Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

undecafluorohexanoic acid, PFHxA [1]; sodium undecafluorohexanoate, NaPFHx [2]; ammonium undecafluorohexanoate, APFHx [3]; other inorganic salts of undecafluorohexanoic acid [4]

EC Number: 206-196-6[1]; 220-881-7[2]; 244-479-6[3]; - [4]
CAS Number: 307-24-4[1]; 2923-26-4[2]; 21615-47-4[3]; - [4]

CLH-O-0000007429-65-01/F

Adopted

14 March 2024

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ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON UNDECAFLUOROHEXANOIC ACID; [PFHXA] [1] SODIUM UNDECAFLUOROHEXANOATE; [NAPFHX] [2] AMMONIUM UNDECAFLUOROHEXANOATE; [APFHX] [3] OTHER INORGANIC SALTS OF UNDECAFLUOROHEXANOIC ACID [4]

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:
undecafluorohexanoic acid; [PFHxA] [1]
sodium undecafluorohexanoate; [NaPFHx] [2]
ammonium undecafluorohexanoate; [APFHx] [3]
other inorganic salts of undecafluorohexanoic acid [4]
EC number: 206-196-6[1]; 220-881-7[2]; 244-479-6[3]; - [4]
CAS number: 307-24-4[1]; 2923-26-4[2]; 21615-47-4[3]; - [4]
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2023	Germany	Gesamtverband Textil und Mode e.V.	Industry or trade association	1
Comment received				
On the basis of the attached „Statement concerning the proposed classification and labeling of PFHxA and ist inorganic salts" the German Textile and Fashion Association rejects the proposed classification as reproductive toxic category 1B. We fully support the content and conclusion of the EuDICo report (attached). ECHA note – An attachment was submitted with the comment above. Refer to public attachment Stellungnahme_PFHxA_t+m_TEGEWA.pdf				
Dossier Submitter's Response				
Thank you for your comment. The following comments and responses refer to your statement "Stellungnahme_PFHxA_t+m_TEGEWA.pdf". <u>Comment/response on group approach:</u> Comments (4/21, 10/21): "*The authors of the opinion disagree with this **group approach**, based on scientific data. …. physico-chemical properties …. influence resorption and distribution through the body. Also the half live in the body is influenced since renal filtration is influenced by solubility. Therefor a group approach for PFHxA and its inorganic salts is not appropriate*."				

Response:

The acid or salt is expected to dissociate in biological media. Hence, regardless whether the acid or the salt are administered, once absorbed into the blood the PFHx-anion will be formed. From toxicokinetic data available on PFHxA and APFHx it can be concluded that absorption via the oral pathway is rapid (< 1 hour) and complete.

RAC will conclude whether the group approach for PFHxA and its inorganic salts is appropriate.

Comments/responses on test organism and statistical analyses:

Comment (p 5,16-20/21):

"*Reasons to downgrade the studies by Charles River Laboratories are extensively discussed throughout the opinion and include not working according to an OECD guideline, the use of a sub-optimal* ***test organism*** *according to the selected testing guideline, not-robust* ***statistical analyses*** *leading to several re-evaluations leading to different result interpretations due to differing result significances*."

Response on test organism:

Regarding data from reproductive and developmental toxicity studies there are no indications that the mouse is unsuitable for testing APFHx. For APFHx, the mouse appears to be more sensitive than the rat.

Iwai and Hoberman (2014) provided an explanation for choosing the mouse as test species: "*The developmental toxicity of perfluoroalkyl acids (PFAAs) has been extensively studied (Lau et al. 2013, Das et al. 2008). The mouse has been used to evaluate the developmental toxicity of other PFAAs including perfluorooctanic acid (PFOA). Unlike the rat, sex differences in exposure levels in the mouse do not appear to exist (Lau et al. 2006). Although only female mice were evaluated in this study, the mouse appears to be an appropriate and sensitive model for evaluation of PFHxA. Developmental toxicity observed in rodents showed a lack of teratological findings (structural anomalies) from PFHxA. Effects on pup survival and postnatal growth have been consistently observed in both rats and mice in a dose-dependent pattern (Lau et al. 2008)*."

In general, findings in the most sensitive species would be used to determine the classification in which more than one acceptable test is available.

Response on statistical analyses:

With regard to the statistical analyses leading to several re-evaluations of the studies by Charles River Laboratories, the DS described and discussed the disagreement with the approach by Iwai et al. (2019) in the CLH report on page 38 and thus refrained from considering the conclusion of Iwai et al. (2019).

Comment/response on Klimisch scores:

Comment (p 18/21):

"*Even though Klimisch 2 studies may be used in a the 'weight of evidence' approach during the evaluation of a toxicological endpoint, studies with a Klimisch score of 1 should always be preferred. Since the Klimisch 1 studies use the recommended test species and did not see adverse effects on peri- or postnatal mortality, the authors of strongly suspect that the DS are picking the study supporting their argument and purpose*."

Response:

The Klimisch scores assigned to the studies may serve as an indication of the 'quality of the data'. In a weight-of-evidence approach, data of several sources (studies in compliance with OECD test guidelines, studies with some deviations and - depending on the consistency of their observations with those from guideline studies - also studies which are not in compliance with test guidelines can be considered as supporting information). In general, findings in the most sensitive species would be used to determine the classification in which more than one acceptable test is available.

Comments/responses on maternal toxicity:

Comment (p 4/21) :
"*The major criticism regarding the conclusion is that maternal toxicity was recognized in several studies. Therefor it cannot be distinguished whether maternal toxicity or developmental toxicity is the reason for the decreased weights or postnatal survival."*

Comment (p 4/21):

"1. From the Loveless at al., 2009, study the DS mention as reproductive effects a decreased pup weight as well as a decreased maternal weight. When the mother's weight is reduced it is most likely that the dam will birth lighter pups since she is not able to fully provide for them. Therefor the authors of this opinion consider it justified that the decreased pup weight is attributable to maternal toxicity instead of reproductive toxicity."

Comment (p 13/21):

"In table 16 of the CLH report (depicted in Figure 3 for convenience) [referring to Loveless et al., 2009] significant changes in body weights of the F1 generation pups are only observed at 500 mg/kg bw/d. In the parental generation P0 there are no data shown for absolute body weights but only for body weight gains which is also significantly reduced in the 500 mg/kg bw/d dose group for males and for females during the first week of gestation. This is not only supporting the opinion of these authors, but is showing clear evidence that the decreased pup weight is due to maternal toxicity."

Comment (p 14/21):

"Regarding the TG 414 study [referring to Loveless et al., 2009] it is stated by the DS on p. 36 and p. 42 that developmental toxicity occurred due to lower foetal body weights. Even though the body weight is slightly decreased in the 500 mg/kg bw/d dose group according to table 17 of the CLH report (depicted in Figure 4 for convenience) it is not statistically significant. Thus it should not be taken into account for developmental toxicity. In the same dose group maternal body weights are significantly reduced. This strongly supports the conclusion of the authors and provides even more evidence of maternal toxicity effects rather than developmental toxicity."

Response:

In rats, maternal toxicity could have contributed to the lower foetal growth. At 500 mg/kg bw/d significantly reduced body weight parameters in dams (bw: range of -5 % to –7 % on GD 19, 20 and 21) and a reduction in foetal body weight (-9 %) were observed. Effects on viability or other teratological effects were not observed (Loveless et al. 2009).

According to CLP, Annex I, 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 case-by-case basis that the developmental effects are secondary to maternal toxicity*." There exists no 'unequivocal demonstration' that reduced pup weight is secondary to maternal toxicity for this study.

Furthermore, in the key developmental and reproductive toxicity study in mice (Charles River Laboratories 2011a, 2011b, 2012) clear adverse effects on development (peri- and postnatal pup mortality at APFHx ≤ 175 mg/kg bw/d, proposed as developmental LOAEL) were observed in the absence of relevant maternal toxicity. These effects are considered adverse, treatment and dose-related and lead to the proposed classification in Category 1B.

These observations do not support the Commenter's view that "*it cannot be distinguished whether maternal toxicity or developmental toxicity is the reason for the decreased weights or postnatal survival*.".

<u>Comments/responses on mean pup body weights:</u>

Comment (p13/21):

"*Regarding the TG 415 (Loveless et al. 2009) study it is stated on p. 35 and p. 41 that treatment-related effects on mean pup body weights were observed at ≥ 100 mg/kg bw/d. This could not be confirmed by the authors of this opinion. In table 16 of the CLH report (depicted in Figure 3 for convenience) significant changes in body weights of the F1 generation pups are only observed at 500 mg/kg bw/d*."

Response:

Significant treatment-related effects on mean pup body weights were observed only at 500 mg/kg bw/d. Please note that in the CLH report ≥ 100 mg/kg bw/d instead of > 100 mg/kg bw/d was written. We apologise for the inconvenience.

<u>Comments on Table 20:</u>

Comment (p 5/15):

"2. *The DS mention an increased relative liver weight in pups according to the Iwai, Hoberman, 2014, study. According to the study itself the relative liver weight was not increased but actually decreased in the highest dose group of phase I. The absolute liver weights as well as terminal body weights were not significantly reduced in this dose group. Additonally, the DS only state this "fact" in the summary table 20 of the CLH report. Thus the authors of this opinion find it quite confounding to mention this false fact in the summary table which is what most people will look at if they want to gain a first insight on the topic*."

Response (decreased liver weight, Table 20):

The DS mistakenly stated in Table 20 that the relative liver weights of the F1-generation male mice were increased (instead of correctly decreased liver weights) in the highest dose group ($p \leq 0.05$, Iwai and Hoberman, 2014). We apologise for the inconvenience.

Comment (p 5/15):

"3. *The only discussed reason for a classification in reproductive toxicity category 1B in the CLH report is an increased peri- and postnatal pup mortality from Iwai, Hoberman, 2014. In contrast to this, only the decreased postnatal survival, i.e. increased postnatal pup mortality, is mentioned in table 20 as a main reproductive effect of PFHxA*."

Response (peri- and postnatal mortality, Table 20):

Table 20 should not serve as a summary table for the CLH report, but should help to gain a general overview of effect patterns across different perfluorocarboxylic acids (PFCAs). We apologise for any inconvenience or ambiguous information.

Comment (p 13):

"*From the citation in table 20, CLH report, it is unclear whether this is deducted from the one generation reproduction toxicity study (OECD TG 415) or the prenatal developmental toxicity study (OECD TG 414)*."

Response (Loveless, Table 20):

Maternal body weight as well as pup body weight are reduced in both studies by Loveless et al. (2009).

References (cited in Iwai and Hoberman (2014), see here Comment/response on test organism):

Lau C, Thibodeau JR, Hason RG, et al. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse: II. Postnatal evaluation. Toxicol Sci. 2013;74(2):382-392.

Das KP, Grey BE, Zehr RD, et al. Effects of perfluorobutyrate exposure during pregnancy in the mouse. Toxicol Sci. 2008; 105(1):173-181.

Lau C, Thibodeaux JR, Hason RG, et al. Exposure to perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol Sci. 2006;90(2):510-518.

Lau C, Anitole K, Hodes C, et al. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol Sci. 2008;99(2): 366-394.

RAC's response

RAC agrees with the DS that read-across between the acid and neutral salts is appropriate for systemic effects as the salts will be present as PFHx and the respective cations in aqueous solution (see ODD section RAC general comment).

RAC agrees with the DS that studies in mice can be used for classification and that the statistical analyses as reported by Iwai et al. (2019) is inappropriate (see ODD section Adverse effects on development, paragraph "Discussion on statistical analysis of stillborn pup endpoint"). RAC further agrees with the DS on their other comments regarding reproductive toxicity studies.

To review the the comments of the DS on analysis of the stillborn pup data in Iwai et al. (2019), a dose-response analysis was performed based on the raw data as provided in the full study reports (CRL 2011a; 2011b). Dose-response analysis was performed using PROAST software (https://proastweb.rivm.nl/). In summary, the dose-response analysis illustrates that:

1. There is indeed a litter effect that needs to be accounted for in analysis of stillborn pups per litter. Furthermore, also a litter effect is observed in analysing all affected pup data together.
2. The data were better described by dose-response models compared to the null model (horizontal line), indicating a clear dose-response in these data. The same applies when all affected pup data were analyzed together.
3. So all in all, the analysis shows a clear trend in the data, indicating both an increase in the number of stillborn pups with dose and an increase in the number of litters affected with dose.

The details of the dose-response analyses are provided below.

PROAST dose-response analysis of stillborn pups:

Data were fitted specifying a possible litter effect, taking into account study ID as covariate, and using a critical effect size of 0.01 (an increased risk of 1% for stillborn pups). Fitting the data with an additional parameter (alpha) to account for a litter effect, statistically significantly improved the fit of the null model. Estimate of parameter alpha was 0.1474437 (P-value: 2.3115e-12). This illustrates the data is best described by incorporating an additional parameter to account for litter effects.

The data was better described by dose-response models compared to the null model, providing proof of dose-response in the stillborn pup data. Three models were an acceptable fit to the data (gamma-a, Expon. m3-ab, and Hill m3-ab) (see details below).

Fitted models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	sens.subgr	conv
null	2	-112.82	229.64	NA	NA	NA	NA	NA	NA
full	9	-97.58	213.16	NA	NA	NA	NA	NA	NA
two.stage-a	5	-101.65	213.3	no	NA	NA	152	--	no
log.logist-a	5	-101.45	212.9	no	NA	NA	186	--	yes
Weibull-a	5	-101.3	212.6	no	NA	NA	195	--	no
log.prob-a	5	-101.76	213.52	no	NA	NA	174	--	no
gamma-a	5	-101.11	212.22	yes	101	255	194	--	no
LVM: Expon. m3-ab	6	-99.28	210.56	yes	144	NA	170	CRL2011b	yes
LVM: Hill m3-ab	6	-99.4	210.8	yes	142	195	168	CRL2011b	yes

An example of the fit of the data using the gamma-a model is provided in Figure 1a. Applying model averaging (with a critical effect size of 0.01) resulted in a benchmark dose lower bound (BMDL) and upper bound (BMDU) of 95.8-336 mg/kg bw/day based on the sensitive subgroup of CRL 2011a (Figure 1b).

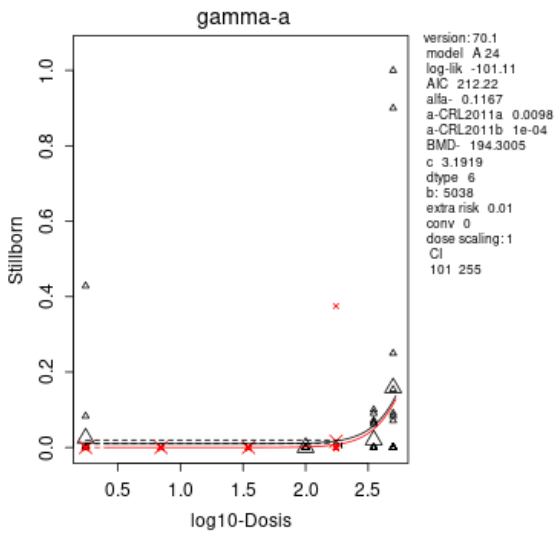


Figure 1a. Plot of stillborn pups per nest expressed against the log10 dosis PFHxA. Black upward triangle represents the data from CRL 2011a, the red cross represents the data from CRL 2011b. Larger symbols represent group means, smaller symbols represent individual nest data.

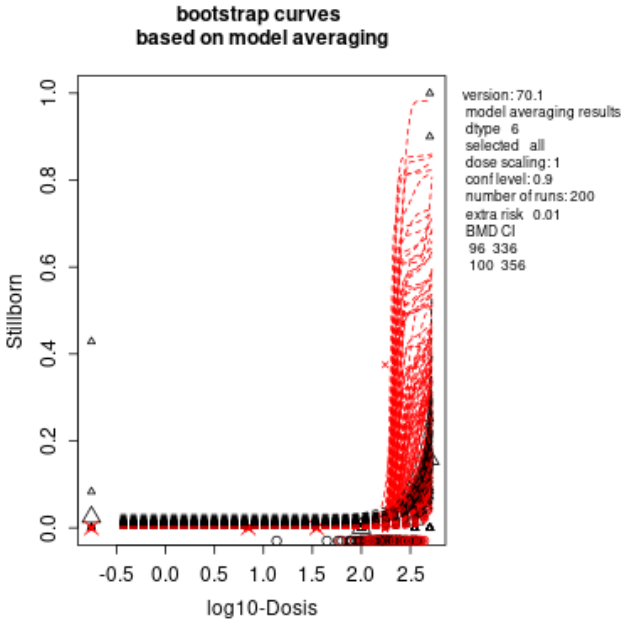


Figure 1b. Model averaging using 200 bootstrap runs. Black upward triangle represents the data from CRL 2011a, the red cross represents the data from CRL 2011b. BMDL-BMDU range for CRL2011a is 95.8-336 mg/kg bw/day.

PROAST dose-response analysis of stillborn pups and neonatal mortality combined:

In this dose-response analysis, the pups that were stillborn, the liveborn pups that died, that went missing or that were ranked as having an unknown vital status, were first summed per nest to represent a number of affected pups per nest.

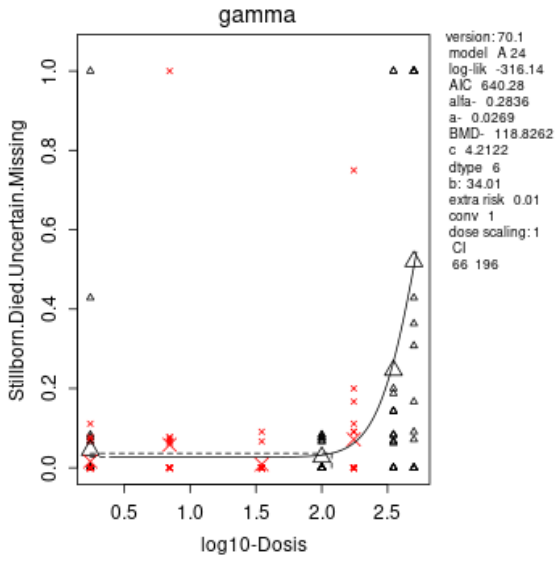
Data were fitted specifying a possible litter effect, taking into account study ID as covariate, and using a critical effect size of 0.01 (an increased risk of 1% for stillborn pups or neonatal mortality). Fitting the data with an additional parameter (alpha) to account for a litter effect, statistically significantly improved the fit of the null model. Estimate of parameter alpha was 0.2851655 (P-value: 0). This illustrates the data is best described by incorporating an additional parameter to account for litter effects.

The data was better described by dose-response models compared to the null model, providing proof of dose-response in the affected pup data. Six models were an acceptable fit to the data (Expon. m3-, Hill m3-, log.logist, Weibull, log.prob, and gamma).

Fitted models

model	No.par	loglik	AIC	accepted	BMDL	BMDU	BMD	sens.subgr	conv
null	2	-344.81	693.62	NA	NA	NA	NA	NA	NA
full	9	-313.15	644.3	NA	NA	NA	NA	NA	NA
two.stage	4	-317.22	642.44	no	NA	NA	66.4	--	yes
log.logist	4	-316.17	640.34	yes	65	191	116	--	yes
Weibull	4	-316.16	640.32	yes	60.1	180	110	--	yes
log.prob	4	-316.19	640.38	yes	76.4	204	126	--	yes
gamma	4	-316.14	640.28	yes	66	196	119	--	yes
LVM: Expon. m3-	4	-316.49	640.98	yes	39.1	154	87	CRL2011a	yes
LVM: Hill m3-	4	-316.48	640.96	yes	39.3	154	87.2	CRL2011a	yes

An example of the fit of the data using the gamma model is provided below (Figure 2a). Applying model averaging (with a critical effect size of 0.01) results in a BMDL and BMDU of 46-188 mg/kg bw/day based on the sensitive subgroup of CRL 2011a (Figure 2b).



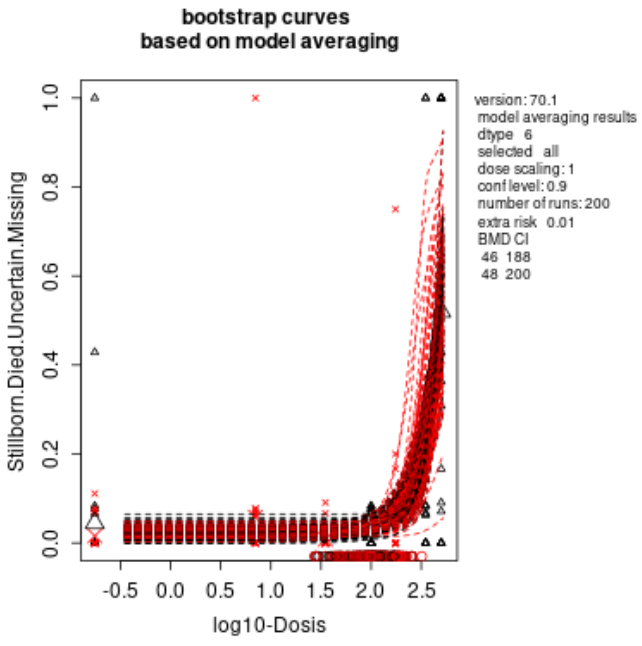


Figure 2a. Plot of affected pups per nest expressed against the log10 dosis PFHxA. Black upward triangle represents the data from CRL 2011a, the red cross represents the data from CRL 2011b. Larger symbols represent group means, smaller symbols represent individual nest data.	**Figure 2b.** Model averaging using 200 bootstrap runs. Black upward triangle represents the data from CRL 2011a, the red cross represents the data from CRL 2011b. BMDL-BMDU range for CRL2011a is 46-188 mg/kg bw/day.

Date	Country	Organisation	Type of Organisation	Comment number
29.06.2023	Sweden		MemberState	2
Comment received				
We thank the German CA for the evaluation of reproductive toxicity and specific target organ toxicity – repeated exposure of PFHxA; NaPFHx; APFHx and other inorganic salts of undecafluorohexanoic acid. The read-across approach from the sodium/ammonium salts to PFHxA and its anion, in line with previous CLH-proposals for the perfluorinated carboxylic acids (PFCAs) perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluoroheptanoic acid (PFHpA), is supported.				
Dossier Submitter's Response				
Thank you for your comment.				
RAC's response				
Thank you for your comment.				

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2023	Germany	Verband TEGEWA e.V.	Industry or trade association	3
Comment received				
Verband TEGEWA e. V. is an association representing chemical companies based in Germany, Switzerland and Netherlands manufacturing and marketing inter alia chemicals for leather and textile production and treatment. Our focus in this contribution is the use of modified polymers with C6 fluorine compounds for water, oil and stain repellency for a limited number of textile and leather applications when safety and personal protection aspects are involved or for high technology applications, especially when future technologies are involved. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Stellungnahme_PFHxA_t+m_TEGEWA.pdf				
Dossier Submitter's Response				
Thank you for your comment. The following comments and responses refer to your statement "Stellungnahme_PFHxA_t+m_TEGEWA.pdf". <u>Comment/response on group approach:</u> Comments (4/21, 10/21): "*The authors of the opinion disagree with this **group approach**, based on scientific data. …. physico-chemical properties …. influence resorption and distribution through the body. Also the half live in the body is influenced since renal filtration is influenced by solubility. Therefor a group approach for PFHxA and its inorganic salts is not appropriate*."				

Response:

The acid or salt is expected to dissociate in biological media. Hence, regardless whether the acid or the salt are administered, once absorbed into the blood the PFHx-anion will be formed. From toxicokinetic data available on PFHxA and APFHx it can be concluded that absorption via the oral pathway is rapid (< 1 hour) and complete.

RAC will conclude whether the group approach for PFHxA and its inorganic salts is appropriate.

Comments/responses on test organism and statistical analyses:

Comment (p 5,16-20/21):

"*Reasons to downgrade the studies by Charles River Laboratories are extensively discussed throughout the opinion and include not working according to an OECD guideline, the use of a sub-optimal* ***test organism*** *according to the selected testing guideline, not-robust* ***statistical analyses*** *leading to several re-evaluations leading to different result interpretations due to differing result significances*."

Response on test organism:

Regarding data from reproductive and developmental toxicity studies there are no indications that the mouse is unsuitable for testing APFHx. For APFHx, the mouse appears to be more sensitive than the rat.

Iwai and Hoberman (2014) provided an explanation for choosing the mouse as test species: "*The developmental toxicity of perfluoroalkyl acids (PFAAs) has been extensively studied (Lau et al. 2013, Das et al. 2008). The mouse has been used to evaluate the developmental toxicity of other PFAAs including perfluorooctanic acid (PFOA). Unlike the rat, sex differences in exposure levels in the mouse do not appear to exist (Lau et al. 2006). Although only female mice were evaluated in this study, the mouse appears to be an appropriate and sensitive model for evaluation of PFHxA. Developmental toxicity observed in rodents showed a lack of teratological findings (structural anomalies) from PFHxA. Effects on pup survival and postnatal growth have been consistently observed in both rats and mice in a dose-dependent pattern (Lau et al. 2008)*."

In general, findings in the most sensitive species would be used to determine the classification in which more than one acceptable test is available.

Response on statistical analyses:

With regard to the statistical analyses leading to several re-evaluations of the studies by Charles River Laboratories, the DS described and discussed the disagreement with the approach by Iwai et al. (2019) in the CLH report on page 38 and thus refrained from considering the conclusion of Iwai et al. (2019).

Comment/response on Klimisch scores:

Comment (p 18/21):

"*Even though Klimisch 2 studies may be used in a the 'weight of evidence' approach during the evaluation of a toxicological endpoint, studies with a Klimisch score of 1 should always be preferred. Since the Klimisch 1 studies use the recommended test species and did not see adverse effects on peri- or postnatal mortality, the authors of strongly suspect that the DS are picking the study supporting their argument and purpose*."

Response:

The Klimisch scores assigned to the studies may serve as an indication of the 'quality of the data'. In a weight-of-evidence approach, data of several sources (studies in compliance with OECD test guidelines, studies with some deviations and - depending on the consistency of their observations with those from guideline studies - also studies which are not in compliance with test guidelines can be considered as supporting information). In general, findings in the most sensitive species would be used to determine the classification in which more than one acceptable test is available.

Comments/responses on maternal toxicity:

Comment (p 4/21) :
"*The major criticism regarding the conclusion is that maternal toxicity was recognized in several studies. Therefor it cannot be distinguished whether maternal toxicity or developmental toxicity is the reason for the decreased weights or postnatal survival."*

Comment (p 4/21):

"1. From the Loveless at al., 2009, study the DS mention as reproductive effects a decreased pup weight as well as a decreased maternal weight. When the mother's weight is reduced it is most likely that the dam will birth lighter pups since she is not able to fully provide for them. Therefor the authors of this opinion consider it justified that the decreased pup weight is attributable to maternal toxicity instead of reproductive toxicity."

Comment (p 13/21):

"In table 16 of the CLH report (depicted in Figure 3 for convenience) [referring to Loveless et al., 2009] significant changes in body weights of the F1 generation pups are only observed at 500 mg/kg bw/d. In the parental generation P0 there are no data shown for absolute body weights but only for body weight gains which is also significantly reduced in the 500 mg/kg bw/d dose group for males and for females during the first week of gestation. This is not only supporting the opinion of these authors, but is showing clear evidence that the decreased pup weight is due to maternal toxicity."

Comment (p 14/21):

"Regarding the TG 414 study [referring to Loveless et al., 2009] it is stated by the DS on p. 36 and p. 42 that developmental toxicity occurred due to lower foetal body weights. Even though the body weight is slightly decreased in the 500 mg/kg bw/d dose group according to table 17 of the CLH report (depicted in Figure 4 for convenience) it is not statistically significant. Thus it should not be taken into account for developmental toxicity. In the same dose group maternal body weights are significantly reduced. This strongly supports the conclusion of the authors and provides even more evidence of maternal toxicity effects rather than developmental toxicity."

Response:

In rats, maternal toxicity could have contributed to the lower foetal growth. At 500 mg/kg bw/d significantly reduced body weight parameters in dams (bw: range of -5 % to –7 % on GD 19, 20 and 21) and a reduction in foetal body weight (-9 %) were observed. Effects on viability or other teratological effects were not observed (Loveless et al. 2009).

According to CLP, Annex I, 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 case-by-case basis that the developmental effects are secondary to maternal toxicity*." There exists no 'unequivocal demonstration' that reduced pup weight is secondary to maternal toxicity for this study.

Furthermore, in the key developmental and reproductive toxicity study in mice (Charles River Laboratories 2011a, 2011b, 2012) clear adverse effects on development (peri- and postnatal pup mortality at APFHx ≤ 175 mg/kg bw/d, proposed as developmental LOAEL) were observed in the absence of relevant maternal toxicity. These effects are considered adverse, treatment and dose-related and lead to the proposed classification in Category 1B.

These observations do not support the Commenter's view that "*it cannot be distinguished whether maternal toxicity or developmental toxicity is the reason for the decreased weights or postnatal survival*.".

<u>Comments/responses on mean pup body weights:</u>

Comment (p13/21):

"*Regarding the TG 415 (Loveless et al. 2009) study it is stated on p. 35 and p. 41 that treatment-related effects on mean pup body weights were observed at ≥ 100 mg/kg bw/d. This could not be confirmed by the authors of this opinion. In table 16 of the CLH report (depicted in Figure 3 for convenience) significant changes in body weights of the F1 generation pups are only observed at 500 mg/kg bw/d*."

Response:

Significant treatment-related effects on mean pup body weights were observed only at 500 mg/kg bw/d. Please note that in the CLH report ≥ 100 mg/kg bw/d instead of > 100 mg/kg bw/d was written. We apologise for the inconvenience.

<u>Comments on Table 20:</u>

Comment (p 5/15):

"2. *The DS mention an increased relative liver weight in pups according to the Iwai, Hoberman, 2014, study. According to the study itself the relative liver weight was not increased but actually decreased in the highest dose group of phase I. The absolute liver weights as well as terminal body weights were not significantly reduced in this dose group. Additonally, the DS only state this "fact" in the summary table 20 of the CLH report. Thus the authors of this opinion find it quite confounding to mention this false fact in the summary table which is what most people will look at if they want to gain a first insight on the topic*."

Response (decreased liver weight, Table 20):

The DS mistakenly stated in Table 20 that the relative liver weights of the F1-generation male mice were increased (instead of correctly decreased liver weights) in the highest dose group ($p \leq 0.05$, Iwai and Hoberman, 2014). We apologise for the inconvenience.

Comment (p 5/15):

"3. *The only discussed reason for a classification in reproductive toxicity category 1B in the CLH report is an increased peri- and postnatal pup mortality from Iwai, Hoberman, 2014. In contrast to this, only the decreased postnatal survival, i.e. increased postnatal pup mortality, is mentioned in table 20 as a main reproductive effect of PFHxA*."

Response (peri- and postnatal mortality, Table 20):

Table 20 should not serve as a summary table for the CLH report, but should help to gain a general overview of effect patterns across different perfluorocarboxylic acids (PFCAs). We apologise for any inconvenience or ambiguous information.

Comment (p 13):

"*From the citation in table 20, CLH report, it is unclear whether this is deducted from the one generation reproduction toxicity study (OECD TG 415) or the prenatal developmental toxicity study (OECD TG 414)*."

Response (Loveless, Table 20):

Maternal body weight as well as pup body weight are reduced in both studies by Loveless et al. (2009).

References (cited in Iwai and Hoberman (2014), see here Comment/response on test organism):

Lau C, Thibodeau JR, Hason RG, et al. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse: II. Postnatal evaluation. Toxicol Sci. 2013;74(2):382-392.

Das KP, Grey BE, Zehr RD, et al. Effects of perfluorobutyrate exposure during pregnancy in the mouse. Toxicol Sci. 2008; 105(1):173-181.

Lau C, Thibodeaux JR, Hason RG, et al. Exposure to perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol Sci. 2006;90(2):510-518.

Lau C, Anitole K, Hodes C, et al. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol Sci. 2008;99(2): 366-394.

RAC's response

RAC agrees with the DS that read-across between the acid and neutral salts is appropriate as the salts will be present as PFHx and the respective cations in aqueous solution (see ODD section RAC general comment).
RAC agrees with the DS that studies in mice can be used for classification and that the statistical analyses as reported by Iwai et al. (2019) is inappropriate (see ODD section

Adverse effects on development, paragraph "Discussion on statistical analysis of stillborn pup endpoint"). Please see the results of the dose-response analysis in the comment box above.

RAC further agrees with the DS on their other comments regarding reproductive toxicity studies.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2023	United Kingdom	Health and Safety Executive	National Authority	4

Comment received

Reproductive Toxicity
On page 23 of the CLH report, an increase in the number of dams with complete litter loss is reported in phase I of the Charles River 2011 reproductive and developmental toxicity study, and on page 24, a significant drop in the viability index is also reported, at the same doses. The toxicokinetics data indicate that PFHxA is detected in breast milk in humans. We would welcome a discussion of the perinatal pup mortality reported between PND 0-4 and whether they may be linked to effects on or via lactation.

Dossier Submitter's Response

Perinatal pup mortality within PND 0-4 can have multiple reasons.
Animal experiments with lactation exposure excluding prenatal exposure, such as cross-fostering experiments, could indicate effects on or via lactation. However, such cross-fostering experiments are not available for PFHxA or APFHx. No data are available that indicate adverse effects on milk production or milk quality. Effects on or via lactation can therefore neither be verified nor excluded. PFHxA was detected in human breast milk samples but often below the limit of quantification (see CLH report p 16).

RAC's response

RAC thanks the UK for pointing out that the substance may cause effects on or via lactation. RAC agrees with the DS that the information available is insufficient for definite conclusion on classification and labelling for lactation.

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2023	Germany	Gesamtverband Textil und Mode e.V.	Industry or trade association	5

Comment received

see attachment „Statement concerning the proposed classification and labeling of PFHxA and ist inorganic salts"

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Stellungnahme_PFHxA_t+m_TEGEWA.pdf

Dossier Submitter's Response

Thank you for your comment. The following comments and responses refer to your statement "Stellungnahme_PFHxA_t+m_TEGEWA.pdf".

<u>Comment/response on group approach:</u>

Comments (4/21, 10/21):

"*The authors of the opinion disagree with this **group approach**, based on scientific data. …. physico-chemical properties …. influence resorption and distribution through the body. Also the half live in the body is influenced since renal filtration is influenced by solubility. Therefor a group approach for PFHxA and its inorganic salts is not appropriate*."

Response:

The acid or salt is expected to dissociate in biological media. Hence, regardless whether the acid or the salt are administered, once absorbed into the blood the PFHx-anion will be formed. From toxicokinetic data available on PFHxA and APFHx it can be concluded that absorption via the oral pathway is rapid (< 1 hour) and complete.

RAC will conclude whether the group approach for PFHxA and its inorganic salts is appropriate.

Comments/responses on test organism and statistical analyses:

Comment (p 5,16-20/21):

"*Reasons to downgrade the studies by Charles River Laboratories are extensively discussed throughout the opinion and include not working according to an OECD guideline, the use of a sub-optimal **test organism** according to the selected testing guideline, not-robust **statistical analyses** leading to several re-evaluations leading to different result interpretations due to differing result significances*."

Response on test organism:

Regarding data from reproductive and developmental toxicity studies there are no indications that the mouse is unsuitable for testing APFHx. For APFHx, the mouse appears to be more sensitive than the rat.

Iwai and Hoberman (2014) provided an explanation for choosing the mouse as test species: "*The developmental toxicity of perfluoroalkyl acids (PFAAs) has been extensively studied (Lau et al. 2013, Das et al. 2008). The mouse has been used to evaluate the developmental toxicity of other PFAAs including perfluorooctanic acid (PFOA). Unlike the rat, sex differences in exposure levels in the mouse do not appear to exist (Lau et al. 2006). Although only female mice were evaluated in this study, the mouse appears to be an appropriate and sensitive model for evaluation of PFHxA. Developmental toxicity observed in rodents showed a lack of teratological findings (structural anomalies) from PFHxA. Effects on pup survival and postnatal growth have been consistently observed in both rats and mice in a dose-dependent pattern (Lau et al. 2008)*."

In general, findings in the most sensitive species would be used to determine the classification in which more than one acceptable test is available.

Response on statistical analyses:

With regard to the statistical analyses leading to several re-evaluations of the studies by Charles River Laboratories, the DS described and discussed the disagreement with the

approach by Iwai et al. (2019) in the CLH report on page 38 and thus refrained from considering the conclusion of Iwai et al. (2019).

Comment/response on Klimisch scores:

Comment (p 18/21):

"*Even though Klimisch 2 studies may be used in a the 'weight of evidence' approach during the evaluation of a toxicological endpoint, studies with a Klimisch score of 1 should always be preferred. Since the Klimisch 1 studies use the recommended test species and did not see adverse effects on peri- or postnatal mortality, the authors of strongly suspect that the DS are picking the study supporting their argument and purpose*."

Response:

The Klimisch scores assigned to the studies may serve as an indication of the 'quality of the data'. In a weight-of-evidence approach, data of several sources (studies in compliance with OECD test guidelines, studies with some deviations and - depending on the consistency of their observations with those from guideline studies - also studies which are not in compliance with test guidelines can be considered as supporting information). In general, findings in the most sensitive species would be used to determine the classification in which more than one acceptable test is available.

Comments/responses on maternal toxicity:

Comment (p 4/21) :
"*The major criticism regarding the conclusion is that maternal toxicity was recognized in several studies. Therefor it cannot be distinguished whether maternal toxicity or developmental toxicity is the reason for the decreased weights or postnatal survival."*

Comment (p 4/21):

"1. From the Loveless at al., 2009, study the DS mention as reproductive effects a decreased pup weight as well as a decreased maternal weight. When the mother's weight is reduced it is most likely that the dam will birth lighter pups since she is not able to fully provide for them. Therefor the authors of this opinion consider it justified that the decreased pup weight is attributable to maternal toxicity instead of reproductive toxicity."

Comment (p 13/21):

"In table 16 of the CLH report (depicted in Figure 3 for convenience) [referring to Loveless et al., 2009] significant changes in body weights of the F1 generation pups are only observed at 500 mg/kg bw/d. In the parental generation P0 there are no data shown for absolute body weights but only for body weight gains which is also significantly reduced in the 500 mg/kg bw/d dose group for males and for females during the first week of gestation. This is not only supporting the opinion of these authors, but is showing clear evidence that the decreased pup weight is due to maternal toxicity."

Comment (p 14/21):

"Regarding the TG 414 study [referring to Loveless et al., 2009] it is stated by the DS on p. 36 and p. 42 that developmental toxicity occurred due to lower foetal body weights. Even

though the body weight is slightly decreased in the 500 mg/kg bw/d dose group according to table 17 of the CLH report (depicted in Figure 4 for convenience) it is not statistically significant. Thus it should not be taken into account for developmental toxicity. In the same dose group maternal body weights are significantly reduced. This strongly supports the conclusion of the authors and provides even more evidence of maternal toxicity effects rather than developmental toxicity."

Response:

In rats, maternal toxicity could have contributed to the lower foetal growth. At 500 mg/kg bw/d significantly reduced body weight parameters in dams (bw: range of -5 % to –7 % on GD 19, 20 and 21) and a reduction in foetal body weight (-9 %) were observed. Effects on viability or other teratological effects were not observed (Loveless et al. 2009).

According to CLP, Annex I, 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 case-by-case basis that the developmental effects are secondary to maternal toxicity*." There exists no 'unequivocal demonstration' that reduced pup weight is secondary to maternal toxicity for this study.

Furthermore, in the key developmental and reproductive toxicity study in mice (Charles River Laboratories 2011a, 2011b, 2012) clear adverse effects on development (peri- and postnatal pup mortality at APFHx ≤ 175 mg/kg bw/d, proposed as developmental LOAEL) were observed in the absence of relevant maternal toxicity. These effects are considered adverse, treatment and dose-related and lead to the proposed classification in Category 1B.

These observations do not support the Commenter's view that "*it cannot be distinguished whether maternal toxicity or developmental toxicity is the reason for the decreased weights or postnatal survival*.".

<u>Comments/responses on mean pup body weights:</u>

Comment (p13/21):

"*Regarding the TG 415 (Loveless et al. 2009) study it is stated on p. 35 and p. 41 that treatment-related effects on mean pup body weights were observed at ≥ 100 mg/kg bw/d. This could not be confirmed by the authors of this opinion. In table 16 of the CLH report (depicted in Figure 3 for convenience) significant changes in body weights of the F1 generation pups are only observed at 500 mg/kg bw/d*."

Response:

Significant treatment-related effects on mean pup body weights were observed only at 500 mg/kg bw/d. Please note that in the CLH report ≥ 100 mg/kg bw/d instead of > 100 mg/kg bw/d was written. We apologise for the inconvenience.

<u>Comments on Table 20:</u>

Comment (p 5/15):

"2. *The DS mention an increased relative liver weight in pups according to the Iwai, Hoberman, 2014, study. According to the study itself the relative liver weight was not*

increased but actually decreased in the highest dose group of phase I. The absolute liver weights as well as terminal body weights were not significantly reduced in this dose group. Additonally, the DS only state this "fact" in the summary table 20 of the CLH report. Thus the authors of this opinion find it quite confounding to mention this false fact in the summary table which is what most people will look at if they want to gain a first insight on the topic."

Response (decreased liver weight, Table 20):

The DS mistakenly stated in Table 20 that the relative liver weights of the F1-generation male mice were increased (instead of correctly decreased liver weights) in the highest dose group ($p \leq 0.05$, Iwai and Hoberman, 2014). We apologise for the inconvenience.

Comment (p 5/15):

"3. *The only discussed reason for a classification in reproductive toxicity category 1B in the CLH report is an increased peri- and postnatal pup mortality from Iwai, Hoberman, 2014. In contrast to this, only the decreased postnatal survival, i.e. increased postnatal pup mortality, is mentioned in table 20 as a main reproductive effect of PFHxA*."

Response (peri- and postnatal mortality, Table 20):

Table 20 should not serve as a summary table for the CLH report, but should help to gain a general overview of effect patterns across different perfluorocarboxylic acids (PFCAs). We apologise for any inconvenience or ambiguous information.

Comment (p 13):

"*From the citation in table 20, CLH report, it is unclear whether this is deducted from the one generation reproduction toxicity study (OECD TG 415) or the prenatal developmental toxicity study (OECD TG 414)*."

Response (Loveless, Table 20):

Maternal body weight as well as pup body weight are reduced in both studies by Loveless et al. (2009).

References (cited in Iwai and Hoberman (2014), see here Comment/response on test organism):

Lau C, Thibodeau JR, Hason RG, et al. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse: II. Postnatal evaluation. Toxicol Sci. 2013;74(2):382-392.

Das KP, Grey BE, Zehr RD, et al. Effects of perfluorobutyrate exposure during pregnancy in the mouse. Toxicol Sci. 2008; 105(1):173-181.

Lau C, Thibodeaux JR, Hason RG, et al. Exposure to perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol Sci. 2006;90(2):510-518.

Lau C, Anitole K, Hodes C, et al. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol Sci. 2008;99(2): 366-394.

RAC's response
RAC agrees with the DS that read-across between the acid and neutral salts is appropriate as the salts will be present as PFHx and the respective cations in aqueous solution (see ODD section RAC general comment). RAC agrees with the DS that studies in mice can be used for classification and that the statistical analyses as reported by Iwai et al. (2019) is inappropriate (see ODD section Adverse effects on development, paragraph "Discussion on statistical analysis of stillborn pup endpoint"). Please see for results of the dose-response analysis the comment box above. RAC further agrees with the DS on other comments regarding reproductive toxicity studies.

Date	Country	Organisation	Type of Organisation	Comment number
29.06.2023	Sweden		MemberState	6

Comment received

Fertility
The Swedish CA supports the proposal of no classification for adverse effects on fertility based on the weight of evidence evaluation and lack of data to support a classification. However, we also note that all studies were performed using the rat as model species, and as for the developmental toxicity of PFHxA (as well as for PFOA and PFNA), the mouse has been shown to be a more sensitive species. It cannot be ruled out that adverse effects on fertility could have been observed if similar studies had been performed using the mouse.

Developmental toxicity
The Swedish CA supports the proposal of classification for adverse effects on development, Repr. 1B, H360D.
Clear adverse effect on development, in the absence of relevant maternal toxicity, were observed in the key developmental and perinatal/postnatal reproductive toxicity study in mice (Charles River Laboratories, 2011a; 2011b), in particular:
- Significantly increased number of stillborn pups at 500 mg/kg bw/day and increased number of stillborn pups at 350 mg/kg bw/day (phase I) as well as significantly increased number of stillborn pups at 175 mg/kg bw/day (phase II) as compared to the respective controls,
- Decreased postnatal viability at PND4: 72.7% and 87.9% vs. 99.1% at 500, 350 and 0 mg/kg bw/day, respectively, as well as at PND20: 61.6% and 80.8% vs. 96.8 at 500, 350 and 0 mg/kg bw/day.
These effects follow the same pattern of increased perinatal mortality and decreased postnatal viability as was observed in studies in mice for the PFCA structural homologues PFHpA, PFOA and PFNA, all leading to Repr. 1B classifications for adverse effects on development for these substances. Similarly, the rat was for these PFCAs shown to be less sensitive than the mouse, likely at least in part due to the more rapid excretion in rats (in particular female rats) as opposed to in mice (Borg and Håkansson, 2012). Thus, the mouse is a more suitable model species than the rat.

Adverse effects on or via lactation
The Swedish CA agrees with the Dossier Submitter that the information available does not support classification under this category.

Other comments:
The references to the Charles River Laboratories (2011a; 2011b) study reports are

sometimes in the text (e.g. page 37) intermixed and not correct.

References:
Borg and Håkansson, 2012. Environmental and Health Risk Assessment of Perfluoroalkylated and Polyfluoroalkylated Substances (PFASs) in Sweden. Table 29: Serum half-lives of PFASs congeners in different species. ISBN 978-91-620-6513-3. Available at: https://www.diva-portal.org/smash/get/diva2:770762/FULLTEXT01.pdf

Dossier Submitter's Response

Thank you for your comment.

We apologise for the inconvenience.

We re-checked all references in the document. References intermixed or not correct are limited to studies by Charles River Laboratories cited on page 37 and page 40.

Page 37

In the second subheading on page 37, the reference Charles River Laboratories (2011b) is incorrectly cited twice. The correct references are Charles River Laboratories (2011a), Charles River Laboratories (2012) and Charles River Laboratories (2011b).

After that, Charles River Laboratories (2011b) is again cited twice in the text. The correct references are Charles River Laboratories (2011a) and Charles River Laboratories (2011b).

At the bottom of the page, Charles River Laboratories (2011b) and Charles River Laboratories (2012) are cited; the correct references here are Charles River Laboratories (2011a), Charles River Laboratories (2011b) and Charles River Laboratories (2012).

Page 40

The heading of Table 19 refers to Charles River Laboratories (2011b) and Charles River Laboratories (2012). The correct references are Charles River Laboratories (2011a) and Charles River Laboratories (2012).

RAC's response

RAC thanks the Swedish CA for pointing out that references are incorrect and thanks the DS for clarification.

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2023	Germany	Verband TEGEWA e.V.	Industry or trade association	7

Comment received

Verband TEGEWA e.V. does not agree with the proposed classification of the three substances as Repr. 1B, H360D. The reason for our objection can be found in the attached "Statement concerning the proposed classification and labelling of PFHxA and its inorganic salts" by EuDiCo GmbH, Leverkusen. TEGEWA fully supports the content and the conclusion of this statement and therefore questions the CLH proposal.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Stellungnahme_PFHxA_t+m_TEGEWA.pdf

Dossier Submitter's Response

Thank you for your comment. The following comments and responses refer to your statement "Stellungnahme_PFHxA_t+m_TEGEWA.pdf".

Comment/response on group approach:

Comments (4/21, 10/21):

"*The authors of the opinion disagree with this **group approach**, based on scientific data. …. physico-chemical properties …. influence resorption and distribution through the body. Also the half live in the body is influenced since renal filtration is influenced by solubility. Therefor a group approach for PFHxA and its inorganic salts is not appropriate*."

Response:

The acid or salt is expected to dissociate in biological media. Hence, regardless whether the acid or the salt are administered, once absorbed into the blood the PFHx-anion will be formed. From toxicokinetic data available on PFHxA and APFHx it can be concluded that absorption via the oral pathway is rapid (< 1 hour) and complete.

RAC will conclude whether the group approach for PFHxA and its inorganic salts is appropriate.

Comments/responses on test organism and statistical analyses:

Comment (p 5,16-20/21):

"*Reasons to downgrade the studies by Charles River Laboratories are extensively discussed throughout the opinion and include not working according to an OECD guideline, the use of a sub-optimal **test organism** according to the selected testing guideline, not-robust **statistical analyses** leading to several re-evaluations leading to different result interpretations due to differing result significances*."

Response on test organism:

Regarding data from reproductive and developmental toxicity studies there are no indications that the mouse is unsuitable for testing APFHx. For APFHx, the mouse appears to be more sensitive than the rat.

Iwai and Hoberman (2014) provided an explanation for choosing the mouse as test species: "*The developmental toxicity of perfluoroalkyl acids (PFAAs) has been extensively studied (Lau et al. 2013, Das et al. 2008). The mouse has been used to evaluate the developmental toxicity of other PFAAs including perfluorooctanic acid (PFOA). Unlike the rat, sex differences in exposure levels in the mouse do not appear to exist (Lau et al. 2006). Although only female mice were evaluated in this study, the mouse appears to be an appropriate and sensitive model for evaluation of PFHxA. Developmental toxicity observed in rodents showed a lack of teratological findings (structural anomalies) from PFHxA. Effects on pup survival and postnatal growth have been consistently observed in both rats and mice in a dose-dependent pattern (Lau et al. 2008)*."

In general, findings in the most sensitive species would be used to determine the classification in which more than one acceptable test is available.

Response on statistical analyses:

With regard to the statistical analyses leading to several re-evaluations of the studies by Charles River Laboratories, the DS described and discussed the disagreement with the approach by Iwai et al. (2019) in the CLH report on page 38 and thus refrained from considering the conclusion of Iwai et al. (2019).

Comment/response on Klimisch scores:

Comment (p 18/21):

"*Even though Klimisch 2 studies may be used in a the 'weight of evidence' approach during the evaluation of a toxicological endpoint, studies with a Klimisch score of 1 should always be preferred. Since the Klimisch 1 studies use the recommended test species and did not see adverse effects on peri- or postnatal mortality, the authors of strongly suspect that the DS are picking the study supporting their argument and purpose*."

Response:

The Klimisch scores assigned to the studies may serve as an indication of the 'quality of the data'. In a weight-of-evidence approach, data of several sources (studies in compliance with OECD test guidelines, studies with some deviations and - depending on the consistency of their observations with those from guideline studies - also studies which are not in compliance with test guidelines can be considered as supporting information). In general, findings in the most sensitive species would be used to determine the classification in which more than one acceptable test is available.

Comments/responses on maternal toxicity:

Comment (p 4/21) :
"*The major criticism regarding the conclusion is that maternal toxicity was recognized in several studies. Therefor it cannot be distinguished whether maternal toxicity or developmental toxicity is the reason for the decreased weights or postnatal survival."*

Comment (p 4/21):

"1. From the Loveless at al., 2009, study the DS mention as reproductive effects a decreased pup weight as well as a decreased maternal weight. When the mother's weight is reduced it is most likely that the dam will birth lighter pups since she is not able to fully provide for them. Therefor the authors of this opinion consider it justified that the decreased pup weight is attributable to maternal toxicity instead of reproductive toxicity."

Comment (p 13/21):

"In table 16 of the CLH report (depicted in Figure 3 for convenience) [referring to Loveless et al., 2009] significant changes in body weights of the F1 generation pups are only observed at 500 mg/kg bw/d. In the parental generation P0 there are no data shown for absolute body weights but only for body weight gains which is also significantly reduced in the 500 mg/kg bw/d dose group for males and for females during the first week of

gestation. This is not only supporting the opinion of these authors, but is showing clear evidence that the decreased pup weight is due to maternal toxicity."

Comment (p 14/21):

"Regarding the TG 414 study [referring to Loveless et al., 2009] it is stated by the DS on p. 36 and p. 42 that developmental toxicity occurred due to lower foetal body weights. Even though the body weight is slightly decreased in the 500 mg/kg bw/d dose group according to table 17 of the CLH report (depicted in Figure 4 for convenience) it is not statistically significant. Thus it should not be taken into account for developmental toxicity. In the same dose group maternal body weights are significantly reduced. This strongly supports the conclusion of the authors and provides even more evidence of maternal toxicity effects rather than developmental toxicity."

Response:

In rats, maternal toxicity could have contributed to the lower foetal growth. At 500 mg/kg bw/d significantly reduced body weight parameters in dams (bw: range of -5 % to –7 % on GD 19, 20 and 21) and a reduction in foetal body weight (-9 %) were observed. Effects on viability or other teratological effects were not observed (Loveless et al. 2009).

According to CLP, Annex I, 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 case-by-case basis that the developmental effects are secondary to maternal toxicity*." There exists no 'unequivocal demonstration' that reduced pup weight is secondary to maternal toxicity for this study.

Furthermore, in the key developmental and reproductive toxicity study in mice (Charles River Laboratories 2011a, 2011b, 2012) clear adverse effects on development (peri- and postnatal pup mortality at APFHx ≤ 175 mg/kg bw/d, proposed as developmental LOAEL) were observed in the absence of relevant maternal toxicity. These effects are considered adverse, treatment and dose-related and lead to the proposed classification in Category 1B.

These observations do not support the Commenter's view that "*it cannot be distinguished whether maternal toxicity or developmental toxicity is the reason for the decreased weights or postnatal survival*.".

<u>Comments/responses on mean pup body weights:</u>

Comment (p13/21):

"*Regarding the TG 415 (Loveless et al. 2009) study it is stated on p. 35 and p. 41 that treatment-related effects on mean pup body weights were observed at ≥ 100 mg/kg bw/d. This could not be confirmed by the authors of this opinion. In table 16 of the CLH report (depicted in Figure 3 for convenience) significant changes in body weights of the F1 generation pups are only observed at 500 mg/kg bw/d*."

Response:

Significant treatment-related effects on mean pup body weights were observed only at 500 mg/kg bw/d. Please note that in the CLH report ≥ 100 mg/kg bw/d instead of > 100 mg/kg bw/d was written. We apologise for the inconvenience.

Comments on Table 20:

Comment (p 5/15):

"2. *The DS mention an increased relative liver weight in pups according to the Iwai, Hoberman, 2014, study. According to the study itself the relative liver weight was not increased but actually decreased in the highest dose group of phase I. The absolute liver weights as well as terminal body weights were not significantly reduced in this dose group. Additonally, the DS only state this "fact" in the summary table 20 of the CLH report. Thus the authors of this opinion find it quite confounding to mention this false fact in the summary table which is what most people will look at if they want to gain a first insight on the topic*."

Response (decreased liver weight, Table 20):

The DS mistakenly stated in Table 20 that the relative liver weights of the F1-generation male mice were increased (instead of correctly decreased liver weights) in the highest dose group ($p \leq 0.05$, Iwai and Hoberman, 2014). We apologise for the inconvenience.

Comment (p 5/15):

"3. *The only discussed reason for a classification in reproductive toxicity category 1B in the CLH report is an increased peri- and postnatal pup mortality from Iwai, Hoberman, 2014. In contrast to this, only the decreased postnatal survival, i.e. increased postnatal pup mortality, is mentioned in table 20 as a main reproductive effect of PFHxA*."

Response (peri- and postnatal mortality, Table 20):

Table 20 should not serve as a summary table for the CLH report, but should help to gain a general overview of effect patterns across different perfluorocarboxylic acids (PFCAs). We apologise for any inconvenience or ambiguous information.

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"*From the citation in table 20, CLH report, it is unclear whether this is deducted from the one generation reproduction toxicity study (OECD TG 415) or the prenatal developmental toxicity study (OECD TG 414)*."

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References (cited in Iwai and Hoberman (2014), see here Comment/response on test organism):

Lau C, Thibodeau JR, Hason RG, et al. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse: II. Postnatal evaluation. Toxicol Sci. 2013;74(2):382-392.

Das KP, Grey BE, Zehr RD, et al. Effects of perfluorobutyrate exposure during pregnancy in the mouse. Toxicol Sci. 2008; 105(1):173-181.

Lau C, Thibodeaux JR, Hason RG, et al. Exposure to perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol Sci. 2006;90(2):510-518.

Lau C, Anitole K, Hodes C, et al. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol Sci. 2008;99(2): 366-394.

RAC's response

RAC agrees with the DS that read-across between the acid and neutral salts is appropriate as the salts will be present as PFHx and the respective cations in aqueous solution (see ODD section RAC general comment).

RAC agrees with the DS that studies in mice can be used for classification and that the statistical analyses as reported by Iwai et al. (2019) is inappropriate (see ODD section Adverse effects on development, paragraph "Discussion on statistical analysis of stillborn pup endpoint"). Please see for results of the dose-response analysis the comment box above. RAC further agrees with the DS on other comments regarding reproductive toxicity studies.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2023	Germany	Gesamtverband Textil und Mode e.V.	Industry or trade association	8

Comment received

see attachment „Statement concerning the proposed classification and labeling of PFHxA and ist inorganic salts"

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Stellungnahme_PFHxA_t+m_TEGEWA.pdf

Dossier Submitter's Response

Thank you for your comment. The following comments and responses refer to your statement "Stellungnahme_PFHxA_t+m_TEGEWA.pdf".

<u>Comment/response on group approach:</u>

Comments (4/21, 10/21):

"*The authors of the opinion disagree with this **group approach**, based on scientific data. …. physico-chemical properties …. influence resorption and distribution through the body. Also the half live in the body is influenced since renal filtration is influenced by solubility. Therefor a group approach for PFHxA and its inorganic salts is not appropriate*."

Response:

The acid or salt is expected to dissociate in biological media. Hence, regardless whether the acid or the salt are administered, once absorbed into the blood the PFHx-anion will be

formed. From toxicokinetic data available on PFHxA and APFHx it can be concluded that absorption via the oral pathway is rapid (< 1 hour) and complete.

RAC will conclude whether the group approach for PFHxA and its inorganic salts is appropriate.

Comments/responses on test organism and statistical analyses:

Comment (p 5,16-20/21):

"*Reasons to downgrade the studies by Charles River Laboratories are extensively discussed throughout the opinion and include not working according to an OECD guideline, the use of a sub-optimal* ***test organism*** *according to the selected testing guideline, not-robust* ***statistical analyses*** *leading to several re-evaluations leading to different result interpretations due to differing result significances*."

Response on test organism:

Regarding data from reproductive and developmental toxicity studies there are no indications that the mouse is unsuitable for testing APFHx. For APFHx, the mouse appears to be more sensitive than the rat.

Iwai and Hoberman (2014) provided an explanation for choosing the mouse as test species: "*The developmental toxicity of perfluoroalkyl acids (PFAAs) has been extensively studied (Lau et al. 2013, Das et al. 2008). The mouse has been used to evaluate the developmental toxicity of other PFAAs including perfluorooctanic acid (PFOA). Unlike the rat, sex differences in exposure levels in the mouse do not appear to exist (Lau et al. 2006). Although only female mice were evaluated in this study, the mouse appears to be an appropriate and sensitive model for evaluation of PFHxA. Developmental toxicity observed in rodents showed a lack of teratological findings (structural anomalies) from PFHxA. Effects on pup survival and postnatal growth have been consistently observed in both rats and mice in a dose-dependent pattern (Lau et al. 2008)*."

In general, findings in the most sensitive species would be used to determine the classification in which more than one acceptable test is available.

Response on statistical analyses:

With regard to the statistical analyses leading to several re-evaluations of the studies by Charles River Laboratories, the DS described and discussed the disagreement with the approach by Iwai et al. (2019) in the CLH report on page 38 and thus refrained from considering the conclusion of Iwai et al. (2019).

Comment/response on Klimisch scores:

Comment (p 18/21):

"*Even though Klimisch 2 studies may be used in a the 'weight of evidence' approach during the evaluation of a toxicological endpoint, studies with a Klimisch score of 1 should always be preferred. Since the Klimisch 1 studies use the recommended test species and did not see adverse effects on peri- or postnatal mortality, the authors of strongly suspect that the DS are picking the study supporting their argument and purpose*."

Response:

The Klimisch scores assigned to the studies may serve as an indication of the 'quality of the data'. In a weight-of-evidence approach, data of several sources (studies in compliance with OECD test guidelines, studies with some deviations and - depending on the consistency of their observations with those from guideline studies - also studies which are not in compliance with test guidelines can be considered as supporting information). In general, findings in the most sensitive species would be used to determine the classification in which more than one acceptable test is available.

Comments/responses on maternal toxicity:

Comment (p 4/21) :
"*The major criticism regarding the conclusion is that maternal toxicity was recognized in several studies. Therefor it cannot be distinguished whether maternal toxicity or developmental toxicity is the reason for the decreased weights or postnatal survival."*

Comment (p 4/21):

"1. From the Loveless at al., 2009, study the DS mention as reproductive effects a decreased pup weight as well as a decreased maternal weight. When the mother's weight is reduced it is most likely that the dam will birth lighter pups since she is not able to fully provide for them. Therefor the authors of this opinion consider it justified that the decreased pup weight is attributable to maternal toxicity instead of reproductive toxicity."

Comment (p 13/21):

"In table 16 of the CLH report (depicted in Figure 3 for convenience) [referring to Loveless et al., 2009] significant changes in body weights of the F1 generation pups are only observed at 500 mg/kg bw/d. In the parental generation P0 there are no data shown for absolute body weights but only for body weight gains which is also significantly reduced in the 500 mg/kg bw/d dose group for males and for females during the first week of gestation. This is not only supporting the opinion of these authors, but is showing clear evidence that the decreased pup weight is due to maternal toxicity."

Comment (p 14/21):

"Regarding the TG 414 study [referring to Loveless et al., 2009] it is stated by the DS on p. 36 and p. 42 that developmental toxicity occurred due to lower foetal body weights. Even though the body weight is slightly decreased in the 500 mg/kg bw/d dose group according to table 17 of the CLH report (depicted in Figure 4 for convenience) it is not statistically significant. Thus it should not be taken into account for developmental toxicity. In the same dose group maternal body weights are significantly reduced. This strongly supports the conclusion of the authors and provides even more evidence of maternal toxicity effects rather than developmental toxicity."

Response:

In rats, maternal toxicity could have contributed to the lower foetal growth. At 500 mg/kg bw/d significantly reduced body weight parameters in dams (bw: range of -5 % to –7 %

on GD 19, 20 and 21) and a reduction in foetal body weight (-9 %) were observed. Effects on viability or other teratological effects were not observed (Loveless et al. 2009).

According to CLP, Annex I, 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 case-by-case basis that the developmental effects are secondary to maternal toxicity*." There exists no 'unequivocal demonstration' that reduced pup weight is secondary to maternal toxicity for this study.

Furthermore, in the key developmental and reproductive toxicity study in mice (Charles River Laboratories 2011a, 2011b, 2012) clear adverse effects on development (peri- and postnatal pup mortality at APFHx ≤ 175 mg/kg bw/d, proposed as developmental LOAEL) were observed in the absence of relevant maternal toxicity. These effects are considered adverse, treatment and dose-related and lead to the proposed classification in Category 1B.

These observations do not support the Commenter's view that "*it cannot be distinguished whether maternal toxicity or developmental toxicity is the reason for the decreased weights or postnatal survival*.".

<u>Comments/responses on mean pup body weights:</u>

Comment (p13/21):

"*Regarding the TG 415 (Loveless et al. 2009) study it is stated on p. 35 and p. 41 that treatment-related effects on mean pup body weights were observed at ≥ 100 mg/kg bw/d. This could not be confirmed by the authors of this opinion. In table 16 of the CLH report (depicted in Figure 3 for convenience) significant changes in body weights of the F1 generation pups are only observed at 500 mg/kg bw/d*."

Response:

Significant treatment-related effects on mean pup body weights were observed only at 500 mg/kg bw/d. Please note that in the CLH report ≥ 100 mg/kg bw/d instead of > 100 mg/kg bw/d was written. We apologise for the inconvenience.

<u>Comments on Table 20:</u>

Comment (p 5/15):

"2. *The DS mention an increased relative liver weight in pups according to the Iwai, Hoberman, 2014, study. According to the study itself the relative liver weight was not increased but actually decreased in the highest dose group of phase I. The absolute liver weights as well as terminal body weights were not significantly reduced in this dose group. Additonally, the DS only state this "fact" in the summary table 20 of the CLH report. Thus the authors of this opinion find it quite confounding to mention this false fact in the summary table which is what most people will look at if they want to gain a first insight on the topic*."

Response (decreased liver weight, Table 20):

The DS mistakenly stated in Table 20 that the relative liver weights of the F1-generation male mice were increased (instead of correctly decreased liver weights) in the highest dose group ($p \leq 0.05$, Iwai and Hoberman, 2014). We apologise for the inconvenience.

Comment (p 5/15):

"3. *The only discussed reason for a classification in reproductive toxicity category 1B in the CLH report is an increased peri- and postnatal pup mortality from Iwai, Hoberman, 2014. In contrast to this, only the decreased postnatal survival, i.e. increased postnatal pup mortality, is mentioned in table 20 as a main reproductive effect of PFHxA*."

Response (peri- and postnatal mortality, Table 20):

Table 20 should not serve as a summary table for the CLH report, but should help to gain a general overview of effect patterns across different perfluorocarboxylic acids (PFCAs). We apologise for any inconvenience or ambiguous information.

Comment (p 13):

"*From the citation in table 20, CLH report, it is unclear whether this is deducted from the one generation reproduction toxicity study (OECD TG 415) or the prenatal developmental toxicity study (OECD TG 414)*."

Response (Loveless, Table 20):

Maternal body weight as well as pup body weight are reduced in both studies by Loveless et al. (2009).

References (cited in Iwai and Hoberman (2014), see here Comment/response on test organism):

Lau C, Thibodeau JR, Hason RG, et al. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse: II. Postnatal evaluation. Toxicol Sci. 2013;74(2):382-392.

Das KP, Grey BE, Zehr RD, et al. Effects of perfluorobutyrate exposure during pregnancy in the mouse. Toxicol Sci. 2008; 105(1):173-181.

Lau C, Thibodeaux JR, Hason RG, et al. Exposure to perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol Sci. 2006;90(2):510-518.

Lau C, Anitole K, Hodes C, et al. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol Sci. 2008;99(2): 366-394.

RAC's response

RAC agrees with the DS that read-across between the acid and neutral salts is appropriate as the salts will be present as PFHx and the respective cations in aqueous solution (see ODD section RAC general comment).

RAC agrees with the DS that studies in mice can be used for classification and that the statistical analyses as reported by Iwai et al. (2019) is inappropriate (see ODD section Adverse effects on development, paragraph "Discussion on statistical analysis of stillborn

pup endpoint"). Please see for results of the dose-response analysis the comment box above.

Date	Country	Organisation	Type of Organisation	Comment number
29.06.2023	Sweden		MemberState	9

Comment received

The Swedish CA supports the Dossier Submitter's conclusion that the effects by PFHxA observed within the dose-ranges for STOT RE-classification does not show sufficient adversity to warrant classification. However, it is also noted that all studies were performed using the rat, which is less sensitive than the mouse, and it cannot be ruled out that adverse effects could have been observed within the dose-ranges for STOT RE-classification if similar studies had been performed in the mouse, as was the case for the closest PFCA homologue PFHpA (STOT RE-1 (liver)). Therefore, in absence of a relevant study on PFHxA the Dossier Submitter could consider to read-across data from PFHpA for classification in STOT RE 1 or 2 for liver effects.

Dossier Submitter's Response

Thank you for your comment.

We consider the repeated dose data from studies with PFHxA as giving sufficient information to decide on the need for classification. In general, data on PFHxA are considered as more relevant than a concern from PFHpA based on read across.

Nevertheless we noted that a 90-day study in mice is not available. At least the mouse developmental study (12-day treatment of mothers with PFHxA up to 500 mg/kg bw/d) by Iwai and Hoberman (2014) did not measure changes of maternal liver weight. Thus this study does not indicate a weight response as a biomarker for liver toxicity. Overall, data do not seem to be sufficient to prioritise the concern from the read across.

RAC's response

RAC agrees with the DS that the information available on PFHxA itself is sufficient to decide that no classification for STOT RE on liver effects is warranted.

PUBLIC ATTACHMENTS
1. Stellungnahme_PFHxA_t+m_TEGEWA.pdf [Please refer to comment No. 3, 7]
2. Stellungnahme_PFHxA_t+m_TEGEWA.pdf [Please refer to comment No. 1, 5, 8]