Response

Thank you for your comment and your support regarding classification proposal.

The Dose rationale provided by the Registrant was that they selected the doses according to available pharmacokinetic and repeated dose studies with this substance or similar substances such as the six and the eight carbon acid (very little amount of data on the seven carbon acid).
It was also reported that usually, the most notable effect of perfluorinated carboxylic acids in mice are liver effects (enlarged due to activation of the PPAR alpha receptor), the potency of this activation appears to be proportional to the chain length.
According to the Registrant, the highest dose selected is high enough to induce enlarged livers in the male mice, and likely to do so in female mice. Regarding the middle dose, it was expected to show no or minimal liver effects in male mice. The lowest dose of 0.5 mg/kg/day was likely to be the NOAEL for all endpoints examined in this study.

Concerning the choice of doses, BE CA agree that the doses were very low. However, necropsy revealed already severe changes in liver. In consequence, BE CA still consider that the doses used in this study, while relatively low (0.5, 10 and 50 mg/kg bw/d), were sufficient to induce treatment-related effects in both generations.

About cleft palates, we confirm this malformation was only reported in found dead pups or in morinbund state or killed in extremis. In the lowest dose group, 6 pups from the same litter were affected by a cleft palate (5 males, 1 female). In none of the pup milk was found in stomach. They were necropsied on Lactation day 1. No other associated necropsy findings were reported.
In the highest dose group, 3 pups were affected, 2 males and 1 female. They were necropsied on lactation day 0. In none of them milk was found in their stomach. In one male, associated effects on the skeleton were reported (on the 7^{th} sternebra which was located between the 5^{th} and the 6^{th} and on the skull where an accessory bone was found). On the other male, no associated effects were noted. On the female, sternebrae were seen to be moderately malaligned (for example, the left half the the third bone was attached to the right half of the fourth).

RAC's response

Considerable liver toxicity was seen in the top dose F0 generation males and females, however, no effects on body weight/body weight gain, food consumption, other organ weights, parameters on sexual function and fertility, or clinical signs were reported in these animals. The observed liver effects are considered relevant for classification as

STOT RE 1, liver, as they demonstrate an irreversible damage to the organ, though, during the period tested, the effects did not appear to have strong impact on the general well being of the animals. This is also expressed by the blood biochemical parameters which were not affected in mated females of the top dose on lactation day 21, but in males and females exposed for 109 days. Such effects might, however, become evident upon longer exposure duration. In their conclusion, RAC considers the tested doses adequate.
RAC notes that OECD TG 422 study is only a screening study, which is normally not sufficient to exclude effects on sexual function and fertility, if the study results are negative. In paragraph 7 of OECD TG 422 it is stated that it provides only initial information on possible effects on male and female reproductive performance due to (amongst other reasons) selectivity of the end points and the short duration of the study. However, as the available screening study also incorporated OECD TG 408 (90 day study) in the test regime, including 90-day pre-mating exposure, post-natal and post-weaning (up until PND 42), exposure was considerably longer than in a normal screening study conducted according to OECD TG 422.

Regarding the observation that cleft palate was only seen in animals that died, it could be possible that the effect was related to death of the animals, however, this was not investigated.

In addition RAC is of the opinion that the observed skeletal changes are most relevant and supportive for a classification as Repr 1B, H360D (in line with the comment provided by the NL CA).

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Belgium	Chemours Netherlands B.V.	Company-Importer	5

Comment received

We strongly disagree with the proposed reproductive toxicity classification in the CLH Proposal as it lacks scientific justification. Further arguments can be found in the attachment "Public comments to the CLH proposal Perfluoroheptanoic acid".

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public comments to CLH proposal Perfluoheptanoic acid_final 24Jan2020.pdf
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential comments to CLH proposal Perfluoheptanoic acid_final24Jan2020.pdf

Dossier Submitter's Response

Thank you for your comment and the historical control data.

Regarding the part 1 of your attachment, "General comments on the scope and applicability of a CLH proposal for a degradation product", see the response to comment No. 1.

In the part 2 of your attachment, "Specific comments on the proposed hazard class of reproductive toxicity", it is mentioned that "In summary, the decreased post-natal survival, decreased pup body weights, and vaginal patency are secondary to the overt maternal toxicity observed at the 50 mg/kg bw/day high dose. The maternal toxicity was considered potent enough to justify a STOT RE liver target organ, so it cannot be claimed,

as stated in the CLH Proposal, that findings in the pups occurred with an "absence of marked maternal toxicity".

The CLP Regulation (table 3.7.1(a)) state that "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. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."

Furthermore, the CLP regulation considers that "Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by- case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies."

Even if the developmental effects (lower postnatal survival, the decreased pup body weight, the cleft palate and the skeletal malformations (mentioned by the NL CA - see comment No. 7 and the response to comment No. 7)) were observed in presence of liver toxicity, BECA still considers a classification as Repr. 1B H360D appropriate.

RAC's response

RAC agrees witht the response provided by the BE CA and also refers to its response to comment number 4.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	United Kingdom	<confidential>	Please select organisation type..	6

Comment received

Section 10.10.6

Repr. 1B; H360D

The MSCA have argued that the available data (decreased postnatal survival, decreased pup body weights, presence of malformations such as cleft palates, delayed sexual maturation in the absence of marked maternal toxicity) are clear evidence of an impact on development of the offspring. With the exception of the comments regarding cleft palate, the incidence of these effects only achieved statistical significance in the offspring of dams receiving 50 mg/kg/day, a dose that causes severe toxicity to the liver of the dams (see comments below re STOT RE). Consequently, the assertion by the MSCA that these effects occurred "in the absence of marked maternal toxicity" is incorrect and, as such, do not support a classification for PFHpA as Repr. Cat. 1B.

The MSCA have argued that an increased incidence of cleft palate was also evidence supporting the classification as Repr. Cat 1B. Cleft palate was reported in 3 pups (2 litters) and 6 pups (1 litters) in the offspring of dams exposed to 50 and 0.5 mg/kg bw/d

PFHpA, respectively. It is noted that this effect did not occur in a dose-dependent manner, suggesting that the effect may not be related to exposure to PFHpA. Furthermore, an increased incidence of cleft palate does not occur in developmental toxicity studies in mice on structurally related perfluorinated alkyl acids (e.g. PFOA – Lau et al, 2006; PFHxA – Iwai et al, 2017). The historical incidence of cleft palate in the strain if mice at the laboratory performing the study is not available to the author. It remains possible that the reported incidences of cleft palate are within the historical range.

Dossier Submitter's Response

BE CA takes note of your comment.

However, the CLP Regulation (table 3.7.1(a)) state that "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. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."

Furthermore, the CLP regulation considers that "Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by- case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies."

Even if the developmental effects (lower postnatal survival, the decreased pup body weight, the cleft palate and the skeletal malformations, as mentioned by the NL CA - see comment No. 7 and its response) were observed in presence of liver toxicity, BECA still considers a classification as Repr. 1B H360D appropriate.

Historical control data regarding cleft palates were not made available to the DS by the registrant in the full study report. Furthermore, the publicly available Charles River HCD report was kindly provided during the public consultation.
It appears that, according to this report, the spontaneous incidence of cleft palates in this strains of mice is 0-2 % in foetuses and 0-5 % in litters (Fetal skeletal abnormalities, pg. 11 https://www.criver.com/sites/default/files/noindex/historical-control-data/hcd-pa-mice.pdf).

Regarding the link with PFOA and PFHpA, BECA wants to remind that PFOA has a harmonised classification (Index number 607-704-00-2) : Acute tox. 4 H302, Acute Tox. 4 H332, Eye dam. 1 H318, Carc. 2 H351, Repr. 1B H360D, Lact. H362 and STOT RE 1 H372 (liver).
Even if cleft palate was not observed in study performed with PFOA, this effect is a supportive effect to our proposal.

RAC's response

RAC largely agrees with the response provided by the BE CA and also refers to its response to comment number 4. In addition RAC agrees with the commenters conclusion that the evidence for classification from the observed cases of cleft palate is not very

strong. Stronger evidence for developmental toxicity comes from the skeletal malformations, i.e. missing digits, malroated forlimbs and small stature.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Netherlands		MemberState	7

Comment received

The NL CA agrees with the 'no classification' for adverse effects on sexual function and fertility.

With respect to the proposed classification for adverse effects on development, the following is noticed:
- Importantly, some relevant adverse effects on development, as described in the study report (Anonymous 2017), are not presented in the CLH-report or its Annex I nor are discussed in relation to the criteria in the CLH-report. These include the higher number of pups observed with digits missing from the left and/or right limbs and malrotation of the forelimbs (mid and high dose group), and small stature (high dose group). Such skeletal malformations are considered severe and relevant for humans. The DS is kindly requested to present a short overview of these findings and to discuss these in relation to the classification criteria. The NL-CA is of the opinion that based on these skeletal malformations, classification in category 1B is warranted. The effects (postnatal survival/lower body weights in the highest dose group and non-dose dependent cleft palates) currently described in the CLH proposal are seen as supportive rather than sufficient evidence for classification in category 1B.
- With respect to the delayed mean age of vaginal patency in the high dose group (33.1 vs 29.9 days), it is noticed that this may have been secondary to lower body weights of the F1.
- Minor comment: Page 18 (section 10.10.2) of the CLH-reports states that "Males of the highest dose exhibited a decrease of the total T4 serum value (6.29, 9.53, 6.50 and 5.61 µg/dL in males respectively at 0, 0.5, 10 and 50 mg/kg bw/d whereas…..)." It is assumed that the value of 9.53 is an error, and this should be 6.53 as presented on page 22 (section 10.10.5; text and table 24).
- Overall, the NL CA considers a Repr. 1B (H360D) classification justified for PFHpA, although this should be based on the skeletal malformations including missing digits and malrotation of the forelimbs as observed in the OECD 422 study.

With respect to classification for effects on/via lactation, the NL CA questions the appropriateness of the 'no classification'. The following is noticed:
- Animal data:
o A decreased postnatal survival (outside the range of HCD at PND4-21) was observed in the OECD 422 study;
o Pup bw was significantly reduced during lactation period in this OECD 422 study. Though male pups showed a reduced bw starting at PND1, this started from PND4 with female pups;
This reduction in bw became smaller after the lactation period;
o Although maternal toxicity was present, i.e. hepatocellular hypertrophy, this is not considered to affect the adverse effects on the pups.
- As noted in the CLH report, recent human data seems to point towards transfer of PFHpA to breast milk and breastfed babies.
- Based on the presented data in the CLH-dossier and taking into account the criteria for classification for lactation, i.e.:
(a) human evidence indicating a hazard to babies during the lactation period; and/or
(b) results of one or two generation studies in animals which provide clear evidence of

adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk,
PFHpA does not meet the first criterion, i.e. there is no human evidence available indicating a hazard to babies.
With respect to the third criterion, PFHpA has, according to the CLH dossier, been detected in human breastmilk. The question then is whether PFHpA is present in breastmilk in potentially toxic levels. The Dossier Submitter is requested to reflect on this issue.
For the second criterion, decreased postnatal survival was observed during PND4-21 and a treatment-related reduction in pup body weight was observed during the lactation period in the OECD 422 study. However, no information is available on the quantity or quality of the milk produced by the dams, nor was the mouse milk analysed for the presence of PFHpA or related metabolites. So a direct link to lactation cannot be made. Other possibilities such as maternal toxicity are considered less likely. Also a direct effect of F1-animals consuming solid food is considered not the cause, given that adverse effects were also noticed when the F1-pups were breast-fed only.

Dossier Submitter's Response

Thank you for your support regarding the 'no classification' proposal for adverse effects on sexual function and fertility.

Concerning the adverse effects on development, an increased incidence of pups with missing digits from the left and/or right limbs and malrotation of the forelimbs (mid and high dose group) and small stature (high dose group) was indeed reported in the study. BE CA agrees with that "*Such skeletal malformations are considered severe and relevant for humans*".
Missing digits were reported in 2/5, 3/2, 3/5 and 14/17 male/female pups exposed to 0, 0.5, 10 and 50 mg/kg bw/d
For more details: (total occurence/N pups (both sexes))

- Missing digit(s) at the right forelimb: 7/3, 2/1, 17/5 and 28/8 from 2, 1, 2 and 5 litters, at 0, 0.5, 10 and 50 mg/kg bw/d, respectively
- Missing digit(s) at the right hindlimb: 4/2, 8/5, 17/7 and 54/25 from 1, 1, 2 and 5 litters, at 0, 0.5, 10 and 50 mg/kg bw/d, respectively
- Missing digit(s) at the left hindlimb: 9/3, 0/0, 4/11, 9/31 from 1, 0, 2 and 5 litters, at 0, 0.5, 10 and 50 mg/kg bw/d, respectively
- Missing digit(s) at the left forelimb: 4/1, 12/3, 0/0 and 40/13 from 1, 1, 0, and 6 litters at 0, 0.5, 10 and 50 mg/kg bw/d, respectively

Small stature was observed in 0/2, 1 /2, 4/4 and 7/8 male/female pups exposed to 0, 0.5, 10 and 50 mg/kg bw/d (1, 2, 3 and 7 litters affected, at 0, 0.5, 10 and 50 mg/kg bw/d, respectively)
Malrotation of the forelimbs was reported in 0/0, 0/0, 1/0 and 3/4 male/female pups exposed to 0, 0.5, 10 and 50 mg/kg bw/d (0, 0, 1 and 4 litters affected, at 0, 0.5, 10 and 50 mg/kg bw/d, respectively)

BE CA agrees these effects warrant a classification as Repr. 1B for developmental toxicity considering the clear dose-related increase in these skeletal malformations and in growth alteration. Maternal toxicity induced by PFHpA have to be discussed as well, however, very few studies are available to explain the link between maternal hepatotoxicity and skeletal effects in the offspring in mice. As the Guidance on CLP criteria states in Annex I Chapter 3.7.2.4.2., "*Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be*

unequivocally demonstrated on a caseby-case basis that the developmental effects are secondary to maternal toxicity."

With these new data in mind, thank you for your support on this proposal to classify PFHpA for adverse effects on development.

Concerning the delayed mean age of vaginal patency in the high dose group (33.1 vs 29.9 days in controls), NL CA notices that this may have been secondary to lower body weights of the F1. We agree with the NL CA, however, the same effect has been observed with other PFAs: for example, in Yang *et al.* (2009), a significant dose-related increase in the mean age of vaginal opening was reported in mice exposed to PFOA at concentrations of 0, 1, 5 and 10 mg/kg bw/d (See Table 1 in Yang *et al*.) In Zhao *et al.* (2012), vaginal opening was significantly delayed in mice exposed to 2.5mg/kg bw/d PFOA (See Table 2 in Zhao *et al*.).
In conclusion, BECA is of the opinion that delayed mean age of vaginal opening is a relevant effect to highlight and it supports our proposal for classification as Repr. 1B for developmental effects.

Regarding the editorial comment on the total T4 serum value, BECA agrees that the value of the low dose group was 6.53 µg/dL and not 9.53 µg/dL.

Regarding the classification for effects on/via lactation, BECA agrees that PFHpA does not meet the first criterion as no human evidence is available showing a hazard to babies. With respect to the second criterion, indeed a decrease in postnatal survival was seen during lactation days 4 to 21 and a treatment-related decrease in pup body weight was also reported during the lactation period.
However, as highlighted by the NL CA, we agree that as no data showed effects on the quantity or quality of the breastmilk, nor was performed a test to detect the presence in the mouse breastmilk PFHpA or its metabolites, a direct link from effects observed on pups during lactating period and lactation cannot be made.

Finally, concerning the last criterion, PFHpA has indeed been detected in human breastmilk as mentioned in the CLH report (Martin *et al.*, 2019; Wang *et al.*, 2016; Monroy *et al.*, 2008; Lee *et al.*, 2018). However, no more data is available about the potential toxicity of PFHpA in breastmilk.

References :

Yang et al., 2009, Differential Effects of Peripubertal Exposure to Perfluorooctanoic Acid on Mammary Gland Development in C57Bl/6 and Balb/c Mouse Strains, Reprod Toxicol. 2009 Jun; 27(3-4): 299–306, doi: 10.1016/j.reprotox.2008.10.003.

Zhao et al., 2012, Perfluorooctanoic acid effects on ovaries mediate its inhibition of peripubertal mammary gland development in Balb/c and C57Bl/6 mice, in Reprod Toxicol. 2012 Jul; 33(4): 563–576, doi: 10.1016/j.reprotox.2012.02.004

Martin J. et al., 2019, Exposure assessment to parabens, bisphenol A and perfluoroalkyl compounds in children, women and men by hair analysis, Science of the Total Environment, Vol 695

Wang Y. et al., 2016, Prenatal exposure to perfluorocarboxylic acids (PFCAs) and fetal and postnatal growth in the Taiwan Maternal and Infant Cohort Study, Environ. Health Perspect., Vol 124, Pg. 1794-1800.

Monroy R. et al., 2008, Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples, Environmental Research, Vol 108, Issue 1, Pg. 56-62.

Lee S. et al., 2018, Perfluoroalkyl substances (PFASs) in breast milk from Korea: Time-course trends, influencing factors, and infant exposure, Science of the Total Environment, Vol. 612, Pg. 286-292.

RAC's response

In line with the DS RAC agrees that the described skeletal findings are relevant findings. In line with the NL CA comment RAC is of the view that the observed cases of cleft palate are incidental findings as they did not show a dose response and were within or at the upper range of the historical control data, provided during the consultation.

Regarding the delay in vaginal patency RAC refers to CLP Annex I, 3.7.1.3 which states that any effect on puberty onset should be covered under sexual function and fertility. PFHpA had no impact on the onset of balanopreputial separation (comparable across groups: PND 30,2, 30,2, 29,5 and 31 in control, low, mid and top dose, respectively). However, time to vaginal opening was significantly prolonged (PND 29,9, 29,4, 30,1 and 33,1* in control, low, mid and top dose, respectively). RAC notes that a delay in this developmental landmark might be explained by the observed decrease in body weight. This does, however, not explain the different response in males and females, as onset of puberty seems to be delayed in females, but not in males, although body weights were clearly lower in the top dose of both sexes.

However, as the effect was accompanied by lowered body weight, RAC did not consider the effect on its own supportive for classification for fertility and reproductive function.

RAC shares the DS's view on lactation.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Germany		MemberState	8

Comment received

The CLH proposal of the BE CA for the classification of PFHpA as Repr. 1B, H360D is based on data from a combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening (similar to OECD 408 and 422, Anonymus 2017), which was performed in mice (CD-1) on oral administration (gavage).

The study design (Anonymous, 2017) is considered appropriate to conclude on classification based on the observed effects. A cursory check of the provided information on the available study reveals some limitations and deficiencies, such as only punctual and limited observations of animals and partially missing information.

If data are available, more detailed documentation would be welcomed:
Table 8, results for F0 generation:
- Please describe more detailed significance of the effects
- Please describe relevant histopathological effects in more detail
- Due to the diversity and extend of the data set, allocation of the effects for males and females to study periods could be improved (prior to mating, mating period for males as well as gestation and lactation phase for females)

Pup survival/Post natal survival index:
• Is there information on the pup survival rates on PND 1 and PND 1-4. Studies for each sex. Other PFAS such as PFHxA show high death rates within the first day of life (PND 1).
• Please evaluate the number of stillborn pups vs. litter size. Studies with other PFAS such as PFHxA show high death rates.
• Mean litter size: What lies behind it? Are there unusually high death rates/litter loss, etc.?

Mean offspring weight data during lactation period: please mention pup number together with the mean values for various PNDs (N = x)

Fertility:
It is noted that the data (Table 13 of the CLH report) show a non-significant decrease in reproductive performance indices in % at 50 mg/kg bw/d only which are outside the HCD for:
• fertility index,
• male copulation index and
• female conception index.

DE CA agrees that no classification is required for fertility. As parameters regarding fertility (fertility index, oestrous cycle length, pre-coital interval, number of implantation sites, gestation length) are not affected, classification as Repr. 1B or 2 is not appropriate.

Developmental toxicity:
In the combined 90-day repeated dose/screening study (Anonymus, 2017) an increased mortality of the offspring is found. The survival rate at PND0 is 98.4% compared to the control in the highest dose group and 89.3% at PND0-4, both values still within the HCD. The body weight of the offspring is also reduced with increasing dose compared to the control. Thus, at PND4 the fetal weight of the male pups in the highest dose group (50 mg/kg bw/d) is 23.2% and of the female pups of the highest dose group 21.6% lower than in the control group. In addition, cleft palates are found in 6 pups from 1 litter of the low dose group (0.5 mg/kg bw/day) and in 3 pups from 2 litters of the highest dose group.

From our point of view on one hand the available data in particular: decreased postnatal survival (mainly during mid and late lactation period), decreased pup body weights (in male pups), presence of malformation (cleft palates in mouse foetuses), and delay in sexual maturation can support the proposal on a classification in Repr. 1B. However, on the other hand, the study has some limitations and deficiencies, which can support classification in Repr. 2. These are for example
(i) only punctual and limited observations of animals,
(ii) missing dose-response relation regarding the presence of cleft palates, and
(iii) lower relevance of cleft palates in mice versus rats.

In our opinion, however, it would be very helpful if data on the structurally similar substances PFOS and APFO were included and discussed before a final evaluation of the reproductive toxicity of PFHpA.

Lactation:
It can be assumed that PFHpA has a lactation effect, which explains reduced weight of the offspring from PND4 to PND21. Although no data on PFHpA in the milk of mice are

available, human data give incidence of PFHpA in breast milk and thus effects due to exposure through breastmilk cannot be excluded.

However, it remains uncertain whether effects on the pups are mediated by quantitative parameters of lactation and/or whether effects on the milk quality were seen.

In conclusion, DE CA agrees with no classification for lactation.

Dossier Submitter's Response

Thank you for your comment and your support regarding the no classification proposal for fertility and lactation.

Please find below a more detailed version of the Table 8 of the CLH report:

Clinical pathology phase :
No significant effect was reported on BW, food consumption, hematology and coagulation, serum chemistry or macroscopic examinations

Main study phase :
F0 :
Mortality: no treatment-related effect on survival. One female exposed to 50 mg/kg bw/d was found dead on day 12 at an advanced stage of autolysis, thus no necropsy could be performed. One male exposed to 0.5 mg/kg bw/d was also found dead at day 103 (hypoactivity and pale body were reported just before death ~30 min), no microscopic or macroscopic findings were observed at necropsy and the cause of death was unknown.
One male exposed to 10 mg/kg bw/d showing hypoactivity, cool and pale body as well as severe weight loss on termination day (-14.6%) and decreased food consumption (between days 14-21) was euthanized in extremis on Day 26. At necropsy, enlarged thymus and an axillary subcutaneous mass (consistent with acute inflammation) were seen as well as a moderate degeneration of the oesophagus muscle. Myeloid hyperplasia in the sternal and femoral bone marrow, increased extramedullary hematopoiesis in the spleen and lymphoid depletion in the thymus were observed.
In the control group, one female was found dead on Lactation day 15, no macroscopic findings were reported at necropsy, but a minimally increased extramedullaryhematopoiesis in the spleen and moderate unilateral periocular haemorrhage were reported.
All other animals survived.

At 50 mg/kg bw/d

Significant increase in ALP (77 and 227** in controls and exposed animals, respectively), ALT (51 and 165* in controls and exposed animals, respectively) and Triglyceride (=Trig., 82 and 153* in controls and exposed animals, respectively) in males
Significant increase in ALP (52 and 152 * in controls and exposed animals, respectively) and Trig. (64 and 161** in controls and exposed animals, respectively) in non-mated females
Significant decrease in thyroid T4 levels in males serum (5.42 and 2.95** in controls and exposed animals, respectively)
Slight increase in precoital interval (2.2 and 2.9 at 0 and 50 mg/kg bw/d, respectively. HCD: 2.7 (2.0-3.3))
Significant increase in liver rel. and abs. weights in both sexes: (See Table 14 of the CLH report)

- **In males:** in controls and exposed animals, respectively
  Final BW (FBW): 36.9 and 37.2 g
  Absolute liver weight (g): 1.8253 and 3.1472**
  Relative liver weight (%): 4.948 and 8.460**
- **In non-mated females:** in controls and exposed animals, respectively
  FBW: 27.8 and 29.1 g
  Absolute liver weight (g): 1.4018 and 1.8879**
  Relative liver weight (%): 5.036 and 6.489**
- **In females, at Lactation day 21:** in controls and exposed animals, respectively
  FBW: 35.6 and 36.7 g

Absolute liver weight (g): 2.0740 and 3.0901**
Relative liver weight (%): 5.799 and 8.415**

Histopathological findings in the liver in both sexes

- **In males, at scheduled necropsy**: See Table 16 of the CLH report for more details
  Mild (9/20 animals) and moderate (11/20 animals) centrilobular hypertrophy of the hepatocytes
  Minimal pigmentation of Kupffer cells (19/20 animals) and minimal hepatocellular necrosis in 19/20 males
- **In females**: See Table 17 of the CLH report for more details
  Non mated at scheduled necropsy: mild centrilobular hypertrophy in 4/4 females and minimal infiltrate of mononuclear cells in 2/4 females
  Mated, at lactation day 21: mild and moderate centrilobular hypertrophy in 8/16 and 10/16 females, respectively
  Minimal hepatocellular necrosis in 7/16 females

<u>At 10 mg/kg bw/d</u>

Significant decrease in ALT levels in lactating females (D21)with 71 and 42* in controls and exposed animals, respectively
Significant decrease in thyroid T4 levels in males serum (5.42 and 3.71** in controls and exposed animals, respectively)
Slight increase in precoital interval (2.2 and 2.7 days at 0 and 10 mg/kg bw/d, respectively)
Significant increase in liver rel. and abs. weights in both sexes (See Table 14 of the CLH report)

- **In males:** in controls and exposed animals, respectively
  FBW: 36.9 and 38.2 g
  Absolute liver weight (g): 1.8253 and 2.1788**
  Relative liver weight (%): 4.948 and 5.689**
- **In non-mated females:**
  Non assessed at this dose level
- **In females, at Lactation day 21:** in controls and exposed animals, respectively
  FBW: 35.6 and 37.5 g
  Absolute liver weight (g): 2.0740 and 2.4908**
  Relative liver weight (%): 5.799 and 6.639**

Histopathological findings in the liver in both sexes

- **In males, at scheduled necropsy**: See Table 16 of the CLH report for more details
  Moderate centrilobular hypertrophy in 13/19 males
- **In females**: See Table 17 of the CLH report for more details
  Non-mated, at scheduled necropsy: not assessed at this dose level
  Mated, at lactation day 21: Mild and moderate centrilobular hypertrophy in 8 and 9/19 females, respectively
  Minimal infiltrate of mononuclear cells in 6/19 females
  Minimal hepatocellular necrosis in 5/19 females

<u>At 0.5 mg/kg bw/d</u>

Slight increase in precoital interval (2.2 and 2.9 at 0 and 0.5 mg/kg bw/d, respectively)
Decrease in in thyroid T4 levels in males serum (5.42 and 4.67 in controls and exposed animals, respectively)
Histopathological findings in the liver in both sexes

- **In males, at scheduled necropsy**: See Table 16 of the CLH report for more details
  minimal and mild centrilobular hypertrophy in 8 and 7/19 males, respectively
  minimal infiltrate of mononuclear cells in 7/19 males
- **In females**: See Table 17 of the CLH report for more details
  Non-mated, at scheduled necropsy: not assessed at this dose level
  Mated, at lactation day 21:
  Minimal and mild centrilobular hypertrophy in 8 and 8/20 females, respectively
  Minimal infiltrate of mononuclear cells in 6/20 animals

About pups survival index (Table 18 in the CLH report), the following data could be added:
Survival index at PND0: 100 % in all groups
Survival index at PND 0 to PND1: 99.6, 95.4, 100 and 100 % at 0, 0.5, 10 and 50 mg/kg bw/d
Survival index at PND1 to PND4: 100, 99.6, 99.6 and 90.8 % at 0, 0.5, 10 and 50 mg/kg bw/d

Regarding the number of stillborn pups vs litter size,

- Mean number born : 11.2, 10.4, 11.9 and 11.2 at 0, 0.5, 10 and 50 mg/kg bw/d
- Mean live litter size at PND0 : 11.2, 10.4, 11.9 and 11.0 at 0, 0.5, 10 and 50 mg/kg bw/d

Regarding mean offspring body weight data during the lactating period (Table 19 in the CLH report): please see the number of pup number in the table below.

Table 1: Pup body weight data (in g ± SD) during the lactation period

Dose level (in mg/kg bw/d)		Males				HCD[A]	Females				HCD[A]
		0	**0.5**	**10**	**50**	♂	**0**	**0.5**	**10**	**50**	♀
PND 1	BW ± SD	1.66 ± 0.121	1.68 ± 0.166	1.68 ± 0.139	1.54* ± 0.136	1.76 (1.63 – 1.91)	1.58 ± 0.142	1.61 ± 0.146	1.59 ± 0.171	1.52 ± 0.153	1.70 (1.53 – 1.82)
	% diff.	/	1.2	1.2	-7.2	/	/	1.9	0.6	-3.8	/
	N	18	20	19	17	/	18	20	19	17	
PND 4 (before selection)	BW ± SD	2.63 ± 0.356	2.74 ± 0.295	2.61 ± 0.267	2.02** ± 0.458	2.70 (2.50 – 3.17)	2.59 ± 0.382	2.66 ± 0.262	2.48 ± 0.310	2.03** ± 0.471	2.60 (2.34 – 3.04)
	% diff.	/	4.2	-0.8	-23.2	/	/	2.7	-4.2	-21.6	/
	N	18	20	19	17	/	18	20	19	17	/
PND 10	BW ± SD	5.95 ± 0.613	6.03 ± 0.566	5.80 ± 0.593	5.00** ± 0.786	6.06 (5.75 – 6.38)	5.85 ± 0.689	5.95 ± 0.466	5.64 ± 0.688	5.04** ± 0.629	5.93 (5.62 – 6.27)
	% diff.	/	1.3	-2.5	-16.0	/	/	1.7	-3.6	-13.8	/
	N	18	20	19	16	/	18	20	19	16	/
PND21	BW ± SD	11.65 ± 1.389	11.55 ± 1.477	10.98 ± 2.031	9.72** ± 1.458	10.66 (8.70 – 13.52)	11.25 ± 1.540	11.09 ± 1.108	10.28 ± 2.144	9.58** ± 1.151	10.24 (7.18 – 13.04)
	% diff.	/	-0.9	-5.8	-16.6	/	/	-1.4	-8.6	-14.8	/
	N	17	20	19	16	/	17	20	19	16	/

* : $p<0.05$; ** : $p<0.01$; [A] : HCD in mouse CD-1 range of study dates 10-97 – 01/15

Concerning read-across data (based on PFOS and APFO), please refer to our response to comment No 9.

Regarding the developmental endpoint, based on the data available in the CLH and the new data mentioned in the NL CA comment No. 7 and the response to this comment, BECA still consider appropriate a classification as Repr. 1B H360D
RAC's response
RAC largely supports the reponse provided by the BECA. In addition RAC agrees that the available study (Anonymous, 2017) has some limitations. However, there is clear evidence for relevant effects (decreased survival, decreased body weights, skeletal malformations) without relevant maternal toxicity / maternal toxicity that could explain the observed effects.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Sweden		MemberState	9
Comment received				
The Swedish CA supports the classification of perfluoroheptanoic acid as Repro. 1B, H360D based on the results of a Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (OECD TG 422) with sodium perfluoroheptanoate via oral gavage in mice. We consider that read-across from perfluorinated carboxylic acids homologues with longer carbon chains could also have been included as supportive information in the WoE assessment to strengthen the conclusion. The developmental toxicity of sodium perfluoroheptanoate observed were consistent with effects reported for APFO/PFOA and longer analogues: - Reduced postnatal survival at 50 mg/kg bw/d (10-12% less than control, not stat. sign.) - Decreased pup body weights at 50 mg/kg bw/d (14-23% less than control, stat. sign.) - Signs of delayed pubertal onset: vaginal patency higher at 50 mg/kg bw/d (stat. sign. compared to control). We note that sodium perfluoroheptanoate was not tested up to doses giving rise to general toxicity (aside from the observed liver toxicity) in the parental animals and we thus consider it plausible that clearer effects of developmental toxicity (and/or fertility) could have been detected at higher concentrations.				
Dossier Submitter's Response				
Thank you for your comment and your support. Concerning the read-across, to clarify some of your concerns, we started the evaluation of the parent compound FS-65 and it can only form PFHpA and lower PFCAs, but not PFOA as degradation products. BE CA is aware of the toxic profile of the longer PFCAs but would like to highlight that toxicity of these PFCAs decreased together with a decrease in chain length (more likely a supposed tendency). Therefore, we asked for a test specifically on PFHpA at the end of the evaluation to better understand if and where the toxic effect stopped when the carbon chain length decreased. Indirectly, it means that we assign less weight to a read-across argument compared to a real toxicity study. Finally, here the opportunity is lacking to interpolate (presume of the effects of a C7 when you have the data for a C6 and a C8), which would be a stronger argument that extrapolation (i.e. use the data from a C10 to presume of the effects of a C8 …), since we do not have the needed toxicity data on perfluorohexanoic acid, for example.				

However, we acknowledge that read-across is more sensible for direct hazards such as liver or reproductive toxicity than for toxicokinetics. We also believe the effects observed and highlighted in the CLH report for reproductive toxicity are consistent with longer carbon chain analoguous substances and agree with Sweden that these effects were observed at low doses.
RAC's response
RAC largely supports the response provided by the BECA. Concerning the dose selection we would like to refer to our response to comment number 4.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Germany		MemberState	10
Comment received				
The liver weight increases in relation to the dose. Liver necrosis starts already at the lowest dose of 0.5 mg/kg bw/d and severity increases with dose gaining a moderate level from 10 mg/kg bw/d onwards. Brown pigmentation of Kupffer cells and hepatocytes could indicate increased histiocytosis of cell debris and are only seen at 50 mg/kg. Marked increases of the triglyceride values at 50 mg/kg could indicate disturbances of fat metabolism, but no clear histopathological effects are seen as corresponding effects. Serious toxicological relevant effects on the liver of F0 and F1 animals of both sexes are found. The activity of liver enzymes ALT and ALP increased (doubling) in the higher dose groups, indicating a modification of organ function. However, as the effect of organ enlargement and the histopathological effects like hypertrophy and necrosis are only minimal to moderate, rather the classification in STOT RE 2, H372 would be justified.				
Dossier Submitter's Response				
Thank you for your comment. BECA still considers that the effective dose is of 10 mg/kg bw/d. Even if minimal necrosis was noted in mice exposed to 10 mg/kg bw/d, this effect was noted in F0 and F1 generations. Regarding the category, BECA considers that a category 2 might be more appropriate. However, BECA wants to highlight that 1 male and 1 female of the F0 generation exposed to 0.5 mg/kg bw/d already exhibited hepatocellular necrosis. In the OECD testing guidance 422, paragraphe 29, it is mentioned that "Two- to four- fold intervals are frequently optimum and addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages." In this study, based on the choice of dose, it is not possible to predict whether a dose between 0.5 and 10 mg/kg bw/d would have caused more liver effects.				
RAC's response				
RAC agrees with the response provided by the BE CA. It is further noted that similar effects were seen in the F1 generation and those animals were exposed even for a shorter period. In addition RAC points out that 3 additional cases of necrosis (minimal) were observed in low dose females (lactation day 21), which were not mentioned in the CLH report (in total 4 incidences of necrosis in the low dose females including 3 minimal and 1 mild).				

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	France		MemberState	11
Comment received				
STOT RE FR agrees with the effective dose of 10 mg/kg proposed by BE. However, according to our calculation, it seems that it could be a mistake in the application of the Haber's law. If C90d x T90d = C109d x T109d, therefore, C90d = (C109d x T109d)/ T90d = (10x109)/90 = 12.1 mg/kg, which would induce a classification as STOT RE 2.				
Dossier Submitter's Response				
Thank you for your comment. We note your support regarding the effective dose proposed at 10 mg/kg bw/d. We agree with your remark: males were exposed for 109-133 doses, and females to at least 109 doses (groups used for gender comparison). In that case, according to Haber's rule, a classification as STOT RE 2 might be more appropriate. However, BECA wants to highlight that 1 male and 1 female of the F0 generation exposed to 0.5 mg/kg bw/d already exhibited hepatocellular necrosis. In the OECD testing guidance 422, paragraphe 29, it is mentioned that "Two- to four- fold intervals are frequently optimum and addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages." In this study, based on the choice of dose, it is not possible to predict whether a dose between 0.5 and 10 mg/kg bw/d would have caused more liver effects.				
RAC's response				
RAC agrees with the response provided by the BE CA. It is further noted that similar effects were seen in the F1 generation and those animals were exposed even for a shorter period. In addition RAC points out that 3 additional cases of necrosis (minimal) were observed in low dose females (lactation day 21), which were not mentioned in the CLH report (in total 4 incidences of necrosis in the low dose females including 3 minimal and 1 mild).				

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	United Kingdom	<confidential>	Please select organisation type..	12
Comment received				
Section 10.12.2 STOT RE 1; H372 (liver) There is clear evidence from the 90-day study of toxicity to the liver in both male and female mice of sufficient severity to warrant classification as STOT RE. However, the dose at which the severe effects occur may not support classification in Cat 1. Classification in				

Category 2 may be more appropriate.

The reported indicators of hepatic toxicity include increases in the blood levels of key liver enzymes, hepatocellular hypertrophy, increases in liver weight and hepatocellular necrosis. Of these effects, hepatocellular necrosis is the key indicator of severe hepatotoxicity. Structurally-related perfluoroalkyl acids such as PFOA are known activators of PPAR a class of chemicals known to cause liver growth and increases in levels of liver enzymes in rodents that have limited relevance to human health hazard assessment (e.g. Elcombe et al, 2010; US NTP, 2019). PFHpA has been shown to activate both mouse and human PPARα in-vitro in transiently transfected COS-1 cells (Wolf et al, 2012).

PPARa activation by PFHpA may have contributed to the effects seen in the current study. Nevertheless, the occurrence of hepatocellular necrosis suggests that significant toxicity has occurred in mice receiving 50 mg/kg/day that is of sufficient severity to warrant classification as STOT RE Cat 2. The argument presented by the MSCA that the response in mice receiving 10 mg/kg/day is of sufficient severity to warrant classification in STOT RE Cat 1 is less convincing.

Dossier Submitter's Response

Well noted, thank you for your comment.

Based on the comment No. 11, BECA considers that a classification in category 2 might be more appropriate. However, BECA still considers that the effective dose is of 10 mg/kg bw/d. Even if minimal necrosis was noted in mice exposed to 10 mg/kg bw/d, this effect was noted in F0 and F1 generations.

BECA wants to highlight that 1 male and 1 female of the F0 generation exposed to 0.5 mg/kg bw/d already exhibited hepatocellular necrosis.

In the OECD testing guidance 422, paragraphe 29, it is mentioned that "Two- to four- fold intervals are frequently optimum and addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages."
In this study, based on the choice of dose, it is not possible to predict whether a dose between 0.5 and 10 mg/kg bw/d would have caused more liver effects.

RAC's response

RAC agrees with the response provided by the BE CA. It is further noted that similar effects were seen in the F1 generation and those animals were exposed even for a shorter period. In addition RAC points out that 3 additional cases of necrosis (minimal) were observed in low dose females (lactation day 21), which were not mentioned in the CLH report (in total 4 incidences of necrosis in the low dose females including 3 minimal and 1 mild).

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Sweden		MemberState	13

Comment received

The Swedish CA supports classification of perfluoroheptanoic acid as STOT RE 1, H372 (liver) based on the results of the Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (OECD TG 422) with sodium perfluoroheptanoate via oral gavage in mice. However, we consider that read-across from

other perfluorinated carboxylic acidshomologues with longer carbon chains could also have been included as supportive information in the WoE assessment to strengthen the database for classification in this hazard class. The liver toxicity of sodium perfluoroheptanoate observed from 10 mg/kg bw/day was consistent with effects reported for APFO/PFOA and longer homologues.

Moreover, we also consider that sodium perfluoroheptanoate was tested only up to 50 mg/kg bw/day without any effects on clinical condition or body weights and testing of higher doses could thus have revealed clearer effects on target organ(s).

Dossier Submitter's Response

Thank you for your support.

Concerning the read-across, please refer to our response to your comment on reproductive toxicity (Comment No. 9). Read-across with only available data on substances with longer carbon chain remains delicate. However, we agree that liver was pointed out as the target organ of PFOA and PFNA (both classified as STOT RE 1, H372 for liver effects).

RAC's response

RAC agrees with the response provided by the BE CA.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2020	Netherlands		MemberState	14

Comment received

STOT RE
The NL-CA agrees with the Dossier Submitter that liver is clearly the target organ. Effects include increased ALP, ALAT and triglyceride levels, increased liver weight, and hepatocellular hypertrophy and necrosis. However, we do not agree with the proposed classification category and consider a classification with STOT RE 2 (H373) as more appropriate.
Increased liver weight and hepatocellular hypertrophy (severity scores ranging from minimal to moderate) as noticed at 0.5 and 10 mg/kg bw/d are considered adaptive in nature and insufficient for classification (these effects do not fulfill CLP-Regulation Annex I: 3.9.2.7.3.(f): morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver)).
At the dose of 50 mg/kg bw/d, increased liver weight and hepatocellular hypertrophy (also scored as minimal to moderate at this dose) were associated with hepatocellular necrosis (clearly shown at 50 mg/kg bw/d in all male animals and half of the female animals (1/4 non-mated females, 9/16 mated females); incidental findings at 10 mg/kg bw/d). This is considered relevant for classification as STOT RE. The severity of the hepatocellular necrosis was mainly scored as minimal with some of the findings scored as mild. However,
an uncertainty is noted in relation to the specification of the type of necrosis in the study report (Anonymous 2017). The incidences of the hepatocellular necrosis was specified as "multifocal/multiple" in a footnote to the tables of the study report. However, it is noted that in the text of the study report and the CLH report, the necrosis is described as "single cell to coalescing". The Dossier Submitter is requested to reflect on this.
Assuming the necrosis would be multi-focal or diffuse, a classification would be justified (according to CLP-Regulation Annex I: 3.9.2.7.3.(e): multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity). The other liver

effects are considered supportive, but not sufficient as stand alone, for a classification as STOT RE. Taking into account the Guidance Value for STOT RE category 2 of 10<C≤100 mg/kg bw/d for a 90-day repeated exposure, and assuming an exposure period of 90-140 days in the OECD 422 study, PFHpA fulfils the criteria for classification as STOT RE 2.

Dossier Submitter's Response

Thank you for your comment.

More information about the incidence of hepatocellular necrosis is described in the table below :

Dose level (mg/kg bw/d)	Degree	0	0.5	10	50
In males					
F0					
Unscheduled death	No effect on the liver				
Scheduled necropsy		0	1	2	20
	Minimal	0	1, M/M	2, FOCAL	19, M/M
	Mild	0	0	0	1, M/M
F1					
Scheduled necropsy PND43		0	0	2	9
	Minimal	0	0	2, M/M	7, M/M
	Mild	0	0	0	1, M/M
	Marked	0	0	0	1, M/M
In females					
F0					
Unscheduled deaths	No effect on the liver				
Non mated		0	NA	NA	1
	Minimal	0	NA	NA	1, M/M
Total litter loss		0	NA	NA	1
	Minimal	0	NA	NA	1, M/M
Failed to deliver		0	0	1	1
	Minimal	0	0	1, FOCAL	0
	Mild	0	0	0	1, M/M
F1					
Scheduled necropsy PND43		0	0	3	8
	Minimal	0	0	3, M/M	1, FOCAL 7, M/M

M/M stands for multiple/multifocal

BECA considers that a classification in category 2 might be more appropriate. However, BECA still considers that the effective dose is of 10 mg/kg bw/d. Even if minimal necrosis was noted in mice exposed to 10 mg/kg bw/d, this effect was noted in F0 and F1 generations.

BECA wants to highlight that 1 male and 1 female of the F0 generation exposed to 0.5 mg/kg bw/d already exhibited hepatocellular necrosis.

In the OECD testing guidance 422, paragraphe 29, it is mentioned that "Two- to four- fold intervals are frequently optimum and addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages."
In this study, based on the choice of dose, it is not possible to predict whether a dose between 0.5 and 10 mg/kg bw/d would have caused more liver effects.

RAC's response

RAC largely agrees with the reponse provided by the BECA. RAC notes that 3 additional cases of necrosis (minimal) were observed in low dose females (lactation day 21), which

were not mentioned in the CLH report (in total 4 incidences of necrosis in the low dose females including 3 minimal and 1 mild).

PUBLIC ATTACHMENTS
1. Public comments to CLH proposal Perfluoheptanoic acid_final 24Jan2020.pdf [Please refer to comment No. 1, 5]

CONFIDENTIAL ATTACHMENTS
1. Confidential comments to CLH proposal Perfluoheptanoic acid_final24Jan2020.pdf [Please refer to comment No. 1, 5]