CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification: Nonadecaflourodecanoic Acid (PFDA) and its ammonium and sodium salts

EC Number: **206-400-3 [1], 221-470-5 [2], - [3]**

CAS Number: **335-76-2 [1], 3108-42-7 [2], 3830-45-3 [3]**

Index Number: **-**

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Version number: 2 **Date: 2015-05-29**

Note on confidential information

Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Nonadecafluorodecanoic acid and its ammonium and sodium salts
Other names (usual name, trade name, abbreviation)	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Nonadecaflourodecanoic acid; Nonadecafluoro-n-decanoic acid; Nonadecafluorodecanoic acid; Perfluoro-n-decanoic acid; Perfluorodecanoic acid, PFDA Ammonium perfluorodecanoate; Ammonium nonadecafluorodecanoate Sodium nonadecafluorodecanoate
ISO common name (if available and appropriate)	*NA*
EC number (if available and appropriate)	206-400-3 [1] 221-470-5 [2] - [3]
EC name (if available and appropriate)	Nonadecafluorodecanoic acid [1] Ammonium nonadecafluorodecanoate [2] Not applicable [3]
CAS number (if available)	335-76-2 [1] 3108-42-7 [2] 3830-45-3 [3]
Other identity code (if available)	*NA*
Molecular formula	$C_{10}HF_{19}O_2$ (free acid)
Structural formula	

	PFDA (free acid)
SMILES notation (if available)	C(=O)(C(C(C(C(C(C(C(C(F)(F)F)(F)F)(F)F)(F)F)(F)F)(F)F)(F)F)(F)F)(F)F)(F)F)O PFDA (free acid)
Molecular weight or molecular weight range	514.084 g/mol (free acid)
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	*NA*
Description of the manufacturing process and identity of the source (for UVCB substances only)	*NA*
Degree of purity (%) (if relevant for the entry in Annex VI)	*Not relevant*

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Nonadecafluorodecanoic acid (CAS no.: 335-76-2)	There is no registration dossier for PFDA. The cited publications in this dossier state that the purity of PFDA is in the range 87.4-98%	No entry in Annex VI Table 3.1	Acute Tox. 3; H301 Acute Tox. 3; H311 Acute Tox. 3; H331 Skin Corr. 1B; H314 Skin Irrit. 2; H315 Eye Dam. 1; H318 Eye Irrit. 2; H319 STOT SE 3; H335 (based on 6 aggregated notfications)
Ammonium nonadecafluorodecanoate (CAS no.: 3108-42-7)	There is no registration dossier for ammonium nonadecafluorodecanoate No information available on the concentration range.	No entry in Annex VI Table 3.1	-
Sodium nonadecafluorodecanoate (CAS no.: 3830-45-3)	There is no registration dossier for sodium nonadecafluorodecanoate No information available	No entry in Annex VI Table 3.1	-

	on the concentration range.		

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
No information available				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
No information available					

Table 5 Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**
PFDA (Nonadecafluorodecanoic acid, CAS no.: 335-76-2)	96%	No information given in the original study report	Test substance from Aldrich Chemical Company in Harris and Birnbaum (1989)
PFDA (CAS no. was not given in the original study report)	87.4% (Gas chromatography of methyl esters, confirmed by mass spectroscopy and proton nuclear magnetic resonance)	Fluorinated-n-decanoic acid with monohydrogen or dihydrogen substitutions	Test substance from Aldrich Chemical Company used in Bookstaff et al., (1990). Purity by titration was 96%
PFDA (CAS no. was not given in the original study report)	No information	No information given in the original study report	Test substance from 3M in Borges et al., (1993)

PFDA (CAS no. was not given in the original study report)	analytical grade (purity not stated)	No information given in the original study report	Test substance from Sigma Aldrich Chemical used in Benninghoff et al., (2012)
PFDA (CAS no.: 335-76-2)	98%	No information given in the original study report	Test substance from ABCR, Germany, used in Kjeldsen and Bonefeld-Jorgensen (2013)

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	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 state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry											
Dossier submitters proposal					Repr. 1B, Lact., Carc. 2	H360Df, H362, H351	GHS08, Dgr	H360Df, H362, H351			
Resulting Annex VI entry if agreed by RAC and COM											

Table 7: Reason for not proposing harmonised classification and status under public consultation

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

3. HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There are no previous discussions on a harmonized classification and labeling of nonadecafluorodecanoic acid (hereafter abbreviated PFDA) or its ammonium and sodium salts.

This classification proposal is based on a read-across from 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid (PFOA, CAS no: 335-67-1) and its ammonium salt, ammoniumpentadecafluorooctanoate (APFO, CAS no: 3825-26-1). PFOA is a structural analogue to PFDA which contains two less carbon and four less fluorines. The RAC has adopted the proposed harmonised classification of PFOA and its ammonium salt (APFO) as Repr. 1B, H360D; H362; Carc. 2, H351; STOT RE 1, H372 (liver); Acute tox. 4, H332; Acute tox. 4, H302 and Eye Dam 1, H318 (RAC Opinion proposing harmonised classification and labelling at Community level of Perfluorooctanoic acid (PFOA), ECHA 2012). Recently, RAC has also adopted an opinion for the harmonised classification of 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Heptadecafluorononanoic acid (PFNA; CAS no: 375-95-1) and its ammonium salt (CAS no: 4149-60-4) and sodium salt (CAS no: 21049-39-8) based on read-across from APFO/PFOA as Repr. 1B, H360Df; H362; Carc. 2, H351; STOT RE 1, H372 (Liver, thymus, spleen); Acute tox. 4, H332; Acute tox. 4, H302 and Eye Dam 1, H318 (RAC Opinion proposing harmonised classification and labelling at Community level of Perfluorononanoic acid (PFNA) and its ammonium and sodium salts, ECHA 2014).

The hazard classes evaluated in this dossier for PFDA and its ammonium and sodium salts are reproductive toxicity and carcinogenicity, which have been adopted for harmonized classification by the RAC for APFO/PFOA. Given that RAC concluded that PFOA warrants the same classification as APFO, the rationale for classifying APFO is included in this dossier. RAC concluded also that the argumentation was valid for PFNA and its ammonium and sodium salts, and hence we propose that the argumentation for read-across from APFO/PFOA is valid for PFDA and its ammonium and sodium salts.

4. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

5. IDENTIFIED USES

PFDA and other perfluorinated carboxylic acids (PFCAs) have been used as plasticizers, lubricants, and water and oil surfactants due to their chemical and thermal stability (Harris et al., 1989). PFDA is used as a lubricant, wetting agent, plasticizer and corrosion inhibitor (ChemSec, 2014).

PFDA was planned for registration on November 30th, 2010 (three pre-registrations at >1000 tonnes/year), May 31st, 2013 (two pre-registrations 100-1000 tonnes/year), and May 31st, 2018 (3 pre-registrations at 10-100 tonnes/year, and 11 pre-registrations at 1-10 tonnes/year).

Ammonium nonadecafluorodecanoate is pre-registered with an envisaged registration deadline on May 31st, 2013

However, no registrations by the industry to ECHA has been made yet and hence, there is no information on registered identified uses within the EU.

6. DATA SOURCES

Open literature: Information on PFDA and its ammonium and sodium salts considered in this report was collected by a literature search last updated on July 2014.

ECHA documents on previous evaluations on the source chemical for read-across (PFOA and its ammonium salt APFO) and additional perfluoroalkylated carboxylic acids have also been referenced throughout the report:

- RAC Opinion proposing harmonised classification and labelling at Community level of Ammoniumpentadecafluorooctanoate (APFO), ECHA 2012
- RAC Opinion proposing harmonised classification and labelling at Community level of Perfluorooctanoic acid (PFOA), ECHA 2012
- RAC Opinion proposing harmonised classification and labelling at Community level of Perfluorononanoic acid (PFNA) and its ammonium and sodium salts, ECHA 2014
- RAC Annex 1 Background document to the Opinion proposing harmonised classification and labelling at Community level of Ammoniumpentadecafluorooctanoate (APFO), ECHA 2012
- RAC Annex 1 Background document to the Opinion proposing harmonised classification and labelling at Community level of Perfluorooctanoic acid (PFOA), ECHA 2012
- RAC Annex 1 Background document to the Opinion proposing harmonised classification and labelling at Community level of Perfluorononanoic acid (PFNA) and its ammonium and sodium salts, ECHA 2014
- Annex XV dossier – Identification of APFO as SVHC, ECHA 2013
- Annex XV dossier – Identification of PFOA as SVHC, ECHA 2013
- Annex XV dossier – Identification of Henicosafluoroundecanoic acid as SVHC, ECHA 2012
- Annex XV dossier – Identification of Tricosafluorododecanoic acid as SVHC, ECHA 2012

Other reports from internationally recognized associations that were taken into consideration include the Ecological screening Assessment report Long-Chain (C9-C20) Perfluorocarboxylic acids, their salts and their precursors, Environment Canada, August 20112.

7. PHYSICOCHEMICAL PROPERTIES

No registration of PFDA or its ammonium or sodium salts by the industry to ECHA has been made yet, there is therefore little available data on the physicochemical properties.

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101.3 kPa	The substance is a solid.		
Melting/freezing point	87.4-88.2	Hare et al., 1954	Measured
Boiling point	218°C	Sigma Aldrich 2004; Kauck and Diesslin 1951	Measured
	203.4	Kaiser et al, 2005	Estimated
	219.4	Kaiser et al, 2005	Estimated
Relative density	No data		
Vapour pressure	-0.64	Arp et al., 2006	Experimental
	0.10	Arp et al., 2006	Experimental
	3.1 to 99.97 kPa (129.6 to 218.9°C)	Kaiser et al., 2005	Calculated
Surface tension	No data		
Water solubility	5.14 g/L	Kauck and Diesslin, 1951	Measured
Partition coefficient n-octanol/water	No data		
Flash point	No data		
Flammability	No data		
Explosive properties	There are no chemical groups present in the molecule associated with explosive properties.		
Self-ignition temperature	The substance is a solid.		
Oxidising properties	No data		
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	2.57512 (dimensionless)	Moroi et al., 2001	Estimated
	-0.22	BD CLH PFNA, ECHA 2014	Estimated (SPARC)
	-5.2	BD CLH PFNA, ECHA 2014	Estimated (ChemID Plus Advance)
Viscosity	No data		

8. EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier.

9. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Male and female Wistar rats PFDA, PFOA; Perfluoroheptanoic acid (PFHA), perfluorohexanoic acid (PFHexA), or PFNA was administered i.v. at a dose of 48.64 mmol/(2.5 ml/kg body weight) for plasma concentration profile and 48.64 µmol/kg body weight for estimation of CL_R.	Plasma concentration profiles show that PFDA slowly decreased from plasma in both male and females. Total clearance of PFDA was 5.2 and 5.3 (ml/day/kg) in males and females respectively. Plasma half-life of PFDA was 339.92 and 58.57 days in males and females respectively Volume of distribution (ml/kg) was 347.7 and 441.1 ml/kg for male and female rats respectively. Clearance rate (ml/day/kg) of the investigated PFCAs were PFHA>PFOA≥PFNA~-PFDA in male rats and PFHA≥PFOA≥PFNA≥PFDA in female rats. There was a close relationship between clearance rate and total clearance (r^2=0.981, p<0.01).	Purity of test substance (PFDA, analytical grade, Sigma Aldrich Japan) unknown. Number of animals not known.	Ohmori K, Kudo N, Katayama K, Kawashima Y. Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. Toxicology. 2003 Mar 3;184(2-3):135-40.
Male and female Wistar rats PFDA, PFHA, PFOA or PFNA were i.p. administered at a dose of 20 mg/ml/kg body weight. Urine and feces were collected every 12 h up to 5 days. For the collection of bile rats were injected .iv. at a dose of 25 mg/kg body weight. Bile samples were collected every 30 min up to 5 h after the injection. To compare rate of urinary excretion rate of the PFCAs rats were i.v. injected at a dose of 25 mg/kg body weight and urine samples collected every 10 minutes.	Urinary and fecal elimination: In male rats 0.2% of the dose of PFDA and 2% of PFNA was eliminated in urine within 120 h after the administration in contrast PFHA and PFOA were eliminated to 92% and 55% of the dose. A similar tendency was observed in female rats for PFDA, but for PFOA and PFNA the urinary elimination was significantly faster than in males. Fecal elimination was a major route of elimination of PFDA in both male and female rats, approx.. >4% of the dose was eliminated by 100 hours. PFOA and PFHA were slowly eliminated in feces in contrast to urinary elimination (approx.. 22.5% of the dose). Concentrations of PFCAs in serum and liver: Concentration of PFDA in blood and liver in female rats 5 days after injection were significantly	Purity of test substance (PFDA, analytical grade, Aldrich Japan) unknown. Number of animals not known.	Kudo N, Suzuki E, Katakura M, Ohmori K, Noshiro R, Kawashima Y. Comparison of the elimination between perfluorinated fatty acids with different carbon chain length in rats. Chem Biol Interact. 2001 Apr 16;134(2):203-16.

	higher (estimated from the graphical presentation to be approx. 50 µg/ml serum and 140 µg/g liver respectively), at least 10-fold compared to PFHA, PFOA and PFNA. In males, concentrations of both PFDA and PFNA were significantly higher ($p<0.05$, compared to the other tested PFCAs) in liver and in blood respectively. Concentrations of PFOA and PFNA were significantly lower ($p<0.05$) in female rats in both liver and serum compared to male rats.		
Male and female Sprague-Dawley rats. [1-^{14}C]PFDA administered a single dose of 9.4 µmol/kg (5 mg/kg) i.p. Samples were collected daily for 28 consecutive days. At designated times post-treatment (2 h, 1,2 ,3 ,4, 7, 14, 28 days for males and 2 h, 1, 4, 7, 28 days for females) four rats were euthanized and tissues and blood were collected for determination of tissue distribution and elimination of PFDA.	Fecal elimination with 51% and 24% of the administered ^{14}C recovered in the feces of males and females by 28 days post-treatment. The cumulative excretion of PFDA-derived ^{14}C in urine was less than 5% of the administered dose in both sexes by 28 days post-treatment. Half-life of PFDA whole body elimination in males: 23 days and in females: 45 days. The liver contained the highest concentration of PFDA-derived ^{14}C in both males and females, followed by the plasma and kidneys.	Purity of [1-^{14}C] PFDA was 99% (synthesized and purified by study authors)	Vanden Heuvel JP, Kuslikis BI, Van Rafelghem MJ, Peterson RE. Disposition of perfluorodecanoic acid in male and female rats. Toxicol Appl Pharmacol. 1991 Mar 1;107(3):450-9.

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

Absorption

There are no studies available on the absorption of PFDA.

Metabolism

The metabolism of [1-^{14}C]PFDA were examined in male and female rats for 28 days after a single ip dose (9.4 µmol/kg, 5 mg/kg) (Vanden Heuvel et al., 1991). Only the parent compound of PFDA was excreted in urine and bile and no urinary or biliary metabolites of PFDA were detected in either sex. Moreover, the daily urinary excretion of fluoride in male and female rats before and after PFDA treatment was similar, suggesting that the parent compound is not defluorinated. The results therefore demonstrate a lack of biotransformation of PFDA in male and female rats.

Distribution

5 days after injection (20 mg/kg bw, i.p.) of PFCAs in rats, significant differences were observed between PFCAs with a tendency that PFCA with longer carbon chain length was higher in serum (Kudo et al., 2001). The concentration of PFDA in serum was approx. (estimated from graphical presentation) 35 µg/ml serum in males and 45 µg/ml serum in females, and 120 µg/g liver and 140 µg/g liver in male and female rats respectively. Serum concentrations of PFOA and PFNA were significantly lower than those in male rats. Such differences were also observed in hepatic concentrations.

In a study by Vanden Heuvel et al (1991) the liver contained the highest concentration of PFDA-derived ^{14}C in both males (2.7% dose of PFDA-derived ^{14}C per gram tissue after 28 days) and females (4.04 % dose of PFDA-derived ^{14}C per gram tissue after 28 days) at all investigated time points up to 28 days, followed by the plasma (0.32% and 0.74 % dose of PFDA-derived ^{14}C per gram tissue after 28 days in males and females respectively) and the kidneys (0.27% and 1.12 % dose of PFDA-derived ^{14}C per gram tissue after 28 days in males and females respectively) after a single ip dose (9.4 µmol/kg, 5 mg/kg) [1-^{14}C]PFDA.

PFDA has been found in human blood and elevated concentrations are observed following specific exposure either via the environment (contaminated drinking water) or occupationally, e.g. ski waxer (Freberg et al., 2010, Nilsson et al., 2010). Concentrations of PFDA (among analysis of 26 per- and polyfluoroalkyl substances) was found in blood samples from pregnant woman in Norway and the concentration was reported to increase with the age of the woman (Berg et al., 2014). Moreover, mothers with low parity had higher levels compared to multiparous woman. This association was also described by Morck et al (2014) investigating the levels perfluoroalkylated and polyfluoroalkylated substances (PFASs) in school children and their mothers in Denmark. Furthermore, measurements of PFDA in mothers and children showed that there was a significant correlation between the levels in children and their mothers and that the levels were significantly higher in the children compared to their mothers. In blood samples from 83 children (30 females, 53 males) in Oswego county, NY, USA the mean value of PFDA was 0.26 ng/ml (maximum 0.82 ng/ml) (Tao et al., 2008).

In a limited study (small, non-randomly selected volunteer samples) 90 human breast milk samples from Japan, Korea and China were analyzed for its content of PFCAs. Mean concentrations in human breast milk of PFDA ranged from <15-21.3 pg/ml. Levels of PFOA, PFNA, PFUnDA and PFDoDA were 51.6-93.5 pg/ml, 14.7-32.1 pg/ml, 16-36.6 pg/ml, and <10 pg/ml, respectively (Fujii et al., 2012). PFDA was also detected in 4 out of 45 samples of breast milk from women in Massachutes, USA in the concentration range <7.72-11.1 pg/ml (Tao et al 2008). However, in most breast milk samples of women living in Barcelona city in Spain PFDA was below the limit of quantification (Llorca et al., 2010).

Elimination

In a comparative study by Ohmori et al (2003) an elimination half-life in serum of 39.9 days in male and 58.6 days in female Wistar rats after a single intra-venous dose of 48.64 mmol/kg bw PFDA was reported. The serum half-life of PFNA in males was 29.5 and in females 2.44 days; for comparison the half-life of PFOA was 5.63 and 0.08 days for male and female rats respectively. The role of urinary excretion in plasma clearance, renal clearance, was determined and the total clearance rate for PFDA was found to be extremely low in this study: 5.2 ml/ (day/kg) in male rats and 5.3 ml/ (day/kg) in female rats. Ranking PFCAs according to the rate

of renal clearance in this study resulted in the following order: PFHA>PFOA>PFNA≈PFDA in male rats, and PFHA≥PFOA>PFNA>PFDA in female rats. The total clearance was significantly highly correlated with the clearance rate (r^2=0.982) suggesting that carbon chain length determines the excretion of PFCAs into urine. Organic anion transport proteins are reported to play a key role in PFCAs (C4 to C10) renal tubular reabsorption (Han et al, 2012) and the rate of renal excretion of PFCAs has been demonstrated to be regulated by testosterone and is determined by carbon chain length (Kudo et al., 2001).

Fecal elimination was demonstrated to be a major route of PFDA in both male and female rats in contrast to the shorter perfluorinated fatty acids (PFHA, PFOA) (Kudo et al., 2001). More than 4% of the injected dose of PFDA was eliminated by 100 hours. In contrast, Kudo et al (2001) reported that the elimination of PFDA in urine was 0.2% of the dose within 120 hours in male rats (after i.v. injection) compared to 92%, 55% and 2% for PFHA, PFOA and PFNA respectively.

A sex difference in the fecal elimination of perfluorodecanoic acid (PFDA) was observed with 51 and 24% of the administered ^{14}C being recovered in the feces of male and female rats, respectively, by 28 days post-treatment after a single i.p. dose (9.4 µmol/kg, 5 mg/kg) of [1-^{14}C]PFDA (Vanden Heuvel et al 1991). Half-life for whole body elimination of [^{14}C]PFDA-derived radioactivity was 23 days in males and 43 days in females. The female rat retained approx. 70% of the dose after 28 days and males retained only 44% of the dose. This difference was demonstrated to be attributed to the faster elimination of PFDA via fecal excretion in males. The concentration of ^{14}C derived from PFDA in blood was also higher in female rats and the PFDA-derived radioactivity was eliminated more slowly in females with a half-life of 29 days compared to 22 days in male rats.

Read-across from the source chemical to fill data gaps on toxicokinetics of PFDA and its ammonium and sodium salts

There is limited toxicokinetic data available for PFDA and its ammonium and sodium salts. Therefore, data on PFDA is supported by read-across from data on PFOA and its ammonium salt APFO.

Justification

The read-across is justified based on structural similarities between PFOA and PFDA. Both substances have a common functional group with only the perfluorinated carbon chain differing in length. In addition, the target and source chemical have physicochemical and toxicological similarities. The toxicokinetics of PFDA and its ammonium and sodium salts is therefore assumed to be predictable on the basis of structural similarities with PFOA and APFO. PFDA and PFOA are relatively strong acids and are expected to dissociate to their respective anionic form at physiological pH. Both acids (PFDA and PFOA) and their salts are expected to be available to cells in the form of their corresponding carboxylate anion (PFD and PFO, respectively) and are therefore considered toxicologically equivalent. Moreover, to further support the read-across the data is discussed in a context of several similar perfluorinated carboxylic acids (PFCAs) with carbon chain length 8-12 (further details on the read-across justification, please refer to the read-across reporting format below). The PFCAs in the supporting chemical category are in the same range of molecular weight and they are lipophilic

with increasing partition coefficient and decreasing water solubility with increasing chain length. Therefore, it is reasonable to assume that the rate of absorption, metabolism, distribution and elimination of PFDA is predictable based on read-across from APFO/PFOA supported by data of PFCAs with carbon chain length 8-12 when data is available.

Source chemical data

Below follows the text from the Background Document for APFO (RAC Annex 1 Background document to the Opinion proposing harmonised classification and labelling at Community level of Ammoniumpentadecafluorooctanoate (APFO), ECHA 2012; page 11).

"A summary of the toxicokinetics of APFO/PFOA is described in the OECD Draft SIDS (2006) Initial Assessment Report of APFO and PFOA and is included below: Limited information is available concerning the pharmacokinetics of PFOA and its salts in humans. Preliminary results of a 5-year half-life study in 9 retired workers indicate that the mean serum elimination half-life of PFOA in these workers was 3.8 years (1378 days, 95% CI, 1131-1624 days) and the range was 1.5 - 9.1 years.
Metabolism and pharmacokinetic studies in non-human primates has been examined in a study of 3 male and 3 female cynomolgus monkeys administered a single i.v. dose of 10 mg/kg potassium PFOA. In male monkeys, the average serum half-life was 20.9 days. In female monkeys, the average serum half-life was 32.6 days. In addition, 4-6 male cynomolgus monkeys were administered APFO daily via oral capsule at 10 or 20 mg/kg-day for six months, and the elimination of PFOA was monitored after cessation of dosing. For the two 10 mg/kg-day recovery monkeys, serum PFOA elimination half-life was 19.5 days, and the serum PFOA elimination half-life was 20.8 days for the three 20 mg/kg-day monkeys.
Studies in adult rats have shown that the ammonium salt of PFOA (APFO) is absorbed following oral, inhalation and dermal exposure. Serum pharmacokinetic parameters and the distribution of PFOA have been examined in the tissues of adult rats following administration by gavage and by i.v. and i.p. injection. PFOA distributes primarily to the liver, serum, and kidney, and to a lesser extent, other tissues of the body. It does not partition to the lipid fraction or adipose tissue. PFOA is not metabolized and there is evidence of enterohepatic circulation of the compound. The urine is the major route of excretion of PFOA in the female rat, while the urine and feces are both main routes of excretion in male rats.

There are gender differences in the elimination of PFOA in adult rats following administration by gavage and by i.v. and i.p. injection. In female rats, following oral administration, estimates of the serum half-life were dependent on dose and ranged from approximately 2.8 to 16 hours, while in male rats estimates of the serum half-life following oral administration were independent of dose and ranged from approximately 138 to 202 hours. In female rats, elimination of PFOA appears to be biphasic with a fast phase and a slow phase. The rapid excretion of PFOA by female rats is believed to be due to active renal tubular secretion (organic acid transport system); this renal tubular secretion is believed to be hormonally controlled. Hormonal changes during pregnancy do not appear to cause a change in the rate of elimination in rats.

Several recent studies have been conducted to examine the kinetics of PFOA in the developing Sprague-Dawley rat. These studies have shown that PFOA readily crosses the placenta and is present in the breast milk of rats. The gender difference in elimination is developmentally regulated; between 4-5 weeks of age, elimination assumes the adult pattern and the gender

difference becomes readily apparent. Distribution studies in the post weaning rat have shown that PFOA is distributed primarily to the serum, liver, and kidney.

Additional information on toxicokinetics will be available in the Annex XV Report (in preparation): PFOA has been found in human blood from all around the world and elevated concentrations are observed following specific exposure either via the environment (contaminated drinking water) or occupationally. The time trend studies show that PFOA levels are significantly associated with the time working as a ski waxer (Freberg et al., 2010, Nilsson et al., 2010b; Nilsson et al., 2010a) and some recent studies strongly indicate that PFOA levels increase with age (Haug et al., 2010, Haug et al., 2011).

PFOA has been shown to be readily transferred to the fetus through the placenta both in laboratory animals and humans. Further, breast milk is an important source of exposure to breast-fed infants and the PFOA exposure for these infants is considerably higher than for adults. Gestational and lactational exposure is of special concern as the foetus and newborn babies are highly vulnerable to toxicant exposure."

Supporting data

In addition, text from the Background Document for PFNA and its sodium and ammonium salts (RAC Annex 1 Background document to the Opinion proposing harmonised classification and labelling at Community level of PFNA and its ammonium and sodium salts, ECHA 2014) is included (below). PFNA-data on its own for read-across purposes to PFDA is not sufficient, however, since PFNA is even closer in structure to PFDA than PFOA the available data support the read-across between PFOA and PFDA.

"In a study by Tatum-Gibbs et al. (2011) Sprague-Dawley rats and CD-1 mice were given a single oral dose of PFNA (dose levels were 1, 3, or 10 mg/kg bw for rats and 1 or 10 mg/kg bw for mice), blood was collected at several time points up until day 50 after treatment when also the liver as well as the kidneys were collected. Serum and tissue concentration of PFNA were determined. The authors of the paper concluded that the serum elimination of PFNA was linear with exposure doses in the rat. Similar to PFOA a major sex difference in the rate of elimination was observed in the rat (estimated half-life of 30.6 days for males and 1.4 days for females). In the mouse, the rate of elimination were non-linear with exposure dose and were slightly faster in females compared to males (estimated serum half-life of 25.8 days (at 1 mg/kg bw) to 68.4 days (at 10 mg/kg bw) in females as compared to 34.3 days (at 1 mg/kg bw) to 68.9 days (at 10 mg/kg bw) in males). For both rats and mice, PFNA was preferentially stored in the liver but not the kidneys. The authors also reported that in mice the hepatic uptake appeared to be more efficient and that the storage capacity was greater in male mice as compared to females.

In a study by Benskin et al. (2009), seven male Sprague-Dawley rats were administered a single gavage dose of 390 µg/kg PFNA (200 µg/kg n-PFNA and 190 µg/kg iso-PFNA). Samples of urine, feces and tail blood were collected over 38 days. The average PFNA concentration in blood after 24 hours was 350 ng/ml n-PFNA and 570 ng/ml iso-PFNA. The first 24 hour blood isomer profiles were primarily an indication of uptake. The half- life for n-PFNA was 40.6 days and 20.7 days for iso-PFNA. These data suggest both a preferential uptake and elimination of iso-PFNA in blood. The daily total average of PFNA's excretion in urine was 32-35% of the given dose and 65-68% of the given dose in feces. Concentrations of PFNA (both iso and n-

PFNA) were analyzed in various tissues. The highest concentrations of PFNA were found in the liver (2.3 ng/g for n-PFNA and 2.7 ng/g for iso-PFNA) followed by kidneys, lungs, heart, spleen, testes, muscle, fat, intestines and brain.

In a study by Henderson and Smith (2006) pregnant mice were exposed to a single gavage dose (30 mg/kg bw) of FTOH (8-2 fluorotelomer alcohol) on GD 8. Whole body homogenates of fetuses at different gestational ages from exposed dams were analyzed for the presence of FTOH or its metabolites (among others PFOA and PFNA). In addition pups from other females exposed in a similar way were crossed fostered and the amounts of FTOH, and its metabolites were analyzed in whole body homogenates/serum/liver of the pups. Since no FTOH was detected in maternal liver or serum nor in fetuses when first analyzed 24 h after dose, FTOH was presumed to have been metabolized by the dam into both PFOA and PFNA. Both PFNA and PFOA (but not FTOH) were found in whole body homogenates of the in utero exposed fetuses as well as in serum and liver of pups from treated dams that following birth had been raised by control dams as well as in pups from control dams that were raised by treated dams. These results show that PFNA and PFOA can cross the placenta and that both compounds are secreted into the milk.

The transfer of PFNA from dam to pup was also shown in a study by Wolf et al. (2010). 129S1/SvlmJ mice were administered PFNA by gavage (0, 0.83, 1.1 1.5 and 2.0 mg/kg) on GD 1-18. Blood was collected at time of weaning from the dams as well as from the weanlings and the concentration of PFNA in serum was measured. The study reported that the concentration in the pups as well as in the dams increased with increasing dose levels. Interestingly, at the time of weaning the serum concentration in the pups were in the same range as the concentration found in the dams (~35 and 25 µg/ml in the dams and pups, respectively, at the high dose level). Furthermore serum concentration of PFNA was higher in non-lactating adult females (29-64 µg/ml depending on dose) as compared to lactating dams (9-35 µg/ml). Even though the design of the study makes it impossible to determine the contribution of placental versus lactational transfer of PFNA, the results suggest transfer of PFNA to the pup via the milk could be substantial."

Conclusion of the read-across data

In rodents, PFOA is demonstrated to be well absorbed orally. >93% of the total ^{14}C was absorbed within 24 hours after a single oral dose of ^{14}C-PFOA (11 mg/kg) in male rats (Gibson and Johnson, 1979). In addition, PFOA/APFO is reported to have high absorption in rodents following inhalation exposure, but limited absorption following dermal exposure. Comparable absorption data for PFCAs C8-C12 are not available, but can be assumed to be high based on their similar physicochemical properties. Thus, PFDA is also assumed to be well absorbed in rodents after oral administration or inhalation.

The highest concentrations of APFO are found in blood, liver, kidney and lung (Hundley et al., 2006; cited in Annex XV dossier PFOA, ECHA 2013). PFNA primarily distributes to the liver in mice (Tatum-Gibbs et al., 2011; cited in BD CLH PFNA, ECHA 2014) and in rats the highest levels are found in liver and kidney (Benskin et al., 2009; cited in BD CLH PFNA, ECHA 2014). A similar distribution pattern was seen in humans as in laboratory animals for PFOA, with the highest concentrations found in lung, kidney, liver and blood (Maestri et al., 2006; cited in Annex XV dossier PFOA, ECHA 2013). In a comparative study by Kudo et al (2001), it

was reported that PFDA highly remains in liver and that concentrations of PFDA compared to PFOA and PFNA was higher in both male and female rats 5 days after injection.

Gestational and lactational transfer of PFNA and PFOA have been demonstrated (Henderson and Smith, 2006; cited in BD CLH PFNA and its ammonium and sodium salts, ECHA 2014) and studies of PFDA indicate that the substance can be secreted into breast milk (Fujii et al., 2012; Tao et al 2008).

There is a species and gender difference in the toxicokinetics of PFCAs. In mice, the difference between the elimination of PFOA in serum between the sexes is small; the substance is slowly eliminated from blood over a long period (Hundley et al., 2006; cited in ECHA Annex XV dossier PFOA, 2013). In contrast, there is a pronounced sex difference in the elimination and half-life of PFOA in rats. The urine is the major route of excretion of PFOA in the female rat, and it is significantly faster excreted in female rats than in male rats (Kudo et al., 2001). The rapid excretion of PFOA by female rats is believed to be due to active renal tubular secretion (organic anion transport system) which is regulated by testosterone and is determined by carbon chain length. Accordingly, PFCAs with carbon chain length ≤10 are mainly excreted via urine in rats with a faster elimination in females compared to male rats (Kudo et al., 2001). The main excretion route of PFDA is via feces for both male and female rats and the half-life of PFDA in rats (39.9 days in males, 58.6 days in females) is longer and the gender difference is smaller compared to the half-life of PFOA (5.63 days in males, 0.08 days in females) and shorter PFCAs.

An elimination half-life around 2-4 years for PFOA has been reported in humans, and in contrast to e.g. rats no sex differences have been observed with respect to the elimination rates. In addition, it should be noted that median human PFNA and PFOA serum concentrations in children are very similar for girls and boys (Schecter et al., 2012; cited in BD CLH PFNA, ECHA 2014), suggesting that the mouse is the preferred animal model for these PFCAs. There are no toxicokinetic studies in mice available for PFDA and there is no data on the elimination of PFDA in humans. However, it is very likely that due to the similarities between PFOA and PFDA with regards to physicochemical properties and long elimination half-lives in exposed animals, that the elimination half-life for PFDA in humans is extremely long and within the same range as the ones recorded for PFOA. If anything, according to the elimination half-life in serum for rats, PFDA is anticipated to be more slowly eliminated as compared to PFOA. Furthermore, considering the long half-life in rats for both male and females, rats may be considered as a relevant model, as well as mouse, for humans to study adverse effects of PFDA.

10. EVALUATION OF HEALTH HAZARDS

Read-across for the purpose of harmonised classification of health hazard classes

Read-across for the purpose of harmonized classification of health hazards of PFDA is based on the structural similarities and functional similarities in physicochemical properties and biological/toxicological properties between the target chemical PFDA and the source chemical PFOA.

The method of chemical categories or grouping is supported in REACH Article 13 - *Information on intrinsic properties of substances may be generated by means other than tests, provided that the conditions set out in Annex XI are met. In particular for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods, for example, in vitro methods or qualitative or quantitative structure-activity relationship models or from information from structurally related substances (grouping or read-across).*

For identification and examination of available information on substances for the purpose of harmonised classification and labelling it is stated in CLP Regulation (EC) No 1272/2008, Article 5.1: *Manufacturers, importers and downstream users of a substance shall identify the relevant available information for the purposes of determining whether the substance entails a physical, health or environmental hazard as set out in Annex I, and, in particular, the following: (c) any other information generated in accordance with section 1 of Annex XI to Regulation (EC) No 1907/2006. Classification based on grouping of substances and read-across approach is supported in REACH regulation (EC) No 1907/2006, Annex XI, section 1.5: Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or "category" of substances. Application of the group concept requires that physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s) within the group by interpolation to other substances in the group (read-across approach). […]*

The similarities may be based on:

1) a common functional group;

2) the common precursors and/or the likelihood of common breakdown products via physical and biological processes, which result in structurally similar chemicals; or

3) a constant pattern in the changing of the potency of the properties across the category.

If the group concept is applied, substances shall be classified and labelled on this basis.

As described in the read-across reporting format below PFDA and PFOA have a common functional group, they are highly similar according to their chemical structure and belong to the same chemical class. PFDA and PFOA are relatively strong acids (c.f. pKa values, estimated range -0.21 to -5.2) and are expected to dissociate to their respective anionic forms at physiological pH. Both acids (PFDA and PFOA) and their salts are expected to be available to cells in the form of their corresponding carboxylate anion (PFD and PFO, respectively) and are therefore considered toxicologically equivalent. The target chemical PFOA have a harmonised classification and is included in the Candidate list, however, there are very few studies on PFOA. Instead read-across from its ammonium salt APFO have been used to fill data gaps for the purpose of harmonised classification. The justification to use APFO data for assessment of PFOA is based on the

expected availability of both substances to cells at physiological pH in the form of the corresponding carboxylate anion (PFO). The main difference between PFOA and its ammonium salt APFO is the initial pH value when coming into contact with body surfaces. However, both PFOA and APFO yield acidic pH values in water. In analogy, PFDA and its ammonium and sodium salts will also be available to cells at physiological pH in the form of their corresponding anion (PFD) which is responsible for the systemic toxicity (oral or inhalation route). Thus, for systemic effects exerted via oral or inhalation route, the read-across is between the two anions PFO and PFD.

Since there is a greater database for APFO/PFOA, which has been extensively reviewed compared to the other long-chain PFCAs and their salts, an analogue approach for chemical grouping was applied to APFO/PFOA and PFDA and its ammonium and sodium salts for the purpose of harmonised classification. To fill data gaps, read-across one-to-one (one analogue used to make estimation for a single chemical) between PFOA and PFDA was performed, and data on PFCAs 8-12 was included to support the read-across robustness. Details on the read-across approach, i.e. showing the trend of physicochemical properties and the structural similarities are given in Table 10.

In this report, the classification proposal focuses on hazards that are evidence-based, not dependent on potency, and hazardous effects that are due to systemic effects, rather than local effects.

Read-across reporting format of an analogue approach

1.

Hypothesis for the analogue approach

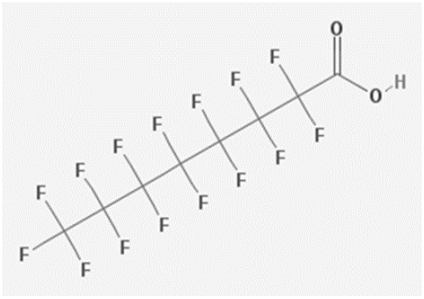
The hypothesis behind the analogue approach is based on the structural similarities between the perfluorinated carboxylic acids: the target substance PFDA and its ammonium and sodium salts and the source substance PFOA and its ammonium salt. The substances also display similarities in physiochemical properties, toxicokinetics and toxicological profiles The selected source chemical (PFOA) with a perfluorinated carbon side-chain length of 8 and the target chemical (PFDA) with a perfluorinated carbon side-chain length of 10 are expected to behave in a predictably similar manner. Thus, the analogue approach can be used to fill data gaps from the source chemical PFOA where data on the target chemical PFDA is lacking.

For the purpose of harmonized classification and labelling the analogue approach was applied to the endpoints reproductive toxicity and cancer.

2.

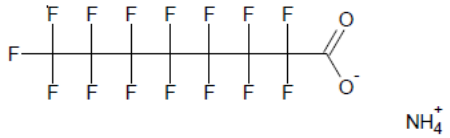
Source chemical

The source chemical is 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid (PFOA)



CAS 335-67-1

And its salt: Ammonium pentadecafluorooctanoate



CAS 3825-26-1

3.

Purity / Impurities

The degree of purity for PFDA is 98% and the impurities are unknown. The degree of purity for PFOA is 98% and the impurities are also unknown.

4.

Analogue approach justification

Structural similarities

PFOA and PFDA are both perfluorinated carboxylic acids (PFCAs) containing a carboxylic acids group and a perfluorinated carbon chain that structurally only differentiate in two added carbons and four fluorines (two CF2-groups). The Organization for Economic Co-operation and Development has defined long-chain perfluorinated carboxylic acids as PFCAs with carbon chains of ≥ 8 carbons (OECD, 2012), and both the target and the source chemical fits into this definition.

The dossier submitter has in this report for the purpose of supporting the read-across further included the target and source chemicals in a supporting chemical category with structurally similar long-chain PFCAs with carbon chain lengths 8-12. The supporting PFCAs and the source and target chemicals with 8-12 CF2-groups belong to the same chemical class in that they all contain a common functional group and they are highly similar according to their chemical structure. Therefore, they are expected to have similar physicochemical properties and to behave in a predictable manner across the defined category spectrum with regards to toxicological properties.

The ammonium salt of the source chemical and the ammonium and sodium salts of the target chemical are included in the read-across and in this classification proposal since it is assumed that PFOA and its ammonium salt APFO are mainly available to cells and tissues (with its physiological pH) in the form of the corresponding carboxylate anion (PFO). In analogy, PFDA and its salts (ammonium nonadecafluorodecanoate (CAS 3108-42-7) and sodium nonadecafluorodecanoate (CAS 73829-36-4)) are also mainly available to cells and tissues in the form of the corresponding carboxylate anion (PFD). Thus, the read-across between the source chemical PFOA and its ammonium salt and the target chemical PFDA and its ammonium and sodium salts is essentially between the two anions: PFO^- and PDF^-. This justifies using the toxicological data from APFO/PFOA for the read-across to PFDA and its ammonium and sodium salts.

Similarities in physicochemical properties

The physicochemical properties of PFOA and PFDA as well as APFO and the ammonium and sodium salts of PFDA are similar or at least in the same range. PFDA has a higher melting/freezing and boiling point than PFOA and with increasing chain length of the C8-12-PFCAs the melting and boiling point increase. The partition coefficient (calculated by COSMOtherm, Wang et al., 2011) is predicted to increase with increasing chain length and the water solubility is predicted to decrease with increasing chain length. This is in agreement with the fact that the polarity of the substances decreases with an increasing chain length. The calculated log Kow of PFOA and PFDA are 5.3 and 6.5 respectively using COSMOtherm (Wang et al., 2011) or 4.81 and 6.15 respectively, estimated using EPI Suite (BD CLH PFNA, ECHA 2014). The measured values for the water solubility of PFOA are 3.4 – 9.5 g/l (dependent on the temperature; the critical micelle concentration = 3.7 g/l for the PFO anion) and 5.14 (at 25°C) for PFDA. The predicted values for water solubility of PFDA and PFOA (see Table 10) are, however, much lower (in the mg/l or µg/l range, depending on prediction model) and indicate a greater difference in solubility between PFOA and PFDA. The reason for the discrepancies between the measured values for PFDA and PFOA and the estimated values are most likely due to the nature of these compounds.

The dissociation constant calculated by SPARC software (BD CLH PFNA, ECHA 2014) indicates high similarities between PFOA and PFDA, and also across the category. Using two different pKA prediction softwares (SPARC and COSMO-RS) Goss (2008) have concluded that the chain length of perfluorinated carboxylic acids seems to have a minimal effect on the pKa values. The assumption would therefore be that

the pKa value for PFOA and PFDA (C10) most likely will be very similar. Indeed, the pKa of PFOA and PFDA as calculated by SPARC (BD CLH PFNA, ECHA 2014) were -0.21 and -0.22 respectively. Using the ChemId plus software the estimated pKa values were somewhat different from the values predicted by the SPARC software (i.e pKa of PFOA and PFDA were -4.2 and -5.2, respectively) (BD CLH PFNA, ECHA 2014).

There are no measured pH values for PFDA or its ammonium or sodium salts. Both PFOA and PFDA are relatively strong acids which are virtually completely protolyzed in water, and thus the pH will only depend on the concentration of the acid. In water the pH values for the ammonium salts will be determined by the ammonium ion and thus it seems reasonable to assume that the pH value of the ammonium salt of PFDA will be the same as the one for the ammonium salt of PFOA (see Table 10). Hence, from a perspective of possible differences in pH, read-across for local effects from the ammonium salt of PFOA (APFO) to the ammonium salt of PFDA would be justified as well as the read-across between PFOA and PFDA.

Similarities in toxicokinetics

The chemical structure of the target and source chemicals renders them both lipid and hydro repellent. Studies in adult rats have shown that the ammonium salt of PFOA (APFO) is absorbed following oral, inhalation and dermal exposure. PFDA has been shown to be absorbed after oral administration (as indicated by liver toxicity, Brewster and Birnbaum 1989) and intraperitoneal injection (as indicated by detectable levels in serum and liver, Kudo et al 2001).

PFOA distributes primarily to the liver, serum, and kidney, and to a lesser extent, other tissues of the body following administration by gavage and by i.v. and i.p. injection in rat. Similarly, after i.p. administration of PFDA in rats the highest concentrations were found in liver, plasma and kidney. Moreover, both substances are detected in human breast milk and blood serum.

Perfluoroalkylated substances are considered to be extremely resistant towards thermal, chemical and biological degradation due to the high carbon-fluorine bond strength and consequently, PFOA and PFDA are not metabolized.

The plasma half-life of PFOA in rats is 5.63 days in males, and 0.08 days in females. For PFDA the plasma half-life in rats is longer in both males and females, 39.9 days and 58.6 days, respectively (Ohmori et al., 2003). Less gender difference in elimination is observed in mouse for PFOA where plasma half-lives in males and females are 19 days and 17 days respectively (Lau et al., 2007). No corresponding data for PFDA in mouse is available.

Similarities of adverse health effects

The mode of action for some of the toxicity caused by APFO/PFOA and PFDA has been identified as the ability of these compounds to activate the peroxisome proliferator-activated receptor α (PPARα). This is recognized to be due to their structural similarities with endogenous fatty acids. However, other mechanisms are also indicated to be involved in e.g. hepatotoxicity and reproductive toxicity. The activity of mouse or human PPARα in transiently transfected cells indicated that with increasing chain length of PFCAs, up to C9 (PFNA), the activity of PPARα increased (Wolf et al., 2008). PFDA, the longest PFCA tested in this study, induced lower activity of mouse PPARα compared to both PFOA and PFNA but higher activity compared to shorter chain perfluoroalkylated acids (PFAAs), and no activation by PFDA of human PPARα was detected in this assay.

Acute toxicity: PFDA and PFOA show low to moderate acute toxicity following oral exposure (Harris et

al., 1989; Glaza et al., 1997 cited in ECHA Background Document for PFOA, 2011). PFDA has an oral LD50 of 120 mg/kg in female mice (Harris et al., 1989) and PFOA has an oral LD50 of 250-500 mg/kg in female rats (Glaza et al., 1997). Moreover, after i.p. administration in male rats LD50 30 days post-treatment for PFOA and PFDA were 189 mg/kg and 41 mg/kg respectively (Olsen Andersen 1983). The mortality of PFDA was demonstrated to be delayed compared to PFOA.

Target organ toxicity: Liver is the main target for PFOA and PFDA and both chemicals cause hepatotoxicity in rodents, mainly manifested as hepatocellular hypertrophy, increased liver weight, and hepatocellular vacuolation.

Other common toxic effects by PFCAs include:

- Decreased body weight
- Effects on lipid metabolism
- Effects on thyroid hormone levels – decreased triiodothyronine (T3) and thyroxine (T4)
- Immunotoxicity (atrophy of thymus and spleen, suppressed antibody responses)

Cancer: In a promoting-activity study in rats PFDA was reported not to increase tumor incidence or altered hepatic foci, indicating that PFDA is not a promoter of hepatocarcinogenesis. However, in rainbow trout (an animal model not responsive to peroxisome proliferation) various PFCAs including PFOA, PFNA and PFDA were shown to enhance liver tumorigenesis (Benninghoff et al., 2012). The source chemical APFO/PFOA has been demonstrated to be a non-genotoxic hepatocarcinogen in rodents which partly can be attributed to PPARα activation and APFO/PFOA has also been reported to increase rates of Leydig cell tumours and of pancreatic acinar cell tumours with unclear mode of action in rodents.

Reproductive toxicity: PFCAs have been shown to possess reproductive toxic properties. Commonly observed effects in the offspring following in utero exposure of PFCAS in the range C8-C12 include:

- Reduced fetal/neonatal body weight and reduced body-weight gain in pups.
- Reduced perinatal/neonatal viability (including mortality).
- Delayed eye opening
- Delayed onset of puberty

5.

Data matrix

The data matrix is constructed by endpoints versus target (PFDA) and source (APFO/PFOA) substance. For support in the discussion of the appropriateness of the read-across approach, additional perfluoroalkylated carboxylic acids are included in the data matrix in the range C8-12 to visualize trends in properties according to carbon chain length. The data matrix is constructed with category endpoints versus members. The members are ordered according to increasing chain length and molecular weight. Data on physicochemical properties and toxicokinetics are included in the matrix, and experimental results on toxicological effects are presented to indicate similar adverse effects and potencies of the category members. For read-across purposes, experimental data on reproductive toxicity and carcinogenicity are listed.

To fill the data gaps on reproductive toxicity and carcinogenicity of PFDA and its ammonium and sodium salts interpolation from measured values of the source chemical PFOA/APFO was used to estimate missing

data points.

A more comprehensive summary of reproductive toxicity studies and carcinogenicity of the source chemical PFOA and its ammonium salt are found in RAC Annex 1 - Background document to the Opinion proposing harmonised classification and labelling at Community level of Ammoniumpentadecafluorooctanoate (APFO), ECHA 2012.

6.

Conclusions

The similarities between PFDA and APFO/PFOA with regards to structure, functional group, physicochemical properties and toxicological effects are concluded to be sufficient to perform a read-across.

Based on the data from the extensively tested source chemical APFO with carbon chain length C8 compared to the target chemical with carbon chain length C10, supported with data of longer PFCAs (C11-12) it is reasonable to assume that the toxicological properties of the less well studied target chemical can be predicted. The source chemical APFO/PFOA and its ammonium salt have harmonised classifications (Index no 607-704-00-2 and 607-703-00-7 respectively, Annex VI to CLP) including Repr. 1B and Carc. 2 and have been identified as Substances of Very High Concern and subsequently been included in the Candidate List pursuant to REACH article 57c (ED/69/2013). Moreover, a RAC opinion concluding on the harmonised classification of the C9 PFCA PFNA and its ammonium and sodium salts was recently agreed based on read-across from APFO/PFOA resulting essentially in an identical classification as APFO/PFOA, with the addition of Repr. 2 H361f and STOT-RE 1 for thymus and spleen.

The available data thus permit an assessment of reproductive toxicity and cancer. PFDA and its ammonium and sodium salts are anticipated to behave in a similar way as the source chemical. Therefore, a harmonised classification of PFDA and its ammonium and sodium salts as Repr. 1B H360Df is warranted. One can assume that the effects of PFDA if anything would be more severe than for APFO/PFOA and PFNA due to the slower elimination of PFDA compared to APFO/PFOA, as demonstrated in rats. In addition, a harmonised classification of PFDA and its ammonium and sodium salts as Carc. 2 is warranted.

Table 10. Data matrix for the analogue read-across: Basic substance information, physical chemical properties and toxicokinetics relevant to justify read-across in the health hazard assessment (reproductive toxicity and carcinogenicity).

	Source chemical		***Support***	***Target chemical***	***Support***	***Support***
	APFO (C8-PFCA)	**PFOA (C8-PFCA)**	**PFNA (C9-PFCA)**	**PFDA (C10-PFCA)**	**PFUnDA (C11-PFCA)**	**PFDoDA (C12-PFCA)**
IDENTIFICATION						
Chemical structure	CF3(CF2)6-COO-NH4+	CF3(CF2)6-COOH	CF3(CF2)7-COOH	CF3(CF2)8-COOH	CF3(CF2)9-COOH	CF3(CF2)10-COOH
CAS no	3825-26-1	335-67-1	375-95-1	335-76-2	2058-94-8	307-55-1
PHYSICOCHEMICAL PROPERTIES						
Molecular weight (g/mol)	431.095	414.07	464.076 (free acid)	514.084	564.0909	614.0984
Physical state at 20°C and 101.3 kPa	solid (according to melting point)	solid (Kirk-Othmer, 1994)	solid (according to melting point)	solid (according to melting point)	solid (according to melting point)	solid (according to melting point)
Melting/ freezing point (C°)	157-165 (decomposition starts above 105°C) (Lines and Sutcliff, 1984) 130 (decomposition) (3M Company, 1987)	54.3 (Lide, 2003) 52 - 54 (Kirk-Othmer, 1994)	65-68 (Oxford University Chemical Safety Data sheet)	87.4-88.2 (Hare et al., 1954)	112 – 114 (Huang et al., 1987)	112 – 114 (Huang et al., 1987)
Boiling point (C°) ***- measured***	Decomposition (Lines and Sutcliff, 1984)	188 at 1013.25 hPa (Lide, 2003) 189 at 981 hPa (Kauck and Diesslin, 1951)	218 at 740 mmHg (Oxford University Chemical Safety Data sheet)	218 (Sigma Aldrich, 2004; Kauck and Diesslin, 1951)		249 (Data from SRC PhysProp Database; Annex XV dossier C12-PFCA, ECHA 2012)
- estimated					238.4 at 101.325 kPa (Kaiser et al., 2005)	

Vapour pressure (Pa) - measured		128 at 59.3 °C (Washburn et al., 2005)	0.10 (Arp et al., 2006)			
- estimated	0.0081 at 20 °C (Washburn et al., 2005) 0.0028 at 25°C (Barton et al., 2009) (cited in Annex XV-dossier APFO, ECHA 2013)	4.2 at 25 °C (Kaiser et al., 2005); (Washburn et al., 2005) 2.3 at 20 °C (Washburn et al., 2005) (cited in Annex XV-dossier PFOA, ECHA 2013)	1.3 to 99.97 kPa (99.6 - 203°C) (Kaiser et al., 2005)	3.1 to 99.97 kPa (129.6 - 218.9°C) (Kaiser et al., 2005)	0.6 to 99.97 kPa (112 - 237.7°C) (Kaiser et al., 2005)	0.9 to 99.96 kPa (127 - 247.7°C) (Kaiser et al., 2005) 1.25 at 25°C (ACD/Labs Software V11.02; Annex XV dossier C12-PFCA, ECHA 2012)
Water solubility (g/L) ***- measured***	14.2 at 2.5°C (Shinoda et al., 1972; cited in Annex XV-dossier APFO, ECHA 2013)	9.5 at 25°C (Kauck and Diesslin, 1951) 4.14 at 22°C (Prokop et al., 1989) (cited in Annex XV-dossier PFOA, ECHA 2013)		5.14 (Kauck and Diesslin, 1951)		
- estimated	43.34 mg/L (WSKOW program, v1.42 (water sol from Kow); BD CLH PFNA, ECHA 2014) 0.00040249 mg/L (WATERNT Program, v1.01 (from fragments); BD CLH PFNA, ECHA 2014)	0.4813 mg/L (WSKOW program, v1.42 (water sol from Kow); BD CLH PFNA, ECHA 2014) 0.0020683 mg/L (WATERNT Program, v1.01 (from fragments); BD CLH PFNA, ECHA 2014)	0.06258 mg/L (WSKOW program, v1.42 (water sol from Kow); BD CLH PFNA, ECHA 2014) 9.942x10-5 mg/L (WATERNT Program, v1.01 (from fragments); BD CLH PFNA, ECHA 2014)	0.008043 mg/L (WSKOW program, v1.42 (water sol from Kow); BD CLH PFNA, ECHA 2014) 4.7238x10-6 mg/L (WATERNT Program, v1.01 (from fragments); BD CLH PFNA, ECHA 2014)	1.2E-4, pH 1 at 25°C 9.0E-4, pH 2 at 25°C 8.5E-3, pH 3 at 25°C 0.056, pH 4 at 25°C 0.14, pH 5 at 25°C 0.16, pH 6- 10 at 25°C (ACD/Labs Software V11.02; Annex XV dossier C11-PFCA, ECHA 2012)	2.9E-5, pH 1 at 25°C 2.2E-4, pH 2 at 25°C 2.0E-3, pH 3 at 25°C 0.014, pH 4 at 25°C 0.034, pH 5 at 25°C 0.039, pH 6 at 25°C 0.040, pH 7 at 25°C 0.041, pH 8-10 at 25°C (ACD/Labs Software V11.02; Annex XV dossier C12-PFCA, ECHA 2012)
Partition coefficient log K_{ow} - measured			2.3 – 2.48 (Annex XV dossier C11-PFCA, ECHA 2012)	2.65 – 2.87 (Annex XV dossier C11-PFCA, ECHA 2012)		
- estimated						10.16 (Annex XV dossier C12-PFCA, ECHA 2012)

		2.69 at pH 7 and 25°C (ACD/Labs Software V11.02) (Annex XV dossier PFOA, ECHA 2013)				logP 9.363±0.888 at 25°C (ACD/Labs Software V11.02; Annex XV dossier C12-PFCA, ECHA 2012)
		6.3 (EPI suite [Syracuse_Research_Corporation, 2000-2008]) (Annex XV dossier PFOA, ECHA 2013)				
	1.94 (EPISuite: KOWWIN Program (v1.68); BD CLH PFNA, ECHA 2014)	4.81 (EPISuite: KOWWIN Program (v1.68); BD CLH PFNA, ECHA 2014)	5.48 (EPISuite: KOWWIN Program (v1.68); BD CLH PFNA, ECHA 2014)	6.15 (EPISuite: KOWWIN Program (v1.68); BD CLH PFNA, ECHA 2014)		
		5.30 (COSMOtherm, Wang et al., 2011)	5.9 (COSMOtherm, Wang et al., 2011)	6.5 (COSMOtherm, Wang et al., 2011)	7.2 (COSMOtherm, Wang et al., 2011)	7.8 (COSMOtherm, Wang et al., 2011)
Dissociation constant pKa ***- measured***	2.80 in 50% aqueous ethanol (Brace, 1962) 2.5 (Ylinen et al., 1990) (cited in Annex XV dossier APFO, ECHA 2013)	2.80 in 50% aqueous ethanol (Brace, 1962) 2.5 (Ylinen et al., 1990) 1.5 - 2.8 (Kissa, 2001) (cited in Annex XV dossier PFOA, ECHA 2013)				
- estimated				2.57512 (dimensionless; Moroi et al., 2001)	0.52±0.10 (Annex XV dossier C11-PFCA, ECHA 2012)	0.52±0.10 (Annex XV dossier C12-PFCA, ECHA 2012)
		-0.21 (SPARC; BD CLH PFNA, ECHA 2014)	-0.21 (SPARC; BD CLH PFNA, ECHA 2014)	-0.22 (SPARC; BD CLH PFNA, ECHA 2014)		
	8.86 (NH4+) (ChemID Plus Advance; BD CLH PFNA, ECHA 2014)	-4.2 (ChemID Plus Advance; BD CLH PFNA, ECHA 2014)	-6.51 (ChemID Plus Advance; BD CLH PFNA, ECHA 2014)	-5.2 (ChemID Plus Advance; BD CLH PFNA, ECHA 2014)		
pH value - measured	Approx. 5 (3M, 1987) (reliability not assignable)	2.6 (1 g/l at 20°C) (Merck, 2005) (reliability not assignable)				

- estimated		3.0 (SPARC; BD CLH PFNA, ECHA 2014)	3.0 (SPARC; BD CLH PFNA, ECHA 2014)	3.0 (SPARC; BD CLH PFNA, ECHA 2014)		
	5.9 (ChemID Plus Advance; BD CLH PFNA, ECHA 2014)	3.0 (ChemID Plus Advance; BD CLH PFNA, ECHA 2014)	3.0 (ChemID Plus Advance; BD CLH PFNA, ECHA 2014)	3.0 (ChemID Plus Advance; BD CLH PFNA, ECHA 2014)		
CLASSIFICATION AND RISK MANAGEMENT						
Candidate list	Toxic for reproduction (Article 57 c); PBT (Article 57 d) ED/69/2013	Toxic for reproduction (Article 57 c); PBT (Article 57 d) ED/69/2013			vPvB (Article 57 e) ED/169/2012	vPvB (Article 57 e) ED/169/2012
CLP Annex VI	607-703-00-7	607-704-00-2	Adopted RAC opinion September 12, 2014	CLH proposal	no CLH	no CLH
CLH	Acute Tox. 4 H302	Acute Tox. 4 H302	Acute Tox. 4 H302			
	Acute Tox. 4 H332	Acute Tox. 4 H332	Acute Tox. 4 H332			
	Eye Dam. 1 H318	Eye Dam. 1 H318	Eye Dam. 1 H318			
	Carc. 2 H351	Carc. 2 H351	Carc. 2 H351	Carc. 2 H351		
	Repr. 1B H360D	Repr. 1B H360D	Repr. 1B H360Df	Repr. 1B H360Df		
	Lact. H362	Lact. H362	Lact. H362			
	STOT RE 1 H372 (liver)	STOT RE 1 H372 (liver)	STOT RE 1 H372 (liver, thymus, spleen)			
TOXICOKINETICS						
distribution ***-rat***	Highest ^{14}C-concentrations in the blood and liver followed by the kidneys, lungs, and skin in male rats after administration of ^{14}C-APFO. Negligible amounts of ^{14}C in organs of female rats. (single oral dose, Hundley et al., 2006)		Preferentially stored in the liver but not the kidneys (single oral dose, Tatum-Gibbs et al., 2011) Highest concentrations were found in the liver followed by kidneys, lungs, heart, spleen, testes, muscle, fat, intestines and brain in male rats (12 weeks, administered by diet, Benskin et al., 2009)	Highest concentration found in liver, plasma, kidneys in both male and female rats after ^{14}C-PFDA administration (single i.p. dose, Vanden Heuvel et al., 1991)		

- mouse	Highest ^{14}C-concentrations in the blood and liver followed by the kidneys, lungs, and skin in both male and female mice after administration of ^{14}C-APFO. (single oral dose, Hundley et al., 2006)		Preferentially stored in the liver but not the kidneys (single oral dose, Tatum-Gibbs et al., 2011)			
half-life in plasma		5.63 days male rats, 0.08 days female rats (rat, i.v., Ohmori et al., 2003)	29.5 days male rats, 2.44 days female rats (rat, i.v., Ohmori et al., 2003) 30.6 days male rats 1.4 days female rats (rat, oral, Tatum-Gibbs et al., 2011)	39.9 days male rats, 58.6 days female rats (rat, i.v., Ohmori et al., 2003)		
-rat						
- mouse		19 days male mice 17 days female mice (Lau et al., 2007)	34–69 days male mice 26–68 days female mice (mouse, oral, Tatum-Gibbs et al., 2011)			
- human		3.8 years (24 males and 2 females; range 1.5 - 9.1 years) (Olsen et al., 2007)				
elimination	Urine major route of excretion in male and female rats. 34.3% and 100% of the total dose was excreted in the urine and feces by 120 h after dosing in male and females respectively. (single oral dose, Hundley et al., 2006)	Urine major route of excretion in the male and female rat. (rat, i.v., Kudo et al., 2001) Major difference in rate of elimination in urine between male and female rats, with slower excretion in males (rats, i.v., Ohmori et al.	Urine major route of excretion in female rats, feces main route of excretion in male rats (rat, i.v., Kudo et al., 2001) Difference in the rate of elimination in urine between male and female rat, with slower excretion	Feces major route of excretion in both male and female rats (rat, i.v., Kudo et al., 2001) Minor difference in the fecal elimination, with 51 and 24% of the administered ^{14}C being recovered in the feces of male and female rats, respectively, by 28 days		
- rat						

		2013)	in males (rat, i.v., Ohmori et al., 2003)	post-treatment. (rat, i.p., Vanden Heuvel et al., 1991)		
- mouse	Urine and feces primary route of excretion in both male and female mice. 11% of the total dose was excreted in the urine and feces by 120 h after dosing in both male and females. (single oral dose, Hundley et al., 2006)					
total clearance - rat		50.5 ml/(day/kg) male, 2233.5 ml/(day/kg) female (rat, i.v., Ohmori et al., 2003)	6.9 ml/(day/kg) male, 105.7 ml/(day/kg) female (rat, i.v., Ohmori et al., 2003)	5.2 ml/(day/kg) male, 5.3 ml/(day/kg) female (rat, i.v., Ohmori et al., 2003)		
clearance rate - rat		<100 ml/kg/day in male rats, approx. 1000 ml/day/kg in female rats (rat, i.v., Ohmori et al., 2003)	Virtually no CLR in males, approx. 100 ml/day/kg in females (rat, i.v., Ohmori et al., 2003)	Virtually no CLR in either male or female rat (rat, i.v., Ohmori et al., 2003)		
gestational and lactational transfer ***-rodents***	Transferred to the foetus through the placenta. The offspring is exposed from breast milk. (rat, oral gavage, Hinderliter et al., 2005 cited in Annex XV-dossier APFO, ECHA 2013)		Can cross the placenta and is secreted into breast milk (mouse, single gavage dose of FTOH, Henderson and Smith 2006; cited in BD CLH PFNA, ECHA 2014) Indications of placental and lactational transfer (mouse, oral gavage, Wolf et al., 2010; cited in BD CLH PFNA, ECHA 2014)			

-human		Detectable concentrations in cord blood. Found to be transferred to infants through breast-feeding. (Annex XV dossier PFOA, ECHA 2013)	Detected in serum, cord blood and breast milk (BD CLH PFNA, ECHA 2014)	Detected in breast milk (Tao et al., 2008; Fujii et al., 2012)		
HUMAN HEALTH HAZARDS						
Acute tox						
- oral	LD50 = 250-500 mg/kg in rats (Glaza, 1997; cited in BD CLH APFO, ECHA 2011)			LD50 = 120 mg/kg in female mice (Harris et al., 1989)		
- inhalation	LC50 = 0.98 mg/L (4 hour exposure) in rat (Kennedy et al., 1986; cited in BD CLH APFO, ECHA 2011) LC50 > 18.6 mg/l (1 hour exposure) in rat (Rusch, 1979; Griffith and Long, 1980; cited in BD CLH APFO, ECHA 2011)					
- i.p.		LD50/30= 189 mg/kg in male rats (Olsen Andersen et al., 1983)		LD50/30= 41 mg/kg in male rats (Olsen Andersen et al., 1983)		
Repeated dose toxicity						

- liver	LOAEL = 30 ppm, based on hepatocellular hypertrophy, hepatocellular degeneration and/or necrosis; cytoplasmic vacuoles; increased absolute and relative liver weight. (mouse, oral via diet for 28 days, Christopher and Marisa, 1977; Griffith and Long, 1980; cited in RAC opinion CLH APFO, ECHA 2011)		Relative liver weight dose dependently increased ($p<0.001$) in dams, starting from lowest dose tested 0.83 mg/kg/day. (mouse, oral gavage, GD 1−18, Wolf et al., 2010)	LOAEL = 1 mg/kg/day based on relative liver weight in dams. (mouse, oral gavage GD 6-15, Harris and Birnbaum, 1989)	LOAEL = 0.3 mg/kg/day based on increased liver weight and centrolobular hypertrophy of hepatocytes (rat, oral gavage, OECD TG 422, Takahashi et al 2014)	LOAEL = 0.5 mg/kg/day based on increased relative liver weight and focal necrosis. (rat, oral gavage, OECD TG 422; Kato et al 2014)
Carcinogenicity						
	Demonstrated to be a non-genotoxic hepatocarcinogen in rodents which partly can be attributed to PPARα activation. Increased rates of Leydig cell tumours and of pancreatic acinar cell tumours with unclear mode of action in rodents. (RAC opinion CLH APFO, ECHA 2011)			Not a promoter of hepatocarcinogenesis (rat, monthly i.p. injections for 9 or 18 months, Borges et al., 1993)		
Adverse effects on sexual function and fertility						

-male reproductive organs		LOAEL = 5 mg/kg/day based on increased incidence of abnormal seminiferous tubules. (mouse, oral gavage 6 weeks; Li et al., 2011).		Marked atrophy of the epithelium of seminal vesicles and marked decreased epithelial height at 80 mg/kg. Marked atrophy of the epithelium of glandular acini of the ventral prostrate at 40 and 80 mg/kg (no information on quantification available). (rat, single dose i.p.; Bookstaff et al., 1990) Atrophy and degeneration of the seminiferous tubules in testes at day 16 and persisted up to day 30 post-administration of 50 mg/kg (no information on quantification available) (rat, single dose i.p; George and Andersen 1986)		Slight histopathological changes in testis, slight to severe findings in epididymis, slight to moderate changes in seminal vesicles and coagulating gland (no statistical significant changes) at 2.5 mg/kg/day (rat, oral gavage, OECD TG 422; Kato et al 2014)
-sperm abnormalities		Sperm morphology abnormalities increased ($p<0.05$) at both doses tested, 1 and 5 mg/kg bw/day. (mouse, oral gavage 6 weeks; Li et al., 2011).	LOAEL = 5 mg/kg/day based on increase in the number of apoptotic spermatogenic cells (rat, oral gavage, 14 days; Feng et al., 2009)			Decreased spermatid and spermatozoa counts at 2.5 mg/kg/day (no statistical significant changes) (rat, oral gavage, OECD TG 422 (Kato et al 2014) Leydig cells, Sertoli cells and spermatogonic cells were displaying apoptotic morphological features at 5 or 10 mg/kg/bw (rat, oral gavage, 14 days; Shi et al., 2007)

-altered hormonal levels		LOAEL = 1 mg/kg bw/day based on reduced plasma testosterone concentration (hPPARα mouse, oral gavage, 6 weeks; Li et al., 2011).	Testosterone levels were increased (87.5%, p<0.01) at 1 mg/kg/day but significantly decreased at 5 mg/kg/day (85.4%, p < 0.01). (rat, oral gavage, 14 days; Feng et al., 2009)	Decreased (p<0.05) plasma testosterone and 5α-dihydrotestosterone concentrations at 40 and 80 mg/kg (rat, single dose i.p.; Bookstaff et al 1990)		Dose-dependent decrease in serum testosterone levels. Markedly decreased (p<0.05) levels at 0.2 mg PFDoA/kg/day and 0.5 mg PFDoA/kg/day (rat, oral gavage 110 days; Shi et al., 2009a)
- ER transactivation in vitro		No ER antagonistic effect, no estrogenic effect (Kjeldsen and Bonefeld-Jorgensen 2013)	No ER antagonistic effect, no estrogenic effect (Kjeldsen and Bonefeld-Jorgensen 2013)	No ER antagonistic effect, weak estrogenic effect (Kjeldsen and Bonefeld-Jorgensen 2013)	No ER antagonistic effect, no estrogenic effect (Kjeldsen and Bonefeld-Jorgensen 2013)	No ER antagonistic effect, no estrogenic effect (Kjeldsen and Bonefeld-Jorgensen 2013)
- AR transactivation (with 25 pM DHT) in vitro		LOEC: 1x10-5 MOEC: 1x10-4 IC50: 1.1x10-5 (Kjeldsen and Bonefeld-Jorgensen 2013)	LOEC: 5x10-5 MOEC: 1x10-4 IC50: 5.2x10-5 (Kjeldsen and Bonefeld-Jorgensen 2013)	LOEC: 1x10-5 MOEC: 1x10-4 IC50: 6.0x10-6 (Kjeldsen and Bonefeld-Jorgensen 2013)	No reported effect (Kjeldsen and Bonefeld-Jorgensen 2013)	No reported effect (Kjeldsen and Bonefeld-Jorgensen 2013)
Adverse effects on the development of the offspring						
- resorptions /litter loss		LOAEL = 5 mg/kg/day based on increased percentage of dams with full litter resorptions (mouse, oral gavage GD 1-17; Lau et al 2006) Increased (p<0.05) percentage of dams with whole litter loss at 5 mg/kg/day (mouse, oral gavage GD 1-17; Wolf et al 2007)	Increased percentage of dams with full litter resorptions or whole litter loss at 2 mg/kg/day (not statistical significant) (mouse, oral gavage GD 1-18; Wolf et al 2010)	Increased % resorptions per litter at 6.4 mg/kg bw/day (19.1%) (not statistical significant) (mouse, oral gavage GD 6-15; Harris and Birnbaum 1989)		
- pup viability		Reduced (p<0.05) percentage of live pups/litter at birth at 0.6 mg/kg Reduced (p < 0.001) pup	Reduced (p< 0.05) number of live pups/litter at birth at 1.1 mg/kg bw. Reduced (p < 0.05) pup survival until PND 21 at	Reduced number of live fetus per litter at 6.3 mg/kg bw/day (not statistical significant) (mouse, oral gavage GD		

		survival PND1–22) at 0.6 mg/kg (mouse, oral gavage GD 1-17, Abbott et al., 2007	1.1 mg/kg bw. (mouse, oral gavage GD 1-18; Wolf et al 2010)	6-15; Harris and Birnbaum 1989)		
- pup body weight		Reduced ($p<0.01$) pup birth weights at 5 mg/kg bw and reduced pup weights at 5 mg/kg/day ($p<0.01$-0.001) on PND 1-22 (mouse, oral gavage GD 1-17; Wolf et al 2007)	No effect on pup weight at birth at any dose level in either male or females. Reduced male pup weight on PND 7, 10 and 14 ($p<0.001$/ 0.01) at 2 mg/kg. Female pup weight was reduced on PND 7, 10, 14 and 21 ($p<0.001$ – 0.05). (mouse, oral gavage GD 1-18; Wolf et al 2010)	Dose dependent decrease ($p<0.05$) in fetal body weight per litter starting from 1.12 mg/kg/day. (mouse, oral gavage GD 6-15; Harris and Birnbaum 1989)	Pup body weight at birth and at 4 days after birth decreased ($p<0.01$) at 1 mg/kg. (rat, oral gavage, OECD TG 422; Takahashi et al 2014)	
- eye opening		Up to 3 days delay in eye opening ($p<0.05$) from 5 mg/kg bw/day (mouse, oral gavage GD 1-17; Lau et al 2006)	Approx. 2 days delay in eye opening ($p<0.01$) at 2 mg/kg bw. (mouse, oral gavage GD 1-18; Wolf et al 2010)			
- mammary gland development		Delayed ($p<0.001$) mammary gland development at 5 mg/kg/day at PND 10 and PND 20 (mouse, oral gavage GD1-17; White et al 2007) Exposure at 5 mg/kg at late fetal (GD 15-17) and early neonatal (exposure via lactation starting PND 1) life resulted in early and persistent mammary gland effects ($p<0.05$) (mouse, oral gavage;				

		White et al., 2009)				
Adverse effects on or via lactation						
		Reduced ($p<0.001$) weight of pups on PND 1-22 exposed in utero and lactationally at 5 mg/kg/day. Reduced ($p<0.01$-0.001) weight of pups on PND 2-22 exposed in utero and lactationally at 3 mg/kg/day. Delay in eye opening ($p < 0.05$) in pups exposed in utero and lactationally at 3 mg/kg/day and 5 mg/kg/day. (mouse, oral gavage GD 1-17; Wolf et al 2007)				

10.1 Acute toxicity

Hazard class not evaluated.

10.2 Skin corrosion/irritation

Hazard class not evaluated.

10.3 Serious eye damage/eye irritation

Hazard class not evaluated.

10.4 Respiratory sensitisation

Hazard class not evaluated.

10.5 Skin sensitisation

Hazard class not evaluated.

10.6 Germ cell mutagenicity

Hazard class not evaluated.

10.7 Carcinogenicity

There are only limited studies of carcinogenicity of PFDA available. Therefore, read-across from PFOA and its ammonium salt was performed for the purpose of classification in the hazard class carcinogenicity of PFDA and its ammonium and sodium salts.

Table 33a: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference

Read-across data: Adapted from BD CLH APFO, ECHA 2012; Table 13					
	Sprague-Dawley rats 50/sex/group Groups of 15 additionally rats/sex were fed 0 or 300 ppm and evaluated after 1 year	APFO	0, 30 or 300 ppm APFO in the diet corresponding to 1.3 and 14.2 mg/kg/day in males and 1.6 and 16.1 mg/kg/day in females 2 years	A dose-related decrease in bw gain in males (high dose -21% by week 6, over 10% through 66 weeks of the study, significant until week 98. Low dose: 5% decrease in bw gain at week 6, little thereafter), and to a lesser extent in females (slightly decreased, maximum 11%, at 92 weeks) was reported, and the decrease was considered treatment related. There were no differences in mortality between treated and untreated groups. Significant decreases in red blood cell counts, hemoglobin concentrations and hematocrit values were observed in the high dose male and female rats. Clinical chemistry changes included slight (<2fold) but significant increases in ALT, AST and AP in both treated male groups from 3-18 months, but only in high dose males at 24 months. Slight (<10%) increases in abs/rel liver and kidney weights were noted in high dose male and female rats at 1 year interim sacrifice and at terminal necropsy. Only the rel liver weights in high dose males were significant ($p<0.05$). Histologic evaluation showed lesions in the liver, testis and ovary. **Liver;** At the 1-year sacrifice a diffuse epatomegalocytosis (12/15) portal mononuclear cell infiltration (13/15) and hepatocellular necrosis (6/15) were reported in the high-dosed males, whereas the incidences in the control group were 0/15, 7/15 and 0/15, respectively. At 2-year sacrifice megalocytosis was found at an incidence of 0%, 12% and 80% in the males, and at 0%, 2% and 16% in the females, in the controls, low- and high dose groups, respectively. Hepatic cystoid degeneration was reported in 14% and 56% of the low and high dose males, as compared to 8% in controls. The incidence of hyperplastic nodules was slightly increased in the high dosed males, 6%, as compared to 0% in controls. **Testis**; At 1-year sacrifice, marked aspermatogenesis was found in 2/15 in high dosed males but not in the controls. At the 2-year sacrifice, testicular masses were found in 6/50high dosed and 1/50low-dosed rats compared to 0/50 in controls. Vascular mineralization was reported in 18% of high dosed males and 6% in low-dosed males, however, not in control males. The testicular effects reached statistically significance in the high-dose group. Furthermore, at 2-year sacrifice a significant increase in the incidence of testicular Leydig cell (LCT) adenomas in the high-dosed group was reported [0/50 (0%), 2/50 (4%) and 7/50 (14%)] in control, low- and high dose group, respectively).	Sibinski, 1987;

				The historical control incidence was 0.82% (from 1 340 Sprague-Dawley rats used in 17 carcinogenicity studies (Chandra et al., 1992). The spontaneous incidence of LCT in 2-year old Sprague-Dawley rats is reported to be approximately 5% (Clegg et al., 1997). **Ovary;** In females at 2-year sacrifice a dose-related increase in the incidence of ovarian tubular hyperplasia was reported, 0%, 14% and 32% in control, low-, and high dose groups, respectively. However, recently the slides of the ovaries were re-evaluated, and more recently nomenclature was used (Mann and Frame, *2004).* The ovarian lesions were diagnosed and graded as gonadal stromal hyperplasia and/or adenomas, which corresponded to the diagnoses of tubular hyperplasia or tubular adenoma by the original study pathologist. With this evaluation no statistically significant increase in hyperplasia (8, 16 and 15 in the control, 30 ppm and 300 ppm group, respectively), adenomas (4, 0 and 2 in the control, 30 ppm and 300 ppm group, respectively or hyperplasia/adenoma combined (12, 16 and 17 in the control, 30 ppm and 300 ppm groups, respectively) were seen in treated groups compared to controls. There was also a significant increase ($P<0.05$) in the incidence of mammary fibroadenomas [10/47 (21%), 19/47 (40%) and 21/49 (43%) in controls, 30 and 300 ppm groups, respectively]. The historical control incidence was 19% observed in 1329 Sprague-Dawley rats used in 17 carcinogenicity studies (Chandra et al., 1992). However, the compared to other historical control data at 24% from a study of 181 female rats terminally sacrificed at 18 month (which was considered an inappropriate historical reference), and the historical control incidence of 37% in 947 female rats in the Haskell laboratory (Sykes, 1987), the evidence of mammary fibroadenomas were considered equivocal.	
	Sprague-Dawley male rats, 76 rats in the treatment group and 80 rats in the control group	APFO	300 ppm APFO 2 years	This study was performed to confirm the induction of LCT, reported in the study by Sibinski, 1987. A significant increase in the incidence of LCT in treated rats (8/76, 11%) compared to controls 0/80 (0%) was reported. The tumours may be a result of endocrine changes, because a induced hepatic aromatase activity (P450-19A1[1], demonstrated in a 14 day study, Liu et al, 1996) and a sustained increase in serum estradiol were reported. In addition, the treated group had a significant increase in the incidence of liver adenomas (2/80 and 10/76 in the control and 300 ppm group, respectively) and pancreatic acinar cell tumours (PACT) (0/80 and 7/76 in the control and 300 ppm group, respectively). There was one pancreatic acinar cell carcinoma in the treated group and none in the control group. Biegel et al., 2001 also	Cook et al., 1994; Biegel et al., 2001 Liu et al, 1996

				studied the temporal relationship between relative liver weights, hepatic β-oxidation, and hepatic cell proliferation and hepatic adenomas following exposure for 1, 3, 6, 9, 12, 15, 18, 21 and 14 months. Relative liver weights and hepatic β-oxidation were increased at all time-points. The liver endpoints (weight, and β-oxidation (but not cell proliferation)) were elevated well before the first occurrence of liver adenomas, which occurred after 12 month of treatment. No effect on peroxisomal β-oxidation in Leydig cells was observed during the study and at the end of study. There were no biologically meaningful differences in serum hormones (testosterone, FSH, prolactin, or LH concentrations) except for serum estradiol concentrations in treated rats. Pancreatic cell proliferation was significantly increased at 15, 18, and 21 months, but no increased proliferation was observed at 9 or 12 months. In the study by Sibinski, 1987, no increase in the incidence of PACT was reported (0/33, 2/34 and 1/34 in the control, 30 and 300 ppm groups, respectively). Therefore, the histological slides from both studies were reviewed by an independent pathologist. This review indicated that PFOA produced increased incidences of proliferative acinar cell lesions in the pancreas in both studies at 300 ppm. The differences reported were quantitative rather than qualitative; more and larger focal proliferative acinar cell lesions and greater tendency for progression of lesions to adenoma of the pancreas were reported in the second study. It was concluded that the difference between pancreatic acinar hyperplasia (reported in Sibinski, 1987) and adenoma (reported in Cook et al., 1994; Biegel et al., 2001) in the rat was a reflection of arbitrary diagnostic criteria and nomenclature by the different pathologists.	

Table 33b: Summary table of human data on carcinogenicity

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference

Table 33c: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
The study was conducted to determine if PFDA, which is a known peroxisome proliferator, has promoting activity in two-stage hepatocarcinogenesis.	PFDA (from 3M, Inc) Purity: not stated	Sprague-Dawley rats Female Five groups with 26 animals each. The control group treated with phenobarbital had 10 animals. Monthly i.p. injections of 0.0, 0.05, 0.50 or 5.0 mg/kg PFDA in corn oil for 9 or 18 months. Rats were given an initiating dose of 10 mg/kg diethylnitrosamine by gavage twenty-four hours after partial hepatectomy. Additional control groups were placed on diets that contained either 0.01% ciprofibrate or 0.05% phenobarbital.	5.0 mg/kg PFDA significantly increased ($p<0.05$) the relative liver weight (23%) at 9 months and increased ($p<0.05$) the activity of the peroxisomal enzyme fatty acyl CoA oxidase at both 9 (100% increase) and 18 months (53% increase). PFDA treatment did not increase the tumor incidence or the number of altered hepatic foci at 9 or 18 months, although the mean volume of foci was dose-dependently increased (3-5 fold, $p<0.01$) at 9 months. An increase (not stat. sign.) in the incidence of hepatocellular carcinoma was observed at 5.0 mg/kg PFDA (2/12) at 9 months but not at 18 months. The results of this investigation indicate that PFDA is not a promoter of hepatocarcinogenesis.	Borges T, Peterson RE, Pitot HC, Robertson LW, Glauert HP. Effect of the peroxisome proliferator perfluorodecanoic acid on the promotion of two-stage hepatocarcinogenesis in rats. Cancer Lett. 1993 Aug 16;72(1-2):111-20.

Mechanistic study in a non-mammalian species	PFDA (from Sigma Aldrich) Purity: analytical grade (not stated)	Trout is an animal model that represents human insensitivity to peroxisome proliferation. A two-stage chemical carcinogenesis model was used in trout to evaluate PFAAs as complete carcinogens or promoters of aflatoxin B(1) (AFB(1))- and/or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced liver cancer. DNA microarray was used to assess hepatic transcriptional response to these dietary treatments in comparison with E2 and the classic peroxisome proliferator, clofibrate (CLOF). Fish (250 fish/treatment) were fed experimental diets containing 5 ppm E2, 2000 ppm PFOA (approximately 50 mg/kg body weight/day), 2000 ppm 8:2 fluorotelomer alcohol (FtOH) or 2000 ppm CLOF ad libitum (2.8–5.6% of body weight) 5 days per week for 6 months. PFNA and PFDA experimental diets were initially administered at 2000 ppm but due to an unexpected number of mortalities early in the study, diet concentrations were reduced to 200 ppm PFDA (5 mg/kg/day) or 1000 ppm PFNA (25 mg/kg/day) for the remainder of the exposure period.	Incidence of liver tumors significantly increased (6.8-fold, $p<0.0001$), multiplicity significantly increased ($p<0.001$) and size of liver tumors significantly increased ($p<0.001$) in trout fed diets containing PFDA compared with AFB(1)-initiated animals fed control diet. PFDA was the most potent promoting agent tested in this study. 200 ppm PFDA increased liver tumor incidence to a greater extent (26% higher) than did a 10-fold higher diet concentration of PFOA. Pearson correlation analyses, unsupervised hierarchical clustering, and principal components analyses showed that the hepatic gene expression profiles for E2 and PFOA, PFNA, PFDA, and PFOS were overall highly similar, though distinct patterns of gene expression were evident for each treatment, particularly for PFNA.	Benninghoff AD, Orner GA, Buchner CH, Hendricks JD, Duffy AM, Williams DE. Promotion of hepatocarcinogenesis by perfluoroalkyl acids in rainbow trout. Toxicol Sci. 2012 Jan;125(1): 69-78

10.7.1 Short summary and overall relevance of the provided information on carcinogenicity

No studies according to OECD test guidelines on the carcinogenic properties of PFDA are available. Two limited studies investigating the potential of PFDA to promote tumorigenesis have been included as supporting data in the current dossier. Borges et al., (2012) investigated the promoting activity of PFDA in two-stage hepatocarcinogenesis in rats after monthly i.p. administration (0.05-5 mg/kg). The results of this investigation indicated that PFDA is not a promoter of hepatocarcinogenesis.

In contrast, in a study in rainbow trout by Benninghoff et al., (2012) PFDA was demonstrated to act as a promoter of liver carcinogenesis. Rainbow trout is an animal model that represents human insensitivity to peroxisome proliferation and was utilized to determine whether various perfluoroalkylated acids (PFAAs), including PFDA, enhance hepatic tumorigenesis in a two-stage chemical carcinogenesis model. The results indicate that PFAAs can promote liver cancer in trout after daily dietary exposure and the authors suggested that the mechanism of promotion may be similar to that of E2 based on gene-transcription profiling.

Read-across from the source chemical to fill data gaps on carcinogenicity of PFDA and its ammonium and sodium salts

To generate information on the carcinogenic properties of PFDA for the purpose of harmonized classification an analogue chemical grouping with read-across from data of APFO/PFOA was used.

Justification

The carcinogenicity of PFDA and its ammonium and sodium salts is assumed to be predictable on the basis of structural similarities with PFOA and APFO. Both substances have a common functional group and only the carbon chain length is differing. PFDA and PFOA are relatively strong acids and are expected to dissociate to their respective anionic forms at physiological pH. Both acids (PFDA and PFOA) and their salts are expected to be available to cells in the form of their corresponding carboxylate anion (PFD and PFO, respectively) and are therefore considered toxicologically equivalent. Moreover, PFDA and its salts and PFOA/APFO have similar predictable physicochemical and toxicological properties. The existing data on PFOA/APFO in the current analogue approach for chemical grouping thus permit an assessment of the carcinogenicity of PFDA and its salts.

Source chemical data

Below, the outcome of the RAC assessment has been included from the Opinion Document for APFO (RAC Opinion proposing harmonised classification and labelling at Community level of Ammoniumpentadecafluorooctanoate (APFO), ECHA 2012):

"There are two carcinogenicity studies on APFO in Sprague-Dawley rats that showed increased liver adenomas, Leydig cell adenomas and pancreatic cell tumors in male rats. Increased rates of mammary fibroadenomas were seen in female rats. However due to high incidence in the control female group evidence for carcinogenic potential of APFO in female rats is equivocal.

Table 13A: *Summary on neoplastic and non-neoplastic lesions from carcinogenicity studies in rats*

Sprague-Dawley rats	*Sibinsky, 1987* *50 rats/sex/group 2 year* *15 rats/sex/group 1 year*			*Cook et al., 1994, Biegel et al., 2001* *76 males at 300 ppm, 80 control males*		*Historical control values for S-D rats#*
Ppm ***Mg/kg bw/d***	***0***	***30*** ***1.3***	***300*** ***14.2***	***0***	***300***	
Liver						
2 year study						
Liver cell adenomas				*2.5%* *(2/80)*	*13%* *(10776)*	

Hyperplastic nodules	*0% / 0%*		*6% / 0%*			
Liver cell megalocytosis	*0% / 0%[$]*	*12% / 2%*	*80% / 16%*			
Cystoid degeneration	*8% / 0%*	*14% / 0%*	*56% / 0%*			
1 year						
Liver cell megalocytosis	*0% / 0%**		*80% / %*			
Portal mononuclear cell infiltration	*47% / 0%*		*80% / 0%*			
Hepatocellular necrosis						
Hepatocellular vacuolation						
Testis						
2-year						
Testicular masses[&]	*0% / -*	*2% / -*	*12% / -*			
Leydig cell adenomas	*0% / -*	*4% / -*	*14% [#/-]*	*0% (0/80)*	*11%**	*5% (Clegg et al 1997)* *0.82%* *Chandra et al., 1992*
Leydig cell hyperplasia				*14% (11/80)*	*46%[#]* *(35/76)*	
Vascular mineralisation	*0% / -*	*6% / -*	*18%# /-*			
1 year						
Aspermatogenesis	*0%/-*		*13% / -*			
Ovary						
2-year						
(Original) Tubular hyperplasia	*- / 0%*	*- / 14%*	*- / 32%[#]*			
§Stromal hyperplasia	*- / 8%*	*- / 16%*	*- / 15%*			
§Stromal adenoma	*- / 4%*	*- / 0%*	*- / 2%*			
§Combined stromal hyperplasia and	*- / 12%*	*- / 16%*	*- / 17%*			

adenoma						
Mamma						
2-year						
Fibroadenoma	*- /21%* *(10/47)*	*- / 40%[#]* *(19/47)*	*- / 43%[#]* *(21/49)*			*18% or 37% Sykes, 1987* *19% Chandra et al., 1992*
Pancreas						
Acinar cell adenoma	*0% / -*	*6%* *(2/34* *Males)*	*3%* *(1/34* *males)*	*0%* *(0/80)*	*9%** *(7/76)*	*0.22%* *Chandra et al., 1992*
Acinar cell carcinoma				*0%* *(0/80)*	*1%* *(1/76)*	
Acinar cell hyperplasia				*18%* *(14/80)*	*39%** *(30/76)*	

*[$]Percentages in males/females, [#]No data from laboratory control values, [§]ovarian lesions rediagnosed in Mann and Frame, 2004, * significantly different from pair-fed control group, p<0.05; [#] significantly different from ad-libitum control group, p<0.05; [&] There is an inconsistency in the OECD SIDS report which says that at the one year sacrifice, testicular masses were found 6/50 high-dose and 1/50 low-dose rats, but not in any of the controls. As no low dose animals were tested at the one year schedule, it is assumed to be a mistake and the effect is related to the 2-year data. No lesions corresponding to the masses were reported in groups of the 1-year sacrifice.*

Liver tumors

Liver tumors in rodents that are conclusively linked to peroxisome proliferation are proposed not to be of relevance for humans (CLP guidance, 3.6.2.3.2 (k)). No evidence on increased hepatic cell proliferation was estimated at interim time points (1 month – 21 months) during the carcinogenicity study (Biegel et al., 2001). While in the original CLH dossier the dossier submitter concluded that there is no (or not yet) evidence on PPARa-related clonal expansion of preneoplastic foci, a recently published study was able to show that administration of APFO to rats leads to hypertrophy and hyperplasia (without any microscopical/biochemical evidence of liver cell toxicity) as a result of early increases in cell proliferation (but no inhibition of apoptosis), which ultimately leads to liver tumor formation (Elcombe et al., 2010). These data clearly demonstrate an early hepatocellular proliferative response to APFO treatment and suggest that the hepatomegaly and tumors observed after chronic dietary exposure of S-D rats to APFO likely are due to a proliferative response to combined activation of PPAR and CAR/PXR. This mode of action is unlikely to pose a human hepatocarcinogenic hazard as demonstrated in studies utilizing mice humanized with respect to the xenosensor nuclear receptors, the activation of the human PPARa, CAR, and PXR does not appear to lead to cell proliferation (Cheung et al. 2004; Gonzalez and Shah 2008; Shah et al. 2007; Ross et al. 2010).

Supporting evidence:

In addition, there was increase in liver weights (partly due to liver cell hypertrophy), but no indication of hepatic cell proliferation and PPARa-activity in a 6-month cynomolgus monkey study (Butenhoff et al., 2002).

Evidence from PPARa-receptor knockout mice to increase liver weight gives some evidence on other modes contributing to the liver tumors. This observation is in line with findings on developmental toxicity from the study of Abbott et al. (2007), where testing in knock-out mice did not abolish the increase in liver weight.

Elcombe et al., 2010 hypothesised that APFO increases mitochondrial mass in rats and monkeys that may in part account for liver weight increase. In monkeys, APFO administration resulted in a marked increase in mitochondrial succinate dehydrogenase (SDH) activity that was thought to explain the dose-related liver weight increases (Butenhoff et al., 2002). However this interpretation is subject to uncertainties since increases in SDH activity did not show dose-dependency in this study. Nevertheless studies show that APFO interferes with mitochondrial activity. Livers from adult male Sprague–Dawley rats that received a 30 mg/kg daily oral dose of APFO for 28 days showed increased PPARγ coactivator-1α (Pgc-1α) protein, a regulator of mitochondrial biogenesis and transcription of mitochondrial genes, leading to a doubling of mtDNA copy number. Further, transcription of genes encoded by mtDNA was 3–4 times greater than that of nuclear encoded genes, suggestive of a preferential induction of mtDNA transcription. Implication of the Pgc-1α pathway is consistent with PPARγ transactivation by PFOA (Walters et al. 2009). Increased mtDNA copy number were already observed 3 days after a single ip injection of 100 mg/kg bw (Berthiaume and Wallace 2002).

PPARγ transactivation by APFO were also concluded from dose-related increase in PPARγ mRNA in PPARα-null mice, while only slightly in hPPARα-mice was observed (Nakagawa et al. 2011) In conclusion, much of the response to APFO can be attributed to PPARa and induction of PPARa regulated genes. The impact of activation of PPARγ-regulated genes that are proposed to interfere with mitochondrial DNA transcription biogenesis and with lipid and glucose metabolism on tumor growth is not known to the rapporteurs.

Beyond the question on whether biological responses related to activation of PPARa are of relevance for humans, there is still some degree of uncertainties with the significance of other nuclear receptor activation on tumor growth and RAC follows argumentation of the dossier submitter that other mode of actions can not fully be excluded.

Leydig cell tumors

RAC agreed with the conclusion of the dossier submitter that there is insufficient evidence to link these tumors to PPARa. Biegel et al. (2001) demonstrated that APFO did not induce peroxisomes in Leydig cells. Another not yet identified mode of action than peroxisome proliferation must be active. Increases in serum estradiol throughout the study (Biegel et al., 2001) may indicate that hormonal mechanism might be involved, while no effect on testosterone biosynthesis has been shown.

14 day gavage administration of APFO up to 40 mg/kg bw/d to rats showed that increases in serum estradiol concentration corresponded to increased hepatic aromatase activity (Liu et al., 1996). However, studies on estrogens demonstrated proliferative effects and tumors of the Leydig cell almost exclusively in the mouse rather than in the rat (Review in Cook et al, 1999).

Pancreatic acinar cell tumors

Increased tumor rates were observed in two carcinogenicity studies. However, the original study of Sibinski reported no significant increase in tumors rather than higher incidences of acinar cell hyperplasia (no details available), while the confirmatory mechanistic carcinogenicity study of Biegel et al. revealed significantly increased rates of acinar cell tumors and of the correspondent hyperplasia.

Dossier submitter proposed that the induction of pancreatic acinar cell tumors is probably related to an increase in serum level of the growth factor, CCK (cholecystokinin-33 [human], cholecystokinin [rat]). Growth factor were also discussed by Biegel et al. (2001) as stimulative for pancreatic acinar cells without giving any proof whether CCK has been changed by treatment. No evidence is given by any of the repeated

dose studies to support hypothesis that APFO enhances cholesterol/triglyceride excretion, thereby increases fat content in the gut and causes tumor growth in pancreatic acinar cells.

It is not clear to which effect pancreatic acinar cells are linked in the liver. Biegel et al. mentioned cholestasis related increases in CCK plasma concentrations for other peroxisome proliferators, but no such effect was reported for APFO. For APFO it can be concluded that at present the mode of action of pancreatic cell adenomas is unknown.

Reference is also given to the EPA Guidance document on PPAR"-Mediated Hepatocarcinogenesis in Rodents and Relevance to Human Health Risk Assessments (EPA, 2003) that stated "In addition to inducing hepatocarcinogenesis in rodents, PPAR" agonists have also been observed to induce pancreatic acinar cell and Leydig cell tumors in rats. Of 15 PPAR" agonists tested to date, nine have been shown to induce all three tumors in non-F344 rat strains but not in mice. In the case of Leydig cell tumor formation, two potential MOAs based on activation of PPAR" have been proposed. One MOA invokes the induction of hepatic aromatase activity leading to an increase in serum estradiol level. The second MOA purports that PPAR" agonists inhibit testosterone biosynthesis. Although agonism of PPAR" may lead to the induction of aromatase or inhibition of testosterone biosynthesis, the data available to date are insufficient to support which, if either, of these two proposed MOAs is operative. For pancreatic acinar cell tumor (PACT) formation, a MOA has been proposed in which PPAR"- agonists cause a decrease in bile acid synthesis and/or change the composition of the bile acid resulting in cholestasis. These steps increase the level of the growth factor cholecystokinin (CCK) which then binds to its receptor, CCKA, leading to acinar cell proliferation. Some evidence exists to support this proposed MOA and there does not appear to be evidence of any other MOA operating in the formation of PACTs after exposure to PPAR" agonists. However, the data are not considered sufficient to establish a MOA with confidence, because it has only been described for two chemicals, PFOA and WY14643, in one laboratory. As a result, the evidence is considered insufficient to infer that this MOA may be generalized to all PACT-inducing PPAR" agonists."

In conclusion, RAC followed the proposal by the dossier submitter, namely that APFO should be classified according to the Directive 67/548/EEC criteria as Carc. Cat. 3; R40, and according to the CLP criteria as Carc. 2 (H351)."

10.7.2 Comparison with the CLP criteria

Based on read-across from the source chemical APFO/PFOA the information is sufficient to fulfil the criteria for carcinogenicity. Very limited data on PFDA and its ammonium or sodium salts is available to support (in addition to) the read-across from APFO/PFOA. Therefore, the reasoning for classification of PFDA and its ammonium and sodium salts will be based on the conclusion for APFO/PFOA (RAC Opinion 2011). As the information available on APFO/PFOA from animal studies are considered to be some evidence, and it cannot be concluded that the mode of action for tumor growth is of no relevance for humans, classification of PFDA and its ammonium and sodium salts as Carc. 2 (suspected of causing cancer) is warranted.

10.7.3 Conclusion on classification and labelling for carcinogenicity

Based on read-across from the source chemicals APFO/PFOA, PFDA and its ammonium and sodium salts should be classified according to the CLP criteria for their potential to cause cancer as Carc. 2; H351.

10.8 Reproductive toxicity

10.8.1 Adverse effects on sexual function and fertility

There is very limited data on adverse effects on sexual function and fertility of PFDA and its ammonium and sodium salts. In this dossier read-across from the structural analogue PFOA/APFO has been utilized where there are no data specifically on PFDA and its ammonium and sodium salts to support harmonised classification. In the adopted RAC opinions for PFOA and APFO it was concluded not to classify PFOA or APFO as having adverse effects on sexual function and fertility. However, the endpoint was determined to be reconsidered by RAC when more data were available. PFNA, the C9 analogue of PFOA and PFDA was recently discussed by the RAC and a RAC opinion concluding on the harmonised classification of PFNA and its ammonium and sodium salts as Repr. 2 (H361f) was agreed based on read-across from PFOA/APFO in combination with additional data for PFNA itself. Therefore, for the purpose of harmonised classification of PFDA and its ammonium and sodium salts read-across from PFOA/APFO in combination with supporting data on PFNA has been used in this proposal. One in vitro mechanistic study on the androgenic and estrogenic effects of PFCAs including PFOA, PFNA and PFDA has been included to further support the classification. In addition, one toxicity study with single i.p. administration of PFDA investigating the androgenic status of rats after PFDA exposure, and two additional acute toxicity studies with i.p administration of PFDA in rats have been included as supporting mechanistic studies.

Table 34a: Summary table of animal studies on adverse effects on sexual function and fertility

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Read-across data: Adapted from BD CLH APFO, ECHA 2012; Table 14						
2 generations		Sprague-Dawley rats (30 rats/group)	APFO	Oral by gavage 0, 1, 3, 10 and 30 mg/kg/Day	***F0 males***: In the highest dose group one male was sacrificed on study day 45 due to adverse clinical signs. No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed. At necropsy a statistically significant reduction in terminal body weight was reported from 3 mg/kg/day (6%, 11%, and 25% decrease from controls in the 3, 10 and 30 mg/kg/day, respectively. Absolute weights of the left and right epididymis, left cauda epididymis, seminal vesicles, prostate, pituitary, left and right adrenals and thymus were statistically significantly reduced at 30 mg/kg /day, however, the organ-to- body weight ratios were either normal or increased. The absolute weight of the liver was significantly increased in all dose groups, and the absolute weights of the kidneys were significantly increased at 1, 3 and 10 mg/kg/day, and significantly deceased at 30 mg/kg/day. Organ weight-to-body weight ratios for the liver and kidneys were significantly increased in all treated groups. No histopathology was performed on the liver and kidney. Dose-related histopathologic changes were reported in the adrenals.	York, 2002; Butenhoff et al., 2004

					No treatment-related effects were reported at necropsy on the reproductive organs, with the exception of increased thickness and prominence of the zona glomerulosa and vacuolisation of the cells of the adrenal cortex in 2/10 males and 7/10 males in the 10 and 30 mg/kg/day dose group. The LOAEL was 1 mg/kg/day based on increased absolute and relative liver weight. ***F0 females:*** No treatment-related effects were reported on oestrus cyclisity, mating or fertility parameters. No treatment-related effects on body weights or organ weights. The NOAEL was 30 mg/kg/day. ***F1 generation:*** At 30 mg/kg/day one pup died on Lactation Day (LD) 1. Additionally, on LD 6 and 8 a significant increase in the numbers of pups found dead were reported at 3 and 30 mg/kg/day. Pup body weight on a per litter basis was significantly reduced up to lactation day 15 in the high dose group (LD 1; 5.5 vs 6.3 in controls, LD 8; 11.9 vs. 13.3 in controls, and LD 15; 22.9 vs. 25.0 in controls). Of the pups necropsied at weaning no absolute or relative organ weight changes were reported. ***F1 males:*** A significant increase in treatment-related deaths (5/60 rats) was reported in the high dose group between day 2-4 post-weaning. Significant increases in clinical signs of toxicity were also reported during most of the post-weaning period at all dose levels. A significant dose-related reduction in mean body weight gain for the entire dosing period (days 1-113). Absolute food consumption was significantly reduced from 10 mg/kg/day during the entire pre-cohabitation period (days 1-70 postweanling), while relative food consumption values were significantly increased.	

					Significant delays in sexual maturation (the average of preputial separation) were reported at 30 mg/kg/day (52.2 days of age vs. 48.5 days of age in controls). When the body weight was co-varied with the time to sexual maturation, the time to sexual maturation showed a dose-related delay that was statistically significant at $p \leq 0.05$. No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed. Necroscopic examination revealed significant effects on the liver and kidney from 3 mg/kg/day. Terminal body weight was significantly dose-related decreased from 1 mg/kg/day (6%, 6%, 11%, and 22% decreased from controls at 1, 3, 10 and 30 mg/kg/day, respectively.The absolute and relative liver weights were significantly increased in all treated groups and were accompanied by histopathological changes. All other organ weight changes reported (thymus, spleen, left adrenal, brain, prostate, seminal vesicles, testes and epididymis) were probably due to body weight reductions, since the relative weights of these organs were either normal or increased. However, the biological significance of the weight changes observed in the adrenal is unclear since histopathological changes were also reported. The NOAEL developmental effects were 3 mg/kg/day and the LOAEL for F1 adult effects was 1 mg/kg/day. ***F1 females***: A significant increase in treatmentrelated deaths (6/60 rats) was reported in the high dose group between day 2-8 post-weaning. Significantly decrease in body weights were reported in the high dose group during post-weaning, precohabitation, gestation and lactation Body weight gain was significantly reduced during day 1-15 posweanling. Decreased absolute food consumption was reported during days 1-	

					22 post-weaning, precohabitation, gestation and lactation in the highest dose group. Relative food consumption values were comparable across all treated groups. Significant delays in sexual maturation (the average of vaginal patency) were reported at 30 mg/kg/day (36.6 days of age vs. 34.9 days of age in controls). When the body weight was co varied with the time to sexual maturation, the time to sexual maturation showed a dose-related delay that was statistically significant at $p \leq 0.05$. No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed. All natural delivery observations were unaffected by treatment at any dose level. No effect on terminal body weights was reported. The absolute weight of the pituitary, the pituitary weight-to-terminal body weight ratio and the pituitary weight-to-brain ration was significantly decreased from 3 mg/kg/day. No histopathologic changes were reported in the pituitary. The NOAEL developmental effects were 10 mg/kg/day and the NOAEL for F1 adult effects was 10 mg/kg/day. ***F2 generation:*** No treatment related adverse clinical signs were reported. Dead or stillborn pups were noted in both the control and treated groups. The deaths occurred on lactation day 1-8 with the majority occurring on days 1-6, however, there was no dose-relationship. No effect on body weights or organ weights, as well as AGD was reported. The NOAEL was set at 30 mg/kg/day.	

Table 34b: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
-	-	-	-	-

Table 34c: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference

No guideline Unknown GLP-status 'Effects of PFDA treatment on the androgenic status of rats' Acute effects on androgen status of male rats in intact rats or in rats castrated and implanted with testosterone capsules. Ex vivo studies of effects of PFDA on decapsulated testes.	PFDA (Aldrich Chemical Co.) Purity: 96% (by titration with sodium hydroxide) 87.4% PFDA as analysed by gas chromatography/mass spectroscopy by the authors.	Rat (Sprague-Dawley) Sexually mature males 10 animals per dosing group of intact animals. 3 or 6 rats per dosing group of castrated animals. 0, 20, 40, 80 mg/kg in propylene glycol/water Single dose, i.p. All rats were killed 7 days after treatment. Pair-fed controls (PFC) rats were weight matched to PFDA-treated rats and provided daily the same amount feed that their partner consumed. Ad libitum-fed control rats (ALC) were also included in the study. 3 or 6 rats from each treatment group were castrated two hours after dosing and testosterone-containing capsule was inserted subcutaneously. For ex vivo tests testes were removed from rats in each treatment group 7 days after dosing and decapsulated. No information on how many animals in each treatment-group.	The cumulative feed consumption was decreased ($p<0.05$) to 44% of that of ad libitum-fed control rats at 80 mg/kg. Body weight was also decreased ($p<0.05$) to 72% of that of ad libitum-fed control rats at 80 mg/kg. At 40 mg/kg PFDA body weight was lower ($p<0.05$) than both the pair-feed control (7% lower) and ad libitum-fed control rats (16% lower). **Testis weight** (no information whether the weight is absolute or relative) slightly (approx. 8% as estimated from the graphical presentation) but statistically significant decreased ($p<0.05$) at 80 mg/kg compared to ad libitum-fed control rats. No histological changes in testes were reported at any dose level. Dose-related decreases in the weights (no information whether the weight is absolute or relative) of **seminal vesicles and ventral prostrates**. At 80 mg/kg weights were reduced ($p<0.05$) to 42% and 49%, respectively, of those in ad libitum-fed control rats. **Marked atrophy of the epithelium of seminal vesicles** at 80 mg/kg. Epithelial height in these PFDA-treated rats was approx. 50% less ($p<0.05$) than control animals (both ad libitum-fed and pair-fed control animals) at 80 mg/kg. **Marked atrophy of the epithelium of glandular acini of the ventral prostrate** at 40 and 80 mg/kg. In rats treated with 40 or 80 mg/kg PFDA about 60% of the prostatic acini were lined by low cuboidal epithelium compared to only about 20% of the acini in ad libitum-fed and pair-fed control animals).	Bookstaff RC, Moore RW, Ingall GB, Peterson RE. Androgenic deficiency in male rats treated with perfluorodecanoic acid. Toxicol Appl Pharmacol. 1990 Jun 15;104(2):322-33.

			Decreased ($p<0.05$) plasma testosterone concentrations at 40 and 80 mg/kg compared to both to ALC (25% and 12% of control levels, respectively) and PFC. Decreased ($p<0.05$) 5α-dihydrotestosterone concentrations at 40 and 80 mg/kg compared to both to ALC (32% and 18% of control levels, respectively) and PFC, measured at day 7 after treatment (N=10) ED50 for decreased plasma androgen concentrations was 30 mg/kg. PFDA treatment had no significant effect on plasma testosterone concentrations in castrated rats with implants of testosterone-containing capsules. Body weights were similar also to intact rats. No difference in ventral prostate weight between castrated and implanted rats and ad libitum fed or pair-fed controls. At 20 and 40 mg/kg PFDA but not at 80 mg/kg PFDA the weights of seminal vesicles were decreased ($p<0.05$) by approx. 30% compared to pair-fed control rats. Decapsulated testis from PFDA treated rats at 80 mg/kg stimulated with human chorionic gonadotropin showed decreased levels of secreted testosterone to 27% of control. The effect was significant already at 40 mg/kg but no effect was observed in testes from rats treated with 20 mg/kg PFDA.	

No guideline Unknown GLP-status Acute effects of PFDA on tissue fatty acids in male rats	PFDA (supplier not specified) Purity: not specified	Rat Fischer rats Adult males *LD50 determination:* 12 doses in the range 40 mg/kg – 500 mg/kg (exact doses administered not stated) i.p. in propylene glycol-water Single dose 10 animals per group *Study of fatty acid composition :* 50 mg/kg ip in propylene glycol-water Single dose 4 animals in each group Pair-fed controls (PFC) rats were weight matched to PFDA-treated rats and provided daily the same amount feed that their partner consumed. Ad libitum-fed control rats (ALC) injected with vehicle were also included in the study. Rats were killed 2, 4, 8 and 16 days after injection.	*LD50 determination:* The LD50 at 30 days was 41 mg/kg, delayed lethality with deaths in second and third week after dosing. The LD50 at 14 days was 64 mg/kg, delayed lethality with deaths in second and third week after dosing. *Study of fatty acid composition :* Decreased food intake started at day 1 and was close to zero from day 7 to 14 after 50 mg/kg PFDA. Mean body weights decreased from 207 to 109 g. At day 6 the decrease in body weight was >20%. The pair-fed controls decreased in body weight from 209 to 131 g. At day 8 after i.p. administration of 50 mg/kg PFDA **mean organ weights of testes** (1.7 g versus 2.8 g in control and 2.2 g in PFC), adrenals and heart were significantly lower than both vehicle control and PFC ($p<0.05$) Mean liver weight was significantly lower than vehicle control (7.6 versus 9.9, $p<0.05$) but not than PFC (3.0 g).	Olson CT, Andersen ME. The acute toxicity of perfluorooctanoic and perfluorodecanoic acids in male rats and effects on tissue fatty acids. Toxicol Appl Pharmacol. 1983 Sep 30;70(3):362-72

No guideline Unknown GLP-status Toxic effects of PFDA in male rats after single administration	PFDA (Aldrich Chemical Co.) 96% straight chain	*LD50 determination 30 days after i.p. injection:* Fischer-344 rats, males and females; Sprague-Dawley rats, males. Number of groups and number of animals per group not stated. Doses tested not stated. *LD50 determination 30 days after oral administration:* Fischer-344 rats, males Number of groups and number of animals per group not stated. Doses tested not stated. *Time course toxicity experiments* Fischer-344 rats, males. Five groups and at least six rats per group. 50 mg/kg single dose i.p. Pair fed controls Rats killed at 4, 8, 12, 16 or 30 days after injection.	*LD50 determination 30 days after i.p. injection:* LD50 at 30 days after i.p. administration in Fischer-344 rats was 41mg/kg for males and 43 mg/kg for females. LD50 at 30 days after i.p. administration in in male Sprague-Dawley rats was 75 mg/kg. A dose of 50 mg/kg i.p. was selected for the time course toxicity study since no mortality was observed within the first 14 days. However, 8 of 24 rats died after 16 or 30 days. Data from animals dying after 14 days were not included in the results. *LD50 determination 30 days after oral administration:* The 30 day oral LD50 in male Fischer-344 rats was 57 mg/kg. *Time course toxicity study* PFDA administration caused decreased food intake within the first days and it was close to zero day 4 to 12. Both control and PFDA-treated rats lost weight until day 13, thereafter the weights remained about the same until day 18-20. 18-20 days after dosing the rats started to gain weight slowly. PFDA-treated rats had greater weight loss compared their pair-fed controls (100 g versus 70 g weight loss at day 16, $p<0.01$).	George ME, Andersen ME. Toxic effects of nonadecafluoro-n-decanoic acid in rats. Toxicol Appl Pharmacol. 1986 Sep 15;85(2):169-80.

			Liver weights and liver to body weights ratios were significantly higher than controls. At 30 days the liver to body weight ratio was almost 50% greater ($p<0.01$) than pair-fed control rats. Histopathological examination revealed **atrophy and degeneration of the seminiferous tubules in testes** which was first seen at day 16 and persisted up to day 30 post-administration (authors report that findings were significant but no quantification is available). Inflammation, hyperkeratosis, edema and some ulceration of the stomach was also reported. Thymic atrophy was seen in treated rats 8 days after exposure and thymic tissue was not found in the majority of treated rats at 12, 16 and 30 days. A uniform and persistent cellular swelling at all times in the liver was observed. Inflammatory cell infiltration and some signs of necrosis were noted at day 8.	

In vitro mechanistic study aimed at investigating interference with steroid hormone receptor functions	PFDA (ABCR, Germany) Purity: 98% (PFHxS, PFOS, PFOA, PFUnA, PFDoA, or Mix were also tested)	*ER transactivation assay*: Stably transfected MVLN cell line derived from the human breast adenocarcinoma MCF-7 cell line carrying an estrogen response element-luciferase reporter vector. The transcriptional activity was measured in response to PFAAs with or without co-treatment of 25 pM E2. Tested concentrations of PFAAs were in the range of $1x10^{-9}$ – $1x10^{-4}$ M. *AR transactivation assay:* Chinese hamster ovary cell line CHO-K1 transiently co-transfected with an MMTV-LUC reporter vector and an AR expression plasmid pSVAR0. The transcriptional activity was measured in response to PFAAs with or without co-treatment of 25 pM DHT. Tested concentrations of PFAAs were in the range of $1x10^{-9}$ – $1x10^{-4}$ M. *Aromatase activity:* Human choriocarcinoma JEG-3 cell line. Aromatization in response to PFAAs was measured by radioactivity derived from the precursor [1β-3H]androst-4-ene-3,17-dione. Tested concentrations of PFAAs were in the range of $1x10^{-8}$ – $1x10^{-4}$ M. Assays were carried out at test concentrations not being cytotoxic.	*ER transactivation assay:* No estrogenic or ER antagonistic effect of PFDA. Weak estrogenic effects of PFHxS, PFOS and PFOA. *AR transactivation assay:* None of the tested PFAAs acted as agonists. Upon co-treatment with 25 pM DHT, five of seven PFAAs (PFOS, PFHxS, PFOA, PFNA, and PFDA) elicited significant ($p<0.05$) concentration-dependent antagonistic effects on DHT-induced AR transactivity. (Cytotoxicity was noted at $\geq 1x10^{-4}$ M for PFDA). IC50=$6x10^{-6}$ M *Aromatase activity:* PFDA weakly decreased the aromatase activity (down to 85% compared to solvent control, $p=0.002$) at $1x10^{-5}$ M (cannot be ruled out that the effects is due to beginning cytotoxicity of PFDA, noted at $\geq 1x10^{-4}$ M for PFDA). No effect of the other tested PFAAs.	Kjeldsen LS, Bonefeld-Jørgensen EC. Perfluorinated compounds affect the function of sex hormone receptors. Environ Sci Pollut Res Int. 2013 Nov;20(11):8031-44.

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

No study on adverse effects on fertility and sexual function of PFDA is available. Indications of anti-androgenic effects of PFDA were reported in Bookstaff et al., 1990. Single doses of PFDA (0, 20, 40 or 80 mg/kg) were administered to rats by i.p. injection and the animals were killed 7 days after treatment. Effects of PFDA treatment on the androgenic status of rats were studied. The cumulative feed consumption was decreased to 44% and body weight to 72% of that of ad libitum-fed control rats at 80 mg/kg. Body weight at termination of study tended (less than 10% as estimated from graphical presentation) to be lower at 40 and 80 mg/kg PFDA compared to the pair-feed control. No mortalities before termination of study were reported. Plasma testosterone and 5α-dihydrotestosterone concentrations were decreased ($p<0.05$) to 12 and 18% of control levels (ad libitum fed rats), respectively at 80 mg/kg PFDA. Dose-related decreases in the weights of seminal vesicles (42%) and ventral prostrates (49%) were observed at 80 mg/kg. Testis weight was slightly (approx. 3% as estimated from graphical presentation) but statistically significant ($p<0.05$) decreased at 80 mg/kg. No histological changes in testes were reported at any dose level. Marked atrophy of the epithelium of seminal vesicles and the epithelial height was about half to that of control animals at 80 mg/kg. Marked atrophy of the epithelium of glandular acini of the ventral prostrate were seen at 40 and 80 mg/kg.

When rats were castrated and implanted with testosterone-containing capsules the effect of PFDA on body weight in castrated and implanted rats was similar to that of intact rats. PFDA-treated (all dose-groups) and control (ad libitum feeded) rats had similar plasma testosterone concentrations and ventral prostate weights, indicating that these effects are secondary to plasma androgen concentrations and that PFDA does not increase the clearance of plasma testosterone concentrations. The weights of seminal vesicles were statistically significant from the pair fed controls at 20 mg/kg and 40 mg/kg PFDA, but not at 80 mg/kg. Furthermore, luteinizing hormone (LH) concentrations were not significantly altered by PFDA treatment, however, it should be noted that the levels of LH in control ad libitum fed rats were close to the limit of detection and any changes may therefore be difficult to detect. An *ex vivo* study with decapsulated testes from PFDA-treated rats demonstrated decreased testosterone secretion after stimulation with the LH analogue human chorionic gonadotropin at 100 mIU/ml. The authors therefore suggested that PFDA decreases testicular responsiveness to LH stimulation.

George and Andersen (1986) reported that a single i.p. administration of 50 mg/kg PFDA in male rats caused atrophy and degeneration of the seminiferous tubules in testes that was first seen at day 16 and persisted up to day 30 post-administration.

In an *in vitro* mechanistic study in stably transfected cell lines (MVLN or CHO-K1) aimed at investigating interference of PFAAs with steroid hormone receptor functions PFDA was reported to significantly antagonize the AR activity in a concentration dependent manner (similar to PFNA, PFOA, PFOS) with an IC50 calculated to be $6x10^{-6}$ M (IC50 for PFOA and PFNA were $1.1x10^{-5}$M and $5.2x10^{-5}$M, respectively) (Kjeldsen et al., 2013). In addition, PFDA weakly decreased the aromatase activity at a high test concentration ($1x10^{-5}$ M) but a potential interference of the results by cytotoxicity could not be excluded according to the authors of the paper.

In summary, there is not sufficient information on PFDA on its own or on its ammonium or sodium salts for classification for adverse effects on sexual function and fertility.

Read-across from the source chemical to fill data gaps on adverse effects on sexual function and fertility of PFDA and its ammonium and sodium salts

To generate information on the potential reproductive toxicity of PFDA for the purpose of harmonized classification an analogue chemical grouping approach was utilized. For APFO/PFOA there are some uncertainties with regards to the conclusion on adverse effects on fertility and sexual function in the RAC opinion (inserted below) mostly based on negative evidence from the two-generation reproductive toxicity study (York, 2002; Butenhoff et al., 2004) and repeated dose toxicity study and therefore no classification was considered warranted. However, the hazard class was recommended by the RAC-rapporteur to be reconsidered due to evidence on sperm abnormalities and reduced testosterone levels (Li et al., 2011). PFNA and its ammonium and sodium salts was recently discussed by the RAC and a RAC opinion concluding on the harmonised classification of PFNA and its ammonium and sodium salts as Repr. 2 H361f was agreed based read-across from data on PFOA/APFO in combination with data on the substance itself. Read-across from data on APFO/PFOA in combination with supporting data on PFNA have therefore been used for the purpose of hazard assessment and classification of PFDA and its ammonium and sodium salts in this proposal.

Justification

The adverse effects on sexual function and fertility of PFDA and its ammonium and sodium salts with no or very little data is assumed to be predictable on the basis of structural similarities with PFOA and APFO. Both substances have a common functional group and only the carbon chain length is differing. PFDA and PFOA are relatively strong acids and are expected to dissociate to their respective anionic forms at physiological pH. Both acids (PFDA and PFOA) and their salts are expected to be available to cells in the form of their corresponding carboxylate anion (PFD and PFO, respectively) and are therefore considered toxicologically equivalent. Moreover, PFDA and its salts and PFOA/APFO have similar predictable physicochemical and toxicological properties. The existing data on PFOA/APFO in the current analogue approach for chemical grouping thus permit an assessment of the adverse effects on sexual function and fertility of PFDA and its salts.

Source chemical data

The outcome of the RAC assessment has been included from the Opinion Document for APFO (RAC Opinion proposing harmonised classification and labelling at Community level of Ammoniumpentadecafluorooctanoate (APFO), ECHA 2012; page 53):

"Based on the previously available data RAC found it conclusive that no proposal to classify for fertility effects was proposed by the dossier submitter. The only effects in the 2-generation study were increased absolute weights of epididymis and seminal vesicles that probably is linked to body weight loss. No relevant effects in male and female animals were reported from the repeated dose toxicity studies and the 2-year carcinogenicity study in rats. The latter study revealed treatment-related testes tumours, which were not related to fertility effects. An additional study on testosterone levels and male reproductive organ effects of APFO were published after submission of the CLH dossier: In male mice, oral APFO-treatment (0, 1 and 5 mg/kg bw/day) for 6 weeks of both wt, null- or humanized PPARα mice showed a statistically significant increase ($p<0.05$) in sperm morphology abnormalities at both concentrations, an increased incidence of abnormal seminiferous tubules and a statistically significant reduction ($p<0.05$) in plasma testosterone

concentration in the wt mice (at 5 mg/kg bw/day) and the hPPARα mice at both concentrations, but none of these effects were observed in the null-mice. In addition, a statistically significant reduction ($p<0.05$) of the reproductive organ (epididymis and seminal vesicle + prostate gland) weight of the wt PPARα mice treated with the highest concentration was seen (Li et al., 2011). The authors reported inconsistencies of PPARα-expressed in interstitial Leydig cells or seminiforous tubule cells of testis in m PPARα-mice, but not in testis of hPPARα-mice (Cheung et al., 2004). The RAC discussed the new study published in 2011 (Li et al., 2011) indicating a potential of adverse effect on the male mice reproductive system.

RAC concluded that evidence on impaired fertility through sperm abnormalities and reduced testosterone levels are not (yet) sufficient to overwrite the negative evidence from the 2- generation study and repeated dose toxicity. Reconsideration of the endpoint is recommended."

Supporting data - PFNA

The assessment of adverse effects on sexual function and fertility of PFNA have been included from the RAC opinion concluding on the harmonised classification and labelling at Community level of PFNA and its ammonium and sodium salts, ECHA 2014 (pages 10-15):

"In the RAC opinions adopted on 2 December 2011 on the classification of APFO and PFOA, which were used as reference substances in a read-across approach for PNFA, PFN-S and PFN-A, no classification for fertility was considered warranted, mostly based on negative evidence from the 2-generation study (York, 2002; Butenhoff et al., 2004). No relevant effects in male and female animals were reported from the repeated dose toxicity studies and the 2-year carcinogenicity study in rats. The latter study revealed treatment related testis tumours, which were not related to fertility effects.

The RAC discussed in 2011 the then recently published study by Li et al. (2011), indicating a potential of adverse effect on the male mice reproductive system. RAC concluded that evidence on impaired fertility through sperm abnormalities and reduced testosterone levels were not (yet) sufficient to override the negative evidence from the 2-generation and repeated dose toxicity studies. However, reconsideration of the endpoint was recommended.

In this RAC opinion, the results of the Li et al. (2011) study are reconsidered followed by a review of a study of Feng et al. (2009), in which rats were exposed by gavage to PFNA.

In the Li et al. (2011) study, aimed at elucidating the mechanism and impact of PPARα on lowering testosterone levels, APFO at doses of 0, 1 or 5 mg/kg/d was orally given daily to mice with different genotypes: 129/sv wild-type (mPPARα), Pparα-null and PPARα-humanized (hPPARα) for 6 weeks. Both low- and high-dose APFO exposure significantly reduced plasma testosterone concentrations in mPPARα and hPPARα, mice respectively. These decreases, according to the authors, may, in part, be associated with decreased expression of mitochondrial cytochrome P450 side-chain cleavage enzyme, steroidogenic acute regulatory protein or peripheral benzodiazepine receptor as well as microsomal cytochrome P450 involved in the steroidogenesis.

Oral APFO-treatment (0, 1 and 5 mg/kg bw/day) of mPPARα, Pparα-null and hPPARαmice for 6 weeks did not affect the epididymal sperm count in any exposed group of mice.

However, APFO treatment at both doses induced a statistically significant increase in sperm morphology abnormalities in mPPARα mice (1.4- and 1.5 fold in comparison with frequency of sperm morphology abnormalities in respective control mice (ca. 7%) and in hPPARα mice (1.3- and 2.6 fold in comparison with frequency of sperm morphology abnormalities in respective control mice (ca. 7%). The types of abnormalities observed were not described.

The APFO dose of 5 mg/kg appeared to increase incidences of abnormal seminiferous tubules with vacuoles or lack of germ cells in mPPARα and hPPARα mice. Necrotic cells were also observed in the testes of mPPARα mice after 5 mg/kg APFO exposure. However, no obvious effects of APFO treatment were morphologically observed in the testes of Pparα-null mice.

Using real-time quantitative polymerase chain reaction, the mRNA levels for several genes associated with testicular cholesterol synthesis, transport and testosterone biosynthesis were examined.

Cholesterol biosynthesis: In Leydig cells of the testes, the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase and HMG-CoA reductase, involved in biosynthesis of testicular cholesterol, which is an essential substrate for testosterone production, were not changed after treatments of APFO in three mouse groups, though the HMG-CoA reductase levels of the untreated, control group were significantly higher in hPPARα mice than mPPARα and Pparα-null mice. The results suggest that enzymes essential for cholesterol biosynthesis in Leydig cells were probably not affected.

Cholesterol transport

Steroidogenic acute regulatory protein (StAR) and peripheral benzodiazepine receptor (PBR) play key regulatory roles in cholesterol transport from the outer to the inner mitochondrial membrane. APFO at doses of 5mg/kg/d inhibited the expression of StAR mRNA in the testis of mPPARα mice, and at the low and high dose in the testis of hPPARα mice. PBR mRNA level was not affected by APFO treatment, except in hPPARα mice exposed to APFO at 5 mg/kg/d, in which PBR mRNA level was decreased. The results suggest that cholesterol transport from the outer to the inner mitochondrial membrane could be reduced by APFO. However, it was noted that PBR mRNA levels in testes of the control groups were higher in hPPARα mice than in mPPARα and Pparα-null mice.

In addition, a statistically significant reduction ($p<0.05$) of the reproductive organ (epididymis and seminal vesicle + prostate gland) weight of the wild-type PPARα mice treated with the highest concentration was seen (Li et al., 2011).

In the Li et al. (2011) study, an increase in abnormal sperms and the incidence of abnormal seminiferous tubules with vacuoles or lack of germ cells were observed in APFO-exposed mPPARα and hPPARα mice. However, these findings were not observed in Pparα-null mice. It shows that activation of peroxisome proliferator-activated receptor alpha (PPARα) by APFO is an essential step in induction of toxicity in testes.

PPARα is expressed in interstitial Leydig cells or seminiferous tubule cells of testis in mPPARα mice, but not in the testis in hPPARα, similarly to Pparα-null mice (Cheung et al., 2004).

Nevertheless, APFO caused reproductive impairment in hPPARα mice similar to that seen in mPPARα mice, suggesting that some toxic molecule(s) such as reactive oxidative species (ROS) molecules due to activation of hepatic PPARα may be produced in the liver and circulated in the body, because a common point between mPPARα and hPPARα mice was that both had PPARα in the liver, and the activation of this receptor in liver produced ROS molecules by induction of the receptor-regulated ROS-generating genes (Nakajima et al., 2010). In the view of the authors (Li et al., 2011), further studies are warranted to assess whether some reactive species which attack mitochondria of the Leydig cells were produced in the liver.

APFO, PFOA, PFNA, PFN-S and PFN-A are agonists of PPARα, which means they are capable of peroxisome induction in cells. Alterations in sperm and testes induced by APFO in the Li et al. (2011) study might thus be related to peroxisome proliferation in the liver. Peroxisomes are cell-organelles which can be induced to a specifically high level in rats and mice under certain conditions, e.g. by repeated exposure to long chain and branched fatty acids. Peroxisome proliferation, which in particular occurs in the liver, causes liver toxicity (e.g. hyperplasia, oxidative stress) and can ultimately, after long-term exposure, also lead to tumours. There is no evidence of e.g. hepatomegaly from clinical studies in humans treated with peroxisome proliferators (Purchase, 1994). Therefore, in the interpretation of these results for classification purposes it should be noted that peroxisome induction/proliferation is listed in section 3.9.2.5.3 of the CLP Guidance among the mechanisms considered not relevant to humans and which should not be considered for classification for STOT RE. This is in line with Section 3.9.2.8.1(e) of Annex I to the CLP Regulation, which states that substance-induced species-specific mechanisms of toxicity, i.e. demonstrated with reasonable certainty to be not relevant for human health, shall not justify classification for STOT RE.

In the mechanistic study of Feng et al. (2009), male SD rats were exposed by gavage to PFNA at doses of 0, 1, 3 and 5 mg/kg bw/d for 14 days. In this study, a dose-dependent increase in the number of apoptotic cells

was observed. No sperm cell counts were done in this study. In the histological examination of testes from rats exposed to 5 mg/kg bw PFNA, the spermatogenic cells exhibited apoptotic features, namely crescent chromatin condensation and chromatin margination. To evaluate the impact of PFNA on germ cell survival, testes sections were examined for DNA fragmentation indicative of cell death using the TUNEL staining (terminal deoxynucleotide transferase mediated dUTP-biotin nick end labeling). Seminiferous tubules of control animals had very few TUNEL-positive cells, indicating very low level germ cell attrition in normal testes. In the 1 mg PFNA/kg/d group, only a few TUNEL-positive cells were observed, but this staining was more pronounced and the TUNEL-positive cells were increased in testes of animals receiving 3 and 5 mg PFNA/kg/d. The TUNEL-positive germ cells were mainly spermatocytes and spermatogonia, and these cells seemed to be initially more susceptible to PFNA toxicity. No quantitative data on numbers of observed TUNEL-positive cells were provided.

In the flow cytometric DNA analysis of spermatogenic cells the percentage of apoptotic cells in the 3 and 5 mg PFNA/kg/d groups ca. 7% and 9%) was increased considerably compared with ca. 1.5% in the control group. No significant differences were detected in the 1 mg/kg/d group.

As reviewed by the authors (Feng et al., 2009), apoptosis during different stages of spermatogenesis is responsible for the maintenance of normal quantity and quality of sperm.

During the process of apoptosis, a family of cysteine proteases (caspases) are activated. Two pathways have been recognized as leading to excessive apoptosis of germ cells. The first pathway links caspase-8 to Fas death receptors belonging to the family of tumor necrosis. In the second pathway, mitochondrial damage induced by extracellular stress causes the releasing of cytochrome c from mitochondria into the cytoplasm, which activates apoptosis.

The following changes in Fas and FasL mRNA expression levels in testis were observed after PFNA exposure: Compared to the control group, expression levels of Fas in the 1 and 3 mg PFNA/kg/d groups were higher, but no statistical differences were documented. In the 5 mg PFNA/kg/d group, Fas expression was markedly upregulated about 90% compared with the control group. Moreover, expression of FasL was significantly down-regulated in the 3 mg PFNA/kg/d dose group; however, no significant differences were observed in the 1 and 5 mg PFNA/kg/d groups.

The effects of PFNA exposure on mRNA expression of genes involved in apoptosis through the mitochondria-dependent pathway in male rats were also determined. Expression levels of Bax gene were increased by 35.7% in the 5 mg PFNA/kg/d group, but no significant differences were observed in the 1 and 3 mg PFNA/kg/d groups compared to the control group. In addition, Bcl-2 expression levels were down-regulated significantly in the 3 and 5 mg PFNA/kg/d groups.

Western blot analysis, used to compare changes in the active caspase-8 and caspase-9 protein levels in total protein extracts from testes, demonstrated that the levels of active caspase-8 were significantly increased in the 3 and 5 mg PFNA/kg/d groups, but PFNA treatment did not affect the levels of active caspase-9 in any of the exposed groups.

The serum estradiol level was 104% higher in the rats exposed to 5 mg/kg bw PFNA than in the control rats, but no significant changes were seen in serum estradiol levels in rats dosed at 1 and 3 mg/kg/day. There was a significant, 1.87-fold increase in testosterone levels in the 1 mg/kg bw PFNA rats compared to the control rats. Testosterone levels were not altered in rats exposed at 3 mg/kg/d, but were significantly decreased, to ca. 15% of the control values, in the 5 mg/kg bw rats.

Neither the Li et al. (2009) study nor the Feng et al. (2009) study, due to the aims of the studies and methodology used, demonstrated that APFO or PFNA produces an adverse effect on sexual function and fertility, such as reduction of mating or fertility indexes or sperm counts. However, they demonstrated that APFO and PFNA may affect morphology of sperm, alter level of sex hormones (testosterone and estradiol) and biochemical processes essential for sperm production or sexual behavior.

In the oral 2-generation reproductive toxicity study using S-111-S-WB in rats (Stump et al., 2008) no effect on fertility was observed. S-111-S-WB (fatty acids C6–C18, perfluoro, ammonium salts, CAS No. 72968-38-

8) is a mixture of perfluorinated fatty acid ammonium salts of different carbon chain lengths that is used a surfactant in polymer manufacturing. The major component of S-111-S-WB is PFNA, although detailed information on content of various constituents was not provided. S-111-S-WB was administered daily via oral gavage to 30 Crl:CD(SD) rats/sex/group at doses of 0.025, 0.125 and 0.6 mg/kg/d over two generations to assess potential reproductive toxicity.

Reproductive performance, mean litter size, pup survival and pup weights were unaffected. No test article-related effects were observed in the F0 and F1 generations on male and female fertility index, estrous cycle length, mean testicular sperm numbers and sperm production rate at any dose. Slightly lower, but statistically significant, mean sperm motility (95.3% of the control value) and progressive motility (94.4% of the control value) was noted for F0 males, but not in F1 males, in the 0.6 mg/kg/d group when compared to the control group values.

Sperm concentration (106/g) in the left epididymis in F0 males was reduced in the 0.025 and 0.6 mg/kg/d groups to 86.4% and 86.5% of control values, respectively, but sperm concentration in the left epididymis was not reduced in the 0.125 mg/kg/d group. In the F1 male generation, sperm concentration (106/g) in the left epididymis was not affected by S-111-S-WB treatment. No pathological changes were observed in histopatological examinations of testes of F0 and F1 male rats.

Lower mean body weights were observed in the 0.6 mg/kg/d group in F0 and F1 males. Higher absolute and relative liver weights were noted in F0 and F1 males in the 0.125 and 0.6 mg/kg/d groups, and in F0 and F1 females in the 0.6 mg/kg/d group. Hepatocellular hypertrophy was observed in F0 and F1 males in the 0.025, 0.125 and 0.6 mg/kg/d groups and in F0 females of the 0.6 mg/kg/d group. The foci of hepatocellular necrosis with associated subacute inflammation were observed in F0 and F1 males of the 0.025, 0.125 and 0.6 mg/kg/d group.

Higher kidney weights were observed for parental males and females in the 0.125 and 0.6 mg/kg/d groups. Hypertrophy of renal tubule cells for F0 males and females in the 0.6 mg/kg/d group correlated with increases in mean absolute and relative kidney weights.

Total S-111-S-WB concentration in the serum of male and female pups was 1.2-1.4-fold higher than in the dams 2 h following administration to the dams on lactation day 13.

The results of the 2-generation study with S-111-S-WB, containing a mixture of perfluoroalkyl acids, primarily of longer carbon chain length than PFOA, with PFNA as a major component, did not provide sufficient evidence of alterations of fertility due to exposure to this mixture at dose levels of 0.125 and 0.6 mg/kg/d. The exposure at these doses elicited clear systemic toxicity due to hepatotoxicity and nephrotoxicity of the mixture, particularly in male rats. Statistically significant, although not dose-related, and quantitatively minor (5-14%) reductions in sperm motility and sperm count in the epididymis of F0 males, but not in F1 males,without histopatological changes in the testes, demonstrated potential for testicular toxicity from exposure to S-111-S-WB. However, these minor alterations in sperm quality could be related to systemic toxicity due to liver and kidney dysfunction.

A dose level of less than 0.025 mg/kg/d was considered to be the NOAEL for F0 and F1 parental systemic toxicity based on microscopic hepatic findings in the males of all test article groups, and a dose level of 0.025 mg/kg/d was considered to be the NOAEL for neonatal toxicity based on higher liver weights in the F1 and F2 pups at 0.125 mg/kg/day and higher.

The proposal for classification of PFNA, PFN-S and PFN-A as Repr. 2, H361f (Suspected of damaging fertility) is further supported by preliminary human data. In the study of Nordström Joensen et al. (2009), a group of 105 young adult men reporting for military draft in Denmark was examined to discover the possible association between the levels in serum of perfluoroalkyl acids (PFAA) and testicular function. The serum level of 10 different PFAA with carbon chain length from C6 to C13 was examined. Out of all examined PFAAs, the highest concentrations were found for perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), PFOA and PFNA (medians of 24.5, 6.6, 4.9, and 0.8 ng/mL, respectively). The high serum concentrations of PFAAs were significantly associated with reduced numbers of normal spermatozoa. In addition, sperm concentration, total sperm count, and sperm motility showed some tendency toward lower

levels in men with high PFAA levels, although not at statistically significant levels. The authors noted that the results from this preliminary study should be corroborated in larger studies.

Taking into account

- *minor effects (small reductions in sperm motility and sperm count in epididymis of F0 males, but not in F1 males) without reductions in mating or fertility indexes with the mixture S-111-S-WB which has PFNA as major constituent, in a 2-generation study (Stump et al., 2008);*
- *increase in serum testosterone levels, decrease in serum estradiol levels and increased frequency of spermatogenic cells with apoptotic features in rats exposed by gavage to 5 mg PFNA/kg/d (Feng et al., 2009);*
- *reduced plasma testosterone concentrations, increased frequency of abnormalities in sperm morphology and vacuolated cells in the seminiferous tubules of 129/sv wild-type (mPPARα) and hPPARα mice exposed orally to APFO for 6 weeks, although these effects could be mediated in part by liver peroxisome proliferation, since they were not observed in similarly exposed Pparα-null mice (Li et al., 2011); and*
- *the supporting preliminary human data,*

RAC is of the opinion that classification of PFNA, PFN-S and PFN-A as Repr. 2, H361f (Suspected of damaging fertility) is warranted.

In the opinion of RAC, the existing evidence is not sufficient to classify PFNA, PFN-S and PFN-A as Repr.1B, H360F (May damage fertility), because the effect on the sperm count was observed only in the F0 generation, but not in F1 males exposed to a mixture of perfluorinated fatty acid ammonium salts of different carbon chain lengths in a 2-generation study (Stump et al., 2008) and the epididymal sperm count was not affected in wild-type, Pparα-null and PPARα-humanized mice exposed orally to APFO for 6 weeks. The fact that PFOA and APFO, were not classified for sexual function and fertility (due to negative results of a 2-generation study with APFO; York, 2002, Butenhoff et al., 2004; and the lack of supporting evidence from repeated dose toxicity studies, which gave no indication of disturbances of fertility) in the RAC opinion (2 December 2011) was also considered."

Supporting data – PFUnDA and PFDoDA

The two longer PFCAs in the supporting chemical category have two very recent studies indicating effects on male reproductive organs in line with the findings reported for PFOA/APFO, PFNA and PFDA. Dose levels for studies of PFUnDA were set based on the results from a dose range-finding study where 20 mg/kg/day PFUnDA caused mortality in 5/5 males and 4/5 females. In a combined repeated dose toxicity study with a reproduction/developmental toxicity screening test (OECD TG 422) there were no adverse histopathological effects on reproductive organs observed in rats dosed with PFUnDA (0, 0.1, 0.3, 1.0 mg/kg/day, oral gavage) for 42 days (males) or 41-16 days (females) (Takahashi et al., 2014). Minimal spermatic granuloma in epididymis was detected at 1.0 mg/kg/day in 1 out of 5 males and mild spermatic granuloma in epididymis was seen at 0.1 mg/kg/day in 1/1 males. No statistical significant effect on reproductive organ weight, however, a slight but statistically significant decrease in the relative testis weight (11%) at high dose 1.0 mg/kg/day was reported (Takahashi et al 2014). Body weight gains in males at 1.0 mg/kg/day decreased (<10% difference compared to control) after day 30 during the dosing period and throughout the recovery period; however, there were no statistically significant changes. Female rats in the satellite group (not mated) displayed a statistically significant ($p \leq 0.05$) reduced body weight (approx. 15% compared to control as estimated from graphical presentation) starting from day 38 and continuing throughout the dosing period (42 days) and recovery period (14 days). No statistical significant changes compared to controls in body weight in the dams were reported. Centrilobular hypertrophy of hepatocytes in

males (3/7 at 0.3 mg/kg/day and 7/7 at 1.0 mg/kg/day) and females (1/12 at 0.3 mg/kg/day and 11/12 at 1.0 mg/kg/day) were observed after treatment for 41-46 days, diffuse vacuolation of hepatocytes in males (3/7), and minimal focal necrosis in males (2/7) and females (2/12) were observed at 1.0 mg/kg/day.

In a similar study by the same research group the PFCA with one additional CF2-group in the carbon chain, PFDoDA, was investigated in rats in a combined repeated dose toxicity study with a reproduction/developmental toxicity screening test (OECD TG422; Kato et al 2014). PFDoDA was administered by oral gavage at doses 0.1, 0.5, or 2.5 mg/kg/day. Body weights in the males (from day 21, approx. 30% less than control at recovery day 14, $p<0.01$), females in the satellite group (from day 35; approx. 30% less than control at recovery day 14, $p<0.01$) and in the dams (through the gestation and lactation period; approx. 30% less than control, $p<0.01$ at GD 20-21) were statistically significant decreased at 2.5 mg/kg/day. 7 of 12 female rats receiving 2.5 mg/kg/day died during late pregnancy. Statistically significant increased incidences compared to control groups of diffuse hepatocyte hypertrophy (slight to moderate) in liver in both males (5/7, $p<0.05$) and females (slight: 6/12, moderate: 6/12, $p<0.01$) at 2.5 mg/kg/day treated for 42 days were reported. Mainly all findings related to reproductive toxicity were reported at the highest dose tested (2.5 mg/kg/bw) and were associated with excessive general toxicity (reduced body weight). Various histopathological changes, including decreased (not statistically significant) spermatid (slight change 2/7) and spermatozoa counts (slight change 2/7; moderate change 1/7; severe change 1/7); slight spermatic granuloma (2/7) and cell debris in the lumen of epididymis (slight to moderate change 3/7); and slight to moderate glandular epithelium atrophy of prostate (4/7), seminal vesicles (4/7), and coagulating gland (4/7) were observed in the male reproductive organs after exposure of 2.5 mg/kg/day PFDoDA for 42 days. The ratio of organ to body weight of testes was increased ($p\leq0.01$) in the group of males that were kept an additional 14 days after 42 days of administration of 2.5 mg/kg/day PFUnDA compared to controls in the recovery group (0.882 compared to 0.671 in control). However, in the same group, the absolute testis weight was 15% lower than control (not statistically significant). Hemorrage on the implantation site and/or congestion on the endometrium were detected in the uterus of all the 7 females that died during the gestation period. Hemorrage at the implantation site was also detected in one female that delivered stillborn pups. Continuous diestrous was observed in the females of the 2.5 mg/kg/day group that were not mated (satellite group) during the administration period (length of estrous cycle could not be determined). During the recovery period, 1 out 5 females in the same group had normal estrous cycle. Female rats that were assigned to the dosing groups to be mated displayed normal estrous cycles and length during the premating period at all doses. Four out of 12 female rats (in addition to those 7 rats who died during late pregnancy) receiving 2.5 mg/kg/day did not deliver live pups, i.e. only one dam delivered pups normally (14 alive, 2 dead). Thus, only 1 litter was examined and data was therefore excluded from statistical evaluation. Delivery index at 0.5 mg/kg/day was slightly but not statistically significant decreased: 89.7% compared to 94.3% in control.

In a number of studies Shi et al have investigated the alterations in gene and protein expression in the testes of rats exposed to PFDoDA for shorter (14 days) or longer periods (110 days) and the results indicate that PFDoDA disrupts testicular steroidogenesis and expression of related genes in male rats. At the highest dose levels tested (5 or 10 mg/kg/bw) where excessive general toxicity were evident (markedly reduced body weight) PFDoDA were reported to induce an apoptotic effect in cells in rat testes: Leydig cells, Sertoli cells and spermatogonic cells were displaying apoptotic morphological features after 14 days treatment of PFDoDA (Shi et al., 2007). The testicular mRNA expression of several genes involved in cholesterol transport and stereoid biosynthesis were significantly reduced at the same dose levels, however, it is unclear to what extent this deteriorated expression is relevant considering the concomitant apoptotic cell death in the tissue. Exposure to PFDoDA for 110 days resulted in a dose-dependent decrease in serum testosterone levels and levels were statistically significant markedly decreased ($p<0.05$) at 0.2 mg PFDoA/kg/day (56% of

control levels) and 0.5 mg PFDoA/kg/day (40% of control levels) (Shi et al., 2009a). PFDoA exposure resulted in significantly decreased ($p<0.05$) protein levels in testes of steroidogenic acute regulatory protein (StAR) at 0.05 mg/kg/day (62.6% of control levels), 0.2 mg/kg/day (50.6% of control levels) and 0.5 mg/kg/day (53% of control levels), and decreased cholesterol side-chain cleavage enzyme (P450scc) at 0.5 mg/kg/day (Shi et al., 2009a). Also in female rats genes responsible for cholesterol transport and steroidogenesis were reported to be effected. The ovarian expression of steroidogenic acute regulatory protein and cholesterol side-chain cleavage enzyme was significantly decreased ($p<0.05$) at 3 mg/kg/day (72% of control levels and 62% of control levels, respectively). 17-beta-hydroxysteroid dehydrogenase was increased ($p<0.05$) from 0.5 mg/kg/day. Furthermore, PFDoDA significantly decreased estradiol levels (60% of control levels) and increased cholesterol levels ($p<0.05$) at 3 mg/kg/day.

Together this shows that PFDoDA, similarly to PFOA and PFDA, have been found to induce testicular toxicity and to decrease testosterone levels, and expressional changes in genes involved in cholesterol transportation and steroidogenesis may play a role in the reduction of testosterone observed after PFDoDA exposure.

Conclusion on read-across data

In summary, sperm abnormalities, effects on male reproductive organs and altered hormonal levels were observed in rodents treated with PFCAs ranging from C8 to C12 indicating that adverse effects on sexual function and fertility is an intrinsic hazard of these substances including PFDA.

10.8.3 Comparison with the CLP criteria

Based on read-across from the source chemical APFO/PFOA, with supporting mechanistic data on the C9 polyfluoroalkylated carboxylic acid PFNA and on the target chemical PFDA, the information is sufficient to fulfil the criteria for classification in reproductive toxicity – adverse effects on sexual function and fertility. There was an absence of effects on fertility and sexual function in a two-generation reproductive toxicity study on the source chemical data APFO (source chemical) and in addition, there were no effects on fertility or sexual function in a two-generation reproductive toxicity study of the mixture S-111-S-WB (containing long chain PFCAs: PFNA was a major constituent, but also PFUnDA and longer chain PFCAs were present). However, there are several studies of the source chemical and supporting long-chain PFCAs presenting adverse effects on fertility and sexual function of PFDA and its ammonium and sodium salts manifested as alterations of male reproductive system, sperm abnormalities and altered hormonal response. These effects are considered as some evidence from experimental animals, but not as clear evidence of an adverse effect on sexual function and fertility. Based on the available data on APFO/PFOA and PFNA RAC was of the opinion that classification of PFNA and its ammonium and sodium salts as Repr. 2, H361f (Suspected of damaging fertility) was warranted, as stated above. PFDA and its ammonium and sodium salts are therefore suspected to be a human reproductive toxicant and a classification in category 2 for reproductive toxicity – adverse effects on sexual function and fertility according to the CLP regulation is proposed. The existing evidence was not considered sufficient to classify PFNA and its ammonium and sodium salts as Repr. 1B, H361F according to RAC (see the conclusion above from the RAC opinion on PFNA and its ammonium and sodium salts): *"In the opinion of RAC, the existing evidence is not sufficient to classify PFNA, PFN-S and PFN-A as Repr.1B, H360F (May damage fertility), because the effect on the sperm count was observed only in the F0 generation, but not in F1 males exposed to a mixture of perfluorinated fatty acid ammonium salts*

of different carbon chain lengths in a 2-generation study (Stump et al., 2008) and the epididymal sperm count was not affected in wild-type, Pparα-null and PPARα-humanized mice exposed orally to APFO for 6 weeks. The fact that PFOA and APFO, were not classified for sexual function and fertility (due to negative results of a 2-generation study with APFO; York, 2002, Butenhoff et al., 2004; and the lack of supporting evidence from repeated dose toxicity studies, which gave no indication of disturbances of fertility) in the RAC opinion (2 December 2011) was also considered." Therefore, based on read-across from the source chemical APFO/PFOA, with supporting information on PFNA and on the target substance itself, evidence is not sufficient to classify PFDA and its ammonium and sodium salts as Repr 1B, H360F.

10.8.4 Adverse effects on development

There is limited data on adverse effects on the development of the offspring of PFDA and its ammonium and sodium salts. In this dossier read-across from the structural analogue PFOA/APFO has been utilized where no data specifically on PFDA and its ammonium and sodium salts to support harmonised classification. One in vitro mechanistic study of PFCAs including PFOA, PFNA and PFDA has been included to further support the classification.

Table 35a: Summary table of animal studies on adverse effects on development

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
No guideline study	The mice were dosed once per day for either 4 consecutive days (GD 10-13) or 10 consecutive days (GD 6-15). Dams were weighed on GD 18 and killed by decapitation	Mouse C57BL/6N Female 10-14 animals in each control and dose group	PFDA, nonadecafluorodecanoic acid; CAS No. 335-76-2 Purity: 96%	Oral gavage in corn oil GD 10-13 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 mg/kg/day Oral gavage in corn oil GD 6-15 0, 0.03, 0.3 1.0, 3.0, 6.4, 12.8 mg/kg/day	*Maternal toxicity* 30% mortality at GD 18 at 12.8 mg/kg/day Net bw change GD 6-18 significantly reduced compared to control 4.9 g ($p<0.01$): 0.4 g at 6.4 mg/kg bw/day, -2.4 g at 12.8 mg/kg bw/day Relative liver weight (organ-to-body weight ratio) significantly increased compared to control 6.7 g ($p<0.01$): 7.9 g at 1 mg/kg bw/day 10.3 g at 3 mg/kg bw/day 13.8 g at 6.4 mg/kg bw/day 15.2 g at 12.8 mg/kg bw/day *Developmental toxicity* Increased % resorptions per litter at 6.3 mg/kg bw/day (19.1%) and at 12.8 mg/kg bw day (41.7%) Full litter resorption at highest doses 1/14 at 6.3 mg/kg bw/day 3/10 at 12.8 mg/kg bw/day	Harris MW, Birnbaum LS. Developmental toxicity of perfluorodecanoic acid in C57BL/6N mice. Fundam Appl Toxicol. 1989 Apr;12(3):442-8.

					Dose-dependent decrease in fetus body weight per litter 1.12 g (96% of ctrl, p<0.05) at 1 mg/kg bw/day, 1.10 g (94% of ctrl, p<0.01) at 3 mg/kg bw/day, 0.90 g (77% of ctrl, p<0.01) at 6.3 mg/kg bw/day, 0.59 g (50% of ctrl, p<0.01) at 12.8 mg/kg bw/day, compared to control 1.17 g. Decreased number of live fetus per litter 5.8 at 6.3 mg/kg bw/day (n.s.), 4.6 at 12.8 mg/kg bw/day (p<0.05) compared to control 7.2.	
Read-across data: Adapted from BD CLH APFO; Table 15						
		Sprague-Dawley rats (22/group)	APFO	Oral by gavage 0, 0.05, 1.5, 5 and 150 mg/kg/day APFO Gestation day 6-15	Maternal toxicity: In the high dose group 3 dams died, and a significant reduction in maternal body weights on gd 9, 12 and 15 was reported. The NOAEL for maternal toxicity was 5 mg/kg/day. Developmental toxicity: No significant differences were found between treated and control groups. The NOAEL for developmental toxicity was 150 mg/kg/day.	Gortner, 1981

		Rabbits (18 /group)	APFO	Oral by gavage 0, 1.5, 5 and 50 mg/kg/day APFO Gestation day 6-18	Maternal toxicity: Six dams died during the study, however, 5 of the 6 deaths were attributed to gavage errors. Transient reduction in body weight gain on gd 6-9, however, they returned to control levels on gd 12-29. No other effects were reported.No clinical or other treatment related signs were reportedThe NOAEL for maternal toxicity was 50 mg/kg/day. Developmental toxicity: A dose-related increase in a skeletal variation, extra ribs or 13th rib, which reached statistically significance at 50 mg/kg/day (38%, 30%, 20% and 16% in the 50, 5, 1.5 mg/kg/day and control group, respectively). The NOAEL for developmental toxicity was 5 mg/kg/day.	Gortner, 1982
		Sprague-Dawley rats (25/group in the first trial, 12/group in the second trial)	APFO	Oral by gavage 0 and 100 mg/kg/day APFO Gestation day 6-15. In trial 1 the dams were sacrificed on gd 21, in trial 2 the dams were allowed to litter and the pups were sacrificed on postpartum day 35.	Trial 1 maternal toxicity: Three dams died at 100 mg/kg/day during gestation (one on GD 11 and two on GD 12). Food consumption and body weight was reduced in treated dams compared to controls. No other effects were reported on reproductive parameters such as maintenance of pregnancy or incidence of resorptions. Trial 1 developmental toxicity: No effects reported. Trial 2 maternal toxicity: The same as in trial 1. Trial 2 developmental toxicity: No effects reported.	Staples et al., 1984

		Sprague-Dawley rats (12/group in trial 1 and 2) inhalation	APFO	0, 0.1, 1, 10 and 25 mg/m3 APFO (whole body dust inhalation), 6 hours/day Respirable particles <10 µm 77% - 90% (MMAD 1.4-3.4 µm±4.3-6.0)*	Gestation day 6-15. In trial 1 the dams were sacrificed on gd 21, in trial 2 the dams were allowed to litter and the pups were sacrificed on postpartum day 35 Trial 1 maternal toxicity: Treatment-related clinical signs were reported in the two highest dose groups (chromodacryorrhea, chromorhinorrhea, a general unkempt appearance, and lethargy in four dams in the high dose group only). 3 dams died in the high dose group on gd 12, 13 and 17. In the two highest dose groups a statistically significant reduction in food consumption was reported, however, no significant differences were seen between treated and pair-fed groups. In the highest dose group a statistically significant reduction in body weight and increase in mean liver weight was reported. The NOAEL for maternal toxicity was 1 mg/m3. Trial 1 developmental toxicity: A statistically significant reduction in mean foetal body weight. was reported at 25 mg/m3 and in the control group pairfed 25 mg/m3. However, interpretation of the decreased foetal body weight is difficult due to mortality in dams The NOAEL for developmental toxicity was 10 mg/m3. Trial 2 maternal toxicity: Similar as to trial 1. Two dams died during treatment in the highest dose group. Trial 2 developmental toxicity: A statistically significant reduction in pup body weight on day 1 post partum (PP) (6.1 g at 25 mg/m3 vs 6.8 g in controls). Days 4 and 22 PP pup body weights continued to remain lower than controls, although the difference was not statistically significant.	Staples et al., 1984

					No significant effects were reported following external examinations of the pups or with ophthalmoscopic examination of the eyes. Interpretations of the effects reported are difficult due to the incidence of maternal mortality.The NOAEL for developmental toxicity was 10 mg/m3.	

		CD-1 mice	APFO	Oral by gavage 0 (45), 1 (17), 3 (17), 5 (27), 10 (26), 20 (42) or 40 (9) mg/kg bw/day APFO (number in brackets is number of dams examined) From gestation day 1 to 17, at gestation day 18, some dams were sacrificed for maternal and foetal examination, and the rest were allowed to give birth.	Maternal toxicity: Statistically significant (st sign) reduction in body weight gain in the 20 and 40 mg/kg bw/day dose groups. Maternal body weight including an adjustment for gravid uterine weight and liver weight produced statistically significant differences from controls only at the highest dose (20 mg/kg).The maternal weight gain on GD 18 was approximately 22, 24, 28, 21, 17, 5 and minus 5 gram in the control animals, 1, 3, 5, 10, 20 and 40 mg/kg bw/day exposed groups, respectively. In addition APFO treatment led to a dose-depended st. sign. Increase in liver weight from 1 mg/kg bw/day. The maternal serum level of APFO increased in a dose-dependent manner. No NOAEL for maternal toxicity could be derived. The LOAEL at 1 mg/kg bw/day is based on a st. sign. increased liver weight. Developmental toxicity: No changes in the number of implantations were reported. However, a st. sign. increase in the incidence of full litter resorption from 5 mg/kg bw/day (6.7, 11.8, 5.9, 25.9, 46.1, 88.1 and 100% in the 0, 1, 3, 5, 10, 20 and 40 mg/kg bw/day dose group, respectively) was reported. The number of live foetuses per litter was st. sign. reduced at 20 mg/kg bw/day. The foetal body weight was st. sign. decreased at 20 mg/kg bw/day. Reduced ossification of sternebrae, caudal vertebrae, metacarpals, metatarsals, phalanges, calvaria, supraoccipital and huoid as well as enlarged fontanel was reported as well. The delay in ossification was especially prominent in the 10 and 20 mg/kg bw/day dose groups, but reduced limb ossification sites and reduced ossification of calvaria was observed from 1 mg/kg bw/day.	Lau et al., 2006

					Most offspring were born alive, but the incidence of stillbirth and neonatal mortality was increased markedly, particularly in the 10 and 20 mg/kg bw/day dose groups. At 10 and 20 mg/kg bw/day most of the pups did not survive the first day of life. Postnatal survival was comparable to controls in the two lowest dose groups and significantly lower at ≥5 mg/kg bw/d. Among survivors, a trend towards growth retardation was noted in the APFO- treated neonates, leading to 25-30 % lower body weights from 3 mg/kg bw/day at weanling. Corresponding to the early postnatal growth deficits, development of the mice exposed in utero was impaired, evident as st. sign. delays in eye opening from 5 mg/kg bw/day, by as much as 3 days. The onset of puberty of male pups was markedly advanced. The preputial separation in the 1mg/kg bw/day dose group was almost 4 days earlier than in control pups, and this accelerated pubertal malformation took place despite a body weight reduction of 25-30%. No acceleration in female pubertal onset was reported. No NOAEL for developmental effects could be determined. The LOAEL at 1 mg/kg bw/day is based on increases in the onset of sexual maturation in males. * *Text added to the original report by the rapporteurs*	

		Sprague-Dawley rats (30 rats/group)	APFO	Oral by gavage 0, 1, 3, 10 and 30 mg/kg/ day APFO 2 generations	F0 males: In the highest dose group one male was sacrificed on study day 45 due to adverse clinical signs. No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed. At necropsy a statistically significant reduction in terminal body weight was reported from 3 mg/kg/day (6%, 11%, and 25% decrease from controls in the 3, 10 and 30 mg/kg/day, respectively. Absolute weights of the left and right epididymis, left cauda epididymis, seminal vesicles, prostate, pituitary, left and right adrenals and thymus were statistically significantly reduced at 30 mg/kg (day, however, the organ-to- body weight ratios were either normal or increased. The absolute weight of the liver was significantly increased in all dose groups, and the absolute weights of the kidneys were significantly increased at 1, 3 and 10 mg/kg/day, and significantly deceased at 30 mg/kg/day. Organ weight-to-body weight ratios for the liver and kidneys were significantly increased in all treated groups. No histopathology was performed on the liver and kidney. Dose-related histopathologic changes were reported in the adrenals. No treatment-related effects were reported at necropsy on the reproductive organs, with the exception of increased thickness and prominence of the zona glomerulosa and vacuolisation of the cells of the adrenal cortex in 2/10 males and 7/10 males in the 10 and 30 mg/kg/day dose group. The LOAEL was 1 mg/kg/day based on increased absolute and relative liver weight. F0 females: No treatment-related effects were reported on oestrus cyclisity, mating or fertility parameters. No treatment-related	York, 2002; Butenhoff et al., 2004

					effects on body weights or organ weights. The NOAEL was 30 mg/kg/day. F1 generation: At 30 mg/kg/day one pup died on Lactation Day (LD) 1. Additionally, on LD 6 and 8 a significant increase in the numbers of pups found dead were reported at 3 and 30 mg/kg/day. Pup body weight on a per litter basis was significantly reduced up to lactation day 15 in the high dose group (LD 1; 5.5 vs 6.3 in controls, LD 8; 11.9 vs. 13.3 in controls, and LD 15; 22.9 vs. 25.0 in controls). Of the pups necropsied at weaning no absolute or relative organ weight changes were reported. F1 males: A significant increase in treatment-related deaths (5/60 rats) was reported in the high dose group between day 2-4 post-weaning. Significant increases in clinical signs of toxicity were also reported during most of the post-weaning period at all dose levels. A significant dose-related reduction in mean body weight gain for the entire dosing period (days 1-113). Absolute food consumption was significantly reduced from 10 mg/kg/day during the entire pre-cohabitation period (days 1-70 postweanling), while relative food consumption values were significantly increased. Significant delays in sexual maturation (the average of preputial separation) were reported at 30 mg/kg/day (52.2 days of age vs. 48.5 days of age in controls). When the body weight was co-varied with the time to sexual maturation, the time to sexual maturation showed a dose-related delay that was statistically significant at $p \leq 0.05$. No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed. Necroscopic examination revealed significant effects on the liver and kidney	

					from 3 mg/kg/day. Terminal body weight was significantly dose-related decreased from 1 mg/kg/day (6%, 6%, 11%, and 22% decreased from controls at 1, 3, 10 and 30 mg/kg/day, respectively. The absolute and relative liver weights were significantly increased in all treated groups and were accompanied by histopathological changes. All other organ weight changes reported (thymus, spleen, left adrenal, brain, prostate, seminal vesicles, testes and epididymis) were probably due to body weight reductions, since the relative weights of these organs were either normal or increased. However, the biological significance of the weight changes observed in the adrenal is unclear since histopathological changes were also reported. The NOAEL developmental effects were 3 mg/kg/day and the LOAEL for F1 adult effects was 1 mg/kg/day. F1 females: A significant increase in treatment related deaths (6/60 rats) was reported in the high dose group between day 2-8 post-weaning. Significantly decrease in body weights were reported in the high dose group during post-weaning, precohabitation, gestation and lactation Body weight gain was significantly reduced during day 1-15 posweanling. Decreased absolute food consumption was reported during days 1-22 post-weaning, precohabitation, gestation and lactation in the highest dose group. Relative food consumption values were comparable across all treated groups. Significant delays in sexual maturation (the average of vaginal patency) were reported at 30 mg/kg/day (36.6 days of age vs. 34.9 days of age in controls). When the body weight was co varied with the time to sexual maturation,	

					the time to sexual maturation showed a dose-related delay that was statistically significant at p≤0.05. No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed. All natural delivery observations were unaffected by treatment at any dose level. No effect on terminal body weights was reported. The absolute weight of the pituitary, the pituitary weight-to-terminal body weight ratio and the pituitary weight-to-brain ration was significantly decreased from 3 mg/kg/day. No histopathologic changes were reported in the pituitary. The NOAEL developmental effects were 10 mg/kg/day and the NOAEL for F1 adult effects was 10 mg/kg/day. F2 generation: No treatment related adverse clinical signs were reported. Dead or stillborn pups were noted in both the control and treated groups. The deaths occurred on lactation day 1-8 with the majority occurring on days 1-6, however, there was no dose-relationship. No effect on body weights or organ weights, as well as AGD was reported. The NOAEL was set at 30 mg/kg/day.	

Table 35b: Summary table of human data on adverse effects on development

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference

Cross-sectional study to determine if specific blood perfluoroalkylated susbtances levels are associated with impaired response inhibition in children.		Blood levels of 11 perfluoroalkylated substances were measured in children (N = 83). Blood levels of perfluoroalkylated and polyfluoroalkylated substances (PFAS) were analyzed in relation to the differential reinforcement of low rates of responding (DRL) task. This task rewards delays between responses (i.e., longer inter-response times; IRTs) and therefore constitutes a measure of response inhibition.	Perfluorooctane sulfonate (PFOS), perfluorohexane sulfate (PFHxS), PFOA, PFNA, perfluorooctanesulfonamide (PFOSA), and PFDA were found at detectable levels in most children (87.5% or greater had detectable levels). Mean values in blood were 9.9, 3.23, 0.82, 0.26, 6.06 and 0.75 ng/ml for PFOS, PFOA, PFNA, PFDA, PFHxS and PFOSA respectively. Higher levels of blood PFOS, PFNA, PFDA, PFHxS, and PFOSA were associated with significantly shorter IRTs during the DRL task. 1 standard deviation increase in blood PFDA was associated with a decrease from median IRT with 5.51-1.62 sec for DRL time period 6-10 min and 8.9-3.01 for DRL time period 11-15 min. In addition, 1 standard deviation increase in blood PFDA was associated with an increase from median number of responses with 54.4+22.7 for DRL time period 6-10 min and 33.7+17.2 for DRL time period 11-15 min.	Gump BB, Wu Q, Dumas AK, Kannan K. Perfluorochemical (PFC) exposure in children: associations with impaired response inhibition. Environ Sci Technol. 2011 Oct 1;45(19):8151-9.

Table 35c: Summary table of other studies relevant for developmental toxicity

Type of study/data	**Test substance, reference to table 5**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**

The frog embryo teratogenesis assay-Xenopus (FETAX)	PFOA, PFNA, PFDA, and PFUnDA (Sigma Aldrich; purity not stated)	Teratogenic indices in Xenopus embryos were measured using the frog embryo teratogenesis assay-Xenopus (FETAX). 20-25 embryos were used for each dose group. Doses in the range 10 µM to 2 mM were tested. The expression of organ-specific biomarkers such as xPTB (liver), Nkx2.5 (heart), and Cyl18 (intestine) was analysed in stage 34 to 46 embryos using real-time PCR. Whole mount in situ hybridization was performed in embryos in the tadpole stage (stage 34-36). PFAA-treated and un-treated embryos were randomly selected for histological evaluation. Extraction and analysis of liver from 25 embryos from each group exposed to 130 µM PFAA (40 µM PFUnDA) for 10 days was performed .	All PFCs tested were found to be developmental toxicants and teratogens. The observed toxicity increased with increasing length of the fluorinated carbon chain. PFDA and PFUnDA were more potent developmental toxicants and teratogens compared to the other PFCs that were evaluated. Developmental toxicity and teratogenicity potencies for the tested PFAAs were in the following order: PFUnDA<PFDA<PFNA<PFOA. Various malformations (stunted tadpole length, multiple edemas, gut miscoiling, microcephaly, and skeletal kinking) in embryos exposed to PFDA and PFUnDA were reported. Severe defects resulting from PFDA and PFUnDA exposure were observed in the liver and heart, respectively, using whole mount in situ hybridization, real-time PCR, pathologic analysis of the heart, and dissection of the liver.	Kim M, Son J, Park MS, Ji Y, Chae S, Jun C, Bae JS, Kwon TK, Choo YS, Yoon H, Yoon D, Ryoo J, Kim SH, Park MJ, Lee HS. In vivo evaluation and comparison of developmental toxicity and teratogenicity of perfluoroalkyl compounds using Xenopus embryos. Chemosphere. 2013 Oct;93(6):1153-60.

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

There is limited information on adverse effects on development of the offspring of PFDA. One prenatal developmental toxicity test (no guideline, no GLP) by Harris and Birnbaum (1989) reported decreased net body weight change GD 6-18 at 6.4 mg/kg/day (0.4 g; 1.8% increase) and 12.8 mg/kg/day (-2.4 g; 11% weight loss compared to GD 6) compared to control (4.9 g; 22% increase). Three out of ten dams at 12.8 mg/kg/day died on GD 18 (mortality = 30%). Maternal mortality greater than 10 % is considered excessive and the data for the dose level 12.8 mg/kg/day will therefore not be considered for further evaluation of developmental toxicity of PFDA.

Maternal relative liver weight (organ-to-body weight ratio for the liver) significantly increased from 1.0 mg/kg/day (7.9, 10.3, 13.8, 15.2 g at 1.0, 3.0, 6.4, and 12.8 mg/kg bw/day respectively) compared to control (6.7 g). Since there are no reported findings of adverse histopathological effects in the liver, the observation of the increased liver weight of treated dams cannot convincingly be considered as evidence of maternal toxicity (Guidance on the application of the CLP criteria (Version 4.0, November 2013).

Fetal body weight per litter was decreased in a dose dependent manner from 1.0 mg/kg/day; -4.3%, -6%, -23% and -50% at 1.0, 3.0, 6.4, and 12.8 mg/kg/day respectively. Number of live foetuses per litter was decreased at 6.4 mg/kg/day (5.8, not statistically significant compared to control) and 12.8 mg/kg/day (4.6, $p<0.05$ compared to 7.2 in control) and resorptions per litter increased to 19.1% (not statistically significant) at 6.3 mg/kg/day and 41.7% ($p>0.06$) at 12.8 mg/kg/day compared to control 9.2%. Full litter resorptions were observed at 6.3 mg/kg/day (1/13) and 12.8 mg/kg/day (3/7) in combination with some maternal toxicity (reduced body weight and body weight gain at 6.3 mg/kg/day) and excessive maternal toxicity (30% mortality and body weight loss at 12.8 mg/kg/day).

Very limited human data is available for PFDA. There is one cross-sectional study that aims to determine if specific blood perfluoroalkylated substances levels are associated with impaired response inhibition in children (Gump et al., 2011). The mean content of PFDA in blood samples from 83 children was 0.26 ng/ml (the concentrations for PFOS, PFOA and PFNA were 9.9, 3.23 and 0.82 ng/ml, respectively). The blood levels were analysed in relation to the differential reinforcement of low rates of responding (DRL) task. This task rewards delays between responses (i.e., longer inter-response times; IRTs) and therefore constitutes a measure of response inhibition. The tested PFAAs were associated with impaired response inhibition in children and IRTs for PFDA were in similar range as for the other tested PFAAs.

Read-across from the source chemical to fill data gaps on adverse effects on the development of the offspring of PFDA and its ammonium and sodium salts

To generate information on the potential reproductive toxicity of PFDA for the purpose of harmonized classification an analogue chemical grouping approach was utilized. Read-across from data of APFO/PFOA with support of data of PFNA was used for the purpose of hazard assessment and classification.

Justification

The developmental toxicity of PFDA and its ammonium and sodium salts with no or very little data is assumed to be predictable on the basis of structural similarities with PFOA and APFO. Both substances have

a common functional group and only the carbon chain length is differing. PFDA and PFOA are relatively strong acids and are expected to dissociate to their respective anionic forms at physiological pH. Both acids (PFDA and PFOA) and their salts are expected to be available to cells in the form of their corresponding carboxylate anion (PFD and PFO, respectively) and are therefore considered as toxicologically relevant. The existing data on PFOA/APFO in the current analogue approach for chemical grouping thus permit an assessment of the developmental toxicity of PFDA and its salts.

Source chemical data

Below, the outcome of the RAC assessment has been included from the Opinion Document for APFO (RAC Opinion proposing harmonised classification and labelling at Community level of Ammoniumpentadecafluorooctanoate (APFO), ECHA 2012; page 14):

"Human data

Available biomonitoring indicated that human serum concentrations were lower than those reported for the mice at 5 mg/kg APFO (max. about 50 μg/ml in dams (White et al., 2007) compared to 6.8 μg/ml (max arithmetic mean in workers, see Olsen studies) and median concentrations of 0.0026 μg/ml in maternal samples of a pilot study (Midasch et al., 2007)). Absence of effects are no proof that effects in animals were not relevant for humans, since internal concentrations were much lower and epidemiological studies were not targeted on the effects of interest and of insufficient size for effect detection.

Animal data

Critical for the proposal of Repr. 1B (according to CLP criteria) and against a proposal of Repr. 2 are effects of developmental toxicity from animal studies that were observed at doses at which no (or no indications of marked) maternal toxicity has been observed.

Rat

Relevant effects indicating developmental toxicity were observed at doses without treatment related effects on body/organ weights in dams of the F0 generation during lactation phase (mortalities and reduced growth) and caused delayed sexual maturation later on in the rat offspring of a 2-generation study (York, 2002; Butenhoff et al., 2004). Effects on or via lactation have not been tested on in this species. No treatment-related effects were seen in the F2-generation. Test substance administration to rats during the mid and late gestation period only (GD 6-15/18) did not cause adverse effects on rat offspring except a dose-related increase of rib variations in a study during GD 6-18. There were no developmental studies addressing effects of APFO in rats where treatment started in the early gestational phase.

Mouse

Without any sign of marked maternal toxicity, exposure during the gestational phase was effective in mice to cause developmental deficits; no malformations occurred. This was demonstrated by a number of studies; most recent studies were not present at the TC C&L discussion in 2006.

Full litter resorptions

Most severe effects (whole litter loss in early pregnancy) were seen in the study of Wolf et al. (2007) when treatment with 5 mg/kg APFO started early at GD1. Percentages of dams with full-litter resorptions significantly increased from 5 mg/kg onwards (26% at 5 mg/kg to 100% at 40 mg/kg) (Lau et al., 2006). Body weight gain started early (from GD5 onwards) to be significantly lower in dams at ≥20 mg/kg than in controls and was interpreted to indicate that full-litter resorption must have occurred in early pregnancy. It

could be assumed that liver effects in dams at this early time of gestation are less pronounced than they may be at the end of gestation (as indicated by liver weight increase on GD18, no data on clinical pathology and microscopy). While maternal toxicity (reduced body weight gain) might be discussed to be linked to resorptions for the dams receiving 20 and 40 mg/kg, no effect on body weight was seen for the 5 mg/kg (26% full litter resorption) and 10 mg/kg (46% full litter resorption versus 7% in controls). While these studies revealed (early) full litter resorptions, no such effect was seen up to 10 mg/kg PFOA in the developmental study of Yahia et al. (2010).

Other effects

Other developmental effects (reduced postnatal survival (≥5 mg/kg), severely compromised postnatal survival (≥20 mg/kg), delays in general growth (≥3 mg/kg), and development (delay of eye opening ≥5 mg/kg), as well as sex-specific alterations in pubertal maturation (separable prepuce indicating earlier onset of male puberty ≥1 mg/kg) were reported in the study of Lau et al. (2006). Liver weight increases were seen in dams of all dose groups, but APFO treatment did not change the number of implantations. However, weight gain of dams indicating marked maternal toxicity was markedly reduced at 20 mg/kg bw/d or after correction for gravid uterine weight and liver weight only at 40 mg/kg bw/d (see RCOM doc). Significantly reduced postnatal survival could be discussed as secondary effects at ≥20 mg/kg bw/d. However dose-dependent increases in liver weight from 1 mg/kg onwards alone were not found to be plausibly linked to the adverse effects on pup growth and development in the study of Lau et al. (2006). In utero exposure to 5 mg/kg APFO alone was sufficient to reduce pup growth and developmental delay in the pups (Wolf et al., 2007). Reduced postnatal survival in pups was seen at 5 mg/kg APFO if exposure in utero continued through the lactation period. No detrimental effect on maternal weight and number of live born pups was seen in groups receiving 3 and 5 mg APFO. 23 days after last treatment (on PND 22) there was a dosedependent absolute and relative increase in liver weight in dams. Reduction of body weight of pups on PND 22 was dose-dependent and more severe after continued exposure via milk. This effect may be related to reduced milk production (some indication from the study of White et al. (2007) that showed inhibition of the mammary gland differentiation before birth) or to direct effects of APFO on pups exposed via the milk only. While maternal weight gain was similar between groups of dams exposed to 5 mg/kg APFO and control dams in the White study, mean body weights and diminished (delayed) development of the mammary gland was seen in pups at PND 10 and 20. This means APFO affected the development of the mammary gland during pregnancy and affected development of the mammary gland in pups. In a follow up study (2009) Wolf demonstrated that delayed mammary gland development in pups at 5 mg/kg APFO also occurred under lactational-only dosing. Mean serum concentrations were reported to be similar in mice exposed in utero than in mice exposed via milk. Effects on mammary gland development could also be induced in mice after peripubertal treatment (at 21-50 days of age), however testing revealed some strain specifity (Yang et al., 2009). In these studies no marked maternal toxicity has been observed and developmental effects could not be interpreted to be secondary to the maternal toxicity. The delay in mammary development has been confirmed in the recently published mouse study in pups where the dams received doses of 0, 0.3, 1.0, and 3.0 mg/kg bw/d APFO from GD 1-17 (Maron et al., 2011). This effect persisted until PND 84. Offspring liver weights were significantly increased in all dose groups (no data on dam effects). In a second study mice were administered to 0, 0.01, 0.1, 1.0 mg/kg APFO bw/d in the late gestation phase only (GD 10-17). Stunted mammary epithelial growth was seen at PND 21 in the 0.01 mg/kg dose group, increased offspring liver weight was seen in the 1.0 mg/kg bw/d dose group indicating that the delay in mammary gland development is more sensitive than the liver effect in pups. The RAC discussion focussed on the relevance of liver weight changes for developmental effects. Doses of APFO without any effect on body weight gain in dams (up to 5 mg/kg or even higher) should not be considered as marked maternal toxicity which according to the CLP guidance could justify no classification. Compared to the 28 day study in mice (Christophe and Marisa,

1977) where all mice at 300 ppm (15 mg/kg) died during the study and single premature deaths were seen at 30 (1.5 mg/kg) and 100 ppm, mortalities of dams in the Lau et al. study were not reported up to 40 mg/kg. Guidance to CLP considers developmental effects even in the presence of maternal toxicity to be evidence of developmental toxicity unless it can be unequivocally demonstrated that these effects are secondary to maternal toxicity. In case a specific maternally mediated mechanism has been demonstrated, the guidance says that category 2 may be considered more appropriate than category 1. Developmental toxicity induced by repeated APFO administration were seen in a dose-related manner, also at doses without indication of marked maternal toxicity, appears not to be linked to maternal toxicity and no specific maternally mediated mechanism was identified. Liver weight increase also at low doses without any effect on body weight gain and one might assumed that liver toxicity (if liver weight increase is interpreted as toxic effect) is the primary effect and developmental effects could be interpreted as secondary to liver toxicity. Unfortunately no other data are available from 2-generation and developmental studies on APFO to characterise liver weight increase (by microscopy or clinical pathology) with respect to its degenerative nature or as adaptive enzyme activation. From a number of studies it was demonstrated that liver cell hypertrophy and related liver weight increase is the most sensitive effect and cytotoxicity was observed at higher doses. Hepatocellular hypertrophy and increased mitosis (no quantification available) were observed at all doses (no details on dose-dependency of incidences and severity); single cell necrosis and mild calcification were only seen at 10 mg/kg PFOA (Yahia et al., 2010). Corresponding effects at 10 mg/kg were significantly increased liver transaminases (ALT, AST) and enzyme activities indicating membrane leakage (LDH, ALP). No microscopic degenerative abnormalities were reported for the dams' liver at 5 mg/kg, where fetal body weight and postnatal survival was already reduced. Assumed that at similar doses of APFO no marked liver cell toxicity had occurred, this indicates that developmental toxicity is not a consequence of liver toxicity. The observation of increased cell proliferation at doses without overt liver toxicity in mice (Yahia et al, 2010) is consistent to the observation of Elcombe et al. (2010) of increased cell proliferation of liver cells at a non-cytotoxic dose in rats. This is considered to reflect the mitogenic nature of effect rather than a regenerative proliferation response at non-cytotoxic doses.

RAC recognises that there are signs of marked maternal toxicity at high doses. However liver weight increase alone could not be plausibly linked to developmental effects in pups. Dose dependent increases in liver weight were seen in dams (and pups) most likely as a direct effect of APFO caused by liver cell hypertrophy with major contribution of PPARα-related peroxisome proliferation. Newer study clearly demonstrated that liver toxicity (single cell toxicity) started at higher doses than hypertrophic response. Therefore the observed developmental effects were not considered to be a secondary non-specific consequence of the maternal (liver) toxicity. Studies in mice allow conclusion that gestational administration of APFO was sufficient to impair neonatal growth and development and that developmental toxicity was linked to the gestational phase of exposure. Mechanistic studies using PPAR knock-out mice demonstrated that some effects (complete litter loss and liver weight increase in dams and pups) seem to be independent of PPARα expression (Abbott et al., 2007). Others such as increased postnatal pup mortality, reduction in pup body weight and postnatal growth and development (delayed eye opening) indicated interference/contribution of PPARα expression most likely as a direct effect of APFO (which is not mediated via liver cell response to PPARα). The observation that liver weight increases are similar in wild type dams and in PPARα-knock out dams and their respective offspring questioned the importance of PPARα expression for the liver effects. PPARα-related effects may contribute, but other modes of action must also be active. In addition the relevance of PPARα expression for humans is well established for the liver, however much less is known for the relevance of PPARα-related effets in other organs and effects in the offspring and juvenile."

Supporting data – PFNA

Below, the outcome of the RAC assessment has been included from the RAC Opinion proposing harmonised classification and labelling at Community level of PFNA and its ammonium and sodium salts, ECHA, 2014 (page 15):

"In Annex VI to the CLP Regulation, APFO and PFOA, used as reference substances in read-across approach for PNFA, PFN-S and PFN-A, have been classified as Repr. 1B, H360D.

There are two developmental toxicity studies for PFNA (Lau et al.2009, Wolf et al. 2010)

In a study by Lau et al. (2009), CD-1 mice were dosed orally GD 1-17 with PFNA at 0, 1, 3 or 5 mg/kg/day (10 mg/kg/day was also used but this dose level was not continued due to severe maternal toxicity including mortality (no further details provided)). One cohort of animals was necropsied on GD 17 and uterine data was evaluated whereas pup survival, growth and development of the offspring were examined in another cohort of animals. PFNA did not affect maternal weight gains (GD 1-17), number of implantations, fetal viability, fetal weight or number of viable fetuses at c-section at dose levels up to and including 5 mg/kg/day. However decreased pup viability was already observed at the first examination after birth in the 5 mg/kg/day dose group. Over the course of the first 12 day after birth there was a continuous loss of pups, and at PND 12, ~80 % of the pups had died. In written communication with Dr. Lau, the dossier submitter were further informed that one group of CD-1 mice had been administered 10 mg/kg bw PFNA (this dose produced maternal toxicity including mortality) but that "every dam lost the entire pregnancy (full litter resorption). So, like APFO, PFNA at a high enough dose will cause full litter resorption."

In the study of Wolf et al. (2010), pregnant 129S1/SvlmJ wild-type (WT) and PPARα knockout (KO) mice were given PFNA by oral gavage once daily on GD 1−18 at 0, 0.83, 1.1, 1.5 and 2 mg/kg/day. Maternal weight gain, implantation, litter size, and pup weight at birth were unaffected in both strains. PFNA exposure reduced the number of live pups at birth and survival of offspring to weaning in the 1.1 and 2 mg/kg groups in WT mice. Eye opening was delayed (mean delay 2.1 days) and pup weight at weaning was reduced in WT mice pups at 2 mg/kg. These developmental endpoints were not affected in the KO mice. Relative liver weight was increased in a dose-dependent manner in dams and pups of the WT mice strain at all dose levels, but only slightly increased in the highest dose group in the KO mice strain. In summary, PFNA altered liver weight of dams and pups, pup survival, body weight, and development in the WT mice pups, while only inducing a slight increase in relative liver weight of dams and pups at 2 mg/kg in KO mice. These results suggest that PPARα is an essential mediator of PFNA-induced developmental toxicity in the mouse.

The available information indicates that exposure to PFNA during gestation reduces pup viability, pup body weight gain, delays puberty as well as the onset of eye opening, increases both dam and pup liver weight (absolute and relative liver weight) and causes full litter resorptions at higher doses. These effects are very similar to the effects reported for APFO/PFOA.

It is noted that one of the mechanisms implicated in the toxicity of the PFNA is the activation of PPARα (Wolf et al., 2010). PPARα is a nuclear receptor that plays a role in regulating lipid and glucose homeostasis, cell proliferation and differentiation, and inflammation. However, the role of PPARα in mediating developmental toxicity effects in humans cannot be excluded.

Taking into account that exposure to PFNA in mice during gestation reduces pup viability, pup body weight gain, delays puberty as well as onset of eye opening, increases both dam and pup absolute and relative liver weight, and induces full litter resorptions/loss at high doses as well as that the developmental toxicity of PFNA in mice are qualitatively and quantitatively similar to developmental toxicity of PFOA (reduced pup viability, full litter resorption and delay in the onset of eye opening)

RAC is of the opinion that PFNA and its ammonium and sodium salts should be classified as Repr. 1B, H360D.*"*

Supporting data – PFUnDA and PFDoDA

Perfluoroalkylated carboxylic acids (PFCAs) with carbon chain length 11 and 12 were studied in reproduction/developmental toxicity screening tests (OECD TG 422; dosing beginning 14 days before mating and ending day 4 of lactation) in rats (Takahashi et al., 2014; Kato el al., 2014). Based on findings in a 14-day dose range-finding study where 9/10 animals (males and females) died after administration (oral gavage) of PFUnDA at 20 mg/kg/day doses at 0.1, 0.3, and 1.0 mg/kg/day were selected for further studies. PFUnDA statistically significantly reduced $p \leq 0.01$) rat pups body weights at birth (13.4% in male pups and 12.5% in female pups, $p \leq 0.01$) at 1 mg/kg, and body weight gain was still reduced to the same extent at 4 days after birth (19.1% in male pups and 16% in female pups, $p \leq 0.01$) in absence of statistical significant effects on maternal body weight (Takahashi et al 2014). No other significant changes in reproductive or developmental parameters were reported.

The same research group also studied PFDoDA in a similar manner (Kato et al 2014). No reproductive or developmental parameters were changed at 0.1 or 0.5 mg/kg/day. No effect on rat pup body weights was noted (only in one litter in high dose; body weight in these pups were not taken into account). Delivery index at 0.5 mg/kg/day was slightly but not statistically significantly decreased: 89.7% compared to 94.3% in control. Body weight in the dams was significantly decreased at 2.5 mg/kg/day through the gestation period (approx. 30% less than control, $p<0.01$ at GD 20-21). 7 of 12 female rats receiving 2.5 mg/kg/day died during late pregnancy while four other females in this group did not deliver live pups (Kato et al 2014).

Conclusion on read-across data

In summary, reduced pup viability and pup body weight gain and increased dam and pup liver weights (absolute and relative liver weights) were observed in rodents treated with APFO/PFOA during gestation. PFCAs ranging from C8 to C12, including PFDA, have effects that are reported to resemble those reported for APFO/PFOA.

No studies on post-natal effects of PFDA are available and it is therefore not possible to make a comparison between PFDA and PFOA/APFO for these effects. Studies with APFO/PFOA demonstrated delayed onset of eye opening and puberty (Lau et al., 2006; Abbott et al., 2007). Moreover, White et al., 2007 and 2009, Macon et al., 2011 reported that effects of APFO on mammary gland development in the mouse pups appeared to be the most sensitive endpoint for developmental toxicity with a NOAEL below 0.01 mg/kg for the dosing period GD1-17 or GD 10-17.

The observed developmental effects in studies with PFOA/APFO are not associated with adverse maternal toxicity: no statistical significant effect on body weight gain during gestation or on maternal weight at necropsy at any dose level (Wolf et al., 2007; Lau et al., 2006). However, there was a dose dependent statistical significant increase in the relative liver weight of both dams and pups in these studies (Wolf et al., 2007 and Lau et al., 2006). Statistical significant effects of developmental toxicity (decreased number of viable fetus per litter, increased resorptions, full litter resorptions and decreased fetal body weight) of PFDA were associated with excessive maternal toxicity. The pre-natal developmental study of PFDA in mice by Harris and Birnbaum (1989) revealed a decrease in net body weight change during gestation, significantly increased maternal relative liver weight (organ-to-body weight ratio for the liver) and at high dose also

mortality. Nevertheless, the data from the pre-natal developmental toxicity study gives an indication of the similarity of the adverse effects of PFDA with those described for the source chemical. Thus, based on read-across from the structurally similar PFOA/APFO, with support from shorter and longer PFCAs, and data on the substance itself there are strong reasons to assume that developmental toxicity is an intrinsic property PFDA.

10.8.6 Comparison with the CLP criteria

Based on read-across from the source chemical APFO/PFOA, with supporting mechanistic data of the C9 polyfluoroalkylated carboxylic acid PFNA and on the target chemical PFDA, the information is sufficient to fulfil the criteria for classification in reproductive toxicity – adverse effects on development of the offspring.

RACs rationale for classification of APFO in Repr. 1B (H360D) included from the Opinion Document for APFO (RAC Opinion proposing harmonised classification and labelling at Community level of Ammoniumpentadecafluorooctanoate (APFO), ECHA 2012):

"Human data do not sufficiently give evidence to conclude on whether Repr. 1A is appropriate. Repr. 2 would be appropriate if there is some, but less convincing evidence on adverse development effects. Overall there is no convincing evidence that developmental effects in pups are exclusively secondary to maternal (liver) toxicity. For APFO there is clear evidence on developmental effects from perinatal studies in mice. Mechanistic considerations allow contribution of some effects to a PPARα-related mode of action. However other modes appear to be active and developmental effects could not be attributed to liver toxicity as a secondary mechanism. Also the role of PPARα-related mode of action is not fully elucidated for the developmental effects. A contribution to some effects is assumed based on their lack of expression in knock-out mice.

Therefore RAC decided to follow the proposal of the dossier submitter that evidence is sufficiently convincing to classify for developmental effects as Repr.1B (H360D) according to the CLP criteria and as Repr. Cat. 2; R61 according to DSD."

Rationale for classification of PFDA and its ammonium and sodium salt in Repr. 1B (H360D): In a weight of evidence approach, based on read-across-data from an analogue approach of chemical grouping of the structurally similar PFOA/APFO (source chemical) with support from existing data on the target chemical itself it is concluded that developmental toxicity is an intrinsic and hazardous property of PFDA manifested mainly as reduced pup weight, reduced number of fetuses, and full litter resorptions/loss. The similarity in structure and effects between the target chemical PFDA and the source chemical PFOA/APFO is further substantiated with data from closely related perflourinated carboxyl acids that also support that this effect is an intrinsic property of PFDA.

The available data provide clear evidence of an adverse effect on the development of the offspring and are not considered to be secondary to other maternal toxic effects. Moreover, there is no conclusive mechanistic evidence to indicate that the observed effects on reproduction and development are not relevant for human.

A classification Repr. 1B – H360D is therefore warranted. It is proposed not to specify route of exposure in the hazard statement.

Classification in Repr 1A is not appropriate as it should be based on human data and no human data specific of PFDA or its salts is available. Moreover, human data on reproductive and developmental toxicity of

PFOA/APFO included in the category is not sufficient and thus read-across for data gap filling is not applicable.

Classification in Repr 2 is not appropriate as the read-across is considered robust and appropriate for the endpoint and applicable for PFDA, and all the existing experimental data on adverse effects on the development of the offspring available for the source chemical are considered reliable based on existing classification and the level of evidence is considered as clear evidence and not as some evidence.

Adverse effects on or via lactation

There is limited data on adverse effects on or via lactation of PFDA and its ammonium and sodium salts. In this dossier read-across from the structural analogue PFOA/APFO have been utilized where data on PFDA and its ammonium and sodium salts on itself are missing and for the purpose of harmonised classification.

Table 36a: Summary table of animal studies on effects on or via lactation

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results
-	-	-	-	-	

Table 36b: Summary table of human data on effects on or via lactation

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
-	-	-	-	

Table 36c: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference

PFCA levels in human breast milk samples from Japan, Korea, and China were analysed to evaluate the geographical differences.	Not applicable.	In a limited study (small, non-randomly selected volunteer samples) 90 human breast milk samples from Japan, Korea and China (30 samples each) that were stored in the Human Specimen Bank of Kyoto University were analyzed for its content of PFCAs. The extracts were analyzed by gas chromatography–mass spectrometry.	Mean concentrations in human breast milk of PFDA ranged from <15-21.3 pg/ml. Levels of PFOA, PFNA, PFUnDA and PFDoDA were 51.6-93.5 pg/ml, 14.7-32.1 pg/ml, 16-36.6 pg/ml, and <10 pg/ml, respectively.	Fujii Y, Yan J, Harada KH, Hitomi T, Yang H, Wang P, Koizumi A. Levels and profiles of long-chain perfluorinated carboxylic acids in human breast milk and infant formulas in East Asia. Chemosphere. 2012 Jan;86(3):315-21.
Analysis of the content of nine PFCAs in human breast milk samples from Massachusetts, U.S.A	Not applicable.	Nine PFCs were measured in 45 human breast milk samples collected in 2004 from Massachusetts, U.S.A. Sample extracts were analysed with high-performance liquid chromatography coupled with an electrospray triple-quadrupole mass spectrometer.	PFDA was detected above the limit of quantification in 4 out of 45 samples of breast milk in the concentration range <7.72-11.1 pg/ml	Tao L, Kannan K, Wong CM, Arcaro KF, Butenhoff JL. Perfluorinated compounds in human milk from Massachusetts, U.S.A. Environ Sci Technol. 2008 Apr 15;42(8):3096-101.

Analysis of human breast milk, milk infant formulas and cereals baby food to determine the content of six PFCs with an analytical method based on alkaline digestion and solid phase extraction (SPE) followed by liquid chromatography-quadrupole-linear ion trap mass spectrometry (LC-QqLIT-MS)	Not applicable.	The method was applied to investigate the occurrence of PFCs in 20 samples of human breast milk, and 5 samples of infant formulas and cereal baby food (3 brands of commercial milk infant formulas and 2 brands of cereals baby food). Breast milk samples were collected in 2008 from donors living in Barcelona city (Spain) on the 40 days postpartum.	PFOS and perfluoro-7-methyloctanoic acid (i,p-PFNA) were predominant being present in the 95% of breast milk samples. PFOA was quantified in 8 of the 20 breast milk samples at concentrations in the range of 21-907 ng/L. However, in most breast milk samples PFDA was below the limit of quantification	Llorca M, Farré M, Picó Y, Teijón ML, Alvarez JG, Barceló D. Infant exposure of perfluorinated compounds: levels in breast milk and commercial baby food. Environ Int. 2010 Aug;36(6):584-92.

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

There are no studies available that demonstrate that PFDA and its ammonium and sodium salts interferes with lactation or cause adverse effects to offspring via lactation. However, there are studies indicating the presence of PFDA in blood samples from pregnant women and in breast milk.

In a limited study (small, non-randomly selected volunteer samples) 90 human breast milk samples from Japan, Korea and China were analyzed for its content of PFCAs. Mean concentrations in human breast milk of PFDA ranged from <15-21.3 pg/ml. Levels of PFOA, PFNA, PFUnDA and PFDoDA were 51.6-93.5 pg/ml, 14.7-32.1 pg/ml, 16-36.6 pg/ml, and <10 pg/ml, respectively (Fujii et al., 2012). PFDA was also detected in 4 out of 45 samples of breast milk from women in Massachutes, USA in the concentration range <7.72-11.1 pg/ml (Tao et al 2008). However, in most breast milk samples of women living in Barcelona city in Spain PFDA was below the limit of quantification (Llorca et al., 2010).

Read-across from the source chemical to fill data gaps on adverse effects of PFDA and its ammonium and sodium salts on or via lactation

To generate information on the potential adverse effects of PFDA and its ammonium and sodium salts on or via lactation for the purpose of harmonized classification an analogue chemical grouping approach was utilized. Read-across from data of APFO/PFOA was used for the purpose of hazard assessment and classification.

Justification

The adverse effects of PFDA and its ammonium and sodium salts on or via lactation and its ammonium and sodium salts is assumed to be predictable on the basis of structural similarities with PFOA and APFO. Both substances have a common functional group and only the carbon chain length is differing. PFDA and PFOA are relatively strong acids and are expected to dissociate to their respective anionic forms physiological pH. Both acids (PFDA and PFOA) and their salts are expected to be available to cells in the form of their corresponding carboxylate anion (PFD and PFO, respectively) and are therefore considered toxicologically equivalent.. The existing data on PFOA/APFO in the current analogue approach for chemical grouping thus permit an assessment of the adverse effects of PFDA and its salts on or via lactation.

Source chemical data

Data on adverse effects on or via lactation have been presented above in the assessment by RAC of developmental toxicity of PFOA. Studies by Wolf et al., 2007, White et al., 2007 and 2009, Macon et al., 2011, and Yang et al., 2009 demonstrate that PFOA can induce effects on or via lactation. Wolf et al., 2007 reported that effects on mouse pup survival from birth to weaning were only affected in litters that were exposed to PFOA both in utero and via lactation. In utero exposure, in the absence of lactational exposure, was sufficient to produce postnatal body weight deficits and developmental delay in the pups. Exposure of PFOA during late fetal and early neonatal life in mice was reported by White et al (2007) to delay development of the mammary gland which was evident in pups at PND 10 and 20. The same group further corroborated these findings in mouse by showing that that delayed mammary gland development in pups also occurred under lactational only dosing (White et al., 2009) and that the delay in mammary development is

persistent (up to PND 84) and the most sensitive end point for developmental toxicity of PFOA (Macon et al., 2011).

Furthermore, PFOA have been shown to be readily transferred to infants through breast-feeding and the PFOA exposure for these infants is considerably higher than for adults (Fromme et al., 2009 and 2010; Kärrman et al., 2007; Tao et al., 2008; Völkel et al., 2008; Haug et al., 2011; cited in RAC Opinion CLH PFOA, ECHA 2012; and Annex XV dossier for PFOA, ECHA 2013).

Supporting data – PFNA

Below, the outcome of the RAC assessment has been included from the RAC Opinion proposing harmonised classification and labelling at Community level of PFNA and its ammonium and sodium salts, ECHA, 2014 (page 16):

"In the study of Wolf et al. (2010), PFNA was detected in serum of all animals. Based on a subset of dams exposed to PNFA by gavage on GD 1−18, PFNA serum levels in pups at weaning were comparable to that of their mothers in WT mice strain while the serum concentration in KO mice were higher in pups compared to their mothers. PFNA levels were also higher in pups compared to the dams, based on a subset of dams matched to their existing pups at weaning (KO mice, P < 0.0001; WT mice, P< 0.005). In all dams with nursing pups, levels of PFNA were lower in KO mice compared to WT mice, while in pups levels of PFNA were higher in KO mice compared to WT mice. These data indicate a substantial transfer of PNFA with mother's milk, related with adverse effect on pups survival and development in the WT mice strain, but not in the KO strain.

Similar findings were observed in a cross-foster study with APFO (Wolf et al., 2007) showing that pup survival from birth to weaning was only affected if the pups that had been exposed in utero and via lactation, whereas exposure of the dams to APFO during gestation was sufficient to produce postnatal body weight deficits and developmental delay in the pups.

APFO affects the development of the mammary gland. White et al. (2007 and 2009) performed parallel experiments where groups of CD-1 mice were dosed with 0, 3 and 5 mg/kg APFO during GD 1-17, 8-17, or 12-17 and then the pups were cross-fostered. They reported that the window of mammary gland sensitivity was due to exposure during late fetal and early neonatal life and that the effects on the mammary gland included altered lactational development of maternal mammary glands and halted female pup mammary epithelial proliferation (the latter effect was persistent). A later study from the same lab (Macon et al., 2011) indicated that the effects on mammary gland development in the pups are the most sensitive endpoint for developmental toxicity with a NOAEL below 0.01 mg/kg for the dosing period GD 1-17 or GD 10-17.

PFNA has been detected in serum, cord blood and human breast milk (Chen et al., 2012, Kärrman et al., 2007, Tao et al., 2008, Liu et al., 2011 and Schecter et al., 2012).

The results of animals studies (Wolf et al., 2010; Wolf et al., 2007; White et al., 2007 and 2009, and Macon et al., 2011) thus provide clear evidence of adverse effect of PFNA or its structural analogs PFOA and APFO in the offspring due to transfer in the milk or adverse effect on the quality of the milk. ***Therefore RAC is of the opinion that PFNA and its ammonium and sodium salts should be classified as Lact., H362.****."*

Conclusion on the read-across

In summary, several studies provide evidence of adverse effects on or vi lactation in rodents treated with PFOA. Moreover, adverse effect on pups survival and development was associated with serum levels of PFNA in pups that were in the same range as their mothers that had been exposed during gestation. PFCAs ranging from C8 to C12, including PFOA, PFNA and PFDA have been detected in breast milk, and the exposure of PFOA to infants via breast feeding have been clearly demonstrated.

10.8.8 Comparison with the CLP criteria

According to CLP Annex I classification of substances for effects on or via lactation can be assigned on the:

(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

Based on read-across from the source chemical APFO/PFOA, with supporting data of the target chemical PFDA, the information is sufficient to fulfil the criteria for classification in reproductive toxicity – adverse effects on or via lactation. PFDA has been found at detectable levels in the blood of mothers and their children, and in breast milk. Data on source chemical (APFO/PFOA) indicate that there is a likelihood that the substance is present in potentially toxic levels in breast milk that may cause concern for the health of a breastfed child. Thus, PFDA and its ammonium and sodium salts should be classified as Lact. H362.

Below, the outcome of the RAC assessment has been included from the Opinion Document for APFO (RAC Opinion proposing harmonised classification and labelling at Community level of Ammoniumpentadecafluorooctanoate (APFO), ECHA 2012):

"PFOA has also been found to be transferred to infants through breast-feeding. Although the criteria from human evidence and/or from results from two generation studies in animals do not provide effects in the offspring due to transfer in the mild or adverse effects on the quality of the milk, there is sufficient evidence from mouse studies with postnatal administration of APFO that indicated adverse effects (delayed/stunted mammary gland development in the offspring) which cause concern for the health of a breastfed child. Classification for effects on or via lactation is independent of whether or not a substance is also classified for reproductive toxicity.

In addition RAC agreed on an additional classification on lactation effects (H362: May cause harm to breast-fed children and R64 May cause harm to breastfed babies)."

10.8.9 Conclusion on classification and labelling for reproductive toxicity

PFDA and its ammonium and sodium salts should be classified according to the CLP criteria for adverse effects on sexual function and fertility as Repr. 2 (H361f).

PFDA and its ammonium and sodium salts should be classified according to the CLP criteria for adverse effects on development as Repr. 1B (H360D).

PFDA and its ammonium and sodium salts should have an additional classification for effects on or via lactation (H362).

10.9 Specific target organ toxicity-single exposure

Hazard class not evaluated.

10.10 Specific target organ toxicity-repeated exposure

Hazard class not evaluated.

10.11 Aspiration hazard

Hazard class not evaluated.

11. EVALUATION OF ENVIRONMENTAL HAZARDS

The environmental hazards of PFDA and its ammonium and sodium salts have not been evaluated.

12. EVALUATION OF ADDITIONAL HAZARDS

Additional hazards of PFDA and its ammonium and sodium salts have not been evaluated.

13. ADDITIONAL LABELLING

Not applicable.

14. DETAILED STUDY SUMMARIES

14.1 PHYSICAL HAZARDS

N/A

14.2 TOXICOKINETICS

Study 1

Study reference:

Ohmori K, Kudo N, Katayama K, Kawashima Y. Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. Toxicology. 2003 Mar 3;184(2-3):135-40.

Detailed study summary and results:

Study type: The study aimed at estimating the total clearance, plasma half-life and renal clearance of perfluoroalkylated carboxyl acids having different chain length and between sexes in rats.

Test material identity: PFDA (Sigma Aldrich). Purity of test substance unknown.

Test subjects: Male and female Wistar rats, number of animals not known.

Route of administration, exposure: PFDA, PFOA; Perfluoroheptanoic acid (PFHA), perfluorohexanoic acid (PFHexA), or PFNA was administered i.v. at a dose of 48.64 mmol/(2.5 ml/kg body weight) for plasma concentration profile determination and at 48.64 µmol/kg body weight for estimation of clearance rate (CLR). Clearance periods were 360 min.

Results: Initial plasma concentration of PFDA was significantly lower than those of PFHA, PFOA and PFNA. At 10 min after an intravenous injection, plasma concentrations of PFHA, PFOA, PFNA and PFDA were 289.59±26.9, 334.79±36.5, 307.59/15.1 and 214.29±14.4, respectively, in male rats.

Plasma concentration profiles up to 60 days after injection show that PFDA slowly decreased from plasma in both male and females. Serum half-life of PFDA was 339.92 and 58.57 days in males and females respectively.

Volume of distribution of PFDA was 347.7 and 441.1 ml/kg for male and female rats respectively.

Total clearance of PFDA was 5.2 and 5.3 ml/day/kg in males and females respectively, but the clearance rate was similar for males and females.

Clearance rate (ml/day/kg) of the investigated PFCAs were PFHA>PFOA≥PFNA~-PFDA in male rats and PFHA≥PFOA≥PFNA≥PFDA in female rats.

The relationship between clearance rate and total clearance was significant, r^2=0.981, ($p<0.01$).

Study 2

Study reference:

Kudo N, Suzuki E, Katakura M, Ohmori K, Noshiro R, Kawashima Y. Comparison of the elimination between perfluorinated fatty acids with different carbon chain length in rats. Chem Biol Interact. 2001 Apr 16;134(2):203-16.

Detailed study summary and results:

Study type: The study aimed at clarifying elimination route and rate of various perfluoroalkylated carboxylic acids in male and female rats.

Test material identity: PFDA (Aldrich Japan). Purity of test substance unknown. Dissolved in propylene glycol:water (1:1, v/v)

Test subjects: Male and female Wistar rats, number of animals not known.

Route of administration, exposure: PFDA, PFHA, PFOA or PFNA were i.p. administered at a dose of 20 mg/ml/kg body weight. Urine and feces were collected every 12 h up to 5 days.

For the collection of bile rats were injected i.v. at a dose of 25 mg/kg body weight. Bile samples were collected every 30 min up to 5 h after the injection.

To compare rate of urinary a excretion rate of the PFCAs rats were i.v. injected at a dose of 25 mg/kg body weight and urine samples collected every 10 minutes.

Results:

Urinary and fecal elimination: In male rats 0.2% of the dose of PFDA and 2% of PFNA was eliminated in urine within 120 h after the administration: In contrast PFHA and PFOA were eliminated to 92% and 55% of the dose. A similar tendency was observed in female rats as in males for PFDA, but for PFOA and PFNA the urinary elimination was significantly faster than in males. Fecal elimination was a major route of elimination of PFDA in both male and female rats, approx. >4% of the dose was eliminated by 100 hours. PFOA and PFHA were slowly eliminated in feces in contrast to urinary elimination (approx. 22.5% of the dose).

Concentrations of PFCAs in serum and liver: Concentration of PFDA in blood and liver in female rats 5 days after injection were significantly higher (estimated from the graphical presentation to be approx. 50 µg/ml serum and 140 µg/g liver respectively), at least 10-fold higher compared to PFHA, PFOA and PFNA. In males, concentrations of both PFDA and PFNA were significantly higher ($p<0.05$, compared to the other tested PFCAs) in liver and in blood respectively. Concentrations of PFOA and PFNA were significantly lower ($p<0.05$) in female rats in both liver and serum compared to male rats.

Study 3

Study reference:

Vanden Heuvel JP, Kuslikis BI, Van Rafelghem MJ, Peterson RE. Disposition of perfluorodecanoic acid in male and female rats. Toxicol Appl Pharmacol. 1991 Mar 1;107(3):450-9.

Detailed study summary and results:

Study type: The study aimed at examining the elimination, tissue distribution, and metabolism of [1-^{14}C]PFDA in male and female rats for 28 days after a single i.p. dose.

Test material identity: [1-^{14}C]PFDA (99% pure) was synthesized and purified by the study authors as described in Reich et al., 1987.

Test subjects: Male and female Sprague-Dawley rats.

Route of administration, exposure:

Elimination and tissue distribution [1-^{14}C]PFDA was administered to male and female rats as a single dose of 9.4 µmol/kg (5 mg/kg) i.p. Samples were collected daily for 28 consecutive days. At designated times post-treatment (2 h, 1,2 ,3 ,4, 7, 14, 28 days for males, and 2 h, 1, 4, 7, 28 days for females) four rats were euthanized and tissues and blood were collected for determination of tissue distribution and elimination of PFDA.

Biliary excretion of PFDA [1-^{14}C]PFDA was administered to male and female rats as a single dose of 9.4 µmol/kg (5 mg/kg) i.p. Start of collection of bile samples were 2 hours later and then collected at 1 hour intervals for 6 hours.

Fluoride determinations in urine and plasma Male and female rats were dosed with 97 µmol/kg, i.p. PFDA. Daily urine samples were collected 1 day prior to dosing and for four days thereafter. Blood was collected prior to dosing and at 1 and 4 days postdosing.

Results: Fecal elimination with 51% and 24% of the administered ^{14}C was recovered in the feces of males and females respectively by 28 days post-treatment.

The cumulative excretion of PFDA-derived ^{14}C in urine was less than 5% of the administered dose in both sexes by 28 days post-treatment.

Half-life of PFDA of whole body elimination in males was 23 days and in females 45 days. Tissue elimination half-life of the liver was 35 days in males and 45 days in females; plasma was 22 days in males and 27 days in females; kidneys was 24 days in males and 31 days in females; blood was 22 days in males and 29 days in females.

The liver contained the highest concentration of PFDA-derived ^{14}C in both males and females at all time points up to 28 days, followed by the plasma and kidneys.

14.3 HEALTH HAZARDS

14.3.1 Acute oral toxicity - animal data

14.3.2 Acute oral toxicity - human data

14.3.3 Acute oral toxicity - other data

14.3.4 Acute dermal toxicity - animal data

14.3.5 Acute dermal toxicity - human data

14.3.6 Acute dermal toxicity - other data

14.3.7 Acute inhalation toxicity - animal data

14.3.8 Acute inhalation toxicity - human data

14.3.9 Acute inhalation toxicity - other data

14.3.10 Skin corrosion/irritation - animal data

14.3.11 Skin corrosion/irritation toxicity - human data

14.3.12 Skin corrosion/irritation - other data

14.3.13 Eye damage/eye irritation - animal data

14.3.14 Eye damage/eye irritation - human data

14.3.15 Eye damage/eye irritation - other data

14.3.16 Respiratory sensitisation - animal data

14.3.17 Respiratory sensitisation - human data

14.3.18 Respiratory sensitisation - other data

14.3.19 Skin sensitisation - animal data

14.3.20 Skin sensitisation - human data

14.3.21 Skin sensitisation - other data

14.3.22 Germ cell mutagenicity - animal data

14.3.23 Germ cell mutagenicity - human data

14.3.24 Germ cell mutagenicity - *in vitro* data

14.3.25 Germ cell mutagenicity - other data (e.g. studies on mechanism of action)

14.3.26 Carcinogenicity - animal data

Not applicable.

14.3.27 Carcinogenicity - human data

Not applicable.

14.3.28 Carcinogenicity - *In vitro* data (e.g. in vitro germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

Not applicable.

14.3.29 Carcinogenicity - other data (e.g. studies on mechanism of action)

Study 1

Study reference:
Borges T, Peterson RE, Pitot HC, Robertson LW, Glauert HP. Effect of the peroxisome proliferator perfluorodecanoic acid on the promotion of two-stage hepatocarcinogenesis in rats. Cancer Lett. 1993 Aug 16;72(1-2):111-20.

Detailed study summary and results:

Test type
No guideline study. The study was conducted to determine if PFDA, which is a known peroxisome proliferator, has promoting activity in two-stage hepatocarcinogenesis in rats. 2 weeks after acclimatization, rats were subjected to 70% partial hepatectomies. Twenty-four hours later the rats were given an initiating dose of 10 mg/kg diethylnitrosamine (DEN) in saline by gastric gavage. Two weeks later, 140 rats were randomly divided into six treatment groups of 26 rats each (control, three PFDA-treatment groups, one ciprofibrate treatment group) and one group of 10 rats comprising a phenobarbital-treatment group. At the same time, four of the six treatment groups received the first monthly i.p. injections of PFDA in corn oil. The remaining two groups were placed on unrefined diets that contained either 0.01% ciprofibrate or 0.05% phenobarbital. Ciprofibrate (peroxisome proliferator) and phenobarbital are known tumor-promoting agents and were included as positive controls. The number and volume of altered hepatic foci were determined after 9 and 18 months as the primary end-point. The histochemical markers GGT, ATPase and G6Pase were used for this purpose.

Test substance
PFDA (from 3M, Inc). Purity: not stated . Impurities not stated. Batch number not stated.

Test animals
- Sprague-Dawley rats, female.

- Five groups with 26 animals each. The control group treated with phenobarbital had 10 animals.
- Age and weight at the study initiation not reported.

Administration/exposure

- Route of administration: intraperitoneal injections of PFDA. Positive control groups were orally exposed via diet.
- Duration of test/exposure period: 9 months or 18 months
- Doses/concentration levels, rationale for dose level selection: PFDA-dose groups: 0.0, 0.05, 0.50, 5.0 mg PFDA/kg/injection. Vehicle: corn oil. In a previous study by the same authors (Borges et al., 1992) the authors found that two-weekly doses of 10 mg/kg resulted in weight loss after months, whereas a dose of 3 mg/kg did not reduce body weight but still produced significant increases in peroxisome enzymes. The study authors therefore chose a similar dose for the highest does and used a monthly injection schedule to avoid adverse consequences from too frequent i.p. injections.
- Frequency of treatment: once a month
- Control group and treatment: Additional control groups were placed on diets that contained either 0.01% ciprofibrate or 0.05% phenobarbital.

- Post exposure observation period: the body weights of all animals were recorded every two weeks. The number and volume of altered hepatic foci were determined after 9 and 18 months as the primary end-point, using the histochemical markers gamma-glutamyl transpeptidase (GGT), glucose6-phosphatase (G6Pase) and ATPase.
- Nine months after the start of the study, three rats from the phenobarbital-treatment group and 12 rats from each of the other treatment groups were sacrificed. The remaining rats in each group continued to receive treatment and were sacrificed at 18 months. At both times, rats were killed 10 days after the final injection of PFDA.
- Vehicle (identification, concentration and volume used, justification of choice of vehicle (if other than water)): corn oil - no further details on the vehicle were available.
- Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation: no information available in the original study report
- Actual doses (mg/kg bw/day): not reported in the original study report
- Satellite groups and reasons they were added: not applicable

Results and discussion

- Mortality and time to death (indicate number died per sex per dose and time to death): no mortality reported
- Clinical signs: not reported in the original study report.
- Body weight gain: not significantly affected by any of the treatments
- Food/water consumption: not reported in the original study report
- Ophthalmoscopic examination: not reported in the original study report
- Clinical chemistry: not reported in the original study report
- Haematology: not reported in the original study report
- Urinalysis: not reported in the original study report
- Organ weights: Spleen and kidney weights not significantly affected by any treatment. At nine months liver to body weight ratios of rats fed diets containing either 0.01% ciprofibrate or 0.05% phenobarbital were significantly increased (66% and 44% increase respectively, compared to control levels, $p<0.01$) and in the PFDA-treatment group 5.0 mg/kg/injection (23% increase compared to control levels, $p<0.05$). At 18 months the liver to body weight ratio was not significantly increased in any of the PFDA-treated groups
- Necropsy findings: nature and severity: not reported in the original study report
- Tissue parameters: 5.0 mg/kg PFDA significantly increased ($p<0.05$) the activity of the peroxisomal enzyme fatty acyl CoA oxidase at both 9 (100% increase compared to control) and 18 months (53% increase compared to control).

- Histopathological findings: Liver sections from most rats in all treatment groups showed chronic inflammation, fibrosis, and bile duct proliferation at both 9 and 18 months. Neither PFDA nor ciprofibrate treatment increased the number of foci/cm^3, the number of foci/liver nor the volume fraction of the foci relative to control rats at either 9 or 18 months. All three doses of PFDA significantly increased the mean focal volume at 9 months in a dose-dependent manner 3-5-fold (0.025 mm^3, 0.049 mm^3, 0.053 mm^3 at 0.05 mg/kg, 0.5 mg/kg, and 5.0 mg/kg respectively, compared to control 0.011 mm^3, $p<0.01$), but there was no increase at 18 months.
- Tumour incidence data by sex, dose and tumour type: PFDA treatment did not significantly increase the tumor incidence, see Table IV. An increase (not stat. sign.) in the incidence of hepatocellular carcinoma was observed at 5.0 mg/kg PFDA (2/12 (17%)) at 9 months but not at 18 months.
- Local or multi-site responses: not applicable (only liver was examined)
- Progression of lesions to malignancy: not applicable
- Gender and/or species-specific responses: not applicable (only female rats included in the study)
- Mode of action (genotoxic, non-genotoxic): not applicable
- Tumour latency: not applicable
- Statistical methods and results (unless already described with specific test results above): statistical evaluations were made using analysis of variance and Dunnett's multiple comparison test. Values were considered significant when $p<0.05$

Table IV. Tumor incidence (%) at 9 and 18 months (Adapted from original report)

Group	Neoplastic nodules	Hepatocellular carcinomas
9 months		
Control	6/11 (55)	0/11 (0)
0.05 mg PFDA	3/12 (25)	0/12 (0)
0.5 mg PFDA	4/12 (33)	0/12 (0)
5.0 mg PFDA	3/12 (25)	2/12 (17)
Ciprofibrate	6/12 (50)	2/12 (17)
Phenobarbital	3/3 (100)	0/3 (0)
18 months		
Control	9/10 (90)	3/10 (30)
0.05 mg PFDA	10/12 (83)	1/12 (8)
0.5 mg PFDA	7/7 (100)	1/7 (14)
5.0 mg PFDA	11/11 (100)	1/11 (9)
Ciprotibrate	7/9 (78)	9/9 (100)*
Phenobarbital	5/5 (100)	1/5 (20)
*Significantly different from control (P < 0.01).		

Study 2

Study reference:

Benninghoff AD, Orner GA, Buchner CH, Hendricks JD, Duffy AM, Williams DE. Promotion of hepatocarcinogenesis by perfluoroalkyl acids in rainbow trout. Toxicol Sci. 2012 Jan;125(1):69-78

Detailed study summary and results:

Study type: Mechanistic study in a non-mammalian species. Trout is an animal model that represents human insensitivity to peroxisome proliferation. A two-stage chemical carcinogenesis model was used in trout to evaluate PFAAs as complete carcinogens or promoters of aflatoxin B(1) (AFB(1))- and/or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced liver cancer. DNA microarray was used to assess hepatic transcriptional response to these dietary treatments in comparison with E2 and the classic peroxisome proliferator, clofibrate (CLOF).

Identity of the test substance: PFDA (from Sigma Aldrich). Purity: analytical grade (not stated)

Test subjects: Rainbow trout were used in the study. Approximately 3500 fry were initiated at 10 weeks postspawn with an aqueous exposure to 10 ppb AFB1 or 0.01% EtOH (noninitiated sham controls) for 30 min; a second cohort of about 1000 fry was AFB1 or sham initiated at 15 weeks of age. To determine whether the expected tumor-promoting effects of PFOA and related compounds are carcinogen or target organ dependent, a third cohort of about 1000 fry was initiated at 10 weeks postspawn with a 30-min aqueous exposure to 35 ppm MNNG, a multiorgan carcinogen in trout (Hendricks et al., 1995), or 0.01% dimethyl sulfoxide (noninitiated sham control). After initiation, fry were fed Oregon Test Diet (OTD), a semipurified casein-based diet, for 1 month (Lee et al., 1991). Then, within each initiation cohort, trout were randomly distributed into dietary treatment groups with 125 animals assigned to duplicate tanks (250 fish/treatment)

Route of administration, exposure: Fish (250 fish/treatment) were fed experimental diets containing 5 ppm E2, 2000 ppm PFOA (approximately 50 mg/kg body weight/day), 2000 ppm 8:2 fluorotelomer alcohol (FtOH) or 2000 ppm CLOF ad libitum (2.8–5.6% of body weight) 5 days per week for 6 months. PFNA and PFDA experimental diets were initially administered at 2000 ppm but due to an unexpected number of mortalities early in the study, diet concentrations were reduced to 200 ppm PFDA (5 mg/kg/day) or 1000 ppm PFNA (25 mg/kg/day) for the remainder of the exposure period. PFDA (as most of the test compounds) was added directly to the oil portion of the test diet.

Results:
Incidence of liver tumors significantly increased (6.8-fold compared to ABF1/control, $p<0.0001$), multiplicity significantly increased (data not shown in the article) and size of liver tumors significantly increased (compared with ABF1/control, $p<0.001$) in trout fed diets containing PFDA compared with AFB(1)-initiated animals fed control diet.

PFDA was the most potent promoting agent tested in this study. 200 ppm PFDA increased liver tumor incidence to a greater extent (26% higher) than did a 10-fold higher diet concentration of PFOA.

Pearson correlation analyses, unsupervised hierarchical clustering, and principal components analyses showed that the hepatic gene expression profiles for E2 and PFOA, PFNA, PFDA, and PFOS were overall highly similar, though distinct patterns of gene expression were evident for each treatment, particularly for PFNA.

14.3.30 Reproductive toxicity - animal data

Study 1

Study reference:

Harris MW, Birnbaum LS. Developmental toxicity of perfluorodecanoic acid in C57BL/6N mice. Fundam Appl Toxicol. 1989 Apr;12(3):442-8.

Detailed study summary and results:

Test type
No guideline was followed. Prenatal developmental toxicity study. The mice were dosed once per day for either 4 consecutive days (GD 10-13) or 10 consecutive days (GD 6-15). Dams were weighed on GD 18 and killed by decapitation. Maternal and developmental toxicity of PFDA were assessed.

Test substance
PFDA, from Aldrich Chemical Company (refer to Table 5 for details)

Test animals
- Mouse, C57BL/6N, Female
- 10-14 animals in each control and dose group
- Female mice were obtained at 6-8 weeks of age and held in breeding colony for at least two weeks prior to mating. No information on weight at the study initiation in the original study report

Administration/exposure
- Route of administration – oral gavage (10 ml/kg body weight)
- Dosing once daily on GD 10-13 or GD 6-15
- Doses/concentration levels: GD 10-13: 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 mg/kg/day; GD 6-15: 0, 0.03, 0.3 1.0, 3.0, 6.4, 12.8 mg/kg/day. No rationale given for dose level selection in the first study, GD10-13, that was designed to investigate dioxin-like toxicity of PFDA and to contain the sensitive window for cleft palate and hydronephrosis development. In the second study PFDA was administered during the entire period of organogenesis (GD 6-15) and the doses were based on the results of the first study.
- Control group received 10 ml/kg bw corn oil
- No historical control data available
- Vehicle: corn oil. No further detail given in the original study report
- A stock solution of 3.2 mg/ml was prepared in fresh corn oil and its concentration and stability, as well as that of selected dilutions, were checked by titration prior to administration
- Test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation: no details given in the original study report
- Actual doses (mg/kg bw/day): not reported in the original study report

Description of test design:
- Details on mating procedure: 2 females per male were housed overnight, females were checked for vaginal plugs the next morning; the day on which a plug was observed was denoted as day 0 of gestation. On GD 6 or 10, females were weighed and randomly assigned to treatment groups.
- Dosing schedules: The mice were dosed once per day for either 4 consecutive days (GD 10-13) or 10 consecutive days (GD 6-15). Dams were weighed on GD 18 and killed by decapitation.
- Standardization of litters: not applicable
- Parameters assessed in dams: The dams were checked daily for signs of toxicity. The dams were weighed on GD 18 prior to decapitation. Maternal liver weights, live fetus weights, and the number of live and dead foetuses were recorded at necropsy. Maternal body weight gain was defined as the gain in weight of the dam between the firs gestation day of treatment and GD 18 minus the weight of the uterus and its contents. The uteri of nonpregnant mice were examined for the presence of implantation sites.
- Organs examined at necropsy in offspring: The foetuses were first examined for gross external abnormalities and then randomly assigned in approximately equal number for soft tissue examination (organs not specified) and skeletal staining.
- Post exposure observation period: not applicable

Results and discussion

- Actual dose received by dose level by sex: not known in females, not applicable in males
- Statistical treatment of results: The litter was considered the experimental unit. The nonparametric ANOVA Kruskal-Wallis was used to determine if there was a significant difference between groups. Where a significant difference was observed, pairwise comparisons of parameters from exposure group to control group were made by the Mann-Whitney U test. All reported p values are two-tailed. The significance of dose-response trends was analysed by Jonckheere's test.

For P (per dose):

- Number of animals at the start of the test and matings: not known. When the dosing started at GD 10 there were 11-14 dams in each treatment group; or at GD 6 there were 10-14 dams in each treatment group.
- Time of death during the study and whether animals survived to termination: there was no mortality in dams that were dosed GD 10-13; among the dams dosed GD 6-15 at the highest dose (12.8 mg/kg/day) there were three dams that died on GD 18 after exhibiting marked weight loss.
- Body weight data for P and F1 animals selected for mating: Body weight of females or males that were mated not known. Body weight of dams at the start of treatment, see tables I and III.

Table I. (Adapted from original report) Reproductive parameters in mice receiving PFDA on GD 10-13

	PFDA (mg/kg/day)								
	0.0	0.25	0.5	1.0	2.0	4.0	8.0	16	32.0
Number of dams	13	12	10	13	14	13	11	13	12
Maternal bw GD 10 (mean ± SEM)	25.6± 0.3	25.2± 0.4	24.5± 0.4	26.3± 0.9	25.3± 0.4	25.1± 0.5	25.1± 0.4	25.9± 0.9	25.1± 0.5
Net body weight change GD 10-13 (mean ± SEM) §	2.8 ± 0.3	2.5 ± 0.5	2.2 ± 0.2	2.0 ± 0.8	2.4 ± 0.4	3.5 ± 0.2	2.8 ± 0.8	1.1 ± 0.7	5.4 ± 0.5
Maternal liver weight (mean ± SEM) §	1.9 ± 0.04	1.8 ± 0.09	1.8 ± 0.08	2.1 ± 0.05	2.2 ± 0.09	2.8 ± 0.05	3.2 ± 0.1	3.2 ± 0.1	2.8 ± 0.2
Maternal relative liver weight (mean ± SEM)	6.7 ± 0.1	6.4 ± 0.3	6.9 ± 0.3	7.5 ± 0.1	7.7 ± 0.2	9.7 ± 0.1	11.5 ± 0.5	12.9 ± 0.4	13.8 ± 0.9
Implantations per litter (mean ± SEM)	8.4 ± 0.8	7.5 ± 0.5	8.2 ± 0.2	8.5 ± 0.6	8.4 ± 0.6	8.2 ± 0.7	8.7 ± 0.3	8.0 ± 0.6	8.2 ± 0.3
Live fetuses per litter (mean ± SEM)	7.6 ± 0.9	6.5 ± 0.2	7.8 ± 0.4	8.2 ± 0.7	7.6 ± 0.8	7.6 ± 0.8	7.2 ± 0.8	7.2 ± 0.7	5.2 ± 1.1
% resorptions per litter (mean ± SEM) (N)	13.1 ± 4.6 (10)	15.9 ± 8.4 (12)	5.0 ± 3.3 (4)	5.8 ± 4.1 (3)	11.4 ± 7.1 (11)	10.1 ± 5.2 (7)	16.2 ± 8.8 (17)	12.4 ± 4.9 (10)	35.4 ± 13.8 (36)

Litters with resorptions (No. with 100%)	7 (0)	5 (1)	2 (0)	2 (0)	5 (1)	4 (0)	6 (1)	6 (0)	7 (4)
Percentage litters with resorptions	53.8	42.7	20.0	15.4	35.7	30.8	54.5	46.2	58.3
Fetal bw per litter (mean ± SEM) §	1.25 ± 0.05	1.20 ± 0.03	1.13 ± 0.02	1.13 ± 0.03	1.11 ± 0.03	1.13 ± 0.03	1.04 ± 0.01	0.98 ± 0.03	0.70 ± 0.03

§ p<0.01 Jonckheere's test for trend
* p< 0.05 Mann-Whitney U test, pairwise comparison to control
** p< 0.01 Mann-Whitney U test, pairwise comparison to control

Table III. (Adapted from original report) Reproductive parameters in mice receiving PFDA on GD 6-15

	PFDA (mg/kg/day)							
	0.0	0.03	0.1	0.3	1.0	3.0	6.4	12.8
Number of dams	12	11	12	12	14	12	14	10
Maternal bw GD 6 (mean ± SEM)	21.9± 0.5	22.1± 0.3	22.0± 0.4	22.4± 0.4	22.0± 0.3	21.5± 0.4	21.6± 0.3	21.8± 0.4
Net body weight change GD 6-15 (mean ± SEM) §	4.9 ± 0.5	5.3 ± 0.2	5.8 ± 0.2	5.4 ± 0.2	5.8 ± 0.3	6.3 ± 0.4	0.4 ± 0.6 **	-2.4 ± 0.4 **
Maternal liver weight (mean ± SEM) §	1.8 ± 0.07	1.8 ± 0.06	1.9 ± 0.04	1.9 ± 0.06	2.2 ± 0.02**	2.9 ± 0.1**	3.0 ± 0.1**	2.9 ± 0.09**
Maternal relative liver weight (mean ± SEM) §	6.7 ± 0.2	6.7 ± 0.2	6.9 ± 0.1	6.8 ± 0.2	7.9 ± 0.1 **	10.3 ± 0.4**	13.8 ± 0.3 **	15.2 ± 0.4 **
Implantations per litter (mean ± SEM)	8.1 ± 0.5	8.6 ± 0.5	8.4 ± 0.2	8.7 ± 0.3	8.2 ± 0.4	8.2 ± 0.2	7.4 ± 0.5	8.8 ± 0.6 *
Live fetuses per litter (mean ± SEM) §	7.2 ± 0.3	7.8 ± 0.3	7.9 ± 0.3	8.5 ± 0.3	7.4 ± 0.5	7.3 ± 0.4	5.8 ± 0.7	4.6 ± 1.0
% resorptions per litter (mean ± SEM)	9.4 ± 2.7	8.3 ± 2.3	6.0 ± 3.2	2.8 ± 2.0	8.9 ± 4.3	10.8 ± 3.4	19.1 ± 7.7	41.7 ± 13.5 †
Litters with resorptions (No. with 100%)	7 (0)	7 (0)	3 (0)	2 (0)	5 (0)	8 (0)	7 (1)	7 (3)
Percentage litters with resorptions	58.3	63.6	25.0	16.7	35.7	66.7	50.0	70.0
Fetal bw per litter (mean ± SEM) §	1.17 ± 0.01	1.16 ± 0.02	1.13 ± 0.02*	1.16 ± 0.03	1.12 ± 0.02*	1.10 ± 0.01 **	0.90 ± 0.03**	0.59 ± 0.02**

[b] Litters with live foetuses only
[c] Net body weight change = 18 body weight – gravid uterus weight + empty uterus weight – gd 10 body weight
[d] % resorptions = (dead foetuses plus resorptions/number of total implantation sites) x100
§ p<0.01 Jonckheere's test for trend
* p< 0.05 Mann-Whitney U test, pairwise comparison to control
** p< 0.01 Mann-Whitney U test, pairwise comparison to control

- Body weight at sacrifice: not specified.
- Absolute and relative organ weight data for the parental animals: only available for the liver

- Toxic response data by sex and dose including indices of mating, fertility, gestation, birth, lactation: not applicable
- Toxic or other effects on reproduction, post natal growth of offspring: not applicable
- Clinical observations: description, severity, time of onset and duration: not reported
- Haematological and clinical biochemistry findings if available effects on sperm: not reported/not applicable
- Number of P and F1 females cycling normally and cycle length: not applicable
- Duration of gestation (calculated from day 0 of pregnancy): not applicable
- Precoital interval (number of days until mating and number of estrous periods until mating): not applicable
- Number of implantations, litter size: see tables I and III
- Corpora lutea: not reported
- Number of live births: see table I and III
- Number of pre- and post-implantation loss: not possible to distinguish between pre- and post-implantation; for data on % resorptions per litter see tables I and III.
- Number of dams with abortions, early deliveries, stillbirths, resorptions and/or dead foetuses: number of dams with abortions, early deliveries, and stillbirths were not reported in the original study report; data on resorptions and/or dead foetues per litter– see tables I and III. 4 of 12 dams receiving 32 mg/kg/day GD 10-13 were found to have 100% of their foetuses resorbing or dead, whereas 5 of the 12 dams had no fetal mortality at all.
- Data on functional observations: not reported in the original study report
- Necropsy findings: not reported in the original study report
- Histopathological findings: nature and severity: not reported in the original study report
- Body weight change and gravid uterine weight, including optionally, body weight change corrected for gravid uterine weight: see tables I and III (weight of gravid uterine not available)
- other organ weight changes if available: organ weight for liver reported only

For F1 pups/litters (per dose):

- Mean number of live pups (litter size): see table I and III
- Sex ratio: no information available
- Viability index (pups surviving 4 days/total births): not applicable
- Survival index at weaning: not applicable
- Mean litter or pup weight by sex and with sexes combined: fetal body weight per litter – see table I and III
- External, soft tissue and skeletal malformations and other relevant alterations: no statistical significant changes reported. Variations in ossification of braincase were observed in 100% of foetuses examined in mice receiving 12.8 mg/kg/day at GD 6-15.
- Number and percent of fetuses and litters with malformations (including runts) and/or variations as well as description and incidences of malformations and main variations (and/or retardations): no statistical significant changes reported. In mice exposed at GD 10-13 one fetus with hematoma on left side of neck at 0.25 mg/kg and 1.0 mg/kg, two foetuses with hematoma on right side of neck at 32 mg/kg, one fetus with exencephaly at 1 mg/kg and 16 mg/kg were reported.
- Data on physical landmarks in pups and other post natal developmental data: not applicable
- Data on functional observations: not applicable.

14.3.31 Reproductive toxicity - human data

Not applicable.

14.3.32 Reproductive toxicity - other data (e.g. studies on mechanism of action)

Study 1

Study reference:

Bookstaff RC, Moore RW, Ingall GB, Peterson RE. Androgenic deficiency in male rats treated with perfluorodecanoic acid. Toxicol Appl Pharmacol. 1990 Jun 15;104(2):322-33.

Detailed study summary and results:
Study type: No guideline, unknown GLP-status. Study designed to investigate effects of PFDA treatment on the androgenic status of rats: acute effects on androgen status of male rats in intact rats or in rats castrated and implanted with testosterone capsules; and ex vivo studies of effects of PFDA on decapsulated testes.

Identity of the test substance: PFDA (Aldrich Chemical Co.). Purity: 96% (by titration with sodium hydroxide). 87.4% PFDA as analysed by gas chromatography/mass spectroscopy by the authors.

Test subjects: Rat (Sprague-Dawley), sexually mature males, 10 animals per dosing group of intact animals.
Ad libitum-fed control (ALC) rats were dosed with an equal amount of vehicle (propylene glycol/water, 50/50, v/v; 1 ml/kg, i.p.) as PFDA treated animals.
3 or 6 rats from each treatment group were castrated two hours after dosing and testosterone-containing capsule was inserted subcutaneously. Pair-fed controls (PFC) rats were weight matched to PFDA-treated rats and provided daily the same amount feed that their partner consumed.

For ex vivo tests testes were removed from rats in each treatment group 7 days after dosing and decapsulated. No information on how many animals in each treatment-group.

Route of administration, exposure: I.p., single dose, 0, 20, 40, 80 mg/kg in propylene glycol/water. All rats were killed 7 days after treatment.

Results:
The cumulative feed consumption was decreased ($p<0.05$) to 44% of that of ad libitum-fed control rats at 80 mg/kg. Body weight was also decreased ($p<0.05$) to 72% of that of ad libitum-fed control rats at 80 mg/kg. At 40 mg/kg PFDA body weight was lower ($p<0.05$) than both the pair-feed control (7% lower) and ad libitum-fed control rats (16% lower).

Testis weight (no information whether the weight is absolute or relative) slightly (approx. 8% as estimated from the graphical presentation) but statistically significant decreased ($p<0.05$) at 80 mg/kg compared to ad libitum-fed control rats. No histological changes in testes were reported at any dose level. Dose-related decreases in the weights (no information whether the weight is absolute or relative) of seminal vesicles and ventral prostrates. At 80 mg/kg weights were reduced ($p<0.05$) to 42% and 49%, respectively, of those on ad libitum-fed control rats.

Marked atrophy of the epithelium of seminal vesicles at 80 mg/kg (incidences not reported). Epithelial height in these PFDA-treated rats was approx. 50% less ($p<0.05$) than control animals (both ad libitum-fed and pair-fed control animals) at 80 mg/kg.
Marked atrophy of the epithelium of glandular acini of the ventral prostrate at 40 and 80 mg/kg. In rats treated with 40 or 80 mg/kg PFDA about 60% of the prostatic acini were lined by low cuboidal epithelium compared to only about 20% of the acini in ad libitum-fed and pair-fed control animals).

Decreased ($p<0.05$) plasma testosterone concentrations at 40 and 80 mg/kg compared to both to ALC (25% and 12% of control levels, respectively) and PFC. Decreased ($p<0.05$) 5α-dihydrotestosterone concentrations at 40 and 80 mg/kg compared to both to ALC (32% and 18% of control levels, respectively) and PFC, measured at day 7 after treatment, N=10.
ED50 for decreased plasma androgen concentrations was 30 mg/kg.

PFDA treatment had no significant effect on plasma testosterone concentrations in castrated rats with implants of testosterone-containing capsules. Body weights were similar also to intact rats. No difference in ventral prostate weight between castrated and implanted rats and ad libitum fed or pair-fed controls. At 20 and 40 mg/kg PFDA but not at 80 mg/kg PFDA the weights of seminal vesicles were decreased ($p<0.05$) by approx. 30% compared to pair-fed control rats.

Decapsulated testis from PFDA treated rats at 80 mg/kg stimulated with human chorionic gonadotropin (100 mIU/ml) showed decreased levels of secreted testosterone to 27% of control ($p<0.05$). The effect was significant already at 40 mg/kg (approx.. 70% of control levels, $p<0.05$) but no effect was observed in testes from rats treated with 20 mg/kg PFDA.

Study 2

Study reference:

Olson CT, Andersen ME. The acute toxicity of perfluorooctanoic and perfluorodecanoic acids in male rats and effects on tissue fatty acids. Toxicol Appl Pharmacol. 1983 Sep 30;70(3):362-72

Detailed study summary and results:

Study type: No guideline, unknown GLP-status. Study designed to investigate acute effects of PFDA on tissue fatty acids in male rats.

Identity of the test substance: PFDA (supplier not specified). Purity: not specified

Test subjects: Fischer rats, adult males. 10 animals per dose group for LD50 determination, 4 animals per dose group for fatty acid composition study.

Route of administration, exposure:

LD50 determination:
12 doses in the range 40 mg/kg – 500 mg/kg (exact doses administered not stated) i.p. in propylene glycol-water, single dose.

Study of fatty acid composition :
50 mg/kg ip in propylene glycol-water, single dose. Ad libitum-fed control rats (ALC) were injected with vehicle propylene glycol-water (1:1). The following day additional rats were weight matched to PFDA-treated rats, injected with vehicle propylene glycol-water (1:1) and provided daily the same amount of food that their PFDA-treated partner consumed (pair-fed control).
Day of injection was day 0. Rats were killed 2, 4, 8 and 16 days after injection.

Results:

LD50 determination:
The LD50 at 30 days was 41 mg/kg, delayed lethality with deaths in second and third week after dosing.
The LD50 at 14 days was 64 mg/kg, delayed lethality with deaths in second and third week after dosing.

Study of fatty acid composition:
Decreased food intake started at day 1 and was close to zero from day 7 to 14 after 50 mg/kg PFDA. Mean body weights decreased from 207 to 109 g up to day 16. At day 6 the decrease in body weight was >20%. The pair-fed controls decreased in body weight from 209 to 131 g.
At day 4 after i.p. administration of 50 mg/kg PFDA mean organ weights were only significantly different in heart (0.48 versus 1.70 in control, $p<0.05$).
At day 8 after i.p. administration of 50 mg/kg PFDA mean organ weights of testes (1.7 g versus 2.8 g in control and 2.2 g in PFC), adrenals and heart were significantly lower than both vehicle control and PFC ($p<0.05$)
Mean liver weight was significantly lower than vehicle control (7.6 versus 9.9, $p<0.05$) but not than PFC (3.0 g).
In the livers at day 2 after injection of PFDA there was increase in oleic and linoeic acids and a decrease in stearic, arachidonic and docosahexaenoic acids. By day 4, palmitic and oleic acid fractions were significantly greater, and stearic, arachidonic and docosahexaenoic acids were

reduced. On both day 8 and 16, PFDA-exposed animals exhibited increased fractions of palmitic and oleic acids and decreased fractions of stearic and arachidonic acids.

Study 3

Study reference:
George ME, Andersen ME. Toxic effects of nonadecafluoro-n-decanoic acid in rats. Toxicol Appl Pharmacol. 1986 Sep 15;85(2):169-80.

Detailed study summary and results:

Study type: No guideline, unknown GLP-status. Study aimed at investigating toxic effects of PFDA in male rats after single administration

Identity of the test substance: PFDA (Aldrich Chemical Co.) 96% straight chain. Dissolved in prolylene glycol and water, 1:1 v/v. Concentration adjusted so the dose volume was less than 0.5 ml.

Test subjects:

LD50 determination 30 days after i.p. injection:
Fischer-344 rats, males and females; Sprague-Dawley rats, males.
Number of groups and number of animals per group not stated.

LD50 determination 30 days after oral administration:
Fischer-344 rats, males
Number of groups and number of animals per group not stated.

Time course toxicity experiments
Fischer-344 rats, males.
Five groups and at least six rats per group.

Route of administration, exposure:

LD50 determination 30 days after i.p. injection:
Doses tested not stated.

LD50 determination 30 days after oral administration:
Doses tested not stated.

Time course toxicity experiments
50 mg/kg, i.p., single dose.
24 hours later, control rats were administered with the vehicle. One control rat was matched with each PFDA-treated rat for pair-feeding. Rats were killed at 4, 8, 12, 16 or 30 days after injection and matching pair fed controls were killed the next day.

Results:

LD50 determination 30 days after i.p. injection:
LD50 at 30 days after i.p. administration in Fischer-344 rats was 41mg/kg for males and 43 mg/kg for females.
LD50 at 30 days after i.p. administration in in male Sprague-Dawley rats was 75 mg/kg.
A dose of 50 mg/kg i.p. was selected for the time course toxicity study since no mortality was observed within the first 14 days. However, 8 of 24 rats died after 16 or 30 days. Data from animals dying after 14 days were not included in the results.

LD50 determination 30 days after oral administration:
The 30 day oral LD50 in male Fischer-344 rats was 57 mg/kg.

Time course toxicity study
PFDA administration caused decreased food intake within the first days and it was close to zero day 4 to 12. Both control and PFDA-treated rats lost weight until day 13, thereafter the weights remained about the same until day 18-20. 18-20 days after dosing the rats started to gain weight slowly. At day 16, PFDA-treated rats had greater weight loss compared their pair-fed controls (100 g versus 70 g weight loss, $p<0.01$).
Liver weights and liver to body weights ratios were significantly higher than controls. At 30 days the liver to body weight ratio was almost 50% greater ($p<0.01$) than pair-fed control rats.
Histopathological examination revealed atrophy and degeneration of the seminiferous tubules in testes which was first seen at day 16 and persisted up to day 30 post-administration (authors report that findings were significant but no quantification is available).
Inflammation, hyperkeratosis, edema and some ulceration of the stomach was also reported. Thymic atrophy was seen in treated rats 8 days after exposure, and thymic tissue was absent in the majority of treated rats at 12, 16 and 30 days. A uniform and persistent cellular swelling at all times in the liver was observed. Inflammatory cell infiltration and some signs of necrosis were noted at day 8.

Study 4

Study reference:
Kjeldsen LS, Bonefeld-Jørgensen EC. Perfluorinated compounds affect the function of sex hormone receptors. Environ Sci Pollut Res Int. 2013 Nov;20(11):8031-44.

Detailed study summary and results:
Study type: In vitro mechanistic study aimed at investigating interference with steroid hormone receptor functions.

Identity of the test substance: PFDA (ABCR, Germany). Purity: 98%
(PFHxS, PFOS, PFOA, PFUnA, PFDoA, or Mix were also tested)

Cell culture and exposure:

ER transactivation assay:
Stably transfected MVLN cell line derived from the human breast adenocarcinoma MCF-7 cell line carrying an estrogen response element-luciferase reporter vector. The transcriptional activity was measured in response to PFAAs with or without co-treatment of 25 pM E2. Tested concentrations of PFAAs were in the range of 1x10-9 – 1x10-4 M.

AR transactivation assay:
Chinese hamster ovary cell line CHO-K1 transiently co-transfected with an MMTV-LUC reporter vector and an AR expression plasmid pSVAR0. The transcriptional activity was measured in response to PFAAs with or without co-treatment of 25 pM DHT. Tested concentrations of PFAAs were in the range of 1x10-9 – 1x10-4 M.

Aromatase activity:
Human choriocarcinoma JEG-3 cell line. Aromatization in response to PFAAs was measured by radioactivity derived from the precursor [1β-3H]androst-4-ene-3,17-dione. Tested concentrations of PFAAs were in the range of 1x10-8 – 1x10-4 M.

Assays were carried out at test concentrations not being cytotoxic.

Results:

ER transactivation assay:
No estrogenic or ER antagonistic effect of PFDA.
Weak estrogenic effects of PFHxS, PFOS and PFOA.

AR transactivation assay:
None of the tested PFAAs acted as agonists.
Upon co-treatment with 25 pM DHT, five of seven PFAAs (PFOS, PFHxS, PFOA, PFNA, and PFDA) elicited significant ($p<0.05$) concentration-dependent antagonistic effects on DHT-induced AR transactivity. (Cytotoxicity was noted at ≥1x10-4 M for PFDA)
PFDA IC50=6x10-6 M
PFNA IC50=5.2x10-5 M
PFOA IC50=1.1x10-5 M

Aromatase activity:
PFDA weakly decreased the aromatase activity (down to 85% compared to solvent control, $p=0.002$) at 1x10-5 M (cannot be ruled out that the effects is due to beginning cytotoxicity of PFDA, noted at ≥1x10-4 M for PFDA). No effect of the other tested PFAAs.

14.3.33Specific target organ toxicity (single exposure) - animal data

14.3.34 Specific target organ toxicity (single exposure) - human data

14.3.35 Specific target organ toxicity (single exposure) - other data

14.3.36 Specific target organ toxicity (repeated exposure) - animal data

14.3.37 Specific target organ toxicity (repeated exposure) - human data

14.3.38 Specific target organ toxicity (repeated exposure) - other data

14.3.39 ASPIRATION HAZARD

14.4 ENVIRONMENTAL HAZARDS

Not applicable.

14.5 ADDITIONAL HAZARDS

Not applicable.

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16. ANNEXES