CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification :

Perfluoroheptanoic acid; tridecafluoroheptanoic acid (PFHpA)

EC Number : 206-798-9

CAS Number : 375-85-9

Index Number : not available

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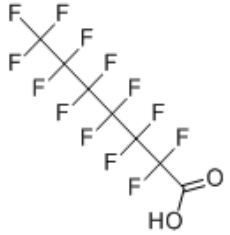
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2,2,3,3,4,4,5,5,6,6,7,7,7-Tridecafluoroheptanoic acid (IUPAC name) Perfluoroheptanoic acid
Other names (usual name, trade name, abbreviation)	PFHpA Tridecafluoroheptanoic acid Heptanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoro- Heptanoic acid, tridecafluoro- Perfluoro-n-heptanoic acid Perfluoroenanthic acid Tridecafluoro-1-heptanoic acid
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	206-798-9
EC name (if available and appropriate)	Perfluoroheptanoic acid
CAS number (if available)	375-85-9
Other identity code (if available)	
Molecular formula	$C_7HF_{13}O_2$
Structural formula	

SMILES notation (if available)	C(F)(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(=O)O
Molecular weight or molecular weight range	364.06 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	No stereoisomerism possible
Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

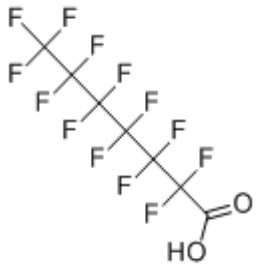
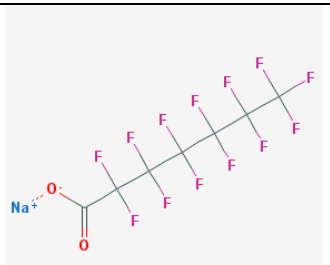
Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**
Perfluoroheptanoic acid; tridecafluoroheptanoic acid EC 206-798-9	80 %	Not listed	The substance is not registered under REACH, but several self classifications exist in the C&L inventory : Acute Tox. 4, H302 Skin Corr. 1B, H314 Eye Dam. 1, H318 Met. Corr. 1, H290 NC

Table 3: Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**
Sodium perfluoroheptanoate	> 99.3 %	Not listed	Not notified in C&L inventory	OECD TG 408 and 422

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
EC 243-518-4				

Table 4: Read-across with sodium salt

	Perfluoroheptanoic acid *	Sodium perfluoroheptanoate **
EC n°	206-798-9	243-518-4
CAS n°	375-85-9	20109-59-5
Structural formula		
Molecular formula	$C_7HF_{13}O_2$	$C_7F_{13}NaO_2$
Molecular weight	364.06 g/mol	386.04 g/mol
Length of carbon chain	7	7
Melting point	30-36 °C (ChemSpider)	159 °C
Boiling point	177 °C (@ 1 atm)	396 °C
Vapour pressure	0.133 mmHg (@ 25 °C)	4.5 x 10^{-7} mmHg
Density	1.735 g/cm³	1.792 g/cm³ (Siegemund G et al, 2000)
W,ater Solubility	4.283 mg/L (consensus value) 3.65 mg/L (EPISuite v4.11)	1936 mg/L
Partition coefficient n-octanol/water	Average 4.91 (range 3.45 to 6.86) 4.15 (EPISuite v4.11)	0.33

Dissociation constant	pKa = -2.29 (estim. ChemSpider) pKa = 2.4 (estim. ACDLabs)	

*Values from US EPA Chemistry Dashboard unless otherwise stated

**Values from US EPA EPISuite, v4.11 unless otherwise stated

Perfluoroheptanoic acid (PFHpA) is a potential degradation product of all substances that contain a perfluorinated linear chain of 6 carbon atoms connected by a terminal perfluorinated carbon atom to another non-fluorinated carbon atom. PFHpA can be expected to constitute a stable degradation product as the fluorinated chain is not degradable at all and a carboxylic acid functionality is the end result of degradation of the non-fluorinated parts of the parent compound. Examples of such substances are FS-65 and FS-61 that are both registered under REACH and that were both selected for Substance Evaluation in 2013.

Perfluoroheptanoate anion is the conjugate base of perfluoroheptanoic acid. Depending on the pH of the environmental matrix in principle both forms can be present and both forms are always in equilibrium with each other. Considering the fact that PFHpA is a strong acid one may accept that in real environmental circumstances the equilibrium will always be shifted nearly completely towards the anion (heptanoate) and the concentration of the acid form will be negligible. In this framework the pK_a value of perfluoroheptanoic acid is the crucial parameter but an experimentally determined value is not available. The estimated value by ACDLabs software is 2.4 while ChemSpider predicts a much stronger acid character (i.e. pK_a = -2.3). In the Annex XV dossier for the analogous substance perfluorooctanoic acid (PFOA), pK_a values between 1.5 and 2.8 are presented. Therefore the estimation based on ACDLabs software seems to be more reliable. Whatever the real pK_a value may be, one can state that **under real environmental conditions only the anion form will be present in relevant concentrations.**

Due to animal welfare reasons, the study was performed with the sodium salt of PFHpA. Using the acid form in the combined OECD 422/408 study would have caused unnecessary animal suffering. Besides, taking into account the near neutral pH value in organs and blood in mammals, effective exposure of the test animals in the study was towards the anion and not to the acid form.

Some physico-chemical properties (e.g. water solubility, vapour pressure, log K_{ow}, …) of the anion form and the acid form differ substantially. Nevertheless, this observation does not prevent applying read-across for the toxicological assessment of PFHpA as these properties do not influence the interactions between test substance and testing animal in the applied test protocol. If the combined 90 day study had been carried out with the acid, it would have been completely transformed into its conjugate anion and so **read-across is appropriate**.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Perfluoroheptanoic acid

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	Perfluoroheptanoic acid	206-798-9	375-85-9							
Dossier submitters proposal	TBD	Perfluoroheptanoic acid; tridecafluoroheptanoic acid	206-798-9	375-85-9	**Repro. 1B** **STOT RE 1**	H360D H372 (liver)	GHS08 Dgr	H360D H372			
Resulting Annex VI entry if agreed by RAC and COM	TBD	Perfluoroheptanoic acid; tridecafluoroheptanoic acid	206-798-9	375-85-9	**Repro 1B** **STOT RE 1**	H360D H372 (liver)	GHS08 Dgr	H360D H372			

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity	Hazard class not assessed in this dossier	No
Reproductive toxicity	Repr. 1B, H360D	Yes
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	STOT RE 1, H372 (liver)	Yes
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Perfluoroheptanoic acid itself is neither registered under REACH nor listed in annex VI of CLP and thus classification and labelling was previously not discussed.

The following self classifications are notified in the C&L inventory for perfluoroheptanoic acid (date 3 January 2019) :

Acute Tox. 4, H302

Skin Corr. 1B, H314

Met. Corr. 1, H290

Eye Dam. 1, H318

Not Classified

Based on the results of the Combined 90-Day Repeated Dose Oral (Gavage) Toxicity Study with the Reproduction/Developmental Toxicity Screening Test with sodium perfluoroheptanoate (EC 243-518-4), PFHpA should be classified as Repr. 1B, H360D and STOT RE 1, H372 (liver).

The sodium salt of perfluoroheptanoic acid is not registered under REACH (1907/2006/EC) and not listed in Annex VI of CLP. Futhermore no notifications are available in the C&L inventory.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Action at community level is needed: the DS disagrees with the current self classification of perfluoroheptanoic acid. Based on the currently available data on its sodium salt, the substance perfluoroheptanoic acid warrants a classification as Repr. 1B and STOT RE 1.

Perfluoroheptanoic acid is a common potential degradation product of all substances that contain a perfluorinated linear chain of six carbon atoms connected by a terminal perfluorinated carbon atom to another non-fluorinated carbon atom and thus also of the substances with trade names FS-65[1] and FS-61[2].

Requirement for harmonised classification by other legislation or process: following the **substance evaluation of FS-65**[1] **and FS-61**[2] a Reproduction/Developmental Toxicity Screening Test in mice (OECD TG 422) was asked with the sodium or potassium salt of the degradation product PFHpA (CAS No 375-85-9; EC No 206-798-9): oral route extended to 90 days for the pre-mating and mating period and extended to 21 days post weaning was (Both SEv Decisions of 31 August 2015). The study was performed on the sodium salt due to animal welfare reasons (irritation/degeneration from continuous administration).

The result of this study warrants classification for the hazard classes “Reproductive Toxicity” and “Specific Target Organ Toxicity- Repeated Exposure”.

5 IDENTIFIED USES

Not available as the substance itself is not registered.

6 DATA SOURCES

- Study report (anonymous, 2017)
- Literature

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20 °C and 101,3 kPa	Solid		
Melting/freezing point	30-36 °C	ChemSpider	Measured
Boiling point	177 °C	US EPA Chemistry Dashboard	Measured
Relative density	1.735 g/cm^3	Siegemund (2000)	Measured
Vapour pressure	0.133 mmHg	US EPA Chemistry Dashboard	Measured
Surface tension	14.6 dyn/cm (range 12.2 to 17.1 dyn/cm)	US EPA Chemistry Dashboard	Estimated
Water solubility	4.283 mg/L	US EPA Chemistry Dashboard	Measured
Partition coefficient n-octanol/water	4.91 (range 3.45 to 6.86)	US EPA Chemistry Dashboard	Estimated
Flash point	55.7 °C (range 51.3 to 60.1)	US EPA Chemistry Dashboard	Estimated
Flammability	No data available		
Explosive properties	No data available		
Self-ignition temperature	No data available		
Oxidising properties	No data available		
Granulometry	No data available		
Stability in organic solvents and identity of relevant degradation products	No data available		
Dissociation constant (pK_a)	-2.29 2.4	ChemSpider ACDLabs	Estimated Estimated
Viscosity	No data available		

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this CLH dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated in this dossier.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Not evaluated in this dossier.

10.2 Acute toxicity - dermal route

Not evaluated in this dossier.

10.3 Acute toxicity - inhalation route

Not evaluated in this dossier.

10.4 Skin corrosion/irritation

Not evaluated in this dossier.

10.5 Serious eye damage/eye irritation

Not evaluated in this dossier.

10.6 Respiratory sensitisation

Not evaluated in this dossier.

10.7 Skin sensitisation

Not evaluated in this dossier.

10.8 Germ cell mutagenicity

Not evaluated in this dossier.

10.9 Carcinogenicity

Not evaluated in this dossier.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 8: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening Mice (CD-1)	Sodium perfluoroheptanoate (purity : > 99.3 %) Vehicle : deionized water	Clinical pathology phase : No significant effect was reported on BW, food consumption, hematology and coagulation, serum chemistry or macroscopic examinations	Anonymous, 2017

Method, guideline, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
F0 : 20 animals/sex/group (except for control and high dose : 20 males and 25 females) Clinical pathology phase : 15/sex/group F1 : 16-20/sex/group Oral : gavage Similar to OECD TG 408 and 422	Doses : 0, 0.5, 10 and 50 mg/kg bw/d Duration of exposure : F0 : 90d prior to mating and during mating period for males (total of 109-113d) and 90d prior to pairing and until lactation d20 for females (total of 130-142). The extra 5 females in the control and high dose group were used for gender comparison and exposed during 109d (not for mating) F1: during PND 22 to 42 (total of 21d)	Main study phase : F0 : **At 50 mg/kg bw/d** Significant increase in ALP, ALT and Trig. in males and in ALP and Trig. in non-mated females Significant decrease in thyroid T4 levels in males serum Slight increase in precoital interval Significant increase in liver rel. and abs. weights in both sexes Histopathological findings in the liver in both sexes **At 10 mg/kg bw/d** Significant increase in ALT levels in lactating females (D21) Significant decrease in thyroid T4 levels in males serum Slight increase in precoital interval Significant increase in liver rel. and abs. weights in both sexes Histopathological findings in the liver in both sexes **At 0.5 mg/kg bw/d** Slight increase in precoital interval Histopathological findings in the liver in both sexes F1 : **At 50 mg/kg bw/d** Decrease in postnatal survival Significant decrease in pups mean BW Trend to increase in F1 females T4 serum levels Cleft palates in 3 pups from 2 litters Significant increase in vaginal patency Significant increase in liver rel. and abs. weights in both sexes Significant increase in adrenal rel. and abs. weights in females Histopathological findings in the liver in both sexes **At 10 mg/kg bw/d** Decrease in the percentage of males/litter Trend to increase in F1 females T4 serum levels Significant increase in liver rel. and abs. weights in males Histopathological findings in the liver in both sexes **At 0.5 mg/kg bw/d** Trend to increase in F1 females T4 serum levels Cleft palate seen in 6 pups from 1 litter Histopathological findings in the liver in both sexes	

No human data or other studies available.

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In a combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening (anonymous, 2017), groups of males and females mice were given by gavage sodium perfluoroheptanoate (purity > 99.3 %) at a concentration of 0, 0.5, 10 or 50 mg/kg bw/d.

The Registrant justified the performance of this study on mice instead of rats. First, the Dossier submitter highlighted that this test is one outcome of a substance evaluation dossier and it was asked to perform the test on mice. It is also stated that mice are a model acknowledged as appropriate for reproductive toxicity studies. Furthermore, the company furnishing animals (Charles River) has reproductive historical control data in the CD-1 mouse. Last but not least, this model is also considered by the Registrant as susceptible to effects induced by reproductive toxicants.

For the main study phase, after an acclimation periof of 9 days, mice were divided into groups of 20 animals per sex per dose. An additional group of 5 females was added in the control and high dose groups. Mice were exposed for 90 days prior to mating to either 0, 0.5, 10 and 50 mg/kg bw/d sodium perfluoroheptanoate. Males were dosed during 90 days throughout mating period until the day before euthanasia (total of 109-133 doses). Females were exposed for 90 days, throughout mating period until lactation day 20 (total of 130-142 doses). In case of delivery failure, administration ended the day prior to euthanasia (post-mating day 23, total of 113 doses). The groups of 5 additional females exposed either to 0 or 50 mg/kg bw/d were not used for mating and euthanasia was performed a the same time than males (total of 109 doses). These last animals were used for gender comparison. Clinical observation, body weight and food consumption were recorded for all animals at regular intervals. FOB and motor activity were studied for 10 F0 males per group during the last week of exposure and for 10 F0 females per group on Lactation Day 21. Clinical pathology examinations (hematology, coagulation, and/or serum chemistry) were analysed for 15 F0 mice per sex per dose group and for the 5 additional non-mated females of control and high-dose groups at necropsies. Thyroid hormones analyses were performed only on males.

Regarding F1 pups, clinical observation, body weights and sexes were observed regularly and AGD was measured at PND 1. F1 pups were exposed until PND 21 through lactation. Afterwards, F1 pups were randomly selected for the F1 generation (1/sex/litter/group) and were directly exposed to the test substance from PND 22 to PND 42. The remaining F1 pups were necropsied on PND 21.

For the clinical pathology phase, after the acclimation period, 15 mice per sex per dose group were selectioned and dosed for 75 days prior to euthanasia. Clinical observation, body weight and food consumption were recorded at regular intervals. A clinical pathology examination (hematology, coagulation, serum chemistry) was performed on all animals on day 75 and all animals were necropsied, whether they died before the end of the dosing period or not.

<u>Clinical pathology phase results</u>

Concerning clinical pathology phase, no treatment-related effects on mortality, clinical signs, body weight, nor on macroscopic examination was observed. One female exposed to 10 mg/kg bw/d was replaced on the second day of exposure due to observed swollen urogenital area after the first dosing. At necropsy, dark red discoloration of the lungs, fluid contents in the uterine cavity and enlarged vagina with thick white contents were reported. The DS considers this euthanasia unrelated to the chemical substance. In the control group, one female was euthanized *in extremis* on D61 due to observed scabbing and dorsal hair loss between Days 13 and 61 and a 5.9 % body weight loss between Days 49 and 56. At necropsy, mottled and rough surfaces on the lungs and cystic ovaries were remarked. All other males and females survived the clinical pathology phase until scheduled euthanasia.

No significant modification was observed in BW, BWG, food consumption, hematology or macroscopic observations in clinical study phase animals of both sexes.

Clinical biochemistry examination (see Table 9 below) revealed enzymes modifications. At the highest dose level, higher AST, ALT and ALP values were noted in both sexes, furthermore higher AST (in females) and ALT values were observed at the mid dose level.

Organ weight and histopathology examinations were not performed in this study phase.

Table 9: Biochemistry data in clinical pathology phase on D75

Dose level (in mg/kg bw/d)	**0**	**0.5**	**10**	**50**
Males				
AST (U/L)	63	67	79	86
ALT (U/L)	47	39	109	122
ALP (U/L)	122	78	68	227
Triglycerides (mg/dL)	103	114	145	148
Females				
AST (U/L)	112	215	258	228
ALT (U/L)	36	47	70	98
ALP (U/L)	101	115	95	166
Triglycerides (mg/dL)	70	70	40	45

Main study phase: F0 results

The F0 generation of the main study phase did not exhibit any clinical sign or treatment-related body weight modification (Table 10). Regarding the clinical biochemistry analysis, males exposed to the highest dose level showed significant higher value of ALP, ALT and Trig. Significant higher ALP and Trig. values were also observed in non-mated females. Serum T4 levels were analysed and exhibited a severe lower value in males of the mid and high dose levels (see table 12). T4 serum levels were not evaluated in F0 females.

Table 10: Body weight data (in g) in F0 animals

Dose level (in mg/kg bw/d)	**0**	**0.5**	**10**	**50**
Males				
D0	28.2 (n=20)	28.1 (n=20)	28.2 (n=20)	27.8 (n=20)
D56	35.9 (n=20)	35.4 (n=20)	37.4 (n=19)	35.4 (n=20)
D109	37.1 (n=20)	36.6 (n=19)	38.2 (n=19)	36.8 (n=20)
Females				
D0	22.6 (n=25)	22.7 (n=20)	22.4 (n=20)	22.3 (n=24)
D56	26.2 (n=25)	26.6 (n=20)	27.1 (n=20)	26.5 (n=24)
D96	28.3 (n=7)^{AB}	28.6 (n=1)	31.0 (n=1)	28.4 (n=6)^{AB}
D109	27.8 (n=5)^{A}	/	/	30.2 (n=5)^{A}
GD0	26.6	27.4	27.6	27.2
GD18	50.0	49.9	53.5	52.0
LD1	33.4	34.0	35.5	34.5
LD21	25.6	36.0	37.5	36.7

A : including 5 females exposed to 0 and 50 mg/kg bw/d not paired and used as gender comparison (no influence of gestation) ;
B : including females paired but not yet mated

Table 11: Biochemistry data in F0 animals

Dose level (in mg/kg bw/d)	**0**	**0.5**	**10**	**50**
Males				
AST (U/L)	88	143	108	167
ALT (U/L)	51	86	41	165 *
ALP (U/L)	77	74	74	227 **
Triglyceride (mg/dL)	82	118	101	153 *
Non-mated Females				
AST (U/L)	102	NA	NA	93
ALT (U/L)	36	NA	NA	41
ALP (U/L)	52	NA	NA	152 *
Triglyceride (mg/dL)	64	NA	NA	161 **
Lactating Females (LD 21)				
AST (U/L)	142	124	101	147
ALT (U/L)	71	49	42 *	56
ALP (U/L)	129	95	87	99
Triglyceride (mg/dL)	88	120	89	137

* P < 0.05; ** P < 0.01

Table 12: Hormone analysis in F0 males at week 15

Dose level (in mg/kg bw/d)	**0**	**0.5**	**10**	**50**
Males				
Total T4 (µg/dl)	5.424	4.674	3.867	2.904
SD	0.915	0.403	0.581	0.344
Females				
Not analysed in F0 females	NA	NA	NA	NA

Examination of the reproductive parameters did not show significant changes (see Table 13). The number of implantation sites was also unaffected by the treatment (11.9, 11.3, 12.8 and 11.8 respectively at 0, 0.5, 10 and 50 mg/kg bw/d). Moreover, gestation length was similar in all groups (19.0, 19.0, 18.9 and 18.9 d at 0, 0.5, 10 and 50 mg/kg bw/d, respectively).

Table 13: Reproductive performance in F0 animals

Dose level (mg/kg bw/d)		**0**	**0.5**	**10**	**50**	**HCD**
Mating index (%)	Male	100.0	100.0	100.0	100.0	97.8 (88.8 – 100.0)[A]
	Female	100.0	100.0	100.0	100.0	99.1 (95.0 – 100.0)[A]
Fertility index (%)	Male	90.0	100.0	94.7	85.0	93.7 (84.0 – 100.0)[A]
	Female	90.0	100.0	95.0	85.0	96.7 (88.0 – 100.0)[A]
Male copulation index (%)		90.0	100.0	94.7	85.0	95.8 (86.7 – 100.0)[A]
Female conception index (%)		90.0	100.0	95.0	85.0	97.2 (88.0 – 100.0)[A]
Estrous cycle length (d)		4.5	5.0	4.9	4.5	5.1 (4.4 – 7.0)[B]
Pre-coital interval (d)		2.2	2.9	2.7	2.9	2.7 (2.0 – 3.3)[B]

[A] : HCD : in mouse CD-1, range of study dates : 10/97 – 07/15
[B] : HCD : in mouse CD-1, range of study dates : 09/96 – 07/15

At the end of the study, animals of the F0 generation were euthanized and necropsied. Males were euthanized following completion of the mating period. Females that delivered were euthanized on lactation day 21, while females that failed to deliver were euthanized on postmating day 23. Macroscopic examinations did not reveal test-substance related changes. Liver weight was significantly increased at the mid and high dose level in both sexes. No other organ weight changes were noted.

Table 14: Organ weight values in F0 males

Dose level (in mg/kg bw/d)		**0**	**0.5**	**10**	**50**
FBW (g)		36.9	36.2	38.2	37.2
Liver (g)	Abs.	1.8253	1.8342	2.1788**	3.1472**
	Rel.	4.948	5.062	5.689**	8.460**
Epididymides (g)	Abs.	0.1004	0.0964	0.1049	0.0972
	Rel.	0.272	0.267	0.276	0.262
Testes (g)	Abs.	0.2448	0.2449	0.2501	0.2373
	Rel.	0.667	0.676	0.657	0.637
Thyroid/parathyroid (g)	Abs.	0.0042	0.0044	0.0041	0.0043
	Rel.	0.011	0.0012	0.011	0.012

* P < 0.05; ** P < 0.01

Table 15: Organ weight values in F0 females

		Non-mated females				**Females lactation d21**			
Dose level (in mg/kg bw/d)		**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
FBW (g)		27.8	NA	NA	29.1	35.6	36.0	37.5	36.7
Liver (g)	Abs.	1.4018	NA	NA	1.8879**	2.0740	2.2033	2.4908**	3.0901**
	Rel.	5.036	NA	NA	6.489**	5.799	6.113	6.639**	8.415**
Ovaries/oviducts (g)	Abs.	0.0251	NA	NA	0.0281	0.0327	0.0347	0.0303	0.0287
	Rel.	0.090	NA	NA	0.096	0.092	0.097	0.081	0.078
Thyroid/parathyroid (g)	Abs.	0.0038	NA	NA	0.0038	0.0051	0.0042*	0.0055	0.0049
	Rel.	0.013	NA	NA	0.013	0.014	0.012*	0.015	0.014
Uterus (g)	Abs.	0.2131	NA	NA	0.1576	0.2390	0.3073	0.2347	0.2051
	Rel.	0.769	NA	NA	0.544	0.674	0.853	0.628	0.562

NA : not applicable ; * P < 0.05 ; ** P < 0.01

In addition to organ weight modifications, microscopic examination revealed severe liver effects. Centrilobular hypertrophy of the hepatocytes were observed in a significative number of males and females at all dose levels. In most severely affected sections, centrilobular hypertrophy extended to the periportal areas. Moreover, single cell to coalescing hepatocellular necrosis was particularly noted at the highest dose. At the highest dose, minimal brown pigmentation was seen in the Kupffer cells and hepatocytes of 19/20 males and 5/19 females. See Tables 16 and 17.

Table 16: Histopathological changes seen in liver in F0 males

Dose level (mg/kg bw/d)		**0**	**0.5**	**10**	**50**
Total number animals examined		20	19	19	20
Number of animals without findings		16	2	2	0
Centrilobular hypertrophy of hepatocytes	Minimal	0	8	2	0
	Mild	0	7	2	9
	Moderate	0	2	13	11

Infiltrate, mononuclear cells	Minimal	4	7	2	2
Brown pigmentation (Kupffer cells and hepatocytes)	Minimal	0	0	0	19
Hepatocellular necrosis	Minimal	0	1	2	19
	Mild	0	0	0	1

Table 17: Histopathological changes seen in liver in F0 females

		Non-mated females				Females lactation d21			
Dose level (mg/kg bw/d)		**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
Total number animals examined		5	0	0	4	17	20	19	16
Number of animals without findings		1	NA	NA	0	16	2	0	0
Centrilobular hypertrophy of hepatocytes	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 cells	Minimal	4	NA	NA	2	1	6	6	5
Brown pigmentation (Kupffer cells and hepatocytes)	Minimal	0	NA	NA	0	0	0	0	5
Hepatocellular necrosis	Minimal	0	NA	NA	1	0	0	5	7
	Mild	0	NA	NA	0	0	1	0	2

Main study phase: F1 results

Each litter was examined and the number of litters was unaffected by the test substance (16, 20, 18 and 16 litters respectively at 0, 0.5, 10 and 50 mg/kg bw/d). The mean litter size at birth did not change (11.2, 10.4, 11.9 and 11.0 pups respectively at 0, 0.5, 10 and 50 mg/kg bw/d). The sex ratio was decreased at the middle dose (54.1, 55.4, 47.3 and 53.8 % of males respectively at 0, 0.5, 10 and 50 mg/kg bw/d). The anogenital distance did not show significant changes (1.85, 1.85, 1.86 and 1.86 mm in males and 1.17, 1.19, 1.18 and 1.20 mm in females respectively at 0, 0.5, 10 and 50 mg/kg bw/d).

However, a trend to decrease in the postnatal survival index was noted (from birth to PND 4 (pre-selection) : 99.6, 95, 99.6 and 89.3 % ; from PND 4 (post-selection) to PND 21 : 99.3, 99.4, 98.7 and 87.8 % respectively at 0, 0.5, 10 and 50 mg/kg bw/d) (Table 18 below). Moreover, mean pup body weight was significantly decreased at the highest dose level (see Table 19) from PND 1 to 21 in males and from PND 4 to 21 in females.

Table 18: Postnatal survival index (in %) in F1

	Dose groups				HCD[A]
Dose level (in mg/kg bw/d)	**0**	**0.5**	**10**	**50**	♂♀
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 (93.0 – 100.0)

[A] : HCD in mouse CD-1 range of study dates 10/97 – 01/15

At PND 21, serum samples were analysed. Males of the highest dose exhibited a decrease of the total T4 serum value (6.29, 9.53, 6.50 and 5.61 µg/dL in males respectively at 0, 0.5, 10 and 50 mg/kg bw/d whereas 6.31, 6.80, 6.81 and 6.47 µg/dL in females respectively at 0, 0.5, 10 and 50 mg/kg bw/d).

Table 19: Pup body weight data (in g ± SD) during the lactation period

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

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

Necropsy was performed on pups which were found dead. Cleft palates were observed in 6 (1) and 3 (2) pups (litters) respectively in the low and high dose levels (1, 8, 3 and 28 examined pups respectively at 0, 0.5, 10 and 50 mg/kg bw/d). Scheduled pups necropsies revealed that one male pup of the high dose group had an enlarged parathyroid gland. Necropsies of nonselected pups showed that only one male pup of the highest dose had an opacity of the left eye. Thyroids and parathyroids weights were recorded and showed a slight decrease in exposed groups (0.0021, 0.0019, 0.0018 and 0.0019 g in males and 0.0021, 0.0020, 0.0018 and 0.0018 g in females respectively at 0, 0.5, 10 and 50 mg/kg bw/d).

Some animals were randomly selected to continue the study and were exposed until PND42. Examination of the balanopreputial separation did not show changes (30.2, 30.2, 29.5 and 31.0 PND respectively at 0, 0.5, 10 and 50 mg/kg bw/d). However, a significant higher vaginal patency was observed (29.9, 29.4, 30.1 and 33.1* PND respectively at 0, 0.5, 10 and 50 mg/kg bw/d).

During the exposure period, body weights were recorded and a significant decrease was observed at 50 mg/kg bw/d in males at PND28 and PND35 and in females from PND22 to PND43. Females pups exposed to 10 mg/kg bw/d also weighted significantly less than the controls on PND43 (see Table 20).

Table 20: Body weight data in F1 (in g) after the lactation period

		Males				Females			
Dose level (mg/kg bw/d)		**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
PND 22	BW	12.6	12.8	12.4	11.1	12.8	12.0	11.7	10.6**
	SD	1.75	1.96	2.01	1.85	1.63	1.50	1.63	1.45
	% diff		1.6	-1.6	-11.9		-6.3	-8.6	-17.2
PND 28	BW	20.8	21.6	20.4	17.5**	18.3	17.8	17.0	15.0**
	SD	2.31	2.43	2.97	2.86	1.72	1.77	1.84	1.77

	% diff		3.8	-1.9	-15.9		-2.7	-7.1	-18.0
PND 35	BW	26.8	27.1	27.0	24.8*	23.2	22.5	21.9	20.5**
	SD	1.99	1.58	2.61	2.53	1.57	1.47	1.65	2.05
	% diff		1.1	0.7	-7.5		-3.0	-5.6	-11.6
PND 43	BW	29.0	29.4	29.4	27.7	24.7	23.7	23.2*	22.1**
	SD	2.61	2.08	2.94	2.78	1.80	1.43	1.79	1.64
	% diff		1.4	1.4	-4.5		-4.0	-6.1	-10.5

* P < 0.05 ; ** P < 0.01

These animals were euthanized and necropsied. Macroscopic examination did not reveal any changes. Adrenal glands and brain weights were significantly affected in females exposed to the highest dose level. Furthermore, liver weight was significantly increased in males of the mid dose and in both sexes of the highest dose (see Table 21).

Table 21: Organ weight data in F1 (in g)

		Males				**Females**			
Dose level (mg/kg bw/d)		**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
FBW		29.0	29.6	29.4	27.7	24.7	23.7	23.2*	22.1**
Adrenal glands	Abs.	0.0062	0.0072	0.0073	0.0075	0.0116	0.0098*	0.0102	0.0081**
	Rel.	0.022	0.025	0.025	0.027	0.047	0.041	0.044	0.036**
Brain	Abs.	0.4651	0.4752	0.4641	0.4607	0.4707	0.4610	0.4580	0.4480*
	Rel.	1.618	1.608	1.590	1.675	1.912	1.951	1.987	2.036
Liver	Abs.	1.8019	1.8571	2.0644*	3.1381**	1.5775	1.5133	1.5513	1.8630**
	Rel.	6.213	6.292	7.013**	11.309**	6.388	6.385	6.709	8.42**
Epididymides	Abs.	0.0571	0.0593	0.0606	0.0561	-	-	-	-
	Rel.	0.197	0.202	0.207	0.203	-	-	-	-
Testes	Abs.	0.1962	0.1994	0.1998	0.1989	-	-	-	-
	Rel.	0.680	0.691	0.678	0.720	-	-	-	-
Ovaries/ovidcuts	Abs.	-	-	-	-	0.0233	0.0202	0.0209	0.0174
	Rel.	-	-	-	-	0.094	0.085	0.090	0.078
Uterus	Abs.	-	-	-	-	0.1740	0.1447	0.1481	0.1368
	Rel.	-	-	-	-	0.704	0.605	0.640	0.613

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

These liver changes were confirmed by the microscopic examination. As in the F0 generation, the F1 generation showed a severe increase of the incidence of centrilobular hypertrophy of the hepatocytes at all dose levels. Moreover, hepatocellular necrosis was noted in the mid and high dose levels. These effects in liver were dose-related (see Table 22).

Table 22: Histopathological changes seen in F1 liver at PND43

	Males				**Females**			
Dose level (in mg/kg bw/d)	**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 (single cell to coalescing)	Minimal	0	0	2	7	0	0	3	8
	Mild	0	0	0	1	-	-	0	0
	marked	0	0	0	1	-	-	0	0

10.10.3 Comparison with the CLP criteria

Category 1 “Known or presumed human reproductive toxicant

Substances are classified in Category for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans.

Category 1A : known human reproductive toxicant

Category 1B : presumed human reproductive toxicant. The classification in this category 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 on 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 mechanisitic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.”

Category 2 : “Suspected human reproductive toxicant

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If defiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

Such effects shall have been observed 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 the other toxic effects.”

No classification is required for fertility.

Since no human studies are available for effects on fertility, classification in Repr. 1A is not appropriate. Furthermore, as parameters regarding fertility (fertility index, estrous cycle length, pre-coital interval, number of implantation sites, gestation length) were not affected, classification in Repr. 1B or 2 is not appropriate.

10.10.4 Adverse effects on development

Table 23: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening		See Table 8	

No information available on human data or other studies relevant for adverse effects on development assessment.

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

In a combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening (anonymous, 2017), groups of males and females mice were given by gavage sodium perfluoroheptanoate (purity > 99.3 %) at a concentration of 0, 0.5, 10 or 50 mg/kg bw/d.

For further details on the study design, please refer to section 10.10.2.

As seen in Table 18 above, postnatal survival was decreased at the highest dose. Pup body weights were significantly decreased in the highest dose group (see Table 20 above).

In F1, thyroid T4 serum levels were decreased at the highest dose group in males (6.29, 6.53, 6.50 and 5.61 ug/dL at 0, 0.5, 10 and 50 mg/kg bw/d, respectively), while the T4 serum levels tended to increase in all female treated groups (6.31, 6.80, 6.81 and 6.47 ug/dL at 0, 0.5, 10, and 50 mg/kg bw/d, respectively). Unfortunately, serum biochemistry was not examined in the offspring, therefore no data on ALT, ALP, Trig. levels are available.

Table 24: Hormone analysis in F1 pups at PND21

Dose level (in mg/kg bw/d)	**0**	**0.5**	**10**	**50**
Males				
Total T4 (µg/dl)	6.286	6.533	6.502	5.612
SD	1.280	1.008	1.041	0.801
Females				
Total T4 (µg/dl)	6.308	6.804	6.806	6.472
SD	1.003	1.218	1.022	1.004

Vaginal patency tended to increase and the augmentation was significant at the highest dose (29.9 ± 2.73, 29.4 ± 2.91, 30.1 ± 3.02 and 33.1* ± 4.87, at 0, 0.5, 10 and 50 mg/kg bw/d).

Furthermore, cleft palates, a rare malformation, were reported in 3 pups (2 litters) and 6 pups (1 litters) in groups exposed to 50 and 0.5 mg/kg bw/d, respectively. This effect has to be taken seriously considering several pups were affected, in different litters, at different dose, even though it did not appear in a dose-dependent way.

Histopathological findings were reported in the liver, in both sexes, at all doses (see Table 21 above). Furthermore, liver relative and absolute weights were significantly increased in both sexes at 50 mg/kg bw/d

and only in males at 10 mg/kg bw/d (see Table 25 below). Adrenal glands absolute and relative weights were significantly decreased at the highest dose, in females only.

Table 25: Organ weight data (in g)

		Males				Females			
Dose level (mg/kg bw/d)		**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
FBW		29.0	29.6	29.4	27.7	24.7	23.7	23.2*	22.1**
Adrenal glands	Abs.	0.0062	0.0072	0.0073	0.0075	0.0116	0.0098*	0.0102	0.0081**
	Rel.	0.022	0.025	0.025	0.027	0.047	0.041	0.044	0.036**
Brain	Abs.	0.4651	0.4752	0.4641	0.4607	0.4707	0.4610	0.4580	0.4480*
	Rel.	1.618	1.608	1.590	1.675	1.912	1.951	1.987	2.036
Liver	Abs.	1.8019	1.8571	2.0644*	3.1381**	1.5775	1.5133	1.5513	1.8630**
	Rel.	6.213	6.292	7.013**	11.309**	6.388	6.385	6.709	8.42**

* P < 0.05; ** P < 0.01

10.10.6 Comparison with the CLP criteria

Category 1 "Known or presumed human reproductive toxicant

Substances are classified in Category for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans.

Category 1A : known human reproductive toxicant

Category 1B : presumed human reproductive toxicant. The classification in this category 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 on 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 mechanisitic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."

Category 2 : "Suspected human reproductive toxicant

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If defiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

Such effects shall have been observed 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 the other toxic effects."

Since no human studies are available for effects on the development, classification as Repr. 1A is not appropriate.

In a combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening (anonymous, 2017), postnatal survival was decreased at 50 mg/kg bw/d. Pup body weights were significantly decreased at the same dose level. The vaginal patency was also significantly higher at the highest dose.

Furthermore, cleft palates, a rare malformation, were reported in 3 pups (2 litters) and 6 pups (1 litters) in groups exposed to 50 and 0.5 mg/kg bw/d, respectively. This effect is considered severe since several pups

were affected, in different litters, at different doses, even though it did not appear in a dose-dependent way. The dossier submitter does not consider this effect as a chance finding.

The Guidance on the application of the CLP criteria (version 5.0 July 2017) states that "Adverse effects on postnatal survival or growth seen only at dose levels causing maternal toxicity may be due to lack of maternal care or other causes such as adverse effects on or via lactation or developmental toxicity. In case postnatal effects are caused by lack of maternal toxicity care classification for developmental effects may not be warranted". Maternal toxicity included effects seen on the liver at 10 and 50 mg/kg bw/d. However, clinical observations and nurturing abilities of the mothers were not reported to be affected by the treatment. Therefore, the liver effects are not regarded as relevant enough to explain the developmental effects. Moreover, these specific developmental effects such as cleft palates and a decrease in postnatal survival have to be given serious attention.

Finally, it should be taken into account that the doses used in this study, while relatively low (0.5, 10 and 50 mg/kg bw/d), were sufficient to induce treatment-related effects in both generations (e.g. on the liver).

Considering the available data (decreased postnatal survival, decreased pup body weights, presence of malformations such as cleft palates, delayed sexual maturation in the absence of marked maternal toxicity) as clear evidence of the substance impact on the development, the dossier submitter proposes a classification in Cat. 1B. The quality of the available study is considered as reliable and convincing enough to support a classification is Cat. 1B instead of Cat. 2.

In light of all these effects, we consider the classification as **Repr. 1B; H360D** warranted.

10.10.7 Adverse effects on or via lactation

See Table 8.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

In a combined 90-day repeated dose toxicity study (see sections 10.10.1, 10.10.2, 10.10.3, 10.10.5) with reproduction/developmental toxicity screening (anonymous, 2017), groups of males and females mice were given by gavage sodium perfluoroheptanoate (purity > 99.3 %) at a concentration of 0, 0.5, 10 or 50 mg/kg bw/d.

Postnatal survival index (see Table 18) was decreased and below HCD in pups exposed at the highest dose in the following periods: 0-4 PND and 4-21 PND. Not data is available on postnatal survival after the lactation period.

Pups body weight (see Table 19) was significantly decreased in males exposed to the highest dose at days 1, 4, 10, 21, 28 and 35. The reduction was not statistically significant at days 22 and 43, but the BW remained lower than in controls. In females of the same dose group, the BW was significantly decreased in comparison with the controls at days 4, 10, 21, 22, 28, 35 and 43, and the reduction was not statistically significant at day 1. The percentage difference of BW increased in both sexes between days 22 and 28 but then rapidly decreased between days 28, 35 and 43 (-11.9, -15.9, -7.5 and -4.5 in males and -17.2, -18.0, -11.6 and -10.5 in females, at days 22, 28, 35 and 43, respectively). Therefore it is not clear if pups BW were lower at the highest dose since birth due to *in utero* exposure to perfluoroheptanoic acid or if they stayed inferior due to exposure *in utero* **and** through breastmilk. In conclusion, effects due to exposure through breastmilk cannot be excluded.

About perfluorohexanoic acid, a few prenatal and reproductive toxicity studies are available in mice and rats (Luz et al., 2019; Iwai et Hoberman, 2014; Loveless et al., 2009), but none of them studied or reported effects during the lactation period.

Concerning human data, it is however acknowledged in the literature that perfluoroheptanoic acid was found in the serum of pregnant women and breastfed infants, in the hair of children, men and women, in cord blood and in human breastmilk (Martin et al., 2019; Wang et al., 2016; Monroy et al., 2008; Lee et al., 2018). A

high transplacental transfer efficiency (range: 0.32 - 18.56, concentration in cord serum divided by concentration in maternal serum) was determined for perfluoroheptanoic acid, which was seen to be the highest of the analysed perfluorocarboxylates (Wang et al., 2019). In a study of Kang et al. (2016), perfluoroheptanoic acid was detected in 67.4 % of breast milk samples, collected from 264 Korean lactating women, at a median concentration of 0.028 ng/mL. A positive correlation ($p<0.001$) was also observed in these breast milk samples between perfluoroheptanoic acid and perfluorooctanoic acid (Kang et al., 2016).

Furthermore, perfluorinated compounds such as perfluorooctanoic acid and perfluorononan-1-oic acid possess a harmonised classification as Lact. H362. It is suggested in the literature that a correlation exists between the duration of the lactation period and the serum concentrations of perfluorinated compounds (Lee et al., 2018b). Mondal et al. (2014) showed that PFOA and PFOS serum concentrations during childhood increased by 6 and 4 %, respectively, per month of breastfeeding. Also, several studies have showed an association between *in utero* exposure and fetal growth restriction and low birth weight (Callan et al., 2016; Chen et al., 2012; Maisonet et al., 2012, Wang et al., 2016) but the association between *in utero* exposure to perfluorinated compounds and postnatal growth (and more largely anthropometry) was inconsistent and unstable over a lifetime (Andersen et al., 2010; Maisonet et al., 2012).

10.10.9 Comparison with the CLP criteria

No toxicokinetic data allow to determine if the test substance (or its metabolites) is found in the milk or alter the quantity or quality of the produced milk.

Perfluoroheptanoic acid has however been detected in human breastmilk.

Due to data lacking, we cannot conclude on this endpoint.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

A classification as **Repr. 1B; H360D** is warranted based on the severe developmental effects observed.

10.11 Specific target organ toxicity-single exposure

Not evaluated in this CLH dossier.

10.12 Specific target organ toxicity-repeated exposure

Table 26: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening		See Table 8	

No information available regarding human data or other studies.

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

In a combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening (anonymous, 2017), groups of male and female mice were given by gavage sodium perfluoroheptanoate (purity > 99.3%) at a concentration of 0, 0.5, 10 or 50 mg/kg bw/d.

For further details on the study design and findings, please refer to section 10.10.2.

At 50 and 10 mg/kg bw/d, a significant increase in liver relative and absolute weights were seen in F0 males, F0 females and F1 generation, as shown above in Tables 14, 15 and 21, respectively.

As aforementioned in section 10.10.2., treatment-related impact on the liver was demonstrated in blood chemistry with a significant increase in ALP, ALT and Trig. in males and in ALP and Trig. in non-mated females in the 50 mg/kg bw/d group. At 10 mg/kg bw/d, a significant increase in ALT was seen in lactating females.

At 0.5, 10 and 50 mg/kg bw/d, associated histopathological findings were reported in the liver of the F0 generation (see Tables 16 and 17 for males and females data, respectively). Indeed, in males, necrosis of the hepatocytes was mild in 1 animal exposed to 50 mg/kg bw/d and minimal in 0, 1, 2 and 19 animals exposed to 0, 0.5, 10 and 50 mg/kg bw/d, respectively. Moreover, centrilobular hypertrophy was detected as minimal in 0, 8, 2 and 0; as mild in 0, 7, 2 and 9 and as moderate in 0, 2, 13 and 11 animals exposed to 0, 0.5, 10 and 50 mg/kg bw/d, respectively.

In non-mated females, minimal necrosis was reported in 0 and 1 animals and mild necrosis in 0 and 1 mice exposed to 0 and 50 mg/kg bw/d, respectively. Mild hepatocellular hypertrophy was reported in 4 mice exposed to 50 mg/kg bw/d.

At lactating day 21, mild necrosis was noted in 0, 1, 0 and 1 females and minimal necrosis in 0, 0, 4 and 7 mice exposed to 0, 0.5, 10 and 50 mg/kg bw/d. Hepatocellular hypertrophy was also reported as moderate in 0, 1, 9 and 10; as mild in 0, 8, 8 and 6 and as minimal in 0, 8, 2 and 0 females exposed to 0, 0.5, 10 and 50 mg/kg bw/d.

Also, Table 22 presents the histopathological findings in the F1 generation. Moderate hepatocytes hypertrophy was seen in 0, 5, 27.8 and 50 % of male and 0, 0, 0, 12.5 % of female pups exposed to 0, 0.5, 10 and 50 mg/kg bw/d, respectively. Mild liver cells hypertrophy was seen in 0, 40, 55.5 and 35.7 % of the males and 0, 25, 16.67 and 56.2 % of females exposed to 0, 0.5, 10 and 50 mg/kg bw/d, respectively. Finally, minimal hepatocytes hypertrohpy was objectified in 0, 40, 11.1 and 7 % of males and 0, 30, 44.4 and 31.2 % of females exposed to 0, 0.5, 10 and 50 mg/kg bw/d, respectively. Concerning necrosis, minimal necrosis was reported on 0, 0, 11.1 and 50 % of male and 0, 0, 16.67 and 50 % of females pups exposed to 0, 0.5, 10 and 50 mg/kg bw/d, respectively.

In brief, test substance-related effects on the liver were seen in this study starting at doses as low as 0.5 mg/kg bw/d, in parental generation and in offsprings.

Table 27: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg bw/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Anonymous, 2017	10	109d	**8.3** mg/kg bw/d	STOT RE CAT. 1

10.12.2 Comparison with the CLP criteria

"Category 1 : Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of:

- reliable and good quality evidence from human cases or epidemiological studies; or

- observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance dose/ concentration values are provided below (see 3.9.2.9), to be used as part of a weight-of-evidence evaluation.

C(oral route) ≤ 10 mg/kg bw/d"

"Category 2 : Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2 (see 3.9.2.6).

10 mg/kg bw/d ≤ C(oral route) ≤ 100 mg/kg bw/d"

According to CLP Regulation (Annex I: 3.9.1.1.), significant effects on health, both reversible and irreversible, that damage the function of an organ immediately or after a delay should be considered for STOT RE classification. Considering the available evidence, it appears that the target organ of perfluoroheptanoic acid is the liver. Results indicate a clear modification of the function and the morphology of the liver (macroscopic and histopathological modifications, organ weight changes). Indeed, effects in liver relative and absolute weights were reported as well as effects on liver enzymes and histopathology (necrosis, centrilobular hepatocytes hypertrophy). The sum of these observations suggests a significant alteration of the liver function.

Irreversibility of these effects could not be completely demonstrated in this study however necrosis was observed. Moreover, both sexes were affected and both generations as well, which supports the significance of targeted effects on the liver of the test substance. Finally, other routes of exposure were not assessed and, therefore, we cannot conclude on one route in particular.

Based on these severe effects a classification for specific organ toxicity after repeated exposure is proposed.

Significant adverse effects on the liver were thus observed after exposure to perfluoroheptanoic acid at doses within the guidance values for STOT RE 1. Indeed, according to the CLP Regulation, the guidance values for STOT RE 2 classification are between 10 and 100 mg/kg bw/day. The effects seen in the liver appeared already significant at 8.3 mg/kg bw/d. Thus, a classification in category 2 is not supported. However, since the observed adverse effects in the 90-d toxicity study are within the guidance values for STOT RE 1 classification (C ≤ 10 mg/kg bw/d), classification into this hazard category is proposed (STOT RE 1; H372 (liver)).

10.12.3 Conclusion on classification and labelling for STOT RE

In conclusion, based on the results of the 90d repeated dose toxicity study with reproduction/developmental toxicity screening, a classification as STOT RE 1; H372 (liver) is proposed.

10.13 Aspiration hazard

Not evaluated in this CLH dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this CLH dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this CLH dossier.

13 ADDITIONAL LABELLING

NA

14 ABBREVIATIONS

Abs. : absolu
AGD : anogenital distance
ALP : alkaline phosphatase
ALT : alanine aminotransferase
AST : aspartate aminotransferase
B : Birth
BW : body weight
Corr. : corrosive
Dam. : damage
DS : dossier submitter
FBW : final body weight
FOB : functional observation battery
HCD : historical control data
Met. metal
NA : not applicable
NC : not classified
OECD : organisation for economic co-operation and development
PFOA: perfluorooctanoic acid
PFOS: perfluorooctane sulfonate
PND : post-natal day
Rel. : relative
Repr. : reproductive toxicity

SEv : substance evaluation process

STOT RE : specific target organ toxicity (repeated exposure)

T4 : thyroxine

TG : test guideline

Tox. : toxicity

Trig. : triglyceride

UVCB : unknown or variable composition, complex reaction products or of biological materials

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16 ANNEXES

Confidential Annex to this CLH report : composition of the substance and references