Committee for Risk Assessment

RAC

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

10 December 2020

CLH-O-0000006908-60-01/F

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

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

Chemical name: **Perfluoroheptanoic acid; tridecafluoroheptanoic acid**

EC Number: **206-798-9**

CAS Number: **375-85-9**

The proposal was submitted by **Belgium** and received by RAC on **24 October 2019.**

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

PROCESS FOR ADOPTION OF THE OPINION

Belgium has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **25 November 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **24 January 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Annemarie Losert**

Co-Rapporteur, appointed by RAC: **Daniel Borg**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **10 December 2020** by **consensus**.

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

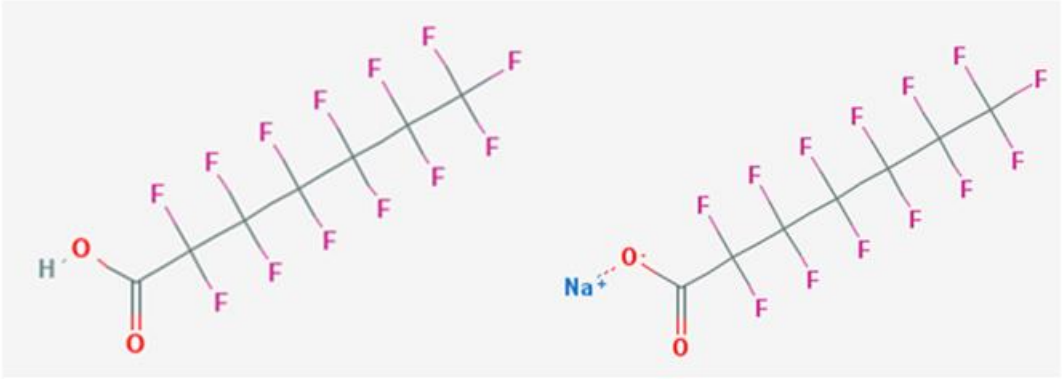
	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Perfluoroheptanoic acid; tridecafluoroheptanoic acid	206-798-9	375-85-9	Repr. 1B STOT RE 1	H360D H372 (liver)	GHS08 Dgr	H360D H372 (liver)			
RAC opinion	TBD	Perfluoroheptanoic acid; tridecafluoroheptanoic acid	206-798-9	375-85-9	Repr. 1B STOT RE 1	H360D H372 (liver)	GHS08 Dgr	H360D H372 (liver)			
Resulting Annex VI entry if agreed by COM	TBD	Perfluoroheptanoic acid; tridecafluoroheptanoic acid	206-798-9	375-85-9	Repr. 1B STOT RE 1	H360D H372 (liver)	GHS08 Dgr	H360D H372 (liver)			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Long-chain perfluoroalkane carboxylic acids such as perfluoroheptanoic acid and their salts are surface-active chemicals which greatly reduce the surface tension of water, aqueous solutions, and organic liquids. They are used as wetting, dispersing, emulsifying, and foaming agents.

Perfluoroheptanoic acid (PFHpA) is a potential degradation product of substances that contain a perfluorinated linear chain of six carbon atoms, connected to a terminal perfluorinated carbon atom on one end and to a non-fluorinated carbon atom on the other end. During degradation, defluorination of one carbon atom can occur and thereby PFHpA is formed. PFHpA is a strong acid, therefore, under most environmental and physiological conditions, it is present in its anionic form.



Two hazard classes were evaluated in the CLH report, repeated dose toxicity and reproductive toxicity, and the Dossier Submitter (DS) proposed read-across from the anionic form to the acidic form, since for practical and animal welfare reasons, the only study available (Anonymous, 2017) was conducted with the anionic form. As PFHpA is present in its anionic form under physiological conditions, RAC supports the proposed read-across from the anionic form to the acidic form for the evaluation of these hazard classes.

The study by Anonymous (2017) is a combined 90-day repeated dose toxicity study with reproductive/developmental toxicity screening (OECD TG 408 & 422) in CD1 mice, which was conducted as part of the REACH substance evaluation process carried out by the Belgian CA for the substances with trade names FS-65 and FS-61, of which PFHpA is a potential degradation product. The preferred species is commonly the rat. However, due to large sex differences in elimination kinetics in the rat for the closely related substance PFOA and PFNA (faster elimination in females than in males), during the substance evaluation process it was concluded that the mouse would be the preferred animal model for testing of PFHpA (ECHA, 2015a). As the conducting laboratory had appropriate historical control data (HCD), which did not indicate any high background incidence of findings that could reduce the value of the study, the mouse is considered by RAC an appropriate species for the conducted study.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

In Anonymous (2017), groups of male and female CD1 mice were treated with 0, 0.5, 10 or 50 mg/kg bw/day PFHpA via gavage in a combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening (OECD TG 408 & 422).

At 10 and 50 mg/kg bw/day significant increases in relative and absolute liver weights were seen in F0 males, F0 females and in the F1 generation. Liver effects were also demonstrated by a significant impact on blood biochemical parameters in top dose males and to a lesser extent in top dose non-mated females, but not in mated females on lactation day 21. Also, significant microscopic liver changes were seen in F0 males and females at all doses tested, showing a dose-related increase in incidence and severity. At the low dose, the major finding was centrilobular hypertrophy, but at the mid and high doses also necrosis was reported, showing dose-related increases in severity and incidence. Similar observations at the same doses were made in the F1 generation examined after exposure via milk and through gavage from PND 22 to PND 42.

No severe general toxicity was observed in the F0 generation. In the F1 animals, viability decreased in the top dose group and body weights were dose-dependently decreased at the mid and top doses. The DS considered the effects seen at 10 mg/kg bw/day to be sufficiently severe (and not secondary to general toxicity) to support a classification as STOT RE (liver). After correction for exposure duration (109 days) an effective dose of 8.3 mg/kg bw/day was calculated by the DS (however, this value was corrected by one MS during the consultation, see below). As this value is below the upper boundary of the guidance value for classification in category 1 (10 mg/kg bw/day, 90-day study), the DS proposed to classify PFHpA as STOT RE 1, with the liver as the target organ.

Comments received during consultation

Four MSCAs supported classification of PFHpA for STOT RE (liver). Three of them were more in favour of category 2, as they considered the effects not severe enough at doses relevant for classification in category 1. One MSCA supporting the proposed classification as STOT RE 1 (liver) and observed that read-across from data on APFO/PFOA would further support this classification. Another MSCA correctly pointed out that Haber's law had not been correctly applied in the CLH report and that the actual effective dose during the 109 days dosing would be 12.1 mg/kg bw/day (instead of 8.3 mg/kg bw/day).

Assessment and comparison with the classification criteria

The DS presented one combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening according to OECD TG 408 & 422 (Anonymous, 2017) in CD1 mice. The test substance (PFHpA, purity > 99.3%) was applied via gavage (vehicle: deionised water) at 0, 0.5, 10 and 50 mg/kg bw/day. The F0 generation consisted of 20 mice/sex/dose with 5 additional female mice in the control and high dose group (for the purpose of gender comparison). Adult animals (~ 6 weeks of age at study initiation) were exposed 90 days prior mating. Males were further exposed during mating, resulting in exposure durations between 109 and 113 days, while females were exposed until day 20 of lactation (i.e. 130 – 142 days). The 5 females in the control and top dose groups introduced for gender comparison, were exposed for 109 days.

In the F0 generation, no clinical signs were observed and there were no effects on survival, body weight/body weight gain, food consumption, reproductive parameters (except a slight increase in pre-coital interval, see the section on reproductive toxicity for details), behaviour in the functional observation battery (FOB) or motor activity. There were also no effects on organ weights, except for statistically significantly increased liver weights in mid and top dose groups (Tables below).

***Table**: Body- and liver weights, F0 males (extracted from the CLH report)*

Dose (mg/kg bw/day)	**0**	**0.5**	**10**	**50**
Final body wt. (g)	36.9	36.2	38.2	37.2
Abs. liver wt. (g)	1.83	1.83	2.18 **	3.15 **
% relative to ctrl.	-	+ 0.3	+ 19	+ 72
Rel. liver wt.	4.95	5.06	5.69 **	8.46 **
% relative to ctrl.	-	+ 2.3	+ 15	+ 71

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

***Table**: Body- and liver weights, F0 females (extracted from the CLH report)*

	Non-mated females				**Females lactation d21**			
Dose (mg/kg bw/day)	**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
Final body wt. (g)	27.8	NA	NA	29.1	35.6	36.0	37.5	36.7
Abs. liver wt. (g)	1.40	NA	NA	1.89 **	2.07	2.20	2.49 **	3.09 **
% relative to ctrl.	-	NA	NA	+ 35	-	+ 6	+ 20	+ 49
Rel. liver wt.	5.04	NA	NA	6.49 **	5.80	6.11	6.64 **	8.42 **
% relative to ctrl.	-	NA	NA	+ 29	-	+ 5	+ 15	+ 45

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

Liver-related blood biochemistry markers were clearly affected in the top dose males (clear increases in aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and triglycerides), but less in non-mated top dose females (increase in ALP & triglycerides) and no effect was seen in top dose females on lactation day 21 (Table below). Liver-related blood biochemistry markers were also affected after 75 days in males and females (Table 9 in the CLH proposal, data not shown here).

***Table**: Clinical biochemistry findings in F0 males and females at study termination (extracted from the CLH report)*

Dose (mg/kg bw/day)	**0**	**0.5**	**10**	**50**
Males				
ALP (U/L)	77	74	74	227 **
ALT (U/L)	51	86	41	165 *
AST (U/L)	88	143	108	167
TG (mg/dL)	82	118	101	153 *
Non-mated females				
ALP (U/L)	52	NA	NA	152 *
ALT (U/L)	36	NA	NA	41
AST (U/L)	102	NA	NA	93
TG (mg/dL)	64	NA	NA	161 **
Females lactation d21				
ALP (U/L)	129	95	87	99
ALT (U/L)	71	49	42 *	56
AST (U/L)	142	124	101	147
TG (mg/dL)	88	120	89	137

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

Significant microscopic liver changes were seen in males and females at all doses tested, showing a dose related-increase in incidence and severity (Tables below). When screening Anonymous

(2017) in detail, RAC identified 3 additional cases of necrosis (minimal) in low dose females exposed up until lactation day 21, which were not reported in the CLH report.

***Table**: Histopathological changes seen in F0 males (extracted from the CLH report)*

Dose (mg/kg bw/day)		**0**	**0.5**	**10**	**50**
Total number animals examined		20	19	19	20
Number of animals without findings		16	2	2	0
Centrilobular hepatocellular hypertrophy	**Minimal**	0	8	2	0
	Mild	0	7	2	9
	Moderate	0	2	13	11
Infiltrate, mononuclear cell	**Minimal**	4	7	2	2
Hepatocellular necrosis	**Minimal**	0	1	2	19
	Mild	0	0	0	1

*: p < 0.05, **: p < 0.01

***Table**: Histopathological changes seen in F0 females (extracted from the CLH report)*

		Non-mated females				**Females lactation d21**			
Dose (mg/kg bw/day)		**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
Total number animals examined		5	0	0	4	17	20	19	19
Number of animals without findings		1	NA	NA	0	16	2	0	0
Centrilobular hepatocellular hypertrophy	**Minimal**	0	NA	NA	0	0	8	3	1
	Mild	0	NA	NA	4	0	8	8	8
	Moderate	0	NA	NA	0	0	1	9	10
Infiltrate, mononuclear cell	**Minimal**	4	NA	NA	2	1	6	6	5
Hepatocellular necrosis	**Minimal**	0	NA	NA	1	0	3	5	7
	Mild	0	NA	NA	0	0	1	0	2

*: p < 0.05, **: p < 0.01

F1 pups were randomly selected for the F1 generation (1/sex/litter/group) resulting in 16-20 pups/sex/group. The remaining pups were necropsied on PND 21. While there were no effects on the number of litters, mean litter size or anogenital distance and there was no evidence for nipple retention in F1 males on PND 13, post-natal survival and pup body weights were reduced (see section on reproductive/developmental toxicity).

Also in the F1-generation, liver weights were significantly increased in males of the mid and top dose groups and in females of the top dose group (Table below). Other organ weight changes included a statistically significant decrease in absolute and relative adrenal gland weight in top dose females, as well as in the low dose group for absolute weight. In addition, absolute brain weight was statistically significantly reduced in top dose females. No histopathological correlates were described for these organs.

***Table**: Body- and liver weights of male and female F1 pups at PND 43 (extracted from the CLH report)*

	Males				**Females**			
Dose (mg/kg bw/day)	**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
Final body wt. (g)	29.0	29.6	29.4	27.7	24.7	23.7	23.2*	22.1 **
Abs. liver wt. (g)	1.80	1.86	2.06 *	3.14 **	1.58	1.51	1.55	1.86 *
% compared to ctrl.	-	+ 3	+ 15	+ 74		- 4	- 1.7	+ 18
Rel. liver wt.	6.21	6.29	7.01 **	11.31 **	6.39	6.39	6.71	8.42 **
% compared to ctrl.	-	+ 1,2	+ 12.9	+ 82	-	-	+ 5	+ 32

*: p < 0.05, **: p < 0.01

The macroscopic liver findings were confirmed by microscopic examination. Centrilobular hypertrophy was seen in all dosed animals, whereas necrosis was seen in mid and top dose males and females. For these observations, a dose related increase was evident (Table below).

***Table**: Histopathological changes at PND43 in the liver of male and female F1 pups (extracted from the CLH report)*

		Males				Females			
Dose level (in mg/kg bw/day)		**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
Total number examined		17	20	18	14	17	20	18	16
Number examined without findings		10	3	1	0	10	8	6	0
Centrilobular hypertrophy of hepatocytes	**Minimal**	0	8	2	1	0	6	8	5
	Mild	0	8	10	5	0	1	3	9
	Moderate	0	1	5	8	0	0	0	2
Infiltrate, mononuclear cell	**Minimal**	7	5	1	3	7	8	5	5
	Mild	0	0	0	0	0	0	1	0
Hepatocellular necrosis	**Minimal**	0	0	2	7	0	0	3	8
	Mild	0	0	0	1	-	-	0	0
	Moderate	0	0	0	1	-	-	0	0

It is also noted that serum T4 levels were decreased in the mid and top dose of F0 males (females not analysed). Also, in F1 males a slight dose-dependent decrease in serum T4 levels was observed, whereas a slight increase was seen in females. No related findings in the thyroid gland were reported.

The DS considered the liver-related effects seen at 10 mg/kg bw/day as sufficiently severe to support a classification as STOT RE. After correction for exposure duration (109 days) an effective dose of 12.1 mg/kg bw/day can be calculated. This value is above the upper guidance value for classification in category 1. It is however noted that necrosis was already seen in one male (minimal) and four females (3 minimal, 1 mild) of the F0 generation exposed to 0.5 mg/kg bw/day. In addition, the dose spacing between 0.5 and 10 mg/kg bw/day is larger than the recommended maximum of 10-fold and the calculated effective dose is only just above the guidance value range for classification in category 1. In addition, exposure of the F1 generation was shorter than 109 days (i.e. during gestation (19 days in CD1 mice), during the first 21 days of life via milk and the following 22 days via gavage), resulting in lower effective doses than those calculated for adult mice (~ 9.1 mg/kg bw/day, which is less than the GV of 10 mg/kg bw/day).

The observed liver effects are adverse and, where necrosis occurred, irreversible and they were seen in males and females in two generations.

As there is only one study available on PFHpA, the effects are only demonstrated in one species. However, as also pointed out by several commenters during the consultation, similar liver toxicity was also demonstrated on the closely related substances PFOA and PFNA, although no in-depth read-across evaluation was presented by the DS.

On the basis of the observed dose-related increase in hepatocellular necrosis, starting at 0,5 mg/kg bw/day, RAC supports the DS's proposal to **classify PFHpA as STOT RE 1; H372 (liver).**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The reproductive toxicity of PFHpA was investigated by Anonymous (2017) in a combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening study (OECD TG 408 & 422) in CD1 mice, receiving 0, 0.5, 10 or 50 mg/kg bw/day PFHpA via gavage.

Sexual function and fertility

In the F0 generation, no effects on survival, body weight/body weight gain, food consumption, clinical signs, mating index, fertility index, implantation sites, gestation, parturition or estrous cycle were reported. Also, regarding the behaviour in the functional observation box (FOB) and motor activity, the animals were comparable across groups. Only a slight increase in pre-coital interval (observed at all dose groups, not statistically significant) was reported (pre-coital interval in control, low, mid and top dose was 2.2d, 2.9d, 2.7d and 2.9d, respectively). The only relevant observations were the effects on liver, which are covered under STOT RE.

On that basis the DS did not propose to classify PFHpA for effects on sexual function and fertility.

Development

The DS considered the observed effects in F1 animals sufficient for classification. These effects included reduced pup survival and pup bodyweights, cleft palate as well as dose-related increases in malformations of the skeleton highlighted during the consultation - i.e. missing digits, malrotated forelimbs and small stature. The DS considered the observed delay in the onset of vaginal patency as supportive evidence.

Regarding the considerable liver toxicity observed in dams, the DS concluded that it was not sufficient to explain the observed developmental effects in the F1 generation. There was no effect on survival, body weight/body weight gain, food consumption or clinical signs that would indicate a strong impact on the adult animals. In this regard the DS also referred to the CLP regulation, table 3.7.1(a) and section 3.7.2.4.2, in order to highlight that even in the presence of toxicity, it needs to be demonstrated that the developmental effects are secondary non-specific consequences of the effects on dams.

The DS considered the available evidence sufficient to support a classification of PFHpA as Repr. 1B, H360D.

Lactation

The DS noted that a decrease in survival was seen during lactation days 4 to 21 and a treatment-related decrease in pup body weight was also noted during the lactation period. However, as there were no data on the quantity or quality of the mouse breastmilk or no investigation of the presence of PFHpA or its metabolites in the breastmilk of the mice, no direct link to effects observed in pups during the lactation period could be made.

The DS further noted that PFHpA had recently been detected in human breast milk but concluded that no conclusions on concentrations of PFHpA in breastmilk leading to adverse effects in babies could be drawn and therefore proposed no classification for effects on or via lactation.

Comments received during consultation

All commenters supported no classification for sexual function and fertility.

3 MSCAs supported classification as Repr. 1B, H360D, based on the reduced pup survival and body weight, the observed cases of cleft palate and the delayed onset of vaginal patency. One MSCA highlighted that an important finding of the available study was not adequately reported in the CLH proposal, i.e. skeletal malformations. This MSCA concluded that the skeletal malformations gave the strongest support for the proposed classification as Repr. 1B, H360D, while the observed cases of cleft palate were clearly considered to be of lower weight. One MSCA considered the case as borderline between Category 1B and 2, because the study had limitations (only punctual and limited observations in animals, missing dose-response relationship for cleft palate, lower human relevance of cleft palate when seen in mice versus rats).

Several MSCAs pointed out that read-across from closely related substances (e.g. PFOA or APFO) would have supported the classification proposal (similar findings had been seen with closely related substances). The DS responded that they were of the view that information from substances with a longer chain length would be less relevant, as no interpolation was possible (decrease of toxicity with decreasing chain length was anticipated).

Also, two companies submitted comments and did not support classification for reproductive toxicity (sexual function and fertility, development, or lactation). Their main argument against the relevance of the developmental findings for classification was that they occurred in the presence of maternal toxicity, demonstrated by severe liver toxicity. They also pointed out that in their view the observed cases of cleft palate were chance findings and provided publicly available HCD from the conducting laboratory. Further, they mentioned that cleft palate was not seen with the closely related substances PFOA and PFHxA, to which the DS responded that they still considered the observed incidences of cleft palate supportive for the classification proposal.

Assessment and comparison with the classification criteria

Sexual function and fertility

Despite dose-dependent liver toxicity observed in male and female mice, the animals did not seem to be severely affected in general. No effects on body weight/body weight gain, food consumption, other organ weights, parameters on sexual function and fertility (despite the slight and not statistically significant increase in pre-coital interval), or clinical signs were reported in these animals. The observed liver effects are still considered relevant for classification as STOT RE 1 (liver), as they demonstrate 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 indicated by the blood biochemical parameters which were not affected in mated females of the top dose group on lactation day 21. Such effects might, however, become evident upon longer exposure duration.

According to the CLP Regulation (Annex I, 3.7.1.3) any effect on the onset of puberty 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 the control, low, mid and top dose groups, respectively). However, time to vaginal opening was significantly prolonged (PND 29.9, 29.4, 30.1 and 33.1* in the control, low, mid and top dose, respectively) (Table below). 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 the onset of puberty was delayed in females, but not in males, although body weights were clearly lower in the top dose of both sexes.

***Table**: Pubertal landmarks in F1 females and males (day of vaginal opening and balanopreputial separation).*

mg/kg bw/day	Ctrl.	0,5	10	50	HCD
Day of vaginal opening	29.9	29.4	30.1	33.1[#]	28.1 ± 2.56 (24.7 - 32.1)
Day of balanopreputial separation*	30.2	30.2	29.5	31	30.5 ± 1.91 (28.5 - 33.8)

[#] Statistically significant ($p < 0.05$)

* None of the differences was statistically significant different from the control group.

However, as the effect was accompanied by lowered body weight, RAC considers the effect on its own not sufficient for classification for sexual function and fertility.

The applied doses did not induce any clinical signs, body weight variations, or other general toxicity, but liver toxicity was seen in dams of all dose groups. RAC therefore concludes that the applied doses were high enough to assess PFHpA's potential to induce effects on sexual function and fertility. It is, however, noted that the OECD TG 422 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, post-natal and post-weaning exposure (up until PND42), exposure was considerably longer than in a normal screening study conducted according to OECD TG 422.

Based on the absence of relevant effects on sexual function and fertility, RAC supports the DS's proposal **not to classify PFHpA for sexual function and fertility**.

Development

Offspring survival

While no effects were observed on the number of litters and mean litter size at birth, there was a decrease in post-natal survival of the pups (Table below). The survival indices from birth to PND 4 were 99.6%, 95%, 99.6% and 89.3% in the control, low, mid and top dose groups, respectively. On PND 21 the indices were 99.3%, 99.4%, 98.7% and 87.8%, respectively, and indicated that a further decrease was seen in the mid and top doses. Effects were outside the HCD in the top dose between PND 4 and 21 only.

***Table**: Postnatal survival index (extracted from the CLH report)*

	Dose groups				HCD
Dose (mg/kg bw/day)	**0**	**0.5**	**10**	**50**	males & females
PND 0	100	100	100	98.4	97.8 (94.1 - 100.0)
PND 0 - 4	99.6	95.0	99.6	89.3	94.1 (87.4 - 98.2)
PND 4 -21	99.3	99.4	98.7	87.8	96.3 (93 - 100.0)

HCD: in CD1 mice, study dates: 10/1997 – 01/2015, number of studies covered: 10.

Offspring body weights

Mean pup body weight was statistically significantly decreased at the top dose from PND 1 in males (except PND 22) and from PND 4 to 21 in females (Tables below). Female pups from the mid dose also had significantly lower body weight compared to the control animals on PND 43. For PNDs 1, 4 and 10 male and female pup body weights at the top dose were outside the HCD (10/1997 – 01/2015; number of studies covered: 12). No HCD was available for PNDs 22, 28, 35 and 43.

***Table**: F1 body weight (g) during and after the lactation period (extracted from the CLH report)*

	Males				Females			
Dose (mg/kg bw/day)	**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
PND 1	1,7	1,7	1,7	1,5 *	1,6	1,6	1,6	1,5
PND 4	2,6	2,7	2,6	2,0 **	2,6	2,7	2,5	2,0 **
PND 10	6,0	6,0	5,8	5,0 **	5,9	6,0	5,6	5,0 **
PND 21	11,7	11,6	11,0	9,8 **	11,3	11,1	10,3	9,6 **
PND 22	12,6	12,8	12,4	11,1	12,8	12,0	11,7	10,6 **
PND 28	20,8	21,6	20,4	17,5 **	18,3	17,8	17,0	15,0 **
PND 35	26,8	27,1	27,0	24,8 *	23,2	22,5	21,9	20,5 **
PND 43	29,0	29,4	29,4	27,7	24,7	23,7	23,2 *	22,1 **

*: $p < 0.05$, **: $p < 0.01$

***Table**: F1 body weight - % difference from control (extracted from the CLH report)*

	Males				Females			
Dose (mg/kg bw/day)	**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
PND 1	-	1,2	1,2	- 7,2	-	1,9	0,6	- 3,8
PND 4	-	4,2	- 0,8	- 23,2	-	2,7	- 4,2	- 21,6
PND 10	-	1,3	- 2,5	- 16	-	1,7	- 3,6	- 13,8
PND 21	-	- 0,9	- 5,8	- 16,6	-	- 1,4	- 8,6	- 14,8
PND 22	-	1,6	- 1,6	- 11,9	-	- 6,3	- 8,6	- 17,2
PND 28	-	3,8	- 1,9	- 15,9	-	- 2,7	- 7,1	- 18,0
PND 35	-	1,1	0,7	- 7,5	-	- 3.0	- 5,6	- 11,6
PND 43	-	1,4	1,4	- 4,5	-	- 4,0	- 6,1	- 10,5

Other findings in the offspring

There were no effects on anogenital distance in male and female pups and no evidence for nipple retention in male pups on PND 13.

Mammary gland development was investigated in control and top dose F1 females on PND 21 and PND 43. A scoring system was applied with 4 scores, 1 being least developed and 4 being most developed. The following results were obtained (Table below), indicating no significant differences between the groups, but slightly higher scores were noted in the control glands.

***Table**: Mammary gland development in F1 females on PND 21 and 43.*

	PND 21		PND 43	
Score	Control	Top dose	Control	Top dose
1	12	14	0	0
2	9	9	12	15
3	5	5	4	1
4	3	0	1	0

Cleft palate

Cleft palate (palatine plates not joined for the entire length) was only found in dead animals (no evidence of milk in stomach, necropsy on PND 0 or 1). There was no dose response relationship: in the low dose, 6 pups of 1 litter were affected (5 males, 1 female) and at the top dose, 3 pups in 2 litters had cleft palate (2 males, 1 female). It is further noted that in the top dose group one male with cleft palate also demonstrated other associated skeletal effects (accessory bones were found on the skull as well as on the 7th sternebra, which was located between the 5th and the 6th sternebra). In the top dose female with cleft palate it was noted that sternebrae were moderately misaligned (for example the left half of the third bone was attached to the right half of the fourth). The second male with cleft palate at that dose did not show associated effects.

During the consultation, HCD from Charles River Laboratories were made available. These data date from 2009 to 2018 and five different ranges of background incidences were reported for cleft palate: for litters between 0 and 14.3%, for foetuses between 0 and 2.1%. No mean values were reported.

The data presented in the Table below show that the incidences of cleft palate were without a dose response relationship and were slightly above historical control values for foetuses in the low dose only. The incidence on a litter basis is within the historical control range.

***Table**: Incidences of cleft palate in F1.*

Doses (mg/kg bw/day)	0	0.5	10	50	HCD
Cleft palate fetus (litter)	0	6 (1)	0	3 (2)	-
Total number of litters	18	20	19	17	-
% litters affected	0	5	0	11.8	0-14.3
Total number of fetuses	201	208	226	190	-
% pups affected	0	2.8%	0	1.6%	0-2.1

Other skeletal malformations

In the mid and top dose groups there was an increase in the number of pups with missing digits (left and/or right limbs) (Table below) and pups with malrotated forelimbs (Table further below). In addition, small stature was observed in mid and top dose pups.

***Table**: Skeletal malformations in F1.*

Dose (mg/kg bw/day)	0	0.5	10	50
Missing digit(s) - total occurrence/N pups, both sexes (litters affected)				
right forelimb	7/3 (2)	2/1 (1)	17/5 (2)	28/8 (5)
left forelimb	4/1 (1)	12/3 (1)	0/0	40/13 (6)
right hindlimb	4/2 (1)	8/5 (1)	17/7 (2)	54/25 (5)
left hindlimb	9/3 (1)	0/0	11/4 (2)	31/9 (5)
Small stature				
male / female	2/5	3/2	3/5	14/17

***Table:** Malrotated forelimbs in F1.*

Doses (mg/kg bw/day)	0	0.5	10	50	HCD
Malrotation of forelimbs					
Total number of litters	18	20	19	17	
% litters affected	-	-	5.3	24	0 - 20.8
Total number of fetuses	201	208	226	190	
% pups affected (m&f)	-	-	0.4	3.2	0 - 1.6

Note: The mark "-" indicates that there were no effects.

RAC considers the observed skeletal malformations in the mid and top doses as relevant findings supporting classification in category 1B. These are malformations considered relevant for humans and there was an increase in their incidence with dose both on a foetus and a litter basis. The observed cases of cleft palate are considered incidental findings as they did not show a dose response relationship and were within or at the upper range of the HCD.

There was a slight dose dependent decrease in pup survival, which was outside the historical control range only in the top dose males and females between PND 4 and 21. Also, pup body weights were clearly affected. A dose dependent and statistically significant decrease was seen in the top dose males on PND 4, PND 10 and PND 21, while in females the decrease started on PND 4. These values were outside the historical control range, except for the findings on PND 21 and were considered supportive evidence for classification.

Developmental toxicity was also seen with the related substances PFOA, PFNA and PFDA. All three substances have a harmonised classification as Repr 1B, H360D, based on recent RAC opinions. However, no in-depth read-across from these substances to PFHpA was presented by the DS.

No general toxicity was seen in the dams in the present study on PFHpA, except for liver toxicity. The observed effects are indicative of irreversible damage to the liver tissue, however, at the time of lactation, liver related blood biochemical parameters were not affected in the dams and no signs of general toxicity were reported (but such effects might become evident upon longer exposure duration). It is further noted that the CLP regulation in table 3.7.1(a) states *"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."*

In section 3.7.2.4.2 the CLP regulation states 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."*

Overall RAC concludes that the available data give clear evidence of adverse effects on development, i.e. dose-related skeletal malformations supported by effects on offspring survival and body-weights, without severe maternal toxicity, which **warrant classification as Repr. 1B; H360D**.

Lactation

RAC concurs with the DS that there are indications of potential effects on or via lactation (reduced survival and body weight from PND 1 / PND 4). However, there are no measurements of amount or quality of the breastmilk in mice and no measurements of PFHpA or its metabolites in the breastmilk of mice. Therefore, it cannot be differentiated whether these effects were induced due to prenatal or postnatal exposure.

The presence of PFHpA in human breastmilk as such is not considered sufficient to support a classification for lactation, as no effective concentrations could be derived that would result in adverse effects on babies.

RAC supports the DS's proposal for **no classification for lactation**.

Additional references

ECHA (2011). RAC opinion on PFOA:
https://echa.europa.eu/documents/10162/02df8dcd-f45c-b8db-6c22-a699b3c10d5c

ECHA (2014). RAC opinion on PFNA:
https://echa.europa.eu/documents/10162/e7038b5d-9d39-d39d-1a54-6c9eb0a23baf

ECHA (2015a). Decision on substance evaluation for PFHpA:
https://echa.europa.eu/documents/10162/520288be-efe9-f8f1-5885-9329ca32e9a9

ECHA (2015b). RAC opinion on PFDA:
https://echa.europa.eu/documents/10162/fc432468-ef7e-0e54-2bef-fde80c703cd0

ANNEXES:

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

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