CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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

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

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ABBREVIATIONS

Acox1	acyl-CoA oxidase 1
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APFHx	ammonium undecafluorohexanoate
AST	aspartate aminotransferase
bw	body weight
CA	competent authority
CAR	PPARα constitutive androstane receptor
CMR	Carcinogenic, Mutagenic or Toxic for Reproduction
Cyp	cytochrome P450
d	day
DS	dossier submitter
ECHA	European Chemical Agency
FTOH	fluorotelomer alcohol
GD	gestation day
GLP	Good Laboratory Practice
HCD	historical control data
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
LDL	low density lipoprotein
LOAEL	lowest observed adverse effect level
NaPFHx	sodium undecafluorohexanoate
NTP	National Toxicology Program (https://ntp.niehs.nih.gov/)
PFAS	poly- and perfluorinated alkyl substance
PFHpA	perfluoroheptanoic acid
PFHxA	undecafluorohexanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
pK_a	negative decadic logarithm of the acid dissociation constant K_a
PPD	day postpartum period
PND	postnatal day
PPARα	peroxisome proliferator-activated receptor alpha
RDT	repeated dose toxicity
SD rat	Sprague Dawley rat
T3	triiodothyronine
T4	thyroxine
TGAb	thyroglobulin antibody
TMAb	thyroid microsomal antibody
T_{max}	time to maximum plasma concentration
TSH	thyroid-stimulating hormone
VLDL	very low density lipoprotein

CONTENTS

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 - Undecafluorohexanoic acid

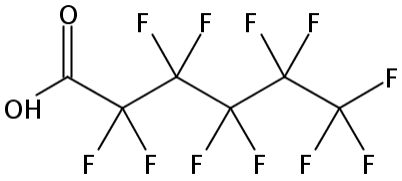
Name(s) in the IUPAC nomenclature or other international chemical name(s)	**Undecafluorohexanoic acid**
Other names (usual name, trade name, abbreviation)	Hexanoic acid, 2,-2,-3,-3,-4,-4,-5,-5,-6,-6,-6-undecafluoro-, PFHxA, Perfluorohexanoic acid
EC number (if available and appropriate)	206-196-6
EC name (if available and appropriate)	Undecafluorohexanoic acid
CAS number (if available)	307-24-4
Molecular formula	$C_6HF_{11}O_2$
Structural formula	
SMILES notation (if available)	C(=O)(C(C(C(C(C(F)(F)F)(F)F)(F)F)(F)F)(F)F)O
Molecular weight or molecular weight range	314.05 g/mol

Table 2: Substance identity and information related to molecular and structural formula of the substance - Sodium undecafluorohexanoate

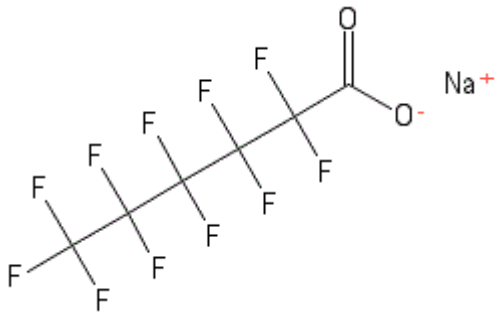
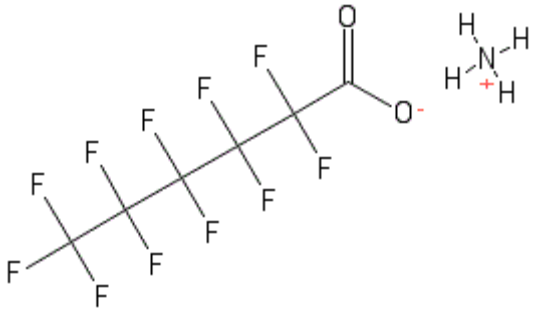
Name(s) in the IUPAC nomenclature or other international chemical name(s)	**Sodium undecafluorohexanoate**
Other names (usual name, trade name, abbreviation)	Hexanoic acid, 2, 2, 3, 3, 4, 4, 5, 5, 6, 6, 6-undecafluoro-,sodium salt (1:1)
EC number (if available and appropriate)	220-881-7
EC name (if available and appropriate)	Sodium undecafluorohexanoate
CAS number (if available)	2923-26-4
Molecular formula	$C_6F_{11}O_2Na$
Structural formula	
SMILES notation (if available)	[Na+].[O-]C(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F
Molecular weight or molecular weight range	336.03 g/mol

Table 3: Substance identity and information related to molecular and structural formula of the substance - Ammonium undecafluorohexanoate

Name(s) in the IUPAC nomenclature or other international chemical name(s)	**Ammonium undecafluorohexanoate**
Other names (usual name, trade name, abbreviation)	Hexanoic acid, 2, 2, 3, 3, 4, 4, 5, 5, 6, 6, 6-undecafluoro-,ammonium salt (1:1)
EC number (if available and appropriate)	244-479-6
EC name (if available and appropriate)	Ammonium undecafluorohexanoate
CAS number (if available)	21615-47-4
Molecular formula	$C_6H_4F_{11}NO_2$
Structural formula	
SMILES notation (if available)	[N+]([H])([H])([H])[H].[O-]C(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F
Molecular weight or molecular weight range	331.08 g/mol

There is no reliable data available for inorganic salts of undecafluorohexanoic acid.

Table 4: 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)	**Other inorganic salts of undecafluorohexanoic acid**
Other names (usual name, trade name, abbreviation)	Not applicable
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	
EC name (if available and appropriate)	
CAS number (if available)	
Other identity code (if available)	
Molecular formula	
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	
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 5: 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)**
Undecafluorohexanoic acid; EC no. 206-196-6, CAS no. 307-24-4	≤ 100	none	Acute Tox. 3, H301 Acute Tox. 3, H311 Acute Tox. 2, H330 Skin Corr. 1B, H314 Skin Corr. 1B, H314, H335 Eye Dam. 1, H318

Table 6: 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)**
Sodium	≤ 100	none	H315

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)
undecafluorohexanoate, EC No. 220-881-7, CAS No. 2923-26-4			H319 H335

Table 7: 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)
Ammonium undecafluorohexanoate, EC No. 244-479-6, CAS No. 21615-47-4	≤ 100	none	Acute Tox. 4, H302 Skin Corr. 1B, H314 Skin Sens. 1, H317 Eye Dam. 1, H318

Table 8: 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)
Other inorganic salts of undecafluorohexanoic acid	≤ 100	none	none

Perfluorohexanoate anion (PFHx) is the conjugate base of undecafluorohexanoic acid (PFHxA). Depending on the pH of the matrix (aqueous solution or biological media) in principle both forms can be present and both forms are always in equilibrium with each other. The free PFHxA is a strong acid with a pKa < 1. Thus, around neutral pH-value the equilibrium will always be shifted nearly completely towards the perfluorohexanoate anion. In standard toxicity studies carried out with PFHxA at adjusted neutral pH-value, the acid will be completely transformed into its conjugate anion.

Some toxicity studies were performed with the sodium salt (sodium perfluorohexanoate) or the ammonium salt (ammonium perfluorohexanoate), as the acid has been shown to be more irritating than the corresponding salts. In aqueous solution, sodium perfluorohexanoate (NaPFHx) is present as PFHx and the sodium cation, while ammonium perfluorohexanoate (APFHx) is present as PFHx and the ammonium cation.

However, regardless of the administered substance, once absorbed into the bloodstream, the anion will be formed. Due to the near neutral pH-value in organs and blood in mammals, the effective exposure of the test animals in a toxicity study with either PFHxA and/or its sodium or ammonium salt is towards the anion.

Therefore, the read-across approach from the sodium salt and ammonium salt to PFHxA is appropriate and is applied here for the toxicological assessment of PFHxA.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 9: Proposed harmonised classification and labelling according to the CLP criteria.

	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 entry in Annex VI										
Dossier submitters proposal	tbd	undecafluorohexanoic acid; PFHxA [1] sodium undecafluorohexanoate; NaPFHx [2] ammonium undecafluorohexanoate; APFHx [3] other inorganic salts of undecafluorohexanoic acid [4]	206-196-6 [1] 220-881-7 [2] 244-479-6 [3] - [4]	307-24-4 [1] 2923-26-4 [2] 21615-47-4 [3] - [4]	Repr. 1B	H360D	GHS08 Dgr	H360D			
Resulting Annex VI entry if agreed by RAC and COM					Repr. 1B	H360D	GHS08 Dgr	H360D			

Table 10: 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)		
Oxidising gases		
Gases under pressure		
Flammable liquids		
Flammable solids		
Self-reactive substances		
Pyrophoric liquids		
Pyrophoric solids		
Self-heating substances		
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids		
Organic peroxides		
Corrosive to metals		
Acute toxicity via oral route		
Acute toxicity via dermal route		
Acute toxicity via inhalation route		
Skin corrosion/irritation		
Serious eye damage/eye irritation		
Respiratory sensitisation		
Skin sensitisation		
Germ cell mutagenicity		
Carcinogenicity		
Reproductive toxicity	**Harmonised classification proposed**	**Yes**
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	**Data conclusive but not sufficient for classification**	**Yes**
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment		
Hazardous to the ozone layer		

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

PFHxA itself is neither registered under REACH (1907/2006/EC) nor listed in Annex VI Table 3 of the Regulation (EC) No. 1272/2008 (CLP Regulation). The following self-classifications are notified in the C&L inventory for PFHxA:

Acute Tox. 3, H301

Acute Tox. 3, H311

Acute Tox. 2, H330

Skin Corr. 1B, H314

Skin Corr. 1B, H314, H335

Eye Dam. 1, H318

APFHx is registered under REACH but not listed in Annex VI Table 3 of the Regulation (EC) No. 1272/2008. The following self-classifications are notified in the C&L inventory for APFHx:

Acute Tox. 4, H302

Skin Corr. 1B, H314

Skin Sens. 1, H317

Eye Dam. 1, H318

NaPFHx is not registered under REACH and not listed in Annex VI of the CLP Regulation. Furthermore, 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 PFHxA and APFHx.
Data on PFHxA, APFHx and NaPFHx are available and used for this CLH proposal. Based on this data, CMR properties (reproductive toxicity) are identified.
PFHxA and its inorganic salts, APFHx and NaPFHx, are expected to dissociate in biological media. Hence, regardless whether the acid or the salt are administered, once absorbed into the blood, the PFHx-anion will be formed.
Based on the results of the reproductive and developmental toxicity study with APFHx in mice, the classification as Repr. 1B, H360D is warranted.
The concerns on reproductive toxicity have already been expressed by RAC in the Opinion on the Annex XV dossier for “undecafluorohexanoic acid (PFHxA), its salts and related substances”, adopted on 8 December 2021 (ECHA, 2021).
Harmonised classification and labelling for CMR and respiratory sensitisation is a community-wide action under Article 36 of the CLP Regulation.

5 IDENTIFIED USES

PFHxA, NaPFHx and other inorganic salts of PFHxA have not been registered yet. The following table gives an overview on registration information for APFHx.

Table 11: Uses of APFHx[1]

	Use(s)
Manufacture	-
Uses as intermediate	-
Formulation	-
Uses at industrial sites	Manufacture of polymers PROC 1, 3, 8a, 8b, 9, 14; PC0: Other: Chemical used in the manufacture of polymers; SU12: Manufacture of plastics products, including compounding and conversion
Uses by professional workers	-
Consumer uses	-
Article service life	-

6 DATA SOURCES

Systematic searches for publications and other relevant data were performed based on the following databases until December 2021 PubMed, Web of Science, Embase, Scopus, Google Scholar.
For APFHx and NaPFHx the databases Wiley, Taylor & Francis, Science Direct and Microsoft Academic were used additionally.

REACH registration dossiers (last modified: 8 July 2021) for APFHx available from ECHA's dissemination database (https://echa.europa.eu/de/registration-dossier/-/registered-dossier/25106) have been analysed for study references, which then have been considered as data sources for this CLH report.

ECHA guidance documents on the application of CLP criteria and on the preparation of dossiers for harmonised classification and labelling were used to compile this report (ECHA, 2014; ECHA, 2017).

[1] (according ECHA's dissemination site, access 24 January 2023)

7 PHYSICOCHEMICAL PROPERTIES

The physicochemical properties of PFHxA are listed in Table 12. PFHxA has not been registered yet. Thus, the physical-chemical data rely on publically available databases which do neither provide detailed information on the software package nor on which form of the substance (dissociated vs. non-dissociated) or which relevant parameters were used for the calculation.

Table 12: Summary of physicochemical properties of PFHxA

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Colorless liquid		
Melting/freezing point	12-14 °C	(Huang, 1987) Huang, Bing Nan; Journal of Fluorine Chemistry 1987, V36(1), P49-62	Experimental
Boiling point	157 °C	(Savu, 2000) Savu PM; Fluorinated Higher Carboxylic Acids. Kirk-Othmer Encyclopedia of Chemical Technology (1999-2015). New York, NY: John Wiley & Sons. On-line Posting Date: 4 Dec 2000	Experimental
Relative density	1.762 g/mL at 20 °C	(Kauck, 1951) Kauck, E. A.; Industrial and Engineering Chemistry 1951, V43, P2332-4	Experimental
Vapour pressure	1.98 mm Hg = 264 Pa at 25 °C	US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.11. Nov, 2012. Available from, as of Jan 11, 2015	Estimated (no experimental value available, unknown reliability of estimated value)
Surface tension	Not available		
Water solubility	15.7 g/L (ca. 22 °C)	Zhao L et al; Chemosphere 114: 51-8 (2014) (Zhao et al., 2014)	Experimental
Partition coefficient n-octanol/water	4.06	calc., COSMOtherm (temp. not specified) (Wang et al., 2011)	Estimated
Dissociation constant	pK_a = -0.16	Zhao L., Bian J., Zhang Y., Zhu L. and Liu Z.; Chemosphere 114, 51-58 (2014) (Zhao et al., 2014)	Comparison of the sorption behaviors and mechanisms of perfluorosulfonates and perfluoro-carboxylic acids on three kinds of clay minerals.

There is no reliable data available for NaPFHx. Please find data for the related substances PFHxA (CAS No. 307-24-4) and APFHx (CAS No. 21615-47-4) in the corresponding tables (Table 12, Table 13) of this CLH report.

Table 13: Summary of physicochemical properties of APFHx

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	White powder	Ota, Y., (2017), Measurement of melting point for APFHx (C-1500N), Report No. 85024	Visual
Melting/freezing point	Decomposition at 135 °C	Ota, Y., (2017), Measurement of melting point for APFHx (C-1500N), Report No. 85024	Experimental according to OECD 102 (capillary tube in a metal block)
Boiling point	Decomposition at 135 °C	Ota, Y., (2017), Measurement of melting point for APFHx (C-1500N), Report No. 85024	Experimental according to OECD 102 (capillary tube in a metal block)
Relative density	1.786 at 20 °C	Ota, Y., (2017), Measurement of density for APFHx (C-1500N), Report No. 85026	Experimental according to OECD 109 (pycnometer method)
Vapour pressure	4.5 mPa at 25 °C	Ota, Y., (2017), Measurement of vapor pressure for APFHx (C-1500N), Report No. 85027	Experimental according to OECD 104 (gas saturation method) followed by calculation
Surface tension	68.4 mN m^{-1} at 20 °C	Yuga, O. (2017), Measurement of surface tension for APFHx (C-1500N), Report No. 85028	Experimental according to OEDC 115 (plate method)
Water solubility	57.6 g L^{-1} at 20 °C	Takeda, M. (2017), Measurement of Critical Micelle Concentration of APFHx (C-1500N), Report No. S414552	Experimental as critical micelle concentration
Partition coefficient n-octanol/water	Log P_{OW} = 2.1 at pH = 2.0 and 25 °C Log P_{OW} = 1.5 at pH = 7.4 and 25 °C	Kawashima, H., (2018), Measurement of 1-octanol/water partition coefficient for PFHxA, Report No. 652-17-P-5533	Experimental according to OECD 117 (HPLC method)
Granulometry	Not available		
Stability in organic solvents and identity of relevant degradation products	Not available		
Dissociation constant	pKa = 3.29 at 20 °C	Kawashima, H., (2018), Measurement of dissociation constants in water for PFHxA, Report No. 652-17-P-5531	Experimental according to OECD 112 (titration method)

There is no reliable data available for inorganic salts of PFHxA. Please find data for the related substances PFHxA (CAS No. 307-24-4) and APFHx (CAS No. 21615-47-4) in the corresponding tables (Table 12, Table 13) of this CLH report.

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

The toxicokinetics of PFHxA and its inorganic salts have been described in detail and summarised previously, e.g. in the PFHxA Annex XV restriction report (submitted 20 December 2019, ECHA (2019a)) and the scientific opinion on the human health risk of PFASs present in food in the EFSA Journal 2020, (EFSA Panel on Contaminants in the Food Chain, 2020). In this section, the key findings of the above mentioned reports and cited literature are presented.

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The free PFHxA is a strong acid with a pKa < 1. Depending on the pH of the matrix in principle PFHxA and its conjugate base PFHx can be present and both forms are always in equilibrium with each other. At neutral pH-values the equilibrium will always be shifted nearly completely towards PFHx. In standard toxicity studies carried out with PFHxA and an adjusted neutral pH-value, the acid will be completely transformed into its conjugate anion.

In available toxicity studies, PFHxA, or various PFHx salts, such as APFHx, and NaPFHx were used as test item. The acid or salt is expected to dissociate in biological media. Hence, regardless whether the acid or the salt are administered, once absorbed into the blood, the PFHx-anion will be formed.

Regarding the known uses of PFHxA and its inorganic salts, oral, dermal and inhalation routes of exposure are conceivable. Different sources of exposure to PFHxA and its precursors in the general population are described, such as oral administration via drinking water and food intake.

Absorption

Data for oral absorption in humans are not available. PFHxA has been detected in human blood, serum, plasma, breast milk or urine samples which indicates absorption in humans.

Absorption of PFHxA and its inorganic salts is reported to be rapid and extensive in mammals after oral administration. The time to maximum plasma concentration (T_{max}) was observed to be 0.7-0.8 hours in male Sprague Dawley rats and 0.3-0.5 hours in CD-1 mice (male and female) and female Sprague Dawley rats after oral (gavage) administration of 2 and 100 mg/kg bw sodium [1-^{14}C]-perfluorohexanoate (Gannon et al., 2011). Absorption and excretion of up to 90% of the administered dose within 24 hours after single and repeated oral administration of 50 mg/kg APFHx to male and female rats and mice was shown by Iwai (2011). In male rats approximately 90% and in female rats about 70-100% of the administered daily dose of PFHxA was recovered in the urine during 24 hours post dosing (Chengelis et al., 2009a). Thus, it can be concluded that absorption via the oral pathway is rapid and complete.

No data are available for dermal absorption in experimental animals or humans. PFHxA contains an acid group which might counteract the dermal absorption.

No data are available for absorption following inhalation in experimental animals or humans. However, absorption of PFHxA is indicated in a study on ski wax technicians, when concentrations up to 12 ng PFHxA/mL whole blood were detected during the exposure period at World Cup season 2007/2008 (Nilsson et al., 2010).

Distribution

Results from animal studies and human monitoring data support the conclusion that PFHxA is distributed to multiple organs.

In rats and mice, perfluorohexanoate was mainly detected in plasma, kidney, liver, lungs, skin, bladder, and uterus after oral administration of sodium [1-^{14}C]-perfluorohexanoate (Gannon et al., 2011), but to lesser extent also in brain, bone, testes, and ovary. A generally higher (1.6- to 3-fold) plasma concentration in male Sprague Dawley rats compared to females was shown for PFHxA, administered by gavage for 28 days (NTP, 2019). In pigs (German Landrace breed) PFHxA was mainly detected in plasma, fat and muscle tissue, liver and kidney (Numata et al., 2014).
After repeated oral (gavage) administrations (13 daily doses) of 50 mg/kg bw APFHx followed by a single oral administration of 50 mg/kg bw [^{14}C]-labelled APFHx, radioactivity was detected in blood and liver of male and female Sprague Dawley rats and CD-1 mice, seven days after the final dose. In the liver, the radioactivity (0.61-1.16% of administered dose) was about 4-8 times higher than in the circulating whole blood (0.15-0.17% of administered dose). Radioactivity in most tissues was very low or below the limit of detection (Iwai, 2011).

PFHxA was detected in human serum, urine and breast milk samples, however, in many studies, often reported below the limit of detection or limit of quantification. PFHxA was detected in the following autopsy tissues from twenty individuals of Tarragona (Spain): lung, brain, liver, kidney, bone, brain and liver (Pérez et al., 2013). The highest concentrations of PFHxA were detected in the brain and liver, with median PFHxA-concentrations of 141 ng/g brain wet weight (mean 180 ng/g brain wet weight) and median 68.3 ng/g liver wet weight (mean 115 ng/g liver weight weight). In lung tissues median PFHxA-concentrations of 207 ng/g lung wet weight (mean: 50.1 ng/g lung wet weight) were measured (Pérez et al., 2013).

A strong protein binding of PFHxA was shown, with more than 99% of PFHxA bound to bovine serum albumin (Bischel et al., 2011). Tissue distribution might be facilitated due to a strong protein affinity. Binding to certain transporter proteins is still under investigations, but may impact the toxicokinetics of PFHxA. If so, a sex and species dependent expression of transporter proteins may explain observed differences in the half-lives between sexes and species.

Metabolism

Studies on metabolism of PFHxA in humans are not available.

Neither metabolism of sodium [1-^{14}C]-perfluorohexanoate in rat or mouse hepatocytes was observed nor metabolites were detected after oral dosing in either rodent species (Gannon et al., 2011). It is concluded that sodium-perfluorohexanoate is a highly stable substance that is not metabolised to a detectable extent.

However, it is known that PFAS as precursors, such as 6:2 FTOH, are able to be transformed, i.e. in rat-, mouse- and human hepatocytes, to PFHxA, besides others (Russell et al., 2015). For further details about FTOHs as precursor for PFHxA, see EFSA Panel on Contaminants in the Food Chain (2020), Gannon et al. (2012) as well as (Russell et al., 2015).

Elimination

In animal studies it was shown that the primary route of elimination of PFHxA or APFHx is the renal excretion via urine. A minor route of elimination is via faeces. In humans, PFHxA was detected in urine (Hartmann et al., 2017; Kim et al., 2014).

More than 99% of the oral dose of sodium [1-^{14}C]-perfluorohexanoate was eliminated within 24 hours in rats (male and female) and in male mice and within 48 hours in female mice (Gannon et al., 2011). The excretion via faeces was negligible. Within 24 hours, after single and repeated (14 days) oral administration of ^{14}C-APFHx to male and female rats and mice, up to 90.2% of the single dose and up to 83.4% of the repeated dose were excreted with the urine, whereas up to 15.5% of the single dose and 12.9% of the repeated dose is excreted via faeces (Iwai, 2011). Sex specific differences were observed in elimination half-lives of rats, as female rats eliminated PFHxA about two to three times faster. Recently, in male rats a 1.6- to 3-fold higher PFHxA plasma concentration was observed compared to female rats at the end of the experiment (NTP, 2019) which may be attributed, at least in part, to sex specific differences in elimination kinetics described above.

The half-life of PFHxA in humans was estimated as 32 days by Russell et al. (2013) using monitoring data from Nilsson et al. (2010) on blood samples of ski wax technicians, who applied fluorinated ski wax containing PFHxA during ski season. A re-evaluation of the Nilsson-data estimated a serum elimination half-life of 5.1 days (Luz et al., 2019)

Across the species, the reported elimination half-life rates were slowest in humans (5.1-32 days), followed by domestic pig (4.1 days) (Numata et al., 2014), mouse (0.9-1.2 hours) (Russell et al., 2013) and rats (0.4-4.3 hours) reported by EFSA Panel on Contaminants in the Food Chain (2020). Elimination half-lives for PFHxA in mammals are lower compared to perfluorinated carboxylic acids with longer chain, such as PFOA, however, accumulation might take place depending on the frequency of exposure.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Evaluation not performed for this substance.

10.2 Acute toxicity - dermal route

Evaluation not performed for this substance.

10.3 Acute toxicity - inhalation route

Evaluation not performed for this substance.

10.4 Skin corrosion/irritation

Evaluation not performed for this substance.

10.5 Serious eye damage/eye irritation

Evaluation not performed for this substance.

10.6 Respiratory sensitisation

Evaluation not performed for this substance.

10.7 Skin sensitisation

Evaluation not performed for this substance.

10.8 Germ cell mutagenicity

Evaluation not performed for this substance.

10.9 Carcinogenicity

Evaluation not performed for this substance.

10.10 Reproductive toxicity

Data on PFHxA, APFHx and NaPFHx are used as the basis for this CLH proposal. PFHxA and its inorganic salts, are expected to dissociate in biological media. Hence, regardless whether the acid or the salt are administered, once absorbed into the blood, the PFHx-anion will be formed.

Table 14: Summary table of animal studies on adverse effects on sexual function and fertility and development

Method, guideline, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results			Reference
One-generation reproduction toxicity study **OECD TG 415, GLP** Key study, Klim. 1 (reliable without restriction) NaPFHx (100%) Rat (Crl:CD(SD)) ♂+♀, n/sex/group: 20 Exposure: Oral (gavage) Vehicle: NANOpure water Doses (analytic. verified): 0, 20, 100, 500 mg/kg bw/d, 70 days prior to cohabitation until weaning P0 ♀: Treatment approx. 126 days P0 ♂: Treatment approx. 110 days *Abbreviation used* *PND: Postnatal day*	**Parental generation (P0)**			(Loveless et al. 2009) REACH Registration APFHx (ECHA dissemination: Toxicity to reproduction 001 key study)
		♀	♂	
	General toxicity			
	Mortality	Mortality observed at 500 mg/kg bw/d • 5/20 (25%) on day 4 (1♀, hunched over prior to death, no pathological findings), on day 9 (1♀, dehydrated, stained fur, no pathological findings), on day 52 (1♀, mechanical trauma of paw), on day 84 (1♀, mechanical trauma of esophagus), on day 114 (1♀, signs of dehydration prior to death, no pathological findings), all reported as non-treatment related No mortality at ≤ 100 mg/kg bw/d No information on mortality of controls	Mortality observed at 20 mg/kg bw/d • 1/20 (5%) on day 23 (kidney, urinary bladder, and prostate abnormalities), reported as non-treatment related No mortality in ≥ 100 mg/kg bw/d No information on mortality of controls	
	Body weight	<u>Gestation period</u> Significant ↓ -30% bw gain at 500 mg/kg bw/d, 1st week of gestation ($p < 0.05$), transient <u>Lactation period</u> Significant ↑ bw gain at 500 mg/kg bw/d (lack of expected decrease in bw gain) ($p < 0.05$), transient **For data on bw see Table 16** Numerical data on litter size and pup weight are not available	Significant ↓ -12% bw gain at 100 mg/kg bw/d, ($p < 0.05$) Significant ↓ -29% bw gain at 500 mg/kg bw/d, ($p < 0.05$) **For data on bw see Table 16**	

Clinical signs	Stained skin/fur • 3/20 at 500 mg/kg bw/d • 0/20 at control	Stained skin/fur • 13/20 at 500 mg/kg bw/d • 0/20 at control
Reproductive function/performance		
Function	No effects on oestrous cycle No further information available	No effects on sperm measures No further information available
Performance	No effect on mating, fertility, gestation length and number of implantation sites Numerical data or reproductive indices are not available	No effects on mating and fertility Numerical data or reproductive indices are not available
Target system/organ toxicity		
Histopathological findings	No effects observed	No effects observed
F1 generation		
General toxicity		
Mortality	No effects observed	No effects observed
Body weight	<u>PND 0</u> • ↓ -11% mean bw at 100 mg/kg bw/d • Significant ↓ -18% mean bw at 500 mg/kg bw/d ($p < 0.05$) <u>Lactation period (PND 7, 14, 21)</u> • ↓ -5 to -11% mean bw at 100 mg/kg bw/d • Significant ↓ -17 to -18% mean bw at 500 mg/kg bw/d ($p < 0.05$)	
	<u>Postweaning day 0-39 (PND 21-60)</u> Mean bw • ↓ -12% mean bw at 500 mg/kg bw/d, during first week of post weaning • Mean bw at 500 mg/kg bw/d similar to control from day 14 postweaning (PND35) bw gain • No data on bw gain during the first week of postweaning • Overall bw gain (day 0-39 postweaning) at 500 mg/kg bw/d comparable to control **For further details on bw see Table 16**	<u>Postweaning day 0-39 (PND 21-60)</u> Mean bw • Significant ↓ mean bw at 500 mg/kg bw/d, throughout postweaning, ↓ -7 to -16% at PND 21-49, (p-value not reported) • Mean bw at 500 mg/kg bw/d similar to control from day 28 postweaning (PND 49) bw gain • ↓ -16% bw gain at 500 mg/kg bw/d, during the first week of postweaning • Overall bw gain (day 0-39 postweaning) at 500 mg/kg bw/d comparable to control **For further details on bw see Table 16**

	Organ weight in F1 adults	No effects observed	<u>Testes:</u> Significant ↓ in rel. testis weight (rel. to bw) • At 20 mg/kg bw/d (↓ -7%) • At 500 mg/kg bw/d (↓ -11%) (p-values not reported, original data not available, documentation insufficient) <u>Epididymides:</u> • Significant ↓ rel. (to bw) weights at 20 mg/kg bw/d • Significant ↓ rel. (to brain weight) weights at 100 mg/kg bw/d (p-values not reported, original data not available, documentation insufficient)	
	Clinical signs	No effects observed	No effects observed	
	Developmental toxicity			
		No test substance-related effects on litter size, sex ratio, pup survival at birth, sexual maturation or F1 adult developmental landmarks at any dose according to study authors. No numerical data or further details reported.		
Prenatal developmental toxicity study **OECD TG 414, GLP** Key study, Klim. 2 (reliable with restriction) NaPFHx (100%) Rat (Crl:CD(SD)) ♀, n/sex/group: 22 Exposure: Oral (gavage) Vehicle: NANOpure water Doses: 0, 20, 100, 500 mg/kg bw/d GD 6-20 *Abbreviation used* *GD: Gestation day*	**Parental generation (P0) - maternal**			(Loveless et al. 2009) REACH Registration APFHx (ECHA dissemination: developm. tox / teratogenicity, 002 key study)
	♀			
	General toxicity			
	Mortality	No mortality observed		
	Body weight	Significant ↓ mean bw at 500 mg/kg bw/d on GD 19, GD 20, GD 21 (p-values not reported) **For data see Table 17** Decrease in bw gain • ↓ -51% at 500 mg/kg bw/d on GD 12-14, • ↓ -30% at 500 mg/kg bw/d on GD 18-20, • ↓ -25% at 500 mg/kg bw/d on GD 20-21 Decrease in overall weight gain • ↓ -19% at 500 mg/kg bw/d on GD 6-21, Decrease in overall net weight gain (weight gain excluding the gravid uterus weight) • ↓ -26% at 500 mg/kg bw/d on GD 6-21 **For further details on bw see Table 17**	
	Clinical signs	No effects reported at 0, 20, 100 mg/kg bw/d • Effects observed in 1/22 animals at 500 mg/kg bw/d, transient		

		(nasal discharge, lung noise, and wet/stained skin/fur, which occurred concomitantly with periods of body weight loss and markedly decreased food consumption) No further information available.		
	Reproductive performance/ maternal developmental toxicity			
		No effects observed with regard to number of abortions, pre- and post-implantation loss, total litter losses by resorption, early or late resorptions, dead foetuses, pregnancy duration. Numerical data or reproductive indices are not available. Changes in number of pregnant was not examined.		
	Target system/organ toxicity			
	Histopathological findings	Not examined		
	F1 generation			
	♀+♂			
	General toxicity			
	Viability	No effects observed		
	Body weight	Decrease in mean bw • ↓ -10% at 500 mg/kg bw/d **Details on bw see Table 17**		
	Litter size	Litter size was not reported Changes in litter size and weights were not examined		
	Clinical signs	Not reported		
	Developmental toxicity			
	Sex	No effects observed with regard to changes in sex ratio		
	Malformations	The number and type of variations observed (no details reported) were similar for all groups and were common to this strain and developmental age according to study authors. No treatment related malformations according to study authors.		
Combined RDT study with reproduction/developmental toxicity screening test **OECD TG 422, GLP** Key study, Klim. 1 (reliable without restriction)	**Parental generation (P0)**			(WIL Research Laboratories, 2005)
		♀	♂	
	General toxicity			
	Mortality	Effects observed, treatment related 6/15 (40%) at 450/300 mg/kg bw/d until scheduled necropsies (deaths/moribundity of 4 ♀ until day 4)	Effects observed, treatment related 5/15 (33%) at 450/300 mg/kg bw/d until scheduled necropsies (deaths/moribundity of 4 ♂ until day 4)	

PFHxA (98.5%)

Rat (SD)
♂+♀, n/sex/group: 10

Exposure: Oral (gavage),
Vehicle: Deionized water, no pH adjustment reported
Doses: 0, 50, 150, 450/300* mg/kg bw/d

Dosing regimen:
♀: 14 daily doses prior to pairing; dosed through lactation day 3; total of 39-44 doses; euthanized on lactation day 4.
Females with no evidence of mating or that failed to deliver dosed for total of 39-52 doses.

♂: 14 daily doses prior to mating, dosed throughout mating period, until day prior to euthanasia; total of 32-34 doses.

Recovery:
14 d non-dosing period at end of treatment period (then , euthanized), ♀ not used for mating
♂+♀, n/sex/group: 5
Doses: 0, 450/300* mg/kg bw/d
♀ total of 40 doses
♂ total of 35 doses

*reduced on day 4 from 450 to 300 mg/kg bw/d due to ↑ mortality within the first 4 days of dosing

Abbreviation used
GD: Gestation day

	For details on cases and stated cause of death see Table 21	**For details on cases and stated cause of death see Table 21**
Body weight	<u>Mating period</u> Decrease in mean bw at 450/300 mg/kg bw/d • Significant ↓ -7.6% on day 4 (p<0.01) • ↓ -3.9% on day 7 No effects on mean bw in ≤150 mg/kg bw/d <u>Gestation</u> Mean bw • ↓ -5.8% mean bw at 450/300 mg/kg bw/d on GD 20 • Mean bw in 450/300 mg/kg bw/d similar to control on GD 0-17 • No effects on mean bw in ≤150 mg/kg bw/d bw gain • Significant ↓ bw gain at 450/300 mg/kg bw/d on GD17-20 (p <0.01, no further data available) - No effects on bw gain in ≤150 mg/kg bw/d <u>Lactation</u> No effects on mean bw and bw gain at any dose	Decrease in mean bw at 450/300 mg/kg bw/d • Significant ↓ -10.3% on day 4 (p<0.01) • ↓ -7.8% on day 13 • ↓ -7.5% on day 32 Mean bw in 450/300 mg/kg bw/d comparable to control on day 49 in recovery group No effects at ≤150 mg/kg bw/d
	Note: Treatment-related lower mean food consumption observed in ♀ (significant, p<0.01) and ♂ (significant, p-value not reported) at 450/300 mg/kg bw/d during day 0-7 correlates to bw loss during day 0-4 in ♀ and ♂. No further details available.	
Clinical signs	Effects observed, treatment related, reversible **For further information on clinical observations, clinical chemistry, haematology, hepatic parameters, see Table 21**	
Reproductive function/performance		
Performance	No treatment related effects on performance Reproductive performance indices	

	Mating ♀&♂	Fertility ♀&♂	♀ conception	♂ copulation
Control	100%	100%	100%	100%
50 mg/kg bw/d	90%	90%	100%	100%
150 mg/kg bw/d	100%	90%	90%	90%
450/300 mg/kg bw/d	100%	100%	100%	100%

PND: Postnatal day *Data tables (summary data, individual data) and historical control data were not available*			
		Precoital interval: No effects observed in any treatment group compared to control <u>Gestation length and parturition</u> Mean gestation length**:** No effects observed in any treatment group compared to control Dystocia: No	
	Target system/organ toxicity		
	Histopathological findings	<u>Adrenal cortex</u> Hyperplasia in zona fasciculata at 450/300 mg/kg bw/d in 2/4 ♀ that died /were euthanized until day 5 No effects in animals examined at scheduled necropsy (end of treatment period) **For further pathological findings see Table 21**	
	F1 generation		
	♀+♂		
	General toxicity		
	Viability/mortality	No effects on viability No effects on postnatal survival Number of pups (litters) found dead during PND 0-4: 6(4) in control, 6(6) at 50 mg/kg bw/d, 7(3) at 150 mg/kg bw/d , 4(3) at 450/300 mg/kg bw/d	
	Body weight	No effects on pup bw and bw changes observed (no values available)	
	Litter size	Mean number of pups born • At 450/300 mg/kg bw/d: 15.2 per dam (reduced compared to control, not reduced compared to historical control) • At ≤150 mg/kg bw/d : unaffected (stated by study author, no data available) • In control: 17.1 per dam • In historical control: 14.5 per dam (stated by study author, no data available) Live litter size • At 450/300 mg/kg bw/d: 14.8 per dam (reduced compared to control, not reduced compared to historical control) • At ≤150 mg/kg bw/d : unaffected (stated by study author, no data available) • In control: 16.9 per dam • In historical control: 14.2 per dam (stated by author, no data available)	
	Developmental toxicity		
	Sex	No effects observed with regard to changes in sex ratio	
	Other findings	No treatment related findings at scheduled pup necropsies (PND4) or necropsies of pups found dead	

Reproductive and developmental toxicity
Study phase I

No guideline, GLP

Key study, Klim. 1 (reliable without restriction)

APFHx (93.4%)

Mouse (Crl: CD I (ICR))
♀, n/sex/group: 20

Exposure: oral (gavage)
Vehicle: deionized water
Doses: **0, 100, 350, 500** mg/kg bw/d
daily, GD 6-18

F1 was not directly exposed and observed until PPD 20 or PPD 41

Abbreviation used
GD: Gestation day
PPD: Day postpartum period

Note: The study was designed to evaluate ICH Harmonised Tripartite Guideline stages C through F of the reproductive process and to detect effects on gestation, parturition, lactation and maternal behaviour in female mice, and on the development of the offspring of the treated female mice.

Parental generation (P0)

♀

General toxicity

Mortality

Mortality observed
3/20 (15%) in control (n=1 at lactation day 14, n=2 at lactation day 16),
6/20 (30%) at 100 mg/kg bw/d (4/1/1 at lactation day 13/14/16, respectively),
1/20 (5%) at 350 mg/kg bw/d (n=1 at gestation day 13),
3/20 (15%) at 500 mg/kg bw/d (n=1 at gestation day 8, n=2 at lactation day 13)

Body weight

Effects observed

Gestation
Mean bw: No effects observed
bw gain: No effects observed

Lactation
Mean bw: No effects observed

bw (g)	Mean±S.D.			
	Control	100 mg/kg bw/d	350 mg/kg bw/d	500 mg/kg bw/d
PPD 0	34.0 ± 1.8	34.9 ± 2.1	34.5 ± 3.0	35.3 ± 3.0
PPD 1	35.3 ± 2.4	36.2 ± 2.2	35.0 ± 2.4	35.8 ± 2.5
PPD 2	36.6 ± 3.1	37.8 ± 2.3	36.5 ± 2.7	36.8 ± 2.6
PPD 3	38.0 ± 3.4	39.5 ± 2.6	37.2 ± 2.8	37.2 ± 2.3
PPD 4	39.8 ± 3.2	40.3 ± 2.8	37.9 ± 3.4	38.4 ± 2.3

bw gain on PPD 0-4:
• Significant ↓ bw gain: ↓ -33% at 350 mg/kg bw/d ($p \leq 0.05$) and ↓ -55% at 500 mg/kg bw/d ($p \leq 0.01$)

bw changes (g)	Mean±S.D.			
	Control	100 mg/kg bw/d	350 mg/kg bw/d	500 mg/kg bw/d
PPD 0-4	+5.7 ± 2.1	+5.4 ± 1.8	+3.8 ± 2.6*	+2.8 ± 2.0**
PPD 4-7	+3.3 ± 2.1	+3.8 ± 1.8	+3.9 ± 2.5	+2.4 ± 2.0
PPD 7-14	+2.9 ± 3.3	+3.3 ± 1.6	+3.7 ± 3.0	+5.3 ± 1.7
PPD 14-20	-2.9 ± 4.7	-4.2 ± 2.2	-1.8 ± 3.6	-5.1 ± 4.5
PPD 0-20	+8.7 ± 2.7	+8.5 ± 1.9	+9.6 ± 4.8	+5.6 ± 4.2

* Significantly different from the control group value ($p \leq 0.05$)
** Significantly different from the control group value ($p \leq 0.01$)

Clinical signs

Excess salivation during gestation period
• Slight in 3/20 at 350 mg/kg bw/d
• Slight to moderate in 6/20 at 500 mg/kg bw/d

(Charles River Laboratories, 2011a)

(Charles River Laboratories, 2012) (report amendment)

(Iwai and Hoberman, 2014)

Reproductive function/performance

Performance

	Pregnant	Litters delivered	Dams with stillborn pups
Control	19/20	19/19	2/19 (10%)
100 mg/kg bw/d	19/20	19/19	0/19 (0%)
350 mg/kg bw/d	20/20	19/20	5/19 (26%)
500 mg/kg bw/d	18/20	17/18	7/17 (41%)

Dams with all pups dying in PPDs 0-3 (complete litter loss):
• 1/19 (5%) in control,
• 0/19 (0%) at 100 mg/kg bw/d,
• 2/19 (10.5%) at 350 mg/kg bw/d,
• 5/17 (31%) at 500 mg/kg bw/d

Dams with all pups dying in PPDs 4-20 (complete litter loss):
none

Duration of gestation (mean ± S.D.)
• 19.9 ± 0.6 in control,
• 19.9 ± 0.2 at 100 mg/kg bw/d,
• 19.9 ± 0.6 at 350 mg/kg bw/d,
• 20.2 ± 1.1 at 500 mg/kg bw/d

Duration of gestation > 20 days
• 1/19 duration of 22 days in control,
• 1/19 duration of 22 days at 350 mg/kg bw/d,
• 2/17 duration of 22 days and 1/17 gestation length of 23 days at 500 mg/kg bw/d

For further details on litters, see Table 19

Target system/organ toxicity

Histopathological findings

Necropsy observations
• 1/20 sternum bent proximal to xiphoid process in control,
• 1/20 sternum bent proximal to xiphoid process at 100 mg/kg bw/d,
• 1/20 liver lobe tan area at 350 mg/kg bw/d,
• 5/20 liver lobe tan area, 1/20 intestines distended with gas at 500 mg/kg bw/d

F1 generation

♀+♂

General toxicity

Viability/mortality

Effects observed, treatment related

<u>Vital status</u>
Stillborn pups: Significant ↑ at 500 mg/kg bw/d ($p \leq 0.01$)

Viability

• Significant ↓ at 500 mg/kg bw/d (p≤0.01) on PPD 0
• Significant ↓ in at 350 mg/kg bw/d (p≤0.01) and at 500 mg/kg bw/d (p≤0.01) on PPD 1-4
• Significant ↓ in at 350 mg/kg bw/d (p≤0.01) and at 500 mg/kg bw/d (p≤0.01) on PPD 0-7

	Day 4 viability index[a]	Day 7 viability index[b]
Control	215/217 (99.1%)	214/217 (98.6%)
100 mg/kg bw/d	247/250 (98.8%)	246/250 (98.4%)
350 mg/kg bw/d	204/232 (87.9%)	201/232 (86.6%) (sign., p≤0.05)
500 mg/kg bw/d	109/150 (72.7%)**	109/150 (72.7%)**

[a]Number of live pups on PPD 4/number of liveborn pups on PPD 0
[b]Number of live pups on PPD 7/number of liveborn pups on PPD 0
** Significantly different from the control group value (p≤0.01)

Significant ↑ number of mice with all pups dying on PPD 0 to 3 at 500 mg/kg bw/d compared to control (p ≤ 0.01)

For further details on F1 observations, see Table 19

Body weight

Effects observed, treatment related

PPD 0
• Significant ↓ mean bw in all treatment groups (p≤0.01)
PPD 4
• Significant ↓ mean bw at 350 mg/kg bw/d and at 500 mg/kg bw/d (p≤0.01)
PPD 7
• Significant ↓ mean bw at 350 mg/kg bw/d (p≤0.01)
PPD 20
• ↓ mean bw: Average pup weights/litter were 89% (at 100 mg/kg bw/d), 80% (at 350 mg/kg bw/d) and 88% (at 500 mg/kg bw/d) of the control group value
PPD 21
• ♂ and ♀: Significant ↓ mean bw: at 100 mg/kg bw/d and 350 mg/kg bw/d (p≤0.05)
PPD 28
• ♀: Significant ↓ mean bw: at 100 mg/kg bw/d and 350 mg/kg bw/d (p≤0.05 to p≤0.01)
PPDs 28-35
• ♂: ↑ bw gain at 100 mg/kg bw/d and 350 mg/kg bw/d (p≤0.05 to p≤0.01)
PPD 35 and 41
• ♀: ↓ mean bw: at 350 mg/kg bw/d (p≤0.05)

For details on F1 observations, see Table 19

	Litter size	Effects observed At birth and throughout the lactation period • ↓ 500 mg/kg bw/d PPD 4 • Significant ↓ at 500 mg/kg bw/d ($p \leq 0.05$) **For further details on litters, see Table 19**	
	Developmental toxicity		
	Sex	No effects on sex ratio observed	
	Other findings	<u>Physical development</u> Eye opening delay at 350 mg/kg bw/d and at 500 mg/kg bw/d observed on PPD 14 <u>Sexual maturation</u> No effects on preputial separation or vaginal patency <u>Liver</u> Liver weight to terminal bw (ratio): Not affected	
Reproductive and developmental toxicity Study phase II **No guideline, GLP)** Key study, Klim. 1 (reliable without restriction) APFHx (93.4%) Mouse (Crl: CD I (ICR)) ♀, n/sex/group: 20 Exposure: Oral (gavage) Vehicle: Deionized water Doses: **0, 7, 35, 175 mg/kg bw/d** daily, GD 6-18	**Parental generation (P0)**		(Charles River Laboratories, 2011b) (Iwai and Hoberman, 2014)
	♀		
	General toxicity		
	Mortality	No treatment-related effects observed Sacrificed: 1/20 at 7 mg/kg bw/d on GD 17 at delivery of litter, 1/20 at 35 mg/kg bw/d on day 2 of lactation due to no surviving pups	
	Body weight	No effects observed (mean bw and bw gain during gestation and lactation period in all groups examined)	
	Clinical signs	No treatment-related effects observed	
	Reproductive function / performance		
	Performance		

	Pregnant	Litters delivered	Dams with stillborn pups
Control	20/20	20/20	0/20 (0%)
7 mg/kg bw/d	17/20	17/20	0/17 (0%)
35 mg/kg bw/d	20/20	20/20	0/20 (0%)
175 mg/kg bw/d	20/20	20/20	1/20 (5%)

Dams with all pups dying in PPDs 0-3:

Abbreviation used
GD: Gestation day
PPD: Day postpartum period

Note: The study was designed to evaluate ICH Harmonised Tripartite Guideline stages C through F of the reproductive process and to detect effects on gestation, parturition, lactation and maternal behaviour in female mice, and on the development of the offspring of the treated female mice.

	• 1/17 (5.9%) at 7 mg/kg bw/d • None in control • None at ≥ 35 mg/kg bw/d Dams with all pups dying in PPDs 4-20: • None Duration of gestation (Mean ± S.D.) • Control: 19.6 ± 0.5 • 7 mg/kg bw/d: 19.8 ± 0.8 • 35 mg/kg bw/d: 19.8 ± 0.4 • 175 mg/kg bw/d: 19.7 ± 0.5 Duration of gestation > 20d • None **For further details on litters, see Table 18**
Target system/organ toxicity	
Histopathological findings	<u>Necropsy observations</u> • Control: Normal appearance • 7 mg/kg bw/d: 1/20 all lobes of liver, numerous clear fluid-filled cysts; 1/20 both horns of uterus, walls, thick • 35 mg/kg bw/d: 1/20 left kidney, surface of capsule, clear fluid-filled cyst; 1/20 both horns of uterus, clear fluid-filled cysts • 175 mg/kg bw/d: Normal appearance
F1 generation	
♀+♂	
General toxicity	
Mortality	Effects observed, treatment related <u>Vital status</u> Stillborn pups: Significant ↑ at 175 mg/kg bw/d (p≤0.01), 3/241 (↑ +1.2%, compared to control 0/249) <u>Viability</u> Significant ↓ at 175 mg/kg bw/d on PPD 1 (p≤0.01), 4 of 238 pups died until PPD 1 (↓ -1.7%)

	Day 4 viability index[a]	Day 7 viability index[b]
Control	246/249 (98.8%)	245/249 (98.4%)
7 mg/kg bw/d	205/211 (97.2%)	205/211 (97.2%)
35 mg/kg bw/d	230/232 (99.1%)	230/232 (99.1%)
175 mg/kg bw/d	231/238 (97.0%)	228/238 (95.8%)

[a]Number of live pups on PPD 4/number of liveborn pups on PPD 0
[b]Number of live pups on PPD 7/number of liveborn pups on PPD 0

	Lactation index (Number of live pups on PPD 20/no. of live pups on PPD 4) • Significant ↓ at 7 mg/kg bw/d (p≤0.01) **For further details on F1 observations see Table 18**	
Body weight	Effects observed, treatment related • Significant ↓pup weight at 175 mg/kg bw/d (p≤0.05) on PPD 0	
Litter size	No effects observed	
Developmental toxicity		
Sex	No effects on sex ratio observed	
Other findings	Sexual maturation and day of eye opening unaffected Eyes • Corneal opacity: n=2 (1 pup in each of two litters) at 175 mg/kg bw/d • Microphthalmia: n=2 (1 pup in each of two litters) at 175 mg/kg bw/d • Lenticular opacity: n=1 at 175 mg/kg bw/d	

Repeated dose toxicity (RDT)-studies containing relevant information about the effects on sexual function and fertility

RDT 28-day study **Similar to OECD TG 407, GLP** Klim. 1 (reliable without restriction) PFHxA (>99%) Rat (SD) ♂+♀, n/sex/group: 10 Exposure: Oral (gavage) Vehicle: Deionized water with 2% Tween 80, pH adjusted to between 6 and 8 Doses*: 0, 62.5, 125, 250, 500, 1 000 mg/kg bw/d daily for 28 days		♀	♂	(NTP, 2019)
	General toxicity			
	Mortality	No effects observed (all rats survived until the end of the study)		
	Body weight	No effects observed (within 10% of vehicle control bw)	Effects observed, treatment related Decrease in mean bw at 1 000 mg/kg bw/d •↓ -12% on day 22 •↓ -13% on day 29	
	Clinical signs	Clinical observations No treatment related effects	Clinical observations No treatment related effects	
	Reproductive toxicity			
	Function	No apparent effects on oestrous cyclicity *Note: Vehicle control rats did not cycle as expected (mean length of 7.2 days versus normal length of ~4.5 days; disproportionately more time*	Effects on sperm parameters observed Epididymal sperm counts Significant ↓ -25% cauda epididymal sperm counts at 1 000 mg/kg bw/d (p ≤ 0.01) (occurred in the presence of a slight decrease in epididymal weight of 5%) ↓ -18% at 1 000 mg/kg bw/d when normalised to total sperm count/g of cauda epididymis	

*twice daily at one-half dose (total): 31.3 (62.6), 62.5 (125), 125 (250), 250 (500), 500 (1 000) mg/kg bw/d		*spent in dioestrus and less time in oestrus); the PFHxA-treated females cycled as would generally be expected. No conclusions drawn.*	<u>Sperm motility</u> Not affected <u>Spermatid counts</u> Not affected **For reproductive tissue evaluation data see Table 15**	
	Target system	No effects observed <u>Clinical chemistry</u> No effects (thyroid stimulating hormone, total thyroxine, free thyroxine, total triiodothyronine and testosterone) <u>Histopathology</u> No effects <u>Target organ weights</u> No effects	Effects observed <u>Clinical chemistry</u> Significant changes at ≥ 62.5 mg/kg bw/d: • ↓ total thyroxine ($p \leq 0.01$) • ↓ free thyroxine ($p \leq 0.01$) • ↓ total triiodothyronine ($p \leq 0.05$ at 62.5 mg; $p \leq 0.01$ at ≥125 mg) No effects observed with regard to thyroid stimulating hormone and testosterone <u>Histopathology</u> Thyroid gland: No changes Testes: No effect on germinal epithelium, interstitial cell and seminiferous tubule (spermatid retention) Epididymis: No hypospermia, no effect on duct (exfoliated germ cell) and epithelium <u>Target organ weights</u> No effects	
	For further information, see Table 21 on specific target organ toxicity			

Notes: ♂ = male; ♀= female; ↓ = decrease; ↑ = increase; GD = Gestation day; Klim. = Klimisch score; PND = Postnatal day; PPD = Day postpartum period; RDT = repeated dose toxicity; rel. = relative; PFHxA = undecafluorohexanoic acid; APFHx = ammonium undecafluorohexanoate; NaPFHx = sodium undecafluorohexanoate

Human data on reproductive toxicity

Human epidemiological studies on perfluorinated alkyl substances including perfluorohexanoic acid (PFHxA) are rare. The literature search identified two cross-sectional epidemiological studies investigating the association of perfluorinated alkyl substances including PFHxA in human blood serum and sex hormone levels or thyroid markers (Li et al., 2017; Zhou et al., 2016).

Li et al. (2017) investigated the association between eight PFAS and thyroid hormones in serum of the general population in southern China (n=202). No correlation between TSH, free T4 or T3 levels and exposure to PFHxA was found. Exposure to PFHxA was positively associated with two biomarkers of thyroid autoimmune disease – thyroglobulin antibody (TGAb) and thyroid microsomal antibody (TMAb). Relevant information regarding reproductive effects of PFHxA cannot be deduced from these results. Furthermore, exposure to multiple perfluorinated alkyl substances and assuming that perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) accounted for approximately 70%-90% of the total sum of perfluorinated alkyl substances in serum limits the information value with regard to PFHxA. Thyroid effects due to PFHxA exposure cannot be concluded beyond reasonable doubt.

Zhou et al. (2016) investigated the correlation between serum levels of nine perfluorinated alkyl substances and sex hormone levels (e.g., testosterone and estradiol) in Taiwanese teenagers (n=225). An inverse relationship between serum PFHxA and testosterone levels was identified in Taiwanese boys, while no association between PFHxA and estradiol was found for girls. Potential confounding factors such as puberty indicators and diurnal cyclicity, both of which can influence sex hormone levels were not accounted for in the study analysis. Thus a definitive association between PFHxA exposure and sex hormone levels is not demonstrated.

Methodological weaknesses and co-exposures to other perfluorinated alkyl substances limit the value of the both mentioned human epidemiological studies for classification purposes. Thus, the identified epidemiological studies are not taken into further consideration for reproductive toxicity in this report. However, please note that overall concerns are raised with regard to effects on human health due to the co-exposure to other similar PFASs, potential additive or synergistic effects with other chemicals, and the ability for wide-spread tissue distribution via protein binding in blood serum.

There is no information available on developmental effects in humans.

10.10.1Adverse effects on sexual function and fertility

One-generation reproduction toxicity study in rats (OECD TG 415; key study, Klimisch score: 1; Loveless et al. (2009))

In a one-generation reproduction toxicity study in Crl:CD(SD) rats, groups of 20 animals/sex were dosed by oral gavage with NaPFHx (purity 100%) at a dose of 0, 20, 100, or 500 mg/kg bw/d. Females were dosed approximately 70 days prior to cohabitation, through gestation and lactation for a total of approximately 126 days. Male rats were exposed for a total of approximately 110 days. F1 rats were not dosed. The rats were 6-8 weeks old at study initiation and 16-18 weeks old at mating.

Clinical observations, body weight, and food consumption were recorded weekly throughout the study.

Oestrous cycle, sperm parameters, survival, and reproductive performance parameters were examined.

Regarding the litter, number of live and dead pups, individual pup weights and, clinical observations were assessed on day four, and weekly during the lactation period. At weaning, a gross pathological examination of F1 offspring was performed. A subset of F1 generation rats was maintained for six weeks after weaning to assess developmental landmarks. Gross pathological examination was performed and selected reproductive organs were weighed.

There were no substance related effects observed on mating, fertility, gestation length, number of implantation sites, oestrous cyclicity, sperm parameters and litter size, sex ratio, pup clinical observations, pup survival, or F1 adult developmental landmarks at any dose tested.

Mortality observed in 5/20 parental animals was reported as non-treatment related (see Table 14). Two deaths were reported to be attributed to mechanical trauma.
Clinical signs of toxicity included stained skin/fur in males and females at 500 mg/kg bw/d.
Treatment-related effects on body weight parameters were reported. In P0 males, changes in body weight parameters were observed at 100 and 500 mg/kg bw/d. The overall bodyweight gain was reduced by 12% and 29%, respectively, as compared to the control group. In P0 females reductions in mean maternal body weight gain by 30% were shown at 500 mg/kg bw/d during the first but not subsequent weeks of gestation. During the lactation period, expected reduced body weight gain was not observed at 100 and 500 mg/kg bw/d. P0 females at 100 and 500 mg/kg bw/d gained an average of 20 g and 25 g, respectively, throughout lactation, compared to an average gain of 5 g in the control group.

Treatment-related effects on body weight parameters were reported in F1 rats and indicate foetal and postnatal toxicity. For further details, please refer to Table 16 and section 10.10.4.
In the robust study summary it is stated that in F1 adult males "testes weight and relative testis weight (relative to bw) were decreased by 7% and 11% (statistically significant) in the 20 and 500 mg/kg bw/d group, respectively, compared with the control." No statistically significant changes in testes weight in the 100 mg/kg bw/d group were observed. Histopathological examination in F1 males was not performed. The data documentation is scarce and original data are not available to the dossier submitter. Overall, this reduces the validity of the testicular weight data.
NaPFHx may target the testes. In comparison to control values, body weights were significantly reduced in the 500 mg/kg bw/d group throughout postweaning and body weight gain was lower during the first week of postweaning. Therefore, an indirect effect of lower body weight parameters compared to controls as the cause of the changes in testes weights cannot be ruled out.

<u>Prenatal developmental toxicity study in rats (OECD TG 414; key study, Klimisch score: 1; Loveless et al. (2009))</u>
In a prenatal developmental toxicity study in Crl:CD(SD) rats, groups of 22 females were given by gavage NaPFHx (purity 100%) at a concentration of 0, 20, 100, or 500 mg/kg bw/d on gestation days (GD) 6-20 (Loveless et al., 2009). In-life observations were performed twice daily on mortality/viability and clinical signs, once daily on body weight, and every other day on food consumption. Rats were sacrificed on GD 21. All dams underwent a gross external and visceral examination and the foetuses were removed from the uteri by caesarean section. The ovaries and uterine content was examined after termination, including examination of gravid uterus weight, number of corpora lutea, implantations, as well as early and late resorptions. Foetuses were weighed, sexed and examined for morphological alterations. All foetuses were examined for external and skeletal alterations, and approximately 50% of the foetuses were examined for soft tissue and visceral head examinations.

No treatment related effects on reproductive parameters were observed in the parental generation.

NaPFHx-related maternal effects occurred at 500 mg/kg bw/d and consisted of changes in body weight parameters (see Table 17). As a result of decreases in weight gain over time, the overall weight gain (GD 6-21) and overall net weight gain (GD 6-21), weight gain minus the gravid uterus weight) were 19% and 26% lower than controls, respectively. The mean body weight gain was 51% lower

than controls on GD 12-14, as well as 30% and 25% lower than controls on GD 18-20 and 20-21, respectively.
Dams of the 500 mg/kg bw/d treatment group had foetuses with ~10% lower foetal body weight compared to controls.
No substance-related deaths or gross post-mortem findings in dams at any dose were reported.

Treatment-related effects on body weight parameters were reported in F1 rats. For further details, please refer to Table 17 and section 10.10.4.

Combined repeated dose toxicity study with reproduction/developmental toxicity screening test in rats (OECD TG 422; key study, Klimisch score: 1; (WIL Research Laboratories, 2005))

In a combined 28-day repeated dose toxicity study with reproduction/developmental toxicity screening (WIL Research Laboratories, 2005) groups of male and female rats were given by gavage PFHxA (purity 98.5 %) at a dose of 0, 50, 150 or 450/300 mg/kg bw/d. The study was conducted in accordance with the OECD TG 422 but omitting the functional observational battery and motor activity observations (the study presented was conducted as preliminary study to a subchronic study which includes functional observations). Male rats were dosed for 14 days prior to mating, throughout mating until the day prior to euthanasia (total of 32-34 doses). Females were dosed through 14 days prior to mating, throughout mating and pregnancy until lactation day four (total of 39-44 doses); females with no evidence of mating/that failed to deliver were dosed through the day prior to euthanasia (post-mating or post-cohabitation day 25) for a total of 39-52 doses. Due to excessive toxicity noted at 450 mg/kg bw/d PFHxA within the first 4 days of dosing, the dosage level was lowered to 300 mg/kg bw/d on day four. The concurrent control group received the vehicle (deionized water) on a comparable regime. The control group as well as the high-dose group each consisted of 15 animals/sex and group. The low-dose and mid-dose group each consisted of 10 animals/sex and group.
All animals were observed twice daily for mortality and moribundity. Clinical observation, body weight and food consumption were recorded at regular intervals. F_1 clinical observations and body weights were recorded on postnatal days one and four. Clinical pathology evaluations on haematology and serum chemistry were performed on five animals/sex/group during study week four (reproductive phase males) and on lactation day four (reproductive phase females) and on all remaining animals during study week seven (recovery phase males and females). Males and females assigned to the reproductive phase were euthanized following a minimum of 28 doses and on lactation day four, respectively. Animals assigned to the recovery phase were euthanized following completion of the 14-day recovery period. F1 pups were necropsied on postnatal day four.
Complete necropsies were conducted on all animals, and selected organs were weighed. Selected tissues were examined microscopically from all animals in the control and high-dose groups, and all animals that were found dead or euthanized in extremis. Additionally, target organs (liver, kidneys, mandibular and mesenteric lymph nodes, thymus, spleen, glandular and non-glandular stomach, pancreas, sternal bone marrow and adrenal cortex) were examined microscopically in the low and mid-dose groups and the recovery animals.

No treatment-related effects on reproductive performance, such as gonadal function, mating behaviour, conception and parturition was observed at dosage levels up to 450/300 mg/kg bw/d. Precoital interval and gestation length were unaffected by PFHxA treatment at all dosage levels.

Treatment-related systemic toxicity in adults (P0) was shown at 450/300 mg/kg bw/d as evidenced by mortality, reduced body weight, changes in the clinical conditions, haematology, serum chemistry, macroscopic findings, increased absolute and relative liver weights, and microscopic changes (for

details see section 10.12). At 150 mg/kg bw/d, effects were observed on the liver in males and females and on serum cholesterol levels in males only.

Repeated dose 28-day oral toxicity study in rats (NTP; key study, Klimisch score: 1; (NTP, 2019))

In a subacute toxicity study Sprague Dawley (SD) rats received PFHxA (purity > 99%) via gavage, seven days per week for 28 days (NTP, 2019). PFHxA was administered twice daily at one-half the dose for total daily doses of 0, 62.6, 125, 250, 500, or 1 000 mg/kg bw. The control animals received the vehicle (deionized water with 2% Tween 80) only. All dose groups consisted of 10 male and 10 female rats. Rats were observed twice daily. Animals were weighed and clinical findings were recorded on day one, weekly thereafter, and at the end of the studies. For vaginal cytology evaluations, samples were collected for 16 consecutive days prior to the end of the study from females in the 0, 125, 250, and 500 mg/kg/d dose groups. Oestrus cycle stages and oestrus cycle length were evaluated at the end of the study, blood was collected for haematology, clinical chemistry, thyroid hormone and testosterone analyses. Sperm samples were collected from 0, 250, 500, and 1 000 mg/kg/d male rats and evaluations on spermatid heads per testis and per gram testis, spermatid counts, and epididymal motility and concentration were performed. Samples were collected from the median liver lobe of male rats for determination of acyl-CoA oxidase activity and from the left liver lobe of all rats for determination of Acox1, Cyp4a1, Cyp2b1, and Cyp2b2 at study termination. Necropsies were performed on all rats. The organs (right adrenal gland, heart, right kidney, liver, lung, spleen, right testis, thymus, thyroid gland, and uterus/cervix/vagina) from each animal were weighed. Histopathologic examinations were performed on all rats.
No mortality was observed. The mean body weight was unaffected by PFHxA treatment in females. In the 1 000 mg/kg bw/d group, the mean body weight of males was significantly lower (13%) in comparison to the control group.
Cauda epididymal sperm counts in male rats were significantly lower (25%) than controls in the highest dose group and occurred in the presence of a slight decrease in epididymal weight (5%) (Table 15). When the authors normalized to total sperm count/g of cauda epididymis, the counts/g were 18% lower than controls.
Testis weights and spermatid counts in the PFHxA-treated rats were similar to control animals. Seminiferous tubule spermatid retention of the testis was observed in two rats administered 1 000 mg/kg/d and in one rat of the control group. Testosterone levels in males administered PFHxA were similar to those of the controls.

Oestrus cycle stages and oestrus cycle length were evaluated in females in the 0, 125, 250, and 500 mg/kg bw/d groups. PFHxA-treated females cycled as would generally be expected unlike the vehicle control rats showing extended mean cycle length of 7.2 days (versus normal length of ~4.5 days) and disproportionately more time spent in dioestrus and less time in oestrus than expected.
There were no apparent PFHxA-related changes in female testosterone levels.

Table 15: Reproductive tissue evaluation data (mean ± standard error) for PFHxA-treated male rats. ((NTP, 2019)

	Control	250 mg/kg/d	500 mg/kg/d	1 000 mg/kg/d
n	10	10	10	10
Weights (g)				
Necropsy body wt.	331 ± 5	327 ± 5	319 ± 7	287 ± 8**
L. Cauda epididymis	0.190 ± 0.005	0.195 ± 0.005	0.186 ± 0.005	0.176 ± 0.005
L. Epididymis	0.527 ± 0.010	0.523 ± 0.013	0.524 ± 0.011	0.496 ± 0.014
L. Testis	1.814 ± 0.035	1.821 ± 0.028	1.851 ± 0.057	1.798 ± 0.043
Spermatid measurements				
Spermatid heads (10^6/testis)	377.0 ± 7.9	356.5 ± 11.0	376.0 ± 11.6	352.3 ± 12.4
Spermatid heads (10^6/g testis)	208.3 ± 4.8	195.9 ± 5.4	203.9 ± 6.1	196.0 ± 5.0
Epididymal spermatozoal measurements				
Sperm motility (%)	87.9 ± 0.4	87.8 ± 0.3	87.2 ± 0.4	87.4 ± 0.4
Sperm counts (10^6/cauda epididymis)	111.9 ± 8.4	102.5 ± 5.2	105.1 ± 4.3	83.7 ± 4.7**
Sperm counts (10^6/g cauda epididymis)	586.4 ± 34.9	526.7 ± 25.8	566.4 ± 20.2	478.3 ± 28.2

**Significantly different ($p \leq 0.01$) from the vehicle control group by Williams' or Dunnett's test (weights) or Shirley's or Dunn's test (epididymal spermatozoal measurements).

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

In a one generation reproductive toxicity study with NaPFHx in rats, no substance-related effects were observed on mating, fertility, gestation length, number of implantation sites, oestrous cyclicity, sperm parameters or litter size. Alterations of the male reproductive system are indicated by decreases in F1 adult male testes weight and relative testis weight. However, due to limited and insufficiently reported data and potential indirect effects of reduced body weight, specific effects on fertility cannot be concluded here (Loveless et al. (2009).

Alterations of the male reproductive system are also described in a repeated dose 28-day oral toxicity study in rats. Cauda epididymal sperm counts in male rats administered 1 000 mg PFHxA/kg bw/d were significantly lower (25%) than vehicle controls and occurred in the presence of a slight decrease in epididymal weight (5%). Sperm motility and spermatid counts were not affected. Testis weights were similar to control animals (NTP, 2019).

In a guideline study on prenatal developmental toxicity of NaPFHx in rats, there were no substance-related effects on the reproductive performance. Maternal toxicity occurred at 500 mg/kg bw/d and consisted of reductions in bodyweight, shown in Table 17 (Loveless et al. (2009).

In a guideline study on reproduction/developmental toxicity screening in rats no effects on sexual function and fertility are described (WIL Research Laboratories, 2005).

10.10.3 Comparison with the CLP criteria

In a weight-of-evidence approach all data provided in the registration dossier and publically available were considered to conclude on the classification for reproductive toxicity.

Criteria for CATEGORY 1: Known or presumed human reproductive toxicant

Substances are classified in Category 1 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. The

classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Category 1A: Known human reproductive toxicant
The classification of a substance in Category 1A is largely based on evidence from humans.

There is no information available which supports a known adverse effect of PFHxA and its inorganic salts on sexual function and fertility in humans. **Assignment to the classification category 1A (sexual function and fertility) is therefore not appropriate.**

Category 1B: Presumed human reproductive toxicant
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.

Data from animal studies do not provide clear evidence of an adverse effect on sexual function and fertility. Effects on the male reproductive system (i.e. cauda epididymal sperm counts) in the rat were only apparent in one subacute repeated dose toxicity study at fairly high dose levels causing marked body weight reductions. Sperm motility and spermatid counts were not affected. Information on similar effects in other animals are not available. No effects on fertility were observed.

Assignment to the classification category 1B (sexual function and fertility) is therefore not appropriate.

Criteria for 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 deficiencies 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.

Animal studies available indicate that PFHxA and its inorganic salts may target the testes. However, effects on the male reproductive system (i.e. cauda epididymal sperm counts) in the rat were only apparent in one subacute repeated dose toxicity study at fairly high dose levels causing marked body weight reductions. Sperm motility and spermatid counts were not affected. Information on similar effects in other animal species are not available. No effects on fertility were observed.

Effects on male reproductive parameters were also reported for other per- and polyfluoroalkyl substances supporting the general presumption of a pattern. However, the weight of evidence is weak and not sufficient to conclude an adverse effect on sexual function.

Assignment to the classification category 2 (sexual function and fertility) is on the basis of the available data not appropriate.

10.10.4 Adverse effects on development

One-generation reproduction toxicity study in rats (OECD TG 415; key study, Klimisch score: 1; Loveless et al. (2009))

In a one-generation reproduction toxicity study in Crl:CD(SD) rats, groups of 20 animals/sex were dosed by oral gavage with NaPFHx (purity 100%) at 0, 20, 100, or 500 mg/kg bw/d. For further details on the study design, please refer to Table 14 and section 10.10.1.

There were no substance related effects observed on litter size, sex ratio, pup clinical observations, pup survival, or F1 adult developmental landmarks at any dose tested according to the study authors. No further details were reported.

Treatment-related effects on body weight parameters were reported in F1 rats, mainly on mean pup body weights during lactation at ≥100 mg/kg bw/d, persisting up to postweaning (see Table 16; for the sake of completeness, P0 bw data is presented in Table 16). Mean pup weights were significantly lower by 17-18% compared to the control group at 500 mg/kg bw/d during the lactation period (PND 0-21).

Throughout postweaning (PND 21-60) body weights of F1 male were significantly lower compared to the control group at 500 mg/kg bw/d. During the first 4 weeks of post-weaning (PND 21-49), the body weights were 7-16% lower than control group. Body weight was similar to the control group from day 28 postweaning (PND 49). Body weight gain of F1 males was 16% lower than in the control group at 500 mg/kg bw/d during the first week of postweaning (PND 21-28) and the difference was reduced over the following three weeks.
Treatment-related effects on body weight parameters during postweaning were milder in F1 females. Body weights were 11-12% lower compared to the control group during the first week of postweaning (PND 21-28) at 500 mg/kg bw/d. Body weight was similar to the control group from day 14 postweaning (PND 35). Body weight gains in F1 adult female rats were not affected at any level tested.

In F1 adult males, testes weight and relative testis weight (relative to bw) were decreased in the 20 and 500 mg/kg bw/d group compared with the control. No statistically significant changes in testes weight in the 100 mg/kg bw/d group were observed. Histopathological examination in F1 males was not performed. For further details, please refer to section 10.10.1.
No treatment related organ weight changes were observed at any dose in F1 adult females.

There were no substance related adverse effects on sexual function and fertility observed (see section 10.10.1).

Table 16: Mean ±SD body weight gains (g) in NaPFHx-treated P0 rats and non-treated F1 rats and body weights (g) of non-treated F1 pups. (Loveless et al. (2009)

	Day		*P0 Dosage (mg/kg bw/d)*			
			Control	**20**	**100**	**500**
Body weight gain P0 ♂	Test	0 - 105	340 ± 44	329 ± 47	299 ± 43*	241 ± 40*
Body weight gain P0 ♀	Gestation	0 - 7	36 ± 10	38 ± 8	37 ± 10	25 ± 8*
		0 - 21	140 ± 25	145 ± 16	147 ± 23	134 ± 19
	Lactation	0 - 21	5.1 ± 26	7.4 ± 20	20 ± 15	25 ± 12*
			F1, not dosed			
Body weight F1 pups	Postnatal	0	7.1 ± 0.9	6.8 ± 0.6	6.3 ± 0.4	5.8 ± 0.4#
		7	18 ± 2.7	18 ± 2.2	17 ± 1.3	15 ± 1.4#
		14	36 ± 3.4	37 ± 3.0	34 ± 2.6	30.0 ± 2.5#
		21	59.6 ± 5.3	62 ± 5.0	57 ± 5.3	49 ± 4.1#
Body weight gain F1 ♂	Postweaning[a]	0 - 39	320 ± 25	327 ± 42	320 ± 27	321 ± 25
Body weight gain F1 ♀	Postweaning[a]	0 - 39	183 ± 21	178 ± 18	173 ± 21	183 ± 24

[a]Age of animals at postweaning day 0 = 21 days old.
*Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane–Dunnett.
#Statistically significant difference from control at $p < 0.05$ by analysis of covariance and Dunnett–Hsu.

<u>Prenatal developmental toxicity study in rats (OECD TG 414; key study, Klimisch score: 1; Loveless et al. (2009))</u>

In a prenatal developmental toxicity study in Crl:CD(SD) rats, groups of 22 females were given by gavage NaPFHx (purity 100%, in NANOpure water) at a dose of 0, 20, 100, or 500 mg/kg bw/d on GD 6-20 (Loveless et al., 2009). For further details on the study design, please refer to Table 14 and section 10.10.1.

NaPFHx-related developmental toxicity occurred at 500 mg/kg bw/d and consisted of a lower foetal body weight of ~10% compared to controls (see Table 17). No teratological effects were reported. No effects on viability were observed.

Maternal effects were observed at 500 mg/kg bw/d as evidenced by lower body weight parameters (see Table 17).

Table 17: Mean (±SD) maternal body weight, body weight gain in NaPFHx-treated P0 rats and foetal body weights. (Loveless et al. (2009)

Group	Day	P0 Dosage (mg/kg bw/d)			
		Control	**20**	**100**	**500**
Maternal bw (g)					
	GD 19	361.1 ± 22.2	365.8 ± 18.3	353.7 ± 25.9	343.9 ± 25.9*
	GD 20	377.4 ± 24.5	383.0 ± 19.0	371.5 ± 25.5	354.6 ± 28.5*
	GD 21	400.0 ± 27.6	405.6 ± 19.2	392.7 ± 27.3	371.5 ± 32.9*
	GD 21 (net bw[a])	304.1 ± 16.3	308.2 ± 18.2	294.1 ± 21.1	288.1 ± 22.7*
Maternal bw gain (g)					
	GD 6 -21	165 ± 18	167 ± 13	161 ± 17	134 ± 27@
	GD 6 -21[b]	69 ± 10	69 ± 10	62 ± 11	51 ± 20@
Foetal bw (g)		5.8 ± 0.3	5.7 ± 0.3	5.8 ± 0.3	5.3 ± 0.6

[a] Net body weight on gestation day 21 = terminal body weight minus the gravid uterus weight.
[b] Total body weight gain (gestation days 6–21) minus products of conception on day 21.
* Parametric comparison to control (Dunnett/Tamhane-Dunnett) significant (p-value not reported).

[@] Statistically significant from control at p < 0.005 by Dunn´s test (as stated in the registration data, while reported in the publication as being < 0.05) .

Combined Repeated dose toxicity study with reproduction/developmental toxicity screening test in rats (OECD TG 422; key study, Klimisch score: 1; WIL Research Laboratories (2005))

In an OECD TG 422 study, groups of male and female rats were given by gavage PFHxA (purity 98.5 %) at a dose of 0, 50, 150 or 450/300 mg/kg bw/d (WIL Research Laboratories, 2005). For further details on the study design, please refer to Table 14 and section 10.10.1.

No treatment-related effects on litter size, pup body weights, viability and postnatal survival was observed at dosage levels up to 450/300 mg/kg bw/d. No changes in sex ratio were observed either. At scheduled pup necropsies (PND4) or necropsies of pups found dead, no treatment-related effects were observed.

Treatment-related systemic toxicity in adults (P0) was shown at 450/300 mg/kg bw/d as evidenced by mortality, reduced body weight, changes in the clinical conditions, haematology, serum chemistry, macroscopic findings, increased absolute and relative liver weights, and microscopic changes (for details see section 10.12).

Reproductive and developmental toxicity study in mice; key study, Klimisch score 1; Charles River Laboratories (2011b); Charles River Laboratories (2011b)

A study of ammonium salt of perfluorinated hexanoic acid (APFHx) on reproduction and developmental toxicity in mice was performed in two successive, separate experimental phases (phase I and II) and recorded in two individual study reports by Charles River Laboratories (2011b) and Charles River Laboratories (2011b). Additionally, an addendum to the phase I study report was given later in the year 2012 (Charles River Laboratories, 2012). In 2014 the study (phase I and II) was published by Iwai and Hoberman (2014). The study was designed to evaluate ICH Harmonised Tripartite Guideline stages C through F of the reproductive process and to detect effects on gestation, parturition, lactation and maternal behaviour in female mice, and on the development of the offspring of the treated female mice.

In the study phase I, mice were orally treated with 0, 100, 350 and 500 mg/kg bw/d and in phase II with 0, 7, 35 and 175 mg/kg bw/d. 20 presumed pregnant mice were assigned per group. APFHx was administered orally via gavage once daily on day 6 of presumed gestation (GD 6) through GD 18.

After completion of the 20 day postpartum period (PND 20), P0 generation female mice were sacrificed. Mice that did not deliver a litter were sacrificed on GD 23. Additionally, on PND 20, all pups not selected for continued evaluation were sacrificed. F1 generation mice selected for continued evaluation were sacrificed on PND 41.
P0 generation female mice were evaluated for viability, clinical observations, body weights, body weight changes, maternal behaviour, litter observations, natural delivery, pup body weights, dam and pup necropsy observations. F1 generation male and female mice were evaluated for viability, clinical observations, body weights, body weight changes, eye opening, age of sexual maturity and necropsy observations.

Clear adverse effects on prenatal (stillbirth) and postnatal (mortality) development were found in mice treated with APFHx (Charles River Laboratories, 2011b; Charles River Laboratories, 2012) as described in the following.

The number of stillborn pups was significantly increased at 500 mg/kg bw/d with 9% of pups delivered (16 stillborn of 177 pups delivered) compared to controls (phase I; 4/221; 1.8%). This effect is also apparent at lower doses but less pronounced. At 350 mg/kg bw/d, 2% of pups delivered (5/245 pups delivered) were stillborn compared to controls (phase I; 4/221; 1.8%). At 500 mg/kg bw/d as well as at 350 mg/kg bw/d stillbirth occurred in five dams each (see Table 19). At 175 mg/kg bw/d, 1.2% of pups delivered (3/241; significant) were stillborn compared to controls (phase II; 0/249; 0%; see Table 18). This effect to the foetuses indicates exposure to the foetuses during maternal treatment via placental transfer of APFHx.

In a reanalysis of data for the stillbirth endpoint, Iwai et al. (2019) suggested using the individual pup as statistical unit. However, litter dependency (intra-litter likeness) was not considered as recommended for analysis of developmental endpoints in offspring when the individual pup is used as the statistical unit according to European Food Safety Authority (EFSA) (2017); Golub and Sobin (2020); Orelien et al. (2002).

Furthermore, (Iwai et al., 2019) conducted a pooled analysis of the control groups of phase I and phase II. This combination of the control is not in accordance with generally accepted procedures for the combination of historical control data (see e.g Guidance Document 116, ENV/JM/MONO(2011)47, OECD (2014)). According to accepted procedures for the use of historical control data (HCD), the control group with the stillborn pups in phase I should have been replaced by HCD. It is noted that such a replacement has to meet certain requirements including the proof that the concurrent control group of phase I is an outlier. For such a proof the exact number of stillborn and alive pups in each historical study would have been needed, but is not stated in Iwai et al. (2019). Only averages of historical control data and percentages were presented in Iwai et al. (2019). It is concluded here that the combination of the control groups as described in Iwai et al. (2019) is not acceptable. For these reasons the DS disagrees with the reanalysis and refrains from considering the conclusion of Iwai et al. (2019).

The number of liveborn pups dying on the day of delivery (PND 0) was significantly increased at 500 mg/kg bw/d (21/150; 14.0%) and (not significant) at 350 mg/kg bw/d (3/232; 1.3%) in phase I as well as significantly increased at 175 mg/kg bw/d (4/238; 1.7%) in phase II (see Table 18 and Table 19).
The significantly increased number of pups dying on PND 0 at 175 mg/kg bw/d in phase II study may be interpreted as a borderline effect and appears not robust enough to conclude on developmental toxicity.
The number of liveborn pups dying on PNDs 1 to 4 was significantly increased at 350 mg/kg bw/d (25/229; 10.9%) and 500 mg/kg bw/d (20/129; 15.5%) compared to controls (see Table 19). Furthermore, viability indices were significantly reduced at 350 mg/kg bw/d (PND 7) and at 500 mg/kg bw/d (PND 4 and PND 7), shown in Table 14.

Pup body weights were significantly lower on PND 0 in all dosage groups in phase I (100-500 mg/kg bw/d) and at 175 mg/kg bw/d in phase II. Lower body weight persisted in the 350 and 500 mg/kg bw/d groups (see Table 18 and Table 19) and were significantly different from the control group value (with mixed-effects model and post hoc Dunnett test) until PND 20 (see Table 19).

Compared to controls, percentage of pups per litter with open eyes was reduced at 350 and 500 mg/kg bw/d on PND 14 in phase I (results not shown). Effects on other physical landmarks were not reported. As APFHx treatment induced clear effects on body weights, an indirect effect of lower body weight as the cause of the delayed eye opening cannot be ruled out.

Non-treatment related deaths of parental animals occurred in phase I and phase II (see Table 14).

In the female parental animals slight excess salivation in three of 20 mice at 350 mg/kg bw/d and slight to moderate excess salivation in six of 20 mice at 500 mg/kg bw/d was observed during the gestation period, but this was the only clinical observations related to APFHx treatment. No clinical signs were observed during the lactation period. The mean body weight of dams at 500 mg/kg bw/d was slighly higher than in control group on PPD 0. An occasionally lower body weight gain was observed on LD 0-4 in dams at 350 mg/kg bw/d (-33%) and 500 mg/kg bw/d (55%), without affecting the mean body weight of the lactating dams in both treatment groups (see Table 14). The effect on body weight gain was transient in nature and is not considered to be a sign of toxicity. In phase II, body weight gains of dams were unaffected by doses up to 175 mg/kg bw/d during the gestation and lactation periods.

The study authors considered 175 mg/kg bw/d as a maternally toxic dose without giving a justification to support this statement. In fact, the study report of Charles River Laboratories (2011b) and the publication of Iwai and Hoberman (2014) do not indicate maternal toxicity at 175 mg/kg bw/d.

Table 18: F1 observations (naturally delivered pups) according to Charles River Laboratories (2011b), study phase II

APFHx Dosage P0 GD 6-18	**(mg/kg bw/d)**	**Control**	**7**	**35**	**175**
No of dams	N	20	20	20	20
Pregnant dams	N	20	17	20	20
Duration of gestation	Mean±S.D.	19.6 ± 0.5	19.8 ± 0.8	19.8 ± 0.4	19.7 ± 0.5
Duration of gestation > 20d		None	None	None	None
Litters delivered	N	20	17	19	20
Live litter size	Mean±S.D.				
Day 0		12.4 ± 2.5	12.4 ± 3.4	12.2 ± 1.7	11.7 ± 2.8
Day 4		12.3 ± 2.4	12.8 ± 1.7 [16][a]	12.1 ± 1.7	11.6 ± 3.0
Day 7		12.2 ± 2.5	12.8 ± 1.7 [16][a]	12.1 ± 1.7	11.4 ± 3.0
Day 14		12.2 ± 2.5	12.8 ± 1.7 [16][a]	12.1 ± 1.7	11.4 ± 3.0
Dams with no liveborn pups	N	0	0	0	0
Dams with all pups dying PPD 0 - 3	N (%)	0 (0.0)	1 (5.9)	0 (0.0)	0 (0.0)
Dams with all pups dying PPD 4 - 20	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pups delivered (total)	N Mean±S.D.	249 12.4 ± 2.5	213 12.5 ± 3.0	232 12.2 ± 1.7	241 12.0 ± 2.1
Stillborn	N (%) Mean±S.D.	0	0	0	3 (1.2)** 0.2 ± 0.7
Details to litters with stillborn, day 0 postpartum	s= stillborn d= died m=missing (number of pups per litter)				Litter 470: 3s, 2d, 1m (8)
Stillborn in 11 historical control groups (2004-2015)[a]	(%)	0 – 1.8			
Unknown vital status	N	0	2	0	0
Liveborn	N (%) Mean±S.D.	249 (100.0) 12.4 ± 2.5	211 (99.1) 12.4 ± 3.4	232 (100.0) 12.2 ± 1.7	238 (98.8) 11.9 ± 2.5
Liveborn pups found dead or presumed cannibalized on PPD 0	N/N(%)	0/249 (0.0)	0/211 (0.0)	0/232 (0.0)	4/238 (1.7)**

Liveborn pups found dead or presumed cannibalized	N/N(%)				
PPD 1-4		3/249 (1.2)	6/211 (2.8)	2/232 (0.9)	3/234 (1.3)
PPD 5-7		1/246 (0.4)	0/205 (0.0)	0/230 (0.0)	3/231 (1.3)
PPD 8-14		0/245 (0.0)	0/205 (0.0)	0/230 (0.0)	0/228 (0.0)
PPD 15-20		0/245 (0.0)	0/205 (0.0)	0/230 (0.0)	1/228 (0.4)
Sum pups dead until PPD0/pups delivered	N/N(%)	0/249 (0%)	2/213 (0.9%)	0/232 (0%)	7/241 (2.9%)
Sum pups dead until PPD20/pups delivered	N/N(%)	4/249 (1.6%)	8/213 (3.8%)	2/232 (0.9%)	14/241 (5.8%)
Pup weight/litter in Grams	Mean±S.D.				
Day 0		1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.4 ± 0.2*
Day 4		2.8 ± 0.3	2.8 ± 0.3	3.0 ± 0.3	2.7 ± 0.5
Day 7		4.2 ± 0.6	4.2 ± 0.4	4.4 ± 0.4	4.2 ± 0.6
Day 14		6.8 ± 1.2	6.7 ± 0.6	7.0 ± 0.7	6.8 ± 0.9
Day 20		10.2 ± 1.8	10.0 ± 1.2	10.8 ± 1.3	10.4 ± 1.4

Treatment occurred on days 6 through 18 of gestation
GD = Day of gestation, PPD = Day of postpartum period
[ ] = Number of values averaged
a Data from Iwai et al. 2019, Int. J. Toxicology Vol. 38(3) 183-191.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

Table 19: F1 observations (naturally delivered pups) according to Charles River Laboratories (2011b) study phase I; Charles River Laboratories (2012)

APFHx Dosage P0 GD 6-18	**(mg/kg bw/d)**	**Control**	**100**	**350**	**500**
No of dams	N	20	20	20	20
Pregnant dams	N	19	19	20	18
Duration of gestation	Mean±S.D.	19.9 ± 0.6	19.9 ± 0.2	19.9 ± 0.6	20.2 ± 1.1
Duration of gestation > 20d	duration in days: N/N pregnant dams (litter ID number)	22 days: 1/19 (8321)	0/19	22 days : 1/19 (8363)	22 days: 2/17 (8380 and 8383) 23 days: 1/17 (8375)
Litters delivered	N	19	19	19	17
Live litter size	Mean±S.D.				
Day 0		11.4 ± 4.5	13.2 ± 1.6	12.0 ± 3.5	9.9 ± 2.9 [13][a]
Day 4		11.9 ± 3.8 [18][a]	13.0 ± 1.7	12.0 ± 3.6 [17][a]	9.9 ± 2.0* [11][a]
Day 7		11.9 ± 3.8 [18][a]	12.9 ± 1.6	11.8 ± 3.6 [17][a]	9.9 ± 2.0 [11][a]
Day 14		11.9 ± 3.8 [18][a]	12.4 ± 1.4 [15][b]	11.6 ± 3.4 [17][a]	9.9 ± 2.0 [11][a]
Dams with no liveborn pups	N	0	0	0	1
Dams with all pups dying PPD 0 - 3	N (%)	1 (5.3)	0 (0.0)	2 (10.5)	5 (31.3) **
Dams with all pups dying PPD 4 - 20	N (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pups delivered (total)	N Mean±S.D.	221 11.6 ± 4.2	250 13.2 ± 1.6	245 12.9 ± 3.8	177 11.1 ± 2.4
Stillborn	N (%) Mean±S.D.	4 (1.8) 0.2 ± 0.7	0 0.0 ± 0.0	5 (2.0) 0.3 ± 0.4	16 (9.0)** 1.0 ± 2.2
Details to litters with stillborn, day 0 postpartum	s= stillborn d= died u=uncertain (number of pups per litter)	Litter 8317: 1s (12) Litter 8321: 3s (7)		Litter 8353: 1s (16) Litter 8357: 1s (10) Litter 8358: 1s (11) Litter 8360: 1s (14) Litter 8370: 1s (15)	Litter 8375: 3s (3) Litter 8378: 9s, 1d (10) Litter 8383: 2s, 6 d (8)

					Litter 8385: 1s, 6d, 7u (14) Litter 8388: 1s, 1d (11) Litter 8389: 4u (6)
Stillborn in 11 historical control groups (2004-2015) [c]	(%)	0 – 1.8			
Unknown vital status[d]	N	0	0	8	11
Liveborn	N (%) Mean±S.D.	217 (98.2) 11.4 ± 4.5	250 (100.0) 13.2 ± 1.6	232 (94.7) 12.2 ± 3.4	150 (84.7)** 9.4 ± 3.9
Liveborn pups found dead or presumed cannibalized on PPD 0	N/N (%)	0/217 (0.0)	0/250 (0.0)	3/232 (1.3)	21/150 (14.0)**
Liveborn pups found dead or presumed cannibalized	N/N (%)				
PPD 1-4		2/217 (0.9)	3/250 (1.2)	25/229 (10.9)**	20/129 (15.5)**
PPD 5-7		1/215 (0.5)	1/247 (0.4)	3/204 (1.5)	0/109 (0.0)
PPD 8-14		0/214 (0.0)	1/244 (0.4)[e]	3/201 (1.5)	0/109 (0.0)
PPD 15-20		0/214 (0.0)	2/215 (0.9)[e]	0/198 (0.0)	0/109 (0.0)
Sum pups dead until PPD0/pups delivered	N/N (%)	4/221 (1.8%)	0/250 (0%)	16/245 (6.5%)	48/177 (27.11%)
Sum pups dead until PPD20/pups delivered	N/N (%)	7/221 (3.2%)	7/250 (2.8%)	47/245 (19.2%)	68/177 (38.4%)
Pup weight/litter in Grams	Mean±S.D.				
Day 0		1.6 ± 0.2	1.5 ± 0.1*[f]	1.4 ± 0.2** [f, g]	1.4 ± 0.2** [f, g]
Day 4		3.0 ± 0.4	2.8 ± 0.2	2.2 ± 0.6** [f, g]	2.4 ± 0.5** [f, g]
Day 7		4.4 ± 0.8	4.1 ± 0.4	3.6 ± 1.0** [f, g]	3.9 ± 0.8 [g]
Day 14		7.4 ± 1.9	6.8 ± 0.8 [h]	6.4 ± 1.4 [g]	6.8 ± 1.1[g]
Day 20		11.0+3.0	9.8+1.5 [h]	8.8+2.7 [h]	9.7+2.0 [h]

Treatment occurred on days 6 through 18 of gestation
GD = Day of gestation, PPD = Day of postpartum period
[ ] = Number of values averaged
[a] Excludes values for litters that had no surviving pups.
[b] Excludes litters with mortality of pups that remained on study after dam was found dead.
[c] Data from Iwai et al. 2019, Int. J. Toxicology Vol. 38(3) 183-191.
[d] Maternal cannibalization or autolysis precluded identification of vital status at birth.
[e] Excludes mortality of pups that remained on study after dam was found dead.
[f] With pup body weights per litter covaried with litter size per litter, the analyses were not significant.
[g] A significant treatment effect in the mixed-effects model with respect to intralitter likeness, controlling for sex and litter size followed by post hoc Dunnett test for multiple comparison of treatment groups versus controls. Significantly different from the control group value ($p \leq 0.001$).
[h] Significantly different from the control group value ($p \leq 0.05$) with mixed-effects model and post hoc Dunnett test (see [g]).
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$)

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

In a one-generation reproductive toxicity study with NaPFHx in rats dose-related effects on body weight were shown in F1 pups on PND 0 and during lactation (PND 7, 14, 21) at ≥ 100 mg/kg bw/d, persisting during the postweaning at 500 mg/kg bw/d. Mean pup weights were significantly reduced by 17-18% compared to the control group at 500 mg/kg bw/d at PND 0 and during lactation. No substance-related effects were observed on litter size, sex ratio, pup clinical observations, pup survival, or F1 adult developmental landmarks at any dose tested. The only treatment-related effect observed in P0 dams was a lower body weight gain during the first but not subsequent weeks of gestation (-30%) in the 500 mg/kg bw/d dose group. During the lactation period, a clear increased body weight gain was observed at ≥ 100 mg/kg bw/d. Some mortalities at 500 mg/kg bw/d were

observed during the study, reported as non-treatment related. Information on mortality of controls is lacking. No evidence of maternal toxicity was seen at 100 mg/kg bw/d (Loveless et al. (2009).

In a prenatal developmental toxicity study with NaPFHx in rats, 10% reductions in foetal body weight at 500 mg/kg bw/d were reported. Significantly reduced body weight parameters (body weight and body weight gain) were also observed in dams at 500 mg/kg bw/d indicating that maternal toxicity could have contributed to the lower foetal growth (see Table 17). Other teratological effects are not described. No effects on viability were observed (Loveless et al. (2009).

In a combined repeated dose toxicity study with reproduction/developmental toxicity screening (OECD TG 422) conducted with PFHxA in rats, no treatment-related effects on development were observed (WIL Research Laboratories, 2005).

A reproductive and developmental toxicity study in mice showed clear adverse effects on reproduction and postnatal development in mice treated with APFHx. The number of stillborn pups was significantly increased at 500 mg/kg bw/d (16/177) and increased at 350 mg/kg bw/d (5/245) in phase I of the study, as well as significantly increased at 175 mg/kg bw/d (3/241) in phase II of the study. At 350 and 500 mg/kg bw/d stillbirth occurred in multiple dams. The number of pups dying on the day of delivery was significantly increased at 500 mg/kg bw/d (21/150) and increased at 350 mg/kg bw/d (3/232) in phase I as well as significantly increased at 175 mg/kg bw/d (4/238) in phase II. Pup body weights were significantly reduced on postnatal day 0 in all dosage groups in phase I (100-500 mg/kg bw/d) and at 175 mg/kg bw/d in phase II. Dams showed slight excess salivation at 350 mg/kg bw/d (3/20 mice) and slight to moderate excess salivation at 500 mg/kg bw/d (6/20 mice) during the gestation period, but not the lactation period. No other clinical observation or mortalities related to APFHx treatment were observed in dams. Body weight gains of dams were unaffected up to 175 mg/kg bw/d during the gestation and lactation periods. A transient lower body weight gain was observed on lactation day 0-4 at 350 and 500 mg/kg bw/d, without affecting the mean body weight of the lactating dams. No effects on mean body weights were observed in dams during gestation and lactation in phase I and II up to 500 mg/kg bw/d (Charles River Laboratories (2011a); Charles River Laboratories (2012); Charles River Laboratories (2011b)). According to the DS's assessment, there were no signs of maternal toxicity at doses ≤175 mg/kg bw/d (LOAEL for maternal toxicity at 350 mg/kg bw/d body weight gain, without affecting the mean body weight of the lactating dams).

This is in agreement with the findings from Loveless et al. (2009), where maternal toxicty was first observed in the 500 mg/kg bw/d dose groups (NOAEL for maternal toxicity 100 mg/kg bw/d).

This data supports a developmental LOAEL of 175 mg/kg bw/d for the endpoint stillborn pups as well as a developmental LOAEL of 100 mg/kg/d for the endpoint pup body weight.

10.10.6 Comparison with the CLP criteria

In a weight of evidence approach all data provided in the registration dossier and publically available were considered to conclude on the classification for reproductive toxicity.

Category 1A: Known human reproductive toxicant
The classification of a substance in Category 1A is largely based on evidence from humans.

There is no information available which supports a known adverse effect of PFHxA and its inorganic salts on reproduction in humans. Assignment of PFHxA and its inorganic salts to classification category 1A is therefore not appropriate.

Category 1B: Presumed human reproductive toxicant

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.

An increase in both peri- and postnatal pup mortality were observed in a reproduction and developmental toxicity study in mice treated with APFHx. Stillbirth appeared in multiple dams, which makes it less likely to be random. The described developmental effects are considered adverse, treatment- and dose-related. There were no signs of maternal toxicity at ≤ 175 mg/kg bw/d, which is the value proposed as developmental LOAEL for the endpoint stillborn pups.

The same developmental toxicity pattern with regard to reduced pup survival in mice is reported for perfluoroheptanoic acid (C7) and perfluorooctanoic acid (C8) classified as Repr. 1B (H360D) as well as for perfluorononanoic acid (C9) classified as Repr. 1B (H360Df).

Structural abnormality and functional deficiency are not reported in the available data.

Table 20: Similarities and differences of data relevant for harmonised classification for Reproductive toxicity (Repr.) of the closest perfluorocarboxylic acid analogues compared to reproductive toxicity effects observed for PFHxA (first line in grey).

Substance	**Harmonised classification for Repr.**	**Reproductive effects**	
		Main reproductive effects of PFHxA	
PFHxA C6 (available studies on sodium and ammonium salts)	Proposed: Repr. 1B, H360D (classification proposed for acid, sodium, ammonium and other salts)	↓ maternal weight (Loveless et al., 2009) ↓ pup weight (Loveless et al., 2009) ↑ pup rel. liver weight (Iwai and Hoberman, 2014) ↓ postnatal survival (Iwai and Hoberman, 2014)	
		Similarities to PFHxA reproductive effects	**Differences to PFHxA reproductive effects**
PFHpA C7 (available study on sodium salt)	Repr. 1B, H360D (ECHA, 2022) (read-across to sodium salt)	↓ pup weight in mice (Anonymous, 2017) ↑ pup abs. and rel. liver weight in mice (Anonymous, 2017) ↓ postnatal survival in mice (Anonymous, 2017)	↑ cleft palates in mice (Anonymous, 2017) changes in sex ratio in mice (↓ percentage of males per litter) (Anonymous, 2017) ↑ vaginal patency in mice (Anonymous, 2017)
PFOA C8 (available studies on ammonium salt)	Repr. 1B; H360D (classifications for acid and ammonium salt) (ECHA, 2019b; ECHA, 2019c)	↓ maternal weight and weight gain in mice (Lau et al., 2006) ↓ pup weight and weight gain in mice (Lau et al., 2006) ↑ pup abs. and rel. liver weight in mice (Macon et al., 2011)	↑ enlarged fontanel, reduced ossification (sternebrae, calvaria) in mice (Lau et al., 2006) ↑ tail and limb defects in mice (Lau et al., 2006)

Substance	Harmonised classification for Repr.	Reproductive effects	
		Main reproductive effects of PFHxA	
		↓ neonatal and postnatal survival in mice (Abbott et al., 2007; Lau et al., 2006; Song et al., 2018; White et al., 2011)	↓ mammary gland development in mice (Macon et al., 2011; Tucker et al., 2015; White et al., 2011) ↑ litter loss in mice (Abbott et al., 2007)
PFNA C9 (available studies on acid)	Repr. 1B; H360Df (classifications for acid, ammonium and sodium salts) (ECHA, 2019d)	↓ maternal weight in rats (Rogers et al., 2014) ↓ birth weight in rats and mice (Rogers et al., 2014; Wolf et al., 2010) ↓ pup weight in mice (Das et al., 2015; Wolf et al., 2010) ↑ pup rel. liver weight in mice (Das et al., 2015; Wolf et al., 2010) ↓ postnatal survival in mice (Das et al., 2015; Wolf et al., 2010)	No change in maternal weight in mice (Singh and Singh, 2019c; Wolf et al., 2010) ↓ fertility in male mice ↓ fertility parameters in male and female rats (NTP, 2019) ↑ full litter resorption or whole litter loss (Das et al., 2015; Wolf et al., 2010)

Clear evidence of major manifestations of developmental toxicity, i.e. death of the developing organism is provided. Classification in category 1B is therefore considered appropriate.

Criteria for 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 deficiencies 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.

Evidence is sufficiently convincing to place PFHxA and its inorganic salts in Category 1B. Classification in category 2 is therefore considered not appropriate.

10.10.7 Adverse effects on or via lactation

There is no information available providing human evidence indicating a hazard to babies during the lactation period and/or results of studies in animals providing evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk.

Toxicokinetic data indicate that PFHxA is present in breast milk in ng/L levels (see section 9.1). No information is available whether PFHxA and its inorganic salts interfere with lactation or potentially toxic levels in breast milk are reached.

10.10.8 Comparison with the CLP criteria

No information is available whether PFHxA and its inorganic salts interferes with lactation or is present in breast milk in amounts sufficient to cause concern for the health of a breastfed child. Assignment of PFHxA and its inorganic salts to this classification category is therefore not appropriate.

10.10.9 Conclusion on classification and labelling for reproductive toxicity

PFHxA and its inorganic salts have the potential to cause adverse effects in animal models. In a weight of evidence approach the data are presented, summarised and compared against the criteria for classification for reproductive toxicity under CLP Regulation. Based on this assessment it is concluded that PFHxA and its inorganic salts are most appropriately classified under CLP Regulation as:

Repr. 1B (H360D; May damage the unborn child).

10.11 Specific target organ toxicity-single exposure

Evaluation not performed for this substance.

10.12 Specific target organ toxicity-repeated exposure

Data on PFHxA, APFHx and NaPFHx are used as the basis for this CLH proposal. PFHxA and its inorganic salts, are expected to dissociate in biological media. Hence, regardless whether the acid or the salt are administered, once absorbed into the blood, the PFHx-anion will form.

Table 21: Summary table of animal studies on STOT RE

<table>
<tr><th>Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>RDT 28-day study

Similar to OECD TG 407, GLP

Key study, Klim. 1 (reliable without restriction)

PFHxA
(>99%)

Rat (SD)
♀+♂, n/sex/group: 10

Exposure: Oral (gavage)
Vehicle: Deionized water with 2% Tween 80, pH adjusted to between 6 and 8
Doses*: 0, 62.5, 125, 250, 500, 1 000 mg/kg bw/d

*twice daily at one-half dose (total): 31.3 (62.6), 62.5 (125), 125 (250),</td><td>
<table>
<tr><th></th><th>♀</th><th>♂</th></tr>
<tr><td colspan="3">General toxicity</td></tr>
<tr><td>Mortality</td><td>No effects observed</td><td>No effects observed</td></tr>
<tr><td>Body weight</td><td>No effects observed

(within 10% of vehicle control bw)</td><td>Effects observed, treatment related

↓ Mean bw at 1 000 mg/kg bw/d
•↓ -12% on day 22
• Significant ↓ -13% on day 29 (p ≤ 0.01)</td></tr>
<tr><td>Clinical signs</td><td><u>Clinical observations</u>
No treatment related effects

<u>Clinical chemistry</u>
Significant changes (p ≤ 0.01)
at ≥ 500 mg/kg bw/d:
• ↑ alanine aminotransferase (ALT)
↑ +35% at 500 mg/kg bw/d
↑ +43% at 1 000 mg/kg bw/d
• ↑ aspartate aminotransferase (AST)
↑ +11% at 500 mg/kg bw/d
↑ +18% at 1 000 mg/kg bw/d

Significant changes (p ≤ 0.01)
at 1 000 mg/kg bw/d:
• ↓ total protein
• ↓ globulin
• ↑ albumin/globulin ratio
• ↑ alkaline phosphatase (ALP)</td><td><u>Clinical observations</u>
No treatment related effects

<u>Clinical chemistry</u>
Significant changes (p ≤ 0.01 or p ≤ 0.05)
at ≥ 62.5 mg/kg bw/d:
• ↓ total thyroxine
↓ -20% to -59% (p ≤ 0.01), dose-related
• ↓ free thyroxine
↓ -25% to -73% (p ≤ 0.01), dose-related
• ↓ total triiodothyronine
↓ -18% to -29% (p ≤ 0.05 at 62.5 mg; p ≤ 0.01 at ≥125 mg), dose-related
• ↓ cholesterol
↓ -17% to -22% (p ≤ 0.01)

Significant changes (p ≤ 0.01 or p ≤ 0.05)
at ≥ 125 mg/kg bw/d:
• ↓ total protein (p ≤ 0.01)</td></tr>
</table>
</td><td>(NTP, 2019)</td></tr>
</table>

Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results			Reference
250 (500), 500 (1 000) mg/kg bw/d		• ↑ bile salts/acids No significant changes: • thyroid stimulating hormone • total triiodothyronine • total thyroxine • tree thyroxine <u>Haematology</u> Significant changes ($p \leq 0.01$) *at ≥ 250 mg/kg bw/d*: • ↓ haematocrit ↓ -17% at 1 000 mg/kg bw/d • ↓ haemoglobin ↓ -8% at 500 mg/kg bw/d ↓ -19% at 1 000 mg/kg bw/d • ↓ erythrocytes ↓ -10% at 500 mg/kg bw/d ↓ -26% at 1 000 mg/kg bw/d Significant changes ($p \leq 0.01$ or $p \leq 0.05$) *at ≥ 500 mg/kg bw/d*: • ↑ reticulocytes ($p \leq 0.01$) • ↑ mean corpuscular volume ($p \leq 0.05$ at 500 mg; $p \leq 0.01$ at 1 000 mg) Significant changes ($p \leq 0.01$ or $p \leq 0.05$)	• ↓ globulin ($p \leq 0.05$ at 125 mg; $p \leq 0.01$ at ≥250 mg) Significant changes ($p \leq 0.01$) *at ≥ 250 mg/kg bw/d*: • ↑ albumin/globulin ratio Significant changes ($p \leq 0.01$): *at ≥ 500 mg/kg bw/d* • ↑ alanine aminotransferase (ALT) ↑ +27% at 500 mg/kg bw/d ↑ +64% at 1 000 mg/kg bw/d • ↑ aspartate aminotransferase (AST) ↑ +18% at 500 mg/kg bw/d ↑ +37% at 1 000 mg/kg bw/d • ↑ alkaline phosphatase (ALP) ↑ +23% at 500 mg/kg bw/d ↑ +51% at 1 000 mg/kg bw/d <u>Haematology</u> Significant changes *at 62.5 mg/kg bw/d* ($p \leq 0.05$) and *at ≥ 125 mg/kg bw/d* ($p \leq 0.01$): • ↓ haematocrit > -10% at ≥500 mg/kg bw/d • ↓ haemoglobin ↓ -6% at 250 mg/kg bw/d ↓ -19% at 500 mg/kg bw/d ↓ -40% at 1 000 mg/kg bw/d • ↓ erythrocytes ↓ -23% at 500 mg/kg bw/d, ↓ -47% at 1 000 mg/kg bw/d Significant changes ($p \leq 0.05$) *at 250 mg/kg bw/d*: • ↑ mean corpuscular volume Significant changes ($p \leq 0.01$) *at ≥ 500 mg/kg bw/d*:	

Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results			Reference
		at 1 000 mg/kg bw/d: • ↓ mean corpuscular haemoglobin concentration ($p \leq 0.01$) • ↑ platelets ($p \leq 0.05$) <u>Hepatic parameters</u> • Dose-related significant ↑ gene expression of PPARα constitutive androstane receptor (CAR)-related genes (Acox1, Cyp4a1, Cyp2b1, Cyp2b2) ($p \leq 0.01$ or $p \leq 0.05$)	• ↑ reticulocytes • ↑ mean corpuscular volume • ↑ platelets <u>Hepatic parameters</u> • Significant ↑ Acyl-CoA oxidase activity at ≥250 mg/kg/d ($p \leq 0.01$) • Dose-related significant ↑ gene expression of PPARα constitutive androstane receptor (CAR)-related genes (Acox1, Cyp4a1, Cyp2b1, Cyp2b2) ($p \leq 0.01$ or $p \leq 0.05$)	
	Anatomic pathology			
	Macroscopic	<u>Organ weights</u> **Liver** Dose-related ↑ absolute and relative liver weights (rel. to bw) 500 mg/kg bw/d, significant changes ($p \leq 0.01$) • ↑ +15.2% mean rel. liver weights • ↑ +14.1% mean abs. liver weights 1 000 mg/kg bw/d, significant changes ($p \leq 0.01$) • ↑ +47.5% mean rel. liver weights • ↑ +44.2% mean abs. liver weights **Kidney** 1 000 mg/kg bw/d, significant changes ($p \leq 0.01$) • ↑ +11.6% mean rel. kidney weights (rel. to bw) • ↑ +8.8% mean abs. kidney weights	<u>Organ weights</u> **Liver** Dose-related ↑ absolute and relative liver weights (rel. to bw) 250 mg/kg bw/d, significant changes ($p \leq 0.01$) • ↑ +14.3% mean rel. liver weights 500 mg/kg bw/d, significant changes ($p \leq 0.01$) • ↑ +31.6% mean rel. liver weights • ↑ +27% mean abs. liver weights 1 000 mg/kg bw/d, significant changes ($p \leq 0.01$) • ↑ +63.6% mean rel. liver weights • ↑ +42% mean abs. liver weights **Kidney** 500 mg/kg bw/d, significant changes ($p \leq 0.01$) • ↑ +11.8% mean rel. kidney weights (rel. to bw) 1 000 mg/kg bw/d, significant changes ($p \leq 0.01$) • ↑ +18.8% mean rel. kidney weights (rel. to bw) **Thymus** 1 000 mg/kg bw/d, significant changes ($p \leq 0.01$) • ↓ mean absolute thymus weight (26.8%)	

<table>
<tr><th>Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td>
<table>
<tr><td>Microscopic</td><td><u>Nonneoplastic effects</u>
Liver
• Significant ↑ incidence of hepatocyte cytoplasmic alteration in ♀(9/10) and ♂(10/10) at 1 000 mg/kg bw/d (p ≤ 0.01)
• Significant ↑ incidence of hepatocyte hypertrophy in ♀ (9/10) at 1 000 mg/kg bw/d and ♂ (9/10) at 500 mg/kg bw/d, ♂ (10/10) at 1000 mg/kg bw/d (p ≤ 0.01)
• Hepatocyte cytoplasmic alteration and hepatocyte hypertrophy of minimal to mild severity
• Hepatocyte necrosis solely in 1/10 ♂ at 1 000 mg/kg bw/d

Nose
• Significant ↑ olfactory epithelium degeneration at ≥ 250 mg/kg bw/d in ♀(6-9/10) and ♂ (6/10) (p ≤ 0.01)
• Significant ↑ olfactory epithelium hyperplasia at ≥ 250 mg/kg bw/d in ♂ (5-6/10, p ≤ 0.01) and at 250 mg/kg bw/d in ♀(4/10, p ≤ 0.05), at 500 mg/kg bw/d in ♀(7/10, p ≤ 0.01)
• Significant ↑ olfactory epithelium suppurative inflammation at 500 mg/kg bw/d in ♀ (5/10, p ≤ 0.05) and at 1 000 mg/kg bw/d in ♀ (8/10, p ≤ 0.01) and ♂ (6/10, p ≤ 0.01)
Epithelial lesions ranged from minimal to moderate severity.

Spleen
• Significant ↑ extramedullary haematopoiesis at 1 000 mg/kg bw/d in ♀ (9/10, p ≤ 0.01) and at ≥ 500 mg/kg bw/d in ♂(6-10/10, p ≤ 0.01)

Kidney
• Significant ↑ minimal chronic progressive nephropathy in ♀ (8/10) at 1 000 mg/kg/d
(control 2/10 ♀ with nephropathy)</td></tr>
</table>
For information on reproductive parameters see Table 14
</td><td></td></tr>
<tr><td>Combined RDT study with reproduction/ developmental toxicity screening test

OECD TG 422, GLP

PFHxA (98.5%)

Rat (SD)</td><td>
<table>
<tr><th colspan="3">Parental generation (P0)</th></tr>
<tr><td></td><td>♀</td><td>♂</td></tr>
<tr><td colspan="3">General toxicity</td></tr>
<tr><td>Mortality</td><td>Effects observed, treatment related

6/15 at 450/300 mg/kg bw/d until scheduled necropsies</td><td>Effects observed, treatment related

5/15 at 450/300 mg/kg bw/d until scheduled necropsies</td></tr>
</table>
</td><td>(WIL Research Laboratories, 2005)</td></tr>
</table>

Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results			Reference
♀+♂, n/sex/group: 10 Exposure: Oral (gavage), Vehicle: Deionized water, no pH adjustment reported Doses: 0, 50, 150, 450/300* mg/kg bw/d Dosing regimen: ♀: 14 daily doses prior to pairing; dosed through lactation day 3; total of 39-44 doses; euthanized on lactation day 4. Females with no evidence of mating or that failed to deliver dosed for total of 39-52 doses. ♂: 14 daily doses prior to mating, dosed throughout mating period, until day prior to euthanasia; total of 32-34 doses. Recovery: 14 d non-dosing period at end of treatment period (then , euthanized), ♀ not used for mating ♂+♀, n/sex/group: 5 Doses: 0, 450/300* mg/kg bw/d		Cases and stated cause of death: • 1 ♀ found dead on day 2: Papillary necrosis in kidney • 1 ♀ found dead on day 3: Erosion/ ulceration in glandular stomach and oesophagus • 1 ♀ found dead on day 4: Erosion/ ulceration in glandular stomach and duodenum • 1 ♀ euthanized in extremis on day 4: Erosion in glandular stomach and papillary necrosis in kidney • 1 ♀ found dead on day 25: Cause of death undetermined • 1 ♀ found dead on day 38: Cause of death undetermined The author stated that mortality occurred with relationship to treatment only in the 450/300 mg/kg bw/d dose group. No further data reported.	Cases and stated cause of death: • 1 ♂ euthanized in extremis on day 4: Cause of death undetermined • 1 ♂ euthanized in extremis on day 4: Papillary necrosis and tubular vacuolation in kidneys • 1 ♂ euthanized in extremis on day 4: Lesions in the trachea, oesophagus and lungs (probable gavage error) • 1 ♂ found dead on day 4: Papillary necrosis in kidney, ulceration in non-glandular stomach • 1 ♂ found dead on day 19: Lesions in the trachea, oesophagus and lungs (probable gavage error) The author stated that mortality occurred with relationship to treatment only in the 450/300 mg/kg bw/d dose group. No further data reported.	
	Body weight	<u>Mating period</u> ↓ mean bw at 450/300 mg/kg bw/d • ↓ -7.6% on day 4 (sign., p<0.01) • ↓ -3.9% on day 7 No effects on bw at ≤ 150 mg/kg bw/d <u>Gestation</u> Mean bw at 450/300 mg/kg bw/d • similar to control on GD 0-17 • ↓ -5.8% on GD 20 Mean bw gain at 450/300 mg/kg bw/d • Significant ↓ on GD17-20 (p<0.01, no further data available) No effects on bw and bw gain at ≤ 150 mg/kg bw/d	↓ mean bw at 450/300 mg/kg bw/d • significant ↓ -10.3% on day 4 (p<0.01) • ↓ -7.8% on day 13 • ↓ -7.5% on day 32 Mean bw at 450/300 mg/kg bw/d comparable to control on day 49 No effects at ≤ 150 mg/kg bw/d	

Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results			Reference
♀ total of 40 doses ♂ total of 35 doses *reduced on day 4 from 450 to 300 mg/kg bw/d due to excessive toxicity (mortality) within the first 4 days of dosing *Abbreviation used* *GD: Gestation day* *Data tables (summary data, individual data) and historical control data were not available*		<u>Lactation</u> No effects on mean bw and bw gain in all treatment groups		
		Note: Treatment-related sign. lower mean food consumption during day 0-7 correlates to bw loss during day 0-4 in ♀ and ♂.		
	Clinical signs	Effects observed, treatment related, reversible At 450/300 mg/kg bw/d: • Rales, gasping, material/discharge around the urogenital/anogenital, mouth and/or nose and salivation prior to dosing at 150 mg/kg bw/d: • Red material around nose <u>Haematology</u> Changes in ♂ only: • ↓ mean haemoglobin levels at all doses, • Significant ↓ mean haemoglobin levels at 450/300 mg/kg bw/d in study week 4 ($p<0.05$ or $p<0.01$) • Significant ↓ mean corpuscular haemoglobin at 450/300 mg/kg bw/d in study week 4 ($p<0.05$ or $p<0.01$) • Significant ↓ mean corpuscular haemoglobin concentration levels at 450/300 mg/kg bw/d in study week 4 ($p<0.05$ or $p<0.01$) • ↑ reticulocyte counts at 450/300 mg/kg bw/d in study week 4 ($p<0.05$) (numerical data/p-values were not available) <u>Serum chemistry</u> Changes ♂only: *observed at week 4 (end of dosing)* • Significant ↓ mean globulin levels at 150 mg/kg bw/d • Significant ↓ mean cholesterol levels at 150 mg/kg bw/d *observed at week 4 (end of dosing) and week 7 (recovery)* • Significant ↓ mean globulin levels at 450/300 mg/kg bw/d • Significant ↓ mean total protein levels at (likely secondary to lower serum globulin levels) • Significant ↓ mean cholesterol levels at 450/300 mg/kg bw/d (numerical data/p-values were not available)	

Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results		Reference
		All treatment-related clinical findings subsided following cessation of dose administration. All other clinical findings for treatment groups were noted with similar incidence in the control group, were limited to single animals, were not noted in a dose-related manner and/or were common findings for laboratory rats of this age and strain.	
	Anatomic pathology		
	Macroscopic	**Stomach** • Ulceration/erosion exclusively in rats that died/were euthanized during first 5 days, 4/4 ♀ and 2/4♂ at 450/300 mg/kg bw/d All other macroscopic changes were described to be spontaneous and/or incidental in nature and unrelated to test item administration. <u>Organ weights</u> **Liver** • Significant ↑ mean rel. (to final bw) liver weight in ♂ at 150 mg/kg bw/d and 450/300 mg/kg bw/d, not reversible until end of recovery period in 450/300 mg/kg bw/d recovery group ($p \leq 0.01$ or $p \leq 0.05$) • ↑ mean abs. liver weights in ♂ at 150 mg/kg bw/d and 450/300 mg/kg bw/d (at study week 4) ($p \leq 0.01$ or $p \leq 0.05$) • Significant ↑ mean abs. and rel. (to final bw) liver weights in ♀ at 450/300 mg/kg bw/d on lactation day 4 ($p \leq 0.01$ or $p \leq 0.05$) Higher liver weights correlated to hepatocellular hypertrophy **Thymus** • Significant ↓mean abs. thymus weight in ♂ at 450/300 mg/kg bw/d without microscopic correlate ($p \leq 0.05$)	
	Microscopic	Effects observed, treatment related **Adrenal cortex** Hyperplasia in zona fasciculata • at 450/300 mg/kg bw/d in 2/4 ♀ that died/were euthanized until day 5 No effects in animals examined at scheduled necropsy (end of treatment period) **Kidney** Papillary necrosis • at 450/300 mg/kg bw/d in 5/15 ♀ and 2/11 ♂ (2 ♂ and 4 ♀ died until day 5, only 1 ♀ was euthanized at scheduled necropsy on lactation day 4)	

Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results	Reference

Lymphoid organs
Incidence of treatment-related changes in lymphoid organs

	Control		450/300 mg/kg bw/d	
	♀	♂	♀	♂
Unscheduled death				
No. examined	0	0	6	5
Mandibular lymph node				
Necrosis	-	-	2/6	-
Depletion	-	-	2/6	3/5
Mesenteric lymph node				
Necrosis	-	-	4/6	2/5
Depletion	-	-	-	2/5
Spleen				
Necrosis	-	-	4/6	1/5
Depletion	-	-	4/6	3/5
Thymus				
Necrosis	-	-	5/6	4/5
Atrophy	-	-	4/6	3/5
Primary Necropsy				
No. examined	10	10	9	6
Mesenteric lymph node				
Necrosis	-	-	2/9	-
Thymus				
Necrosis	1/10	-	-	-
Atrophy	-	-	3/9	-

Liver
Hepatocellular hypertrophy
• Minimal severity in 3/9 ♀ at 450/300 mg/kg bw/d and 2/9 ♀ in 150 mg/kg bw/d
• Minimal severity in 4/6 ♂ at 450/300 mg/kg bw/d and 2/10 ♂ in 150 mg/kg bw/d
• Mild severity in 2/6 ♂ at 450/300 mg/kg bw/d
(reversible in ♂, recovery not assessed in ♀ due to mortality)
Hepatic changes were investigated only in animals that were euthanized at the end of the dosing period.

Changes in the kidney, stomach, adrenal cortex and lymphoid organs were almost entirely limited to animals that died or were euthanized early.

Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results			Reference
RDT 90-day study No guideline, no GLP Supporting study, Klim. 2 (reliable with restriction) PFHxA (98.5%) Rat Crl,CD(SD), ♀+♂, n/sex/group: 10 (plus 28-day recovery groups control and high-dose) Exposure: Oral (gavage) Vehicle: Deionized water, no pH adjustment reported Doses: 0, 10, 50, 200 mg/kg bw/d		♀	♂	(Chengelis et al., 2009b)
	General toxicity			
	Mortality	No effects observed	No effects observed	
	Body weight	↓ mean bw throughout dosing period (not significant) • ↓ -6% at 50 mg/kg bw/d at study week 13 • ↓ -5% at 200 mg/kg bw/d at study week 13	Significant ↓ mean bw throughout dosing period ($p < 0.05$ or $p < 0.01$) • ↓ -11% at 50 mg/kg bw/d at study week 13 • ↓ -8% at 200 mg/kg bw/d at study week 13	
	Clinical signs	<u>Clinical observations</u> No treatment related effects <u>Functional observational battery and Locomotor activity</u> No treatment related effects observed <u>Clinical chemistry</u> • Significant ↓ globulin in ♀ ($p < 0.01$) and ♂ ($p < 0.05$) at 200 mg/kg bw/d • Significant ↑ in ALT (+237%) and ALP (+34%) in ♂ at 200 mg/kg bw/d ($p < 0.05$) (no longer statistically significant different from control 28 days after cessation of dosing) • Significant ↓ cholesterol in ♂ at 50 mg/kg bw/d ($p < 0.01$) and 200 mg/kg bw/d ($p < 0.05$) • Significant ↓ total protein in ♂ at 200 mg/kg bw/d ($p < 0.05$) • ↑ albumin/globulin ratio in ♂ at 200 mg/kg bw/d <u>Haematology</u> *Red blood cell parameters* (red blood cell count, haemoglobin content, haematocrit) • Significant ↓ (< -10%) in ♂ at 200 mg/kg bw/d ($p < 0.01$) • ↓ (< -10%) in ♀ at 200 mg/kg bw/d (not significant) reversible following a 28-day recovery period *Reticulocyte counts* • Significant ↑ (>+10%) in ♂ at 200 mg/kg bw/d ($p < 0.05$) • ↑ in ♀ at 200 mg/kg bw/d (not sign.) <u>Hepatic parameters</u> • Significant ↑ peroxisome proliferation index in ♂ ($p < 0.01$) at 200 mg/kg bw/d (10 mg/kg bw/d and 50 mg/kg bw/d not examined)		
	Anatomic pathology			

Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results			Reference
	Macroscopic	Gross necropsy No treatment-related macroscopic findings Organ weights **Kidney** • Significant ↑ rel. kidney weight (rel. to final bw) at 50 mg/kg bw/d ($p < 0.01$) • ↑ rel. kidney weight (rel. to final bw) at 200 mg/kg bw/d (not significant) • Abs. kidney weight was not affected	Gross necropsy No treatment-related macroscopic findings Organ weights **Kidney** • Significant ↑ rel. kidney weight (rel. to final bw) at 10 mg/kg bw/d and 50 mg/kg bw/d ($p < 0.05$) and 200 mg/kg bw/d ($p < 0.01$) • Abs. kidney weight was not affected **Liver** • Significant ↑ rel. liver weight (rel.to final bw.) at 200 mg/kg bw/d ($p < 0.01$), reversible in 28 d recovery • ↑ abs. liver weights at 200 mg/kg bw/d	
	Microscopic	Non-neoplastic effects **Liver:** • Minimal centrilobular hepatocellular hypertrophy in 7/10 ♂ at 200 mg/kg bw/d (reversible in 28 d recovery) • Moderate random hepatocellular necrosis in 1/10 ♂ at 200 mg/kg bw/d (multifocal foci of coagulative necrosis with variable number of inflammatory cells within or around the necrosis) • No effects in other dose groups Note: Historical control data not available. **Kidney:** No histopathological correlates of kidney enlargement were observed		
RDT 90-day study		♀	♂	(Loveless et al., 2009)
	General toxicity			

Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results			Reference
OECD TG 408, GLP Key study, Klim.1 (reliable without restriction) NaPFHx (100%) Rat (Crl:CD(SD)) ♀+♂, n/sex/group: 10 Exposure: Oral (gavage) Vehicle: NANOpure water Doses: 0, 20, 100, 500 mg/kg bw/d 30- and 90-day recovery group for control and high-dose	Mortality	Mortality observed at 500 mg/kg bw/d • 1/10 at 500 mg/kg bw/d, found dead on day 5: renal papillary necrosis; • 1/10 at 100 mg/kg bw/d, euthanized in extremis on day 50: Cranial trauma The author stated that all other rats survived until their respective scheduled terminal sacrifices.	No effects observed	REACH Registration APFHx (ECHA dissemination: Repeated dose toxicity, 001 key study)
	Body weight	No effects observed on mean bw or mean bw gain (for 90-day dosing; after 30-day recovery; after 90-day recovery)	Effects observed, treatment-related mean bw •Significant ↓ on day 42- 105 at 500 mg/kg bw/d ($p < 0.05$) •↓-10% on day 91 at 500 mg/kg bw/d mean bw gain • ↓-19% on day 0-91 at 500 mg/kg bw/d No effects observed after 30-day recovery or 90-day recovery	
	Clinical signs	<u>Clinical observations</u> No effects observed <u>Clinical chemistry</u> *Changes in ♂ at 500 mg/kg bw/d* • Significant ↑ alkaline phosphatase ($p < 0.05$) (+161% ALP at 500 mg/kg bw/d) no longer statistically significant different from control 1 month after cessation of dosing Changes in ♂ at ≥ *100 mg/kg bw/d* • Significant ↓ total protein ($p < 0.05$) • Significant ↓ globulin ($p < 0.05$) • Significant ↓ bilirubin ($p < 0.05$) • Significant ↑ aspartate aminotransferase (AST) ($p < 0.05$) (+25% AST at 100 mg/kg bw/d, +39% AST at 500 mg/kg bw/d) no longer statistically significant different from control 3 month after cessation of dosing		

Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results	Reference

Changes in ♂ at ≥ 20 mg/kg bw/d
• Significant ↑ alanine aminotransferase (ALT) ($p < 0.05$)
(+133% ALT at 20 mg/kg bw/d, +44% ALT at 100 mg/kg bw/d, +55% ALT at 500 mg/kg bw/d)
no longer statistically significant different from control 3 month after cessation of dosing

Changes in ♀ at ≥ 100 mg/kg bw/d
• Significant↓ bilirubin ($p \leq 0.05$)

Haematology
Effects observed, treatment-related at 500 mg/kg bw/d on day 93 (♀) and day 92 (♂)

	♀	♂
Red blood cell count	↓ -18% *	↓ -31% *
Haemoglobin	↓-15%*	↓ -36%*
Haematocrit	↓ -13%*	↓ -31%*
Reticulocytes	↑+280%*	↑+210%*
Platelets	↑+125%*	↑+160%*

*statistically significant difference from control at $p < 0.05$
recovery of treatment-related effects at both recovery groups (30- and 90-day) observed

Ophthalmology findings
Effects observed, not treatment-related
• 1/20 ♂and 1/20 ♀ at 20 mg/kg bw/d (keratitis of the cornea, retinal degeneration, shrunken globe-phthisis bulbi)

Functional observational battery
No effects observed

Hepatic parameters
• ↑hepatic beta-oxidation rates in ♂ at 100 mg/kg bw/d, 500 mg/kg bw/d and in ♀ at 500 mg/kg bw/d at 10-day and/or 90-day time point and persisted through 30-day recovery time point, with a similar response in ♂ and ♀ at 500 mg/kg bw/d

Anatomic pathology

<table>
<tr><th>Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure</th><th colspan="3">Results</th><th>Reference</th></tr>
<tr><td></td><td>Macroscopic</td><td>No gross pathological findings

Liver
• Significant ↑ abs. weight and rel. weight (rel. to bw) at 500 mg/kg bw/d (p < 0.05)

Kidney
• Significant ↑ rel. weights (relative to bw) at 500 mg/kg bw/d (p < 0.05)</td><td>Liver
• Significant ↑ abs. weight and rel. weight (rel. to bw) at 500 mg/kg bw/d (p < 0.05)
•↑ abs. weight and rel. weight (rel. to bw) at 100 mg/kg bw/d
• 3/10 ♂ at 500 mg/kg bw/d, pale discoloration of the liver

Kidney
• Significant ↑ rel. weights (relative to bw) at 500 mg/kg bw/d (p < 0.05)
• abs. weight not affected

Other organs
• Significant ↓ spleen abs. weight at 500 mg/kg bw/d (p < 0.05)
• Significant ↑ testes rel. weight at 500 mg/kg bw/d (relative to bw) (p < 0.05)
• Significant ↓ thymus abs. weight at 500 mg/kg bw/d (p < 0.05)

No further gross pathological findings</td><td></td></tr>
<tr><td></td><td>Microscopic</td><td colspan="2"><u>Non-neoplastic effects</u>
Liver
Incidence of hepatocellular hypertrophy
<table>
<tr><td></td><td colspan="4">♀</td><td colspan="4">♂</td></tr>
<tr><td>Dose (mg/kg bw/d)</td><td>0</td><td>20</td><td>100</td><td>500</td><td>0</td><td>20</td><td>100</td><td>500</td></tr>
<tr><td>Number examined</td><td>10</td><td>10</td><td>9-11[a]</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td>Hepatocellular hypertrophy</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Main study</td><td>0</td><td>0</td><td>0</td><td>5</td><td>0</td><td>0</td><td>4</td><td>10</td></tr>
<tr><td>30-day recovery</td><td>0</td><td>-</td><td>-</td><td>4</td><td>0</td><td>-</td><td>-</td><td>9</td></tr>
<tr><td>90-day recovery</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>6</td></tr>
</table>
a: In 100 mg/kg bw day females, the number examined was 11 and 9 for the main study and 90-day recovery groups, respectively

Thyroid gland</td><td></td></tr>
</table>

Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results	Reference
	• Minimal follicular cell hypertrophy in ♀ and ♂ at 500 mg/kg bw/d, reversible after 90 d recovery (not reversible after 30 d recovery) **Haematopoietic system** • ↑ splenic extramedullary haematopoiesis and /or erythroid hyperplasia in bone marrow in ♀ and ♂ at 500 mg/kg bw/d **Kidney** • no microscopic or clinical pathology changes indicative of renal toxicity **Nose** Incidences of test substance-related nasal lesions	

	♀				♂			
Dose (mg/kg bw/d)	0	20	100	500	0	20	100	500
Number examined	10	10	9-11[a]	10	10	10	10	10
Olfactory epithelium degeneration/atrophy								
Main study	0	0	5	4	0	0	4	7
30-day recovery	0	-	-	0	0	-	-	0
90-day recovery	0	0	0	0	0	0	0	0
Adhesions, turbinates								
Main study	0	0	0	3	0	0	0	3
30-day recovery	0	-	-	3	0	-	-	2
90-day recovery	0	0	0	2	0	0	0	5
Respiratory Metaplasia								
Main study	0	0	1	7	0	0	0	4
30-day recovery	0	-	-	8	0	-	-	8
90-day recovery	0	0	0	4	0	0	0	8
Olfactory epithelium intraepithial microcysts								
Main study	0	0	0	0	0	0	0	0
30-day recovery	0	-	-	1	0	-	-	3
90-day recovery	0	0	0	0	0	0	0	4

a: In 100 mg/kg bw/d females, the number examined was 11 and 9 for the main study and 90-day recovery groups, respectively.
-: not evaluated

No neoplastic effects observed

Combined chronic toxicity/	**General toxicity**	(Klaunig et al., 2015)

<table>
<tr><th>Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure</th><th colspan="2">Results</th><th>Reference</th></tr>
<tr><td>carcinogenicity study (104-weeks)

OECD TG 453, GLP

Key study, Klim.1 (reliable without restriction)

PFHxA
(98.1%)

Rat (Crl:CD (SD))
♀+♂, n/sex/group: 60-70

Exposure: Oral (gavage)
Vehicle: Deionized water, no pH adjustment reported
Dosage ♂: 0, 2.5, 15, 100 mg/kg bw/d
Dosage ♀: 0, 5, 30, 200 mg/kg bw/d</td><td>Mortality</td><td>Effects observed

<table><tr><td></td><td colspan="4">♀</td><td colspan="4">♂</td></tr><tr><td>Dose</td><td>0</td><td>5</td><td>30</td><td>200</td><td>0</td><td>2.5</td><td>15</td><td>100</td></tr><tr><td>Week</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>0</td><td>60</td><td>60</td><td>60</td><td>70</td><td>60</td><td>60</td><td>60</td><td>70</td></tr><tr><td>25</td><td>1/59 (2%)</td><td>0/59 (0%)</td><td>0/60 (0%)</td><td>1/67 (1%)</td><td>0/60 (0%)</td><td>0/57 (0%)</td><td>0/57 (0%)</td><td>0/69 (0%)</td></tr><tr><td>50</td><td>4/58 (7%)</td><td>2/59 (3%)</td><td>0/60 (0%)</td><td>3/65 (5%)</td><td>5/60 (18%)</td><td>0/56 (0%)</td><td>1/55 (2%)</td><td>4/55 (7%)</td></tr><tr><td>80</td><td>14/58 (24%)</td><td>13/58 (22%)</td><td>9/60 (15%)</td><td>20/64 (31%)</td><td>13/59 (22%)</td><td>13/56 (23%)</td><td>12/54 (22%)</td><td>15/52 (29%)</td></tr><tr><td>104</td><td>37/58 (64%)</td><td>33/58 (57%)</td><td>40/60 (67%)</td><td>50/64 (78%)</td><td>40/58 (69%)</td><td>32/56 (57%)</td><td>30/53 (57%)</td><td>27/51 (53%)</td></tr></table>Data excluding the accidental deaths (mechanical injury, gavage error or reflux injury)

In ♀: significant dose-related increase in mortality (p-value not reported)
In ♂: treatment and control groups not significantly different</td><td rowspan="3">REACH Registration APFHx (ECHA dissemination: Repeated dose toxicity, 002 key study)</td></tr>
<tr><td>Body weight</td><td>No treatment related effects

bw changes below 10% (occasionally statistically significant): observed in all treatment groups (compared to control), no dose-response relationship
No further data given</td></tr>
<tr><td>Clinical signs</td><td><u>Clinical observations</u>
Effects observed, treatment-related
• Rales and excessive struggling during dosing in high-dose groups
• Yellow material in the ventral trunk, anogenital and/or urogenital areas in highest dose (♀ at 200 mg/kg bw/d; ♂ at 100 mg/kg bw/d)

<u>Functional observational battery and Locomotor activity</u>
No treatment related effects observed

<u>Clinical chemistry</u>
Effects observed
Triglyceride levels:</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure</th><th colspan="2">Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>• Significant ↑ +66.3% in ♀ at 200 mg/kg bw/d in week 26 (p ≤ 0.05)
• Significant ↓ -47.4% in ♂ at 2.5 mg/kg bw/d in week 52 (p ≤ 0.05)
• Significant ↓ -42.8% in ♂ at 100 mg/kg bw/d in week 52 (p ≤ 0.05)
LDL and VLDL
• Significant ↓ -56% in ♀ at 200 mg/kg bw/d in week 26 (p ≤ 0.05)

<u>Haematology</u>
No effects observed in ♂

Effects observed in ♀:
Mean number red blood cells:
• Significant ↓ -8.1% in ♀ at 200 mg/kg bw/d in week 51 (p <0.05)
Haemoglobin:
• Significant ↓ -5.2 % in ♀ at 200 mg/kg bw/d in week 51 (p <0.05)
Reticulocytes:
• Significant ↑ +21.8% in ♀ at 200 mg/kg bw/d in week 25 (p <0.05)
• Significant ↑ +26.3% in ♀ at 200 mg/kg bw/d in week 51 (p <0.05)

<u>Hormone parameters</u>
No treatment-related effects in ♀ and ♂</td><td></td></tr>
<tr><td></td><td colspan="2">Anatomic pathology</td><td></td></tr>
<tr><td></td><td>Microscopic</td><td><u>Non-neoplastic effects</u>
Effects observed, treatment related

Kidney
Papillary necrosis
• in 17/56 ♀ at 200 mg/kg bw/d
Renal tubular degeneration
<table>
<tr><td></td><td colspan="4">♀</td><td colspan="4">♂</td></tr>
<tr><td>Dose</td><td>0</td><td>5</td><td>30</td><td>200</td><td>0</td><td>2.5</td><td>15</td><td>100</td></tr>
<tr><td></td><td>1/39</td><td>0/35</td><td>2/40</td><td>17/56</td><td>4/42</td><td>2/36</td><td>0/37</td><td>3/46</td></tr>
</table>
Note: findings correlate with clinical signs (yellow material at the urogenital and anogenital area), with changes in urine parameters (specific gravity and urine volume)

Liver
Incidence of histopathological findings</td><td></td></tr>
</table>

Method, guideline, deviations if any, test substance, species, strain, sex, no/group, dose levels duration of exposure	Results	Reference

	♀				♂			
Dose	0	5	30	200	0	2.5	15	100
Liver	60	60	60	70	60	60	60	70
Congestion	6	11	4	8	15	15	16	23
Minimal	3	8	4	6	12	8	9	17
Mild	3	3	0	2	3	6	7	6
Severe	0	0	0	0	0	1	0	0
Necrosis, hepatocellular	2	0	3	12	4	3	5	6
Minimal	2	-	1	4	1	0	3	4
Mild	0	-	2	6	2	3	2	2
Moderate	0	-	0	2	1	0	0	0
Severe	0	-	0	2	0	0	0	0
Necrosis, hepatocellular, centrilobular	1	0	6	4	2	3	0	1
Minimal	0	-	1	0	0	0	-	0
Mild	1	0	1	2	2	1	-	0
Moderate	1	-	0	2	0	0	-	0
Severe	0	-	4	0	0	2	-	1

Note: Hepatocellular necrosis primarily in animals that were found dead or euthanized prior to the scheduled necropsy. Hepatocellular necrosis consistent with ischemia resulting from diminished hepatic blood flow. Historical control data not available.

Larynx/pulmonary airway
• Localised inflammation and/or epithelial necrosis in the larynx or pulmonary airway epithelium
• ♂: 0/60 (0%), 2/60 (3.3%), 4/60 (6.7%) and 12/70 (17.1%) males were found dead or euthanized in extremis and assigned “reflux injury” as the cause of death in the 0, 2.5, 15, and 100 mg/kg bw/d group, respectively.
• ♀: 0/60 (0%), 0/60 (0%), 0/60 (0%) and 4/70 (5.7%) females were found dead or euthanized in extremis and assigned “reflux injury” as the cause of death in the 0, 5, 30, and 200 mg/kg bw/d group, respectively.

Neoplastic effects
No effects observed

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

Two oral subacute toxicity studies, one oral subchronic toxicity study and one oral chronic toxicity study of PFHxA in rats and one oral subchronic toxicity study of NaPFHx in rats are available. Treatment-related effects were observed in all studies and included lower body weights and body weight gain in comparison to controls, altered organ weights, an influence of PFHxA on clinical chemistry, haematological parameters and hepatic parameters. Histopathological findings were present in the liver, nose and kidney.

As hepatotoxic effects were described in each study including liver hypertrophy, alterations in hepatic parameters and corresponding changes in clinical chemistry parameters, the liver seems to represent the main target organ for PFHxA/ NaPFHx-related toxicity:
Significant dose-related increases in absolute and relative liver weights were observed in rats of both sexes after subacute exposure (WIL Research Laboratories, 2005) as well after subchronic exposure (Loveless et al., 2009). The incidence of hepatocellular hypertrophy also increased dose-related in rats of both sexes after subacute exposure (WIL Research Laboratories, 2005) and subchronic exposure (Loveless et al., 2009). Liver hypertrophy may reflect an adaptive response to the induction of metabolic enzymes. Hepatocellular hypertrophy correlated with (and is probably attributed to) peroxisome proliferation.
Accordingly, peroxisome induction/proliferation was observed at the same doses as the associated hypertrophy after subchronic exposure (Loveless et al., 2009) as well as at 200 mg PFHxA/kg bw/d in male rats in another subchronic study (Chengelis et al., 2009b). Peroxisome induction/proliferation was observed at 250 mg PFHxA/kg bw/d in male rats after subacute exposure (NTP, 2019). Neoplastic effects were not observed in rats after chronic exposure with PFHxA (Klaunig et al., 2015).
PFHxA treatment related effects on clinical chemistry parameters were described, such as altered liver enzyme levels (ALT, AST, ALP) in both sexes. Increases in ALT activities of two- to threefold of the controls were reached in male rats at 20 mg NaPFHx/kg bw/d and at 200 mg PFHxA/kg bw/d following 90 days of exposure (Chengelis et al., 2009b; Loveless et al., 2009). ALT activity reached the level of controls within subsequent recovery phase of one to three months. Increases in serum ALT activity in the range of two- to threefold when compared with concurrent controls should be considered as indicative of hepatocellular damage. Increases in AST activities were observed to be below 40% after subacute PFHxA exposure and subchronic NaPFHx exposure (Chengelis et al., 2009b; Loveless et al., 2009). Increases in ALP activity of 2.6-fold of the controls were reached in male rats at 500 mg NaPFHx/kg bw/d following 90 days of exposure, which were noted to recover one month after cessation of dosing (Loveless et al., 2009). Increased levels of AST and ALP are supporting indicators of liver cell dysfunctions.
Microscopic examinations revealed scattered findings of hepatocellular necrosis in individual treated rats in 3/5 repeated dose toxicity studies: while only one male rat at 1 000 mg PFHxA/kg bw/d (NTP, 2019) and only one male rat at 200 mg PFHxA/kg bw/d (Chengelis et al., 2009b) were reported, an increased incidence of hepatocellular necrosis most consistent with regional or diffuse ischemia were noted in treated female rats at 30 mg PFHxA/kg bw/d (9/60, 15%) and 200 mg PFHxA/kg bw/d (16/70, 23%) after chronic exposure (Klaunig et al., 2015). In the control group 3/60 (5%) female rats showed hepatocellular necrosis. Incidence of hepatocyte necrosis increased dose-related. Minimal to moderate severity was reported in the control group. Minimal to severe necrosis was observed in 30 and 200 mg PFHxA/kg bw/d dose groups. It was noted by the authors that hepatocellular necrosis was observed primarily in animals that were found dead or were euthanized prior to the scheduled necropsy. Mortality was observed in 78% female rats at 200 mg/kg bw/d after 104 weeks (see Table 21). This may be regarded as confounding factor. No distinct hepatotoxicity is described for survivors. The number of survivors in the lower dose or control group did not fall below 25% in female rats after 104 weeks. Male rats did not develop PFHxA-related liver cell necrosis in this study. The

reported hepatocellular necrosis in female rats appears to be a substance- and dose-related severe effect. No increased incidence of neoplasms related to treatment of PFHxA was observed in either male or female rats. The authors state that no statistically significant effects of PFHxA on tumour incidence were noted. (Klaunig et al., 2015). Hepatocellular necrosis in female rats were not reported in the other available repeated dose toxicity studies with subchronic or subacute exposure. The findings reported in male rats appear to be individual cases.

Significant decreases in thyroid hormone concentrations were observed in one subacute toxicity study at all dose groups of male rats (but not females) without compensatory increases in thyroid stimulating hormone. No histopathologic changes in the thyroid gland were found (NTP, 2019). Evidence for an increase in hepatic UDP-glucuronosyltransferase activity, resulting in accelerated degradation of thyroxine in the liver is not given. In a subchronic toxicity study, minimal follicular cell hypertrophy was present in male and female rats at 500 mg/kg bw/d and was considered potentially adverse. The thyroid hypertrophy was reversible after 90-day recovery. Thyroid hormone levels were not examined. This dose, 500 mg/kg bw/d, induced also liver hypertrophy in both sexes (Loveless et al., 2009). There is no mechanistic information available to determine the underlying mechanism of the described thyroid follicular cell hypertrophy. Thus, conclusions on the relevance to humans cannot be drawn.

PFHxA treatment affected red blood cell parameters in male and/or female rats in all studies and indicate adverse anaemic effects. In one subacute study, regenerative anaemia was observed in male and female rats. The authors did not find indications on haemolysis or haemorrhage (NTP, 2019). A reduction in haemoglobin at ≥ 10% was reached in one subacute study in male rats when dosing at ≥ 500 mg/kg bw/d and in females at 1 000 mg/kg bw/d (NTP, 2019) as well as in one subchronic study in male and female rats when dosing at 500 mg/kg bw/d (Loveless et al., 2009). Reticulocytes were significantly increased in the affected groups in both studies mentioned. Methaemoglobin levels, haemosiderosis and/or clinical signs of hypoxia are not reported. A reduction in haemoglobin at ≥ 20% was observed in the subacute study in male rats when dosing at 1 000 mg/kg bw/d (NTP, 2019) and in the subchronic study in male rats when dosing at 500 mg/kg bw/d (Loveless et al., 2009). The underlying mechanism for regenerative anaemia is not clear with the data available.

Treatment-related increases in kidney weight parameters were observed in rats of both sexes after subacute and subchronic exposure to PFHxA/ NaPFHx. After 28-d exposure to PFHxA, absolute and relative (to bw) kidney weights were significantly increased in 1 000 mg/kg bw/d females and associated with minimal chronic progressive nephropathy (NTP, 2019). After 90-d exposure to PFHxA relative kidney weights were significantly increased in males from 10 mg/kg bw/d upwards and females in the 50 mg/kg bw/d group only. In this study, this was not associated with any histopathological correlates of kidney enlargement. Absolute kidney weight was not affected (Chengelis et al., 2009b). After 90-d exposure to NaPFHx, relative kidney weight increases were observed at 500 mg/kg bw/d in male and female rats, but were not associated with microscopic or clinical pathology changes indicative of renal toxicity. Absolute kidney weight was not affected (Loveless et al., 2009). Papillary necrosis was observed after subacute exposure at 450/300 mg PFHxA /kg bw/d in male rats (WIL Research Laboratories, 2005) and after chronic exposure at 200 mg PFHxA /kg bw/d in female rats (Klaunig et al., 2015). The effects described occurred either without histopathological correlates of kidney toxicity or at highest dosage. Significant effects of adverse nature at generally moderate exposure concentrations are not demonstrated.

Adverse effects on the respiratory tract were observed in both sexes in dose-dependent manner after subacute, subchronic and chronic exposure, e.g. degeneration/hyperplasia/inflammation/atrophy of the olfactory epithelium, nasal turbinate adhesions, respiratory metaplasia in the nose and tracheal epithelial necrosis (Klaunig et al., 2015; Loveless et al., 2009; NTP, 2019). Histopathological examinations of degeneration, hyperplasia and inflammation of the olfactory epithelium did not indicate a gavage-related reflux described in rats (NTP, 2019). Hyperplasia as response to

degeneration seems plausible. The available data do not allow any conclusions to be drawn about the underlying mechanism with regard to the olfactory epithelium. Localised inflammation and/or epithelial necrosis was observed in the larynx or pulmonary airway epithelium in 2/60 (3.3%), 4/60 (6.7%) and 12/70 (17.1%) males in the 2.5, 15, and 100 mg/kg bw/d group, respectively and 4/70 (5.7%) females in the 200 mg/kg bw/d group, which were found dead or euthanized in extremis. "Reflux injury" was assigned as the cause of death. Inflammation and/or epithelial necrosis was not observed in the larynx or pulmonary airway epithelium of males and females in the control group (Klaunig et al., 2015). It is not clear whether the observed effects occur due to application or local irritation or if a substance-specific systemic effect after repeated dosing is present. Erosion/ulceration in glandular stomach/oesophagus/duodenum was also reported by WIL Research Laboratories (2005) and is most likely a result of mucosal irritation due to the acidic nature of PFHxA.

10.12.2 Comparison with the CLP criteria

In a weight of evidence evaluation all data provided in the registration dossier and publically available were considered to conclude on the classification for specific target organ toxicity-repeated exposure. Human data on specific, target organ toxicity arising from a repeated exposure to PFHxA, NaPFHx and/or APFHx are not available. Data from subacute, subchronic and chronic evaluations of treatment-related toxicity in experimental animals are available.

Corrected guidance values for 104 weeks of dosing:
STOT RE 1: ≤ 1.24 mg/kg bw/d
STOT RE 2: ≤ 12.4 mg/kg bw/d
Corrected guidance values for 39 d of dosing:
STOT RE 1: ≤ 23.1 mg/kg bw/d
STOT RE 2: ≤ 231 mg/kg bw/d
Corrected guidance values for 32 d of dosing:
STOT RE 1: ≤ 28.1 mg/kg bw/d
STOT RE 2: ≤ 281 mg/kg bw/d

Table 22: Effects, effect level and comparison with guidance values

Effect	Effect level	Equivalent guidance values	Classification justified
Liver weight (rel.&abs.) increase	450/300 mg PFHxA/kg bw/d (39 days) in female rats	STOT RE 1: ≤ 23.1 mg/kg bw/d STOT RE 2: ≤ 231 mg/kg bw/d	Exceeds guidance values
Hepatocellular hypertrophy	150 mg PFHxA/kg bw/d (39 days) in female rats	STOT RE 1: ≤ 23.1 mg/kg bw/d STOT RE 2: ≤ 231 mg/kg bw/d	Within STOT RE 2 guidance value
	150 mg PFHxA/kg bw/d (32 days) in male rats	STOT RE 1: ≤ 28.1 mg/kg bw/d STOT RE 2: ≤ 281 mg/kg bw/d	Within STOT RE 2 guidance value
	100 mg NaPFHx/kg bw/d (90 days) in male rats	STOT RE 1: ≤ 10 mg/kg bw/d STOT RE 2: ≤ 100 mg/kg bw/d	Within STOT RE 2 guidance value
ALT	20 mg NaPFHx/kg bw/d (90 days) in male rats (reversible 90 days after cessation of dosing)	STOT RE 1: ≤ 10 mg/kg bw/d STOT RE 2: ≤ 100 mg/kg bw/d	Within STOT RE 2 guidance value
ALP	500 mg NaPFHx/kg bw/d (90 days)	STOT RE 1: ≤ 10 mg/kg bw/d	Exceeds guidance values

	in male rats (reversible 30 days after cessation of dosing)	STOT RE 2: ≤ 100 mg/kg bw/d	
Hepatocellular necrosis	30 mg PFHxA/kg bw/d (104 weeks) in female rats	STOT RE 1: 1.24 mg/kg bw/d STOT RE 2: 12.4 mg/kg bw/d	Exceeds guidance values
Anaemia Hb ≥ 10%	500 mg NaPFHx/kg bw/d (90 days) in male/female rats	STOT RE 1: ≤ 10 mg/kg bw/d STOT RE 2: ≤ 100 mg/kg bw/d	Exceeds guidance values
	500 mg NaPFHx/kg bw/d (28 days) in male rats	STOT RE 1: ≤ 30 mg/kg bw/d STOT RE 2: ≤ 300 mg/kg bw/d	Exceeds guidance values
Anaemia Hb ≥ 20%	500 mg NaPFHx/kg bw/d (28 days) in male rats	STOT RE 1: ≤ 30 mg/kg bw/d STOT RE 2: ≤ 300 mg/kg bw/d	Exceeds guidance values

PFHxA-related and dose dependent liver toxicity (including increased activities of liver enzymes indicative of liver cell dysfunctions) was observed in male and female rats after subacute, subchronic and chronic exposure, but findings such as liver hypertrophy and enzyme activities observed at doses below the guidance values for classification were not accompanied by adverse histological changes. Hepatocellular necrosis and anaemic effects were only seen at high doses beyond those relevant for classificaton.

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.

There is no information available which shows significant and/or severe target organ toxic effects, of relevance to human health and produced at generally low exposure concentrations of PFHxA and its inorganic salts.

Assignment to the classification category 1 (STOT RE) is therefore not appropriate.

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.

There is no information available which shows significant target organ toxic effects, of relevance to human health and produced at generally moderate exposure concentrations of PFHxA and its inorganic salts.

Assignment to the classification category 2 (STOT RE) is therefore not appropriate.

10.12.3 Conclusion on classification and labelling for STOT RE

Classification and labelling on STOT RE is not appropriate for PFHxA or its inorganic salts.

10.13 Aspiration hazard

Evaluation not performed for this substance.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Evaluation not performed for this substance.

12 EVALUATION OF ADDITIONAL HAZARDS

Evaluation not performed for this substance.

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