Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

Perfluoroheptanoic acid; tridecafluoroheptanoic acid

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

CLH-O-0000006908-60-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

10 December 2020

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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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PERFLUOROHEPTANOIC ACID; TRIDECAFLUOROHEPTANOIC ACID

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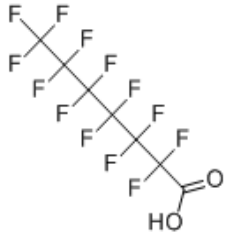
1 IDENTITY OF THE SUBSTANCE

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

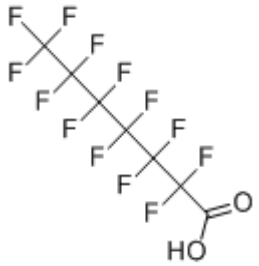
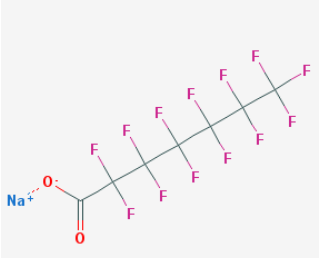
Table 2: Constituents (non-confidential information)

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 EC 243-518-4	> 99.3 %	Not listed	Not notified in C&L inventory	OECD TG 408 and 422

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**.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PERFLUOROHEPTANOIC ACID; TRIDECAFLUOROHEPTANOIC ACID

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			

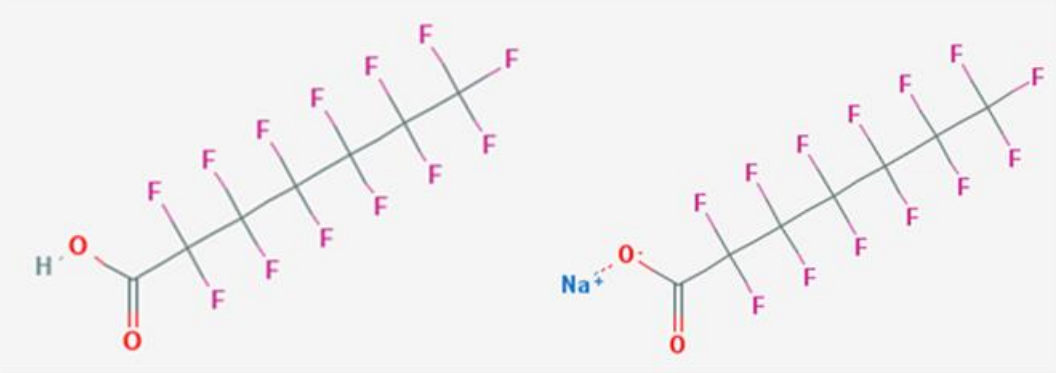
Table 6: Reason for not proposing harmonised classification and status under public consultation

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

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.



acidic form 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.

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.

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**.

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 ($C \leq 10$ 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.

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 a significant increase 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).**

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

Additional references
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Annex I to the 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 (PFHpA)

EC Number : 206-798-9

CAS Number : 375-85-9

Index Number : not available

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Version number: 1 Date: 7 March 2019

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PERFLUOROHEPTANOIC ACID; TRIDECAFLUOROHEPTANOIC ACID

CONTENTS

1 PHYSICAL HAZARDS

Not evaluated in this CLH dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated in this CLH dossier.

3 HEALTH HAZARDS

3.1 Acute toxicity - oral route

Not evaluated in this dossier.

3.2 Acute toxicity - dermal route

Not evaluated in this dossier.

3.3 Acute toxicity - inhalation route

Not evaluated in this dossier.

3.4 Skin corrosion/irritation

Not evaluated in this dossier.

3.5 Serious eye damage/eye irritation

Not evaluated in this dossier.

3.6 Respiratory sensitisation

Not evaluated in this dossier.

3.7 Skin sensitisation

Not evaluated in this dossier.

3.8 Germ cell mutagenicity

Not evaluated in this dossier.

3.9 Carcinogenicity

Not evaluated in this dossier.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening (anonymous, 2017)

Study reference:

Anonymous (2017)

Detailed study summary and results:

Test type

OECD TG 408 and 422

Test substance

- Sodium perfluoroheptanoate
- *Degree of purity :* >99.3%

Test animals

- *Species/strain/sex :* sexually mature male and virgin female crl:CD1(ICR) mice
- *No. of animals per sex per dose :*
    - F0 generation : Main study phase : 20/sex/dose (except for females of the control and the highest dose : 25)

      Clinical pathology phase : 15/sex/dose
    - F1 generation : 16-20/sex/group
- *Age and weight at the study initiation :* approx. 6w old

Administration/exposure

- *Route of administration* : gavage
- *duration and frequency of test/exposure period :* daily
    - F0 : males : 90d prior to mating, throughout mating and until necropsy : total of 113d, approximatively

      Females : 90d prior to pairing, throughout gestation and until lactation d21 : total of 142d, approximatively; except for 5 extra females in control and highest dose groups which were not used for mating (used for gender comparison) : total of 109d (euthanised at the same time point as males)

      For clinical pathology : necropsy after d75
    - F1 : PND 22 to 42 (necropsy PND43)
- *doses/concentration levels :* 0, 0.5, 10 and 50 mg/kg bw/d
- *vehicle:* deionized water

Description of test design:

- 20 F0 animals/sex/group paired to produce F1 litters. Moreover, 5 females in control and highest dose groups were not paired but continued to receive the test substance (for gender comparison).

- Clinical pathology evaluation (hematology, coagulation and serum chemistry) on 15 F0 animals/sex/group and the 5 non-mated females in control and highest dose groups at the scheduled necropsy (week 15)
- F0 males and non-mated females were euthanized at the end of mating period. F0 females were euthanized on lactation d21 for females that delivered or postmating d23 for females that failed to deliver.
- F1 pups randomly selected for the F1 generation (1/sex/litter/group). Remaining pups were necropsied on PND21.

Results and discussion

Clinical pathology phase :

- *time of death during the study and whether animals survived to termination :* no treatment-related effects
- *body weight data :* no effects

Table 1 : Mean body weight data (in g)

	Males				Females			
Dose level (in mg/kg bw/d)	**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
D0	28.4	28.5	28.4	28.5	22.7	22.3	22.5	22.3
D35	33.9	35.2	36.1	33.8	25.8	24.9	25.1	25.2
D75	37.0	38.7	39.3	36.7	28.2	27.4	28.2	26.9

- *food consumption :* no effects
- *Hematology and coagulation :* no treatment-related effects (no significant effect observed)
- *Clinical biochemistry findings (at D75) :*
    - higher AST value in males exposed to the highest dose level and in females at all dose levels (63/112, 67/215, 79/258 and 86/228 U/L in males/females respectively at 0, 0.5, 10 and 50 mg/kg bw/d)
    - higher ALT value in both sexes at the mid and high dose levels (47/36, 39/47, 109/70 and 122/98 U/L in males/females respectively at 0, 0.5, 10 and 50 mg/kg bw/d)
    - higher ALP value in both sexes at the highest dose (122/101, 78/115, 68/95 and 227/166 U/L in males/females respectively at 0, 0.5, 10 and 50 mg/kg bw/d)
- *necropsy findings :* no effects
- *organ weight :* not evaluated in this phase
- *histopathological findings:* not evaluated in this phase

Main study phase :

F0 generation (per dose):

- *clinical observations:* no treatment-related effects

- *time of death during the study and whether animals survived to termination :* no treatment-related effects
- *body weight data :* no treatment related effects

Table 2 : Body weight data (in g)

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 : 5 extra females in control and high dose levels not paired (gender comparison) ; B : females paired but not yet mated

- *ophthalmic findings :* no ophthalmic lesions observed
- *haematological findings :* no effects
- *clinical biochemistry findings :* effects were observed

Table 3 : Clinical biochemistry findings

Dose level (in mg/kg bw/d)	**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

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

- *Thyroid hormone analysis :* Significantly lower T4 values were noted in males of the mid and high dose levels. (Not evaluated in females)
- *Functional observational battery :* no effects

- *toxic response data by sex and dose including indices of mating, fertility, gestation, birth, viability and lactation; indicate the numbers used in calculating the indices :* reproductive parameters were unaffected

Table 4 : Reproductive parameters

Dose level (in mg/kg bw/d)		**0**	**0.5**	**10**	**50**	**HCD**
Mating index (%)	Male	100.0	100.0	100.0	100.0	88.8 – 100.0
	Female	100.0	100.0	100.0	100.0	95.0 – 100.0
Fertility index (%)	Male	90.0	100.0	94.7	85.0	84.0 – 100.0
	Female	90.0	100.0	95.0	85.0	88.0 – 100.0
Male copulation index (%)		90.0	100.0	94.7	85.0	86.7 – 100.0
Female conception index (%)		90.0	100.0	95.0	85.0	88.0 – 100.0

- *toxic or other effects on reproduction, offspring, postnatal growth*
- *number of P and F1 females cycling normally and cycle length* : the estrous cycle length was unaffected by the test substance (4.5, 5.0, 4.9 and 4.5d respectively at 0, 0.5, 10 and 50 mg/kg bw/d) (HCD : 4.4 – 7.0)
- *precoital interval (number of days until mating and number of estrous periods until mating) :* slight increase (2.2, 2.9, 2.7 and 2.9d respectively at 0, 0.5, 10 and 50 mg/kg bw/d) (HCD : 2.0 – 3.3)
- *number of implantations, corpora lutea, litter size :*
    - *mean number of implantation sites :* 11.9, 11.3, 12.8 and 11.8 respectively at 0, 0.5, 10 and 50 mg/kg bw/d
- *number of pre- and post-implantation loss*
- *number of dams with abortions, early deliveries, stillbirths, resorptions and/or dead fetuses*
- *duration of gestation (calculated from day 0 of pregnancy) :* similar in all groups (19.0, 19.0, 18.9 and 18.9d respectively at 0, 0.5, 10 and 50 mg/kg bw/d)
- *number of live births*
- *data on functional observations :* unaffected
- *necropsy findings :* unaffected
- *organ weight changes :* significantly higher liver weight was observed in both sexes

Table 5 : Organ weight values in 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<0.01

Table 6 : Organ weight values in 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

- *histopathological findings: nature and severity :*
  - *liver :*

Table 7 : Histopathological changes seen in liver in males

Dose level (in 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 cell	Minimal	4	7	2	2
Hepatocellular necrosis	Minimal	0	1	2	19
	Mild	0	0	0	1

Table 8 : Histopathological changes seen in liver in females

	Non-mated females				Females lactation d21			
Dose level (in 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	2	0
	Mild	0	NA	NA	4	0	8	8	6
	Moderate	0	NA	NA	0	0	1	9	10
Infiltrate, mononulcear cell	Minimal	4	NA	NA	2	1	6	6	5
Hepatocellular necrosis	Minimal	0	NA	NA	1	0	0	4	7
	Mild	0	NA	NA	0	0	1	0	1

For F1 pups/litters (per dose) :

- *number of litter :* 16, 20, 18 and 16 respectively at 0, 0.5, 10 and 50 mg/kg bw/d
- *mean number of live pups (litter size) : live litter size at PND 0 :* 11.2, 10.4, 11.9 and 11.0 respectively at 0, 0.5, 10 and 50 mg/kg bw/d
- *sex ratio at birth :* % of males per litter : 54.1, 55.4, 47.3 and 53.8 % respectively at 0, 0.5, 10 and 50 mg/kg bw/d
- *anogenital distance :*
    - *males :* 1.85, 1.85, 1.86 and 1.86 mm respectively at 0, 0.5, 10 and 50 mg/kg bw/d
    - *females :* 1.17, 1.19, 1.18 and 1.20 mm respectively at 0, 0.5, 10 and 50 mg/kg bw/d
- *viability index (pups surviving 4 days/total births)*
- *postnatal survival from birth to PND 4 (pre-selection) :* 99.6, 95.0, 99.6 and 89.3 % respectively at 0, 0.5, 10 and 50 mg/kg bw/d
- *postnatal survival from PND 4 (post-selection) to PND 21 (% per litter) :* 99.3, 99.4, 98.7 and 87.8 % respectively at 0, 0.5, 10 and 50 mg/kg bw/d
- *mean pup weight by sex :*

Table 9 : Mean offspring weight data (in g)

	Males				**Females**			
Dose level (in mg/kg bw/d)	**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
PND 1	1.66	1.68	1.68	1.54*	1.58	1.61	1.59	1.52
PND 4	2.63	2.74	2.61	2.02**	2.59	2.66	2.48	2.03**
PND 10	5.95	6.03	5.80	5.00**	5.85	5.95	5.64	5.04**
PND21	11.65	11.55	10.98	9.72**	11.25	11.09	10.28	9.58**

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

- *thyroid hormone analysis (PND 21) :*
    - total T4 value : 6.29, 6.53, 6.50 and 5.61 µg/dL in males respectively at 0, 0.5, 10 and 50 mg/kg bw/d and 6.31, 6.80, 6.81 and 6.47 dL in females respectively at 0, 0.5, 10 and 50 mg/kg bw/d
- *necropsy findings :*

- Necropsies of pups found dead : cleft palate was 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 pup necropsies (PND 21) : one male pup of the highest dose exhibited an enlarged parathyroid gland (17, 19, 18 and 16 examined pups respectively at 0, 0.5, 10 and 50 mg/kg bw/d)
- Necropsies of nonselected pups (PND 21) : one male pup of the highest dose showed opacity of the left eye (46, 44, 39 and 29 examined pups respectively at 0, 0.5, 10 and 50 mg/kg bw/d)

- *organ weight at PND21 :*
  - *Thyroids/parathyroids :* 0.0021, 0.0019, 0.0018 and 0.0019 g in males respectively at 0, 0.5, 10 and 50 mg/kg bw/d 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
- *histopathological findings :* no test substance related effect

For F1 (per dose):

- *data on physical landmarks in pups and other postnatal developmental data*
  - *balanopreputial separation :* 30.2, 30.2, 29.5 and 31.0 PND respectively at 0, 0.5, 10 and 50 mg/kg bw/d
  - *vaginal patency :* 29.9, 29.4, 30.1 and 33.1* PND respectively at 0, 0.5, 10 and 50 mg/kg bw/d
- *body weight :*

Table 10 : Body weight data (in g)

	Males				**Females**			
Dose level (in mg/kg bw/d)	**0**	**0.5**	**10**	**50**	**0**	**0.5**	**10**	**50**
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**

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

- *Thyroid hormone analysis :* not evaluated
- *necropsy findings :* no test substance related effects
- *organ weight :* thyroid weight not evaluated

Table 11 : 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**

	Rela	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*
	Rela	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**
	Rela	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	-	-	-	-
	Rela	0.197	0.202	0.207	0.203	-	-	-	-
Testes	Abs	0.1962	0.1994	0.1998	0.1989	-	-	-	-
	Rela	0.680	0.691	0.678	0.720	-	-	-	-
Ovaries/ovidcuts	Abs	-	-	-	-	0.0233	0.0202	0.0209	0.0174
	Rela	-	-	-	-	0.094	0.085	0.090	0.078
Uterus	Abs	-	-	-	-	0.1740	0.1447	0.1481	0.1368
	Rela	-	-	-	-	0.704	0.605	0.640	0.613

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

- *histopathology findings : (*brain not evaluated)

Table 12 : Histopathological changes seen in liver

		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, mononulcear 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				
	marked	0	0	0	1				

3.10.2 Human data

No available data

3.10.3 Other data (e.g. studies on mechanism of action)

No available data

3.11 Specific target organ toxicity – single exposure

Not evaluated in this CLH dossier.

3.12 Specific target organ toxicity – repeated exposure

See chapter 3.10 above.

3.13 Aspiration hazard

Not evaluated in this CLH dossier.

4 ENVIRONMENTAL HAZARDS

Not evaluated in this CLH dossier.