

Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification
and labelling at Community level of

**Perfluorononan-1-oic acid [1];
(2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-
heptadecafluorononanoic acid (PFNA)
and its sodium (PFN-S) [2] and ammonium (PFN-A)
[3] salts**

EC number: 206-801-3

CAS number: 375-95-1

CLH-O-0000004708-66-03/F

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

Adopted

12 September 2014

CLH report

Proposal for Harmonized Classification and Labeling

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

**Substance Name: 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-
Heptadecafluorononanoic acid and its sodium and
ammonium salts**

EC Number: 206-801-3

**CAS Number: 375-95-1 (acid), 21049-39-8 (sodium salt) and 4149-60-4
(ammonium salt)**

Index Number:

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptafluorononanoic acid and its sodium and ammonium salts
EC number:	206-801-3 (acid) Not applicable (sodium salt) Not applicable (ammonium salt)
CAS number:	375-95-1 (acid), 21049-39-8 (sodium salt) and 4149-60-4 (ammonium salt)
Annex VI Index number:	-
Degree of purity:	97%
Impurities:	No information available

1.2 Harmonized classification and labeling proposal

Table 2: The current Annex VI entry and the proposed harmonized classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	-
Current proposal for consideration by RAC	Carc. 2 - H351; Repr. 1B - H 360D; Lact - H362; STOT RE 1 (liver) – H372; Acute Tox 4 - H332; Acute Tox. 4 - H302, Eye dam 1 - H318
Resulting harmonized classification (future entry in Annex VI, CLP Regulation)	Carc. 2 - H351; Repr. 1B - H 360D; Lact - H362; STOT RE 1 (liver) – H372; Acute Tox 4 - H332; Acute Tox. 4 - H302, Eye dam 1 - H318

1.3 Proposed harmonized classification and labeling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification ¹⁾	Reason for no classification ²⁾
2.1.	Explosives	None		None	Data lacking
2.2.	Flammable gases	None		None	Data lacking
2.3.	Flammable aerosols	None		None	Data lacking
2.4.	Oxidizing gases	None		None	Data lacking
2.5.	Gases under pressure	None		None	Data lacking
2.6.	Flammable liquids	None		None	Data lacking
2.7.	Flammable solids	None		None	Data lacking
2.8.	Self-reactive substances and mixtures	None		None	Data lacking
2.9.	Pyrophoric liquids	None		None	Data lacking
2.10.	Pyrophoric solids	None		None	Data lacking
2.11.	Self-heating substances and mixtures	None		None	Data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Data lacking
2.13.	Oxidizing liquids	None		None	Data lacking
2.14.	Oxidizing solids	None		None	Data lacking
2.15.	Organic peroxides	None		None	Data lacking
2.16.	Substance and mixtures corrosive to metals	None		None	Data lacking
3.1.	Acute toxicity - oral	Acute Tox. - H302		None	
	Acute toxicity - dermal	None		None	Data lacking
	Acute toxicity - inhalation	Acute Tox. 4-H332		None	
3.2.	Skin corrosion / irritation	None		None	Data lacking
3.3.	Serious eye damage / eye irritation	Eye Dam 1-H318		None	
3.4.	Respiratory sensitization	None		None	Data lacking
3.4.	Skin sensitization	None		None	Data lacking
3.5.	Germ cell mutagenicity	None		None	Data lacking
3.6.	Carcinogenicity	Carc. 2-H351		None	
3.7.	Reproductive toxicity	Repr. 1B - H360D H362		None	
3.8.	Specific target organ toxicity	None		None	Data lacking

	–single exposure				
3.9.	Specific target organ toxicity – repeated exposure	STOT. RE1 (liver)-H372		None	
3.10.	Aspiration hazard	None		None	Data lacking
4.1.	Hazardous to the aquatic environment	None		None	Data lacking
5.1.	Hazardous to the ozone layer	None		None	Data lacking

¹⁾ Including specific concentration limits (SCLs) and M-factors

²⁾ Data lacking, inconclusive, or conclusive but not sufficient for classification

Labeling: Pictogram: GHS08, GHS07
Signal word: Danger
Hazard statements: H351, H360D, H362, H372, H302, H332, H318
Precautionary statements: not harmonized

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labeling

There are no previous discussions on a harmonized classification and labeling of 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Heptadecafluorononanoic acid (hereafter abbreviated PFNA) or its sodium and ammonium salts.

2.2 Short summary of the scientific justification for the CLH proposal

This classification proposal is based on a read- across from Perfluorooctanoic acid (PFOA) and its ammonium salt, ammoniumpentadecafluorooctanoate (APFO). PFOA is an analogue to PFNA which contains one less carbon and two less fluorines. The RAC has recently adopted the proposed harmonized classification of PFOA and APFO as Repr. 1B (H360D), Lact (H362) Carc. 2 (H351), STOT RE 1 (liver) (H372), Acute Tox. 4 (H332), Acute Tox. 4 (H302) and Eye Dam 1 (H318) (ECHA Opinion, 2011 a, b). The analogue approach to use read-across data from APFO/PFOA to fill in data gaps for PFNA is supported in the case of developmental toxicity by a scientific study (Wolf et al. 2010) that shows that exposure during gestation to PFNA at dose levels absent of marked maternal toxicity causes an increase in pup mortality, decrease in pup body weight and, delays in eye opening. In addition, there are also similarities between PFNA and APFO/PFOA in toxicokinetics and similarities in repeated dose toxicity. The endpoints evaluated in this dossier for PFNA are the same endpoints as those that have been adopted for harmonized classification by the RAC for APFO/PFOA.

In the APFO/PFOA CLH report it is stated that both substances (PFOA and APFO) are mainly available to cells and tissues (with its physiological pH) in form of the corresponding carboxylate anion (PFO). This is the justification for using the toxicological data from APFO for the read-across to PFOA. PFNA and its salts (sodium heptadecafluorononanoate [CAS 21049-39-8] and ammonium heptadecafluorononanoate [CAS 4149-60-4]) are also mainly available to cells and tissues (with its physiological pH) in form of the corresponding carboxylate anion heptadecafluorononanoate. Therefore all these forms of PFNA are included in this CLH proposal.

1.

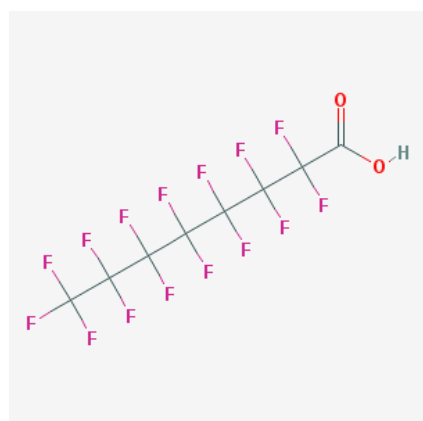
Hypothesis for the analogue approach

The hypothesis behind the analogue approach is based on the structural similarities between PFNA and its analogue PFOA, the similarities in physiochemical, toxicokinetic properties, biological and toxicological (increase in liver weight, activation of PPAR α , pup survival etc.) properties. It is reasonable to use the analogue approach to fill in data gaps of reproductive toxicity from the source chemical APFO/PFOA where data on PFNA is lacking.

2.

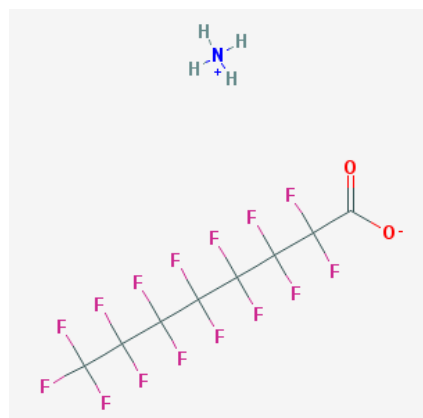
Source chemical

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 PFNA is 97% and the impurities are unknown. The degree of purity for PFOA is 98% and the impurities are also unknown.

4.

Analogue approach justification

PFOA and PFNA are both acids that structurally only differentiate in an added carbon and two fluorines. Both chemicals have a long half-time life in the human body and very similar kinetics in exposed animals. These chemicals bind to proteins in the body in a similar manner and due to the strength of the carbon-fluorine bond; both chemicals are extremely resistant towards thermal, chemical and biological degradation. In addition, the chemical structure of these chemicals renders them both lipid and hydro repellent. The mode of action for some of the toxicity caused by APFO/PFOA and PFNA has been identified as the ability of these compounds to activate the peroxisome proliferator-activated receptor α (PPAR α). Both chemicals cause an increase in liver weight, and decreased pup weight gain. In addition both chemicals can delay eye-opening, decrease pup viability and pup survival. These chemicals are detected in human breast milk, blood serum and cord blood.

5.

Data matrix

The data matrix is constructed by endpoints versus target (PFNA) and source (PFOA) substance. Data for physicochemical properties are included in the matrix are presented to indicate similar adverse effects and potencies between APFO/PFOA and PFNA. For read-across purposes, experimental data on reproductive toxicity are listed in part B, section 4.10.2.1 in table 21.

6.

Conclusions

The similarities between PFNA and APFO/PFOA are sufficient to perform a read-across. With the supportive studies on PFNA, we propose to classify PFNA with the same classification for the same endpoints as APFO/PFOA that has already been adopted by the RAC.

2.3 Current harmonized classification and labeling

2.3.1 Current classification and labeling in Annex VI, Table 3.1 in the CLP Regulation

PFNA is not currently listed in Annex VI in the CLP Regulation.

2.4 Current self-classification and labeling

2.4.1 Current self-classification and labeling based on the CLP Regulation criteria

Self-classification notifications for PFNA by industry are available in the C&L Inventory (<http://echa.europa.eu/information-on-chemicals/cl-inventory-database>).

The industry has submitted 30 C&L notification for PFNA (five notification groups). Two notification groups have classified PFNA as STOT SE 3 (H335), Skin Irrit. 2 (H315) and Eye Irrit. 2 (H319). The third notification group has classified PFNA as Skin Irrit. 2 (H315), Eye Irrit. 2 (H319) and has assigned H335 without specifying the Hazard class associated with this hazard statement (STOT SE3). The 4th group has classified PFNA as Skin Corr. 1C (H314) and Eye Dam.

1 (H318). The last notification group (two notifiers) have only indicated a hazard statement H314 without specification of the Hazard class (Skin Corr 1C).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

PFNA has CMR properties (reproductive toxicity and cancer). There is no harmonized classification for PFNA. Harmonized classification and labeling for CMR and respiratory sensitization is a community-wide action under article 36 of the CLP. This MSCA disagrees with the existing self-classifications notified to the C&L inventory by industry for STOT RE, acute toxicity and eye damage and considers that the harmonised classifications for these endpoints as proposed in this dossier are justified by the information available on this substance.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

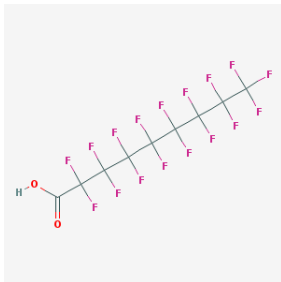
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	206-801-3 (acid) Not applicable (sodium salt) Not applicable (ammonium salt)
EC name:	Perfluorononan-1-oic acid (acid) Not applicable (sodium salt) Not applicable (ammonium salt)
CAS number (EC inventory):	
CAS number:	375-95-1 (acid), 21049-39-8 (sodium salt) and 4149-60-4 (ammonium salt)
CAS name:	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9- heptadecafluorononanoic acid (and its sodium and ammonium salts)
IUPAC name:	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9- heptadecafluorononanoic acid (and its sodium and ammonium salts)
CLP Annex VI Index number:	None
Molecular formula:	C ₉ HF ₁₇ O ₂ (free acid)
Molecular weight range:	464.076 g/mol (free acid)

Structural formula



PFNA (free acid)

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Heptadecafluorononanoic acid (375-95-1)	97%		There is no registration dossier for PFNA. The cited publications in this dossier only state that the purity of PFNA was 97%. No other information is available from the named provider of the substance in the cited publications (i.e. Sigma Aldrich)

Current Annex VI entry: None

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
No data available			

Current Annex VI entry: Not applicable

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No data available				

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

There is no registration dossier for PFNA, and there is therefore little available data on the physicochemical properties of PFNA. The data below comes from Chemical safety data sheets.

1.3 Physicochemical properties

Table 9: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	The substance is a solid.	Oxford University Chemical Safety Data sheet	
Melting/freezing point	65-68 °C	Oxford University Chemical Safety Data sheet	Not specified
Boiling point	218 °C at 740mmHg	Oxford University Chemical Safety Data sheet	Not specified
Relative density	No data		
Vapor pressure	No data		
Surface tension	No data		
Water solubility	No data		
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.	Oxford University Chemical Safety Data sheet	
Self-ignition temperature	The substance is a solid.		
Oxidizing properties	No data		
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	Stable.	Oxford University Chemical Safety Data sheet	
Dissociation constant	No data		
Viscosity	No data		

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

There is no registration dossier for PFNA. However, PFNA is on the list of pre-registered substances with a registration date of 30/11/2010.

2.2 Identified uses

PFNA (375-95-1) is primarily used as a processing aid for the fluoropolymer manufacture, most notably for polyvinylidene fluoride (Prevedouros et al., 2006). PFNA is also used as a lubricating oil additive, surfactant for fire extinguishers, cleaning agent, textile antifouling finishing agent, polishing surfactant, and in liquid crystal display panels (Swerea IVF 2009).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

There is limited toxicokinetic data available for PFNA. Ohmori et al. (2003) reported an elimination half-life of 29.6 days in male and 2.3 days in female Wistar rats after a single intra-venous dose of 48.64 mmol/kg bw PFNA. The total clearance rate for PFNA was in this study: 6.9 ml/ (day/kg) in male rats and 105.7 ml/ (day/kg) in female rats.

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 390µg/kg PFNA (200µg/kg *n*-PFNA and 190µg/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 350ng/ml *n*-PFNA and 570ng/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/SvImJ 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.

Analogue data:

The text below has been copied in from the Background Document for APFO (ECHA Background document, 2011)

“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.”

4.1.2 Human information

There is very limited data regarding human exposure to PFNA. What is known about human exposure to PFNA is that it is 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).

Median human PFNA and PFOA serum concentrations in children were found to be very similar for girls and boys (Schecter et al., 2012). This study collected and analyzed serum concentrations of PFNA and PFOA in children from Texas of zero to 12 years of age. No significant sex-dependent differences in the serum concentrations for PFNA and PFOA were found. Average serum concentrations ranged from 2-3ng/ml for PFOA and 0.6-1.4ng/ml for PFNA from birth to 12 years of age.

The PFOA serum elimination half-life is estimated to 3.8 years (the range was 1.5 – 9.1 years) in 26 retired workers (24 men and 2 women) (Olsen et al., 2007 and Harada et al., 2005).

4.1.3 Summary and discussion on toxicokinetics

PFNA as well as APFO/PFOA are very slowly eliminated from blood and have long durations in mice. It is very likely that due to the similarities between PFOA and PFNA both with regards to physicochemical properties and long elimination half-lives in exposed animals, that the elimination half-life for PFNA 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 CD-1 mice, PFNA is more slowly eliminated as compared to PFOA. The data suggests that there is a major sex difference in the serum elimination of PFNA in the rat; this is also true for PFOA (Ohmori et al., 2003). In mice the difference between the elimination of PFNA and PFOA in serum between the sexes is less pronounced. Median human PFNA and PFOA serum concentrations in children are very similar for girls and boys (Schechter et al., 2012). Altogether this suggests that the mouse is the preferred animal model. Both PFNA and PFOA can cross the placenta and the animal data suggests that the elimination via lactation could be substantial. PFNA (as well as PFOA) has been found in cord blood as well as in human breast milk which indicates that exposure during gestation and lactation also will occur in humans.

4.2 Acute toxicity

4.2.1 Non-human information

There is no available information on acute toxicity for PFNA. PFNA and APFO/PFOA have very similar physico-chemical as well as toxicokinetic properties. This justifies that the classification for PFNA is based on read-across from data for APFO/PFOA. To aid the reader of this CLH report we have included tables from the Background Document for APFO (ECHA Background document, 2011) as well as text from the Opinion Document for APFO (ECHA Opinion, 2011) that was produced during the classification process of APFO and PFOA by the Committee for Risk Assessment (RAC) at ECHA

4.2.1.1 Acute toxicity: oral

Table 10: Acute toxicity-oral (part of Table 2 in Background Document for APFO)

Species	LD ₅₀ (mg/kg)	Observations and Remarks	Reference
CD rats (5/sex/group)	680 (male) 430 (female)	Vehicle: Acetone (40%) and corn oil (60%). The following doses of APFO were tested. 100, 215, 464, 1000 and 2150 mg/kg in a volume of 10ml/kg. Animals were observed for mortality and pharmacotoxic signs during the first four hours after dosing, at 24 hours and daily thereafter for a total of 14 days. The study was performed according to GLP.	Dean and Jessup, 1978; Griffith and Long, 1980
Sprague-Dawley rats (5/sex/group)	>500 (male) Between 250-500 (females)	APFO was tested at doses of 250 and 500 mg/kg in a volume of 10 ml/kg. Vehicle was water. Clinical observations were made at 1, 2.5 and 4 hours after treatment and each day for 14 days. GLP. Yes. The study was performed according to OECD test guidelines (no info on TG used). All animals exhibited body weight gain throughout the study. All animals treated at 250 mg/kg appeared normal during the study except for two females that exhibited red-stained faces and/or wet urogenital area within 24 hours of test material administration. Clinical signs of toxicity observed in the animals treated with 500 mg/kg were: red-stained face, yellow stained or wet urogenital area, hypoactivity, hunched posture, staggered gait, and excessive salivation (clinical findings also cited from Kudo and Kawashima, 2003). There were no test-material related lesions observed at necropsy, although at 250 mg/kg, one male had a cannibalized right flank, one female had multiple dark brown areas in the glandular mucosa of the stomach, and a second female had a clear fluid in the lumen of the bilateral horns of the uterus. No more details regarding mortality was reported.	Glaza, 1997

4.2.1.2 Acute toxicity: inhalation

Table 11: Acute toxicity-inhalation (Table 3 in Background Document for APFO)

Species	LC ₅₀ (mg/l)	Exposure time (h/day)	Observations and Remarks	Reference
Spague-Dawley rats (5/sex/group)	>18.6	1 hour	No mortality was reported in male and female Sprague-Dawley rats following inhalation to 18.6 mg/l APFO for one hour. (18.6 divided with 4 hours = 4.6 mg/l). The animals were observed for abnormal signs at 15-minutes intervals during the exposure, upon removal from the chamber, hourly for 4 hours after removal from test chamber, and daily thereafter for 14 days.	Rusch, 1979; Griffith and Long, 1980
Rat (6/sex/group)	0.98	4	4 hour exposure. APFO was administered to rats by inhalation (head only) as dust. The concentrations of APFO ranged from 0.38 to 5.7 mg/l. All deaths occurred within 48 hours.	Kennedy et al., 1986

4.2.1.3 Acute toxicity: dermal

Table 12: Acute toxicity-dermal (Table 4 in Background Document for APFO)

Species	LD ₅₀ (mg/kg)	Observations and Remarks	Reference
New Zealand white rabbits (5/sex/group)	Greater than 2000	Aqueous paste. Only one dose tested, 2000 mg/kg. No vehicle. The rabbits had their hair clipped from their backs before the appropriate amount of the test substance was applied to intact skin. The area of application was covered with a gauze patch and an occlusive dressing. After 24 hour exposure, the collars and dressings were removed. The test site was washed with tap water. Clinical observations and mortality checks were made at approximately 1, 2.5, and 4 hours after test material application and twice daily thereafter for 14 days. All animals appeared normal and exhibited body weight gains throughout the study. GLP. Yes. The test substance used was identified as T-6342.	Glaza, 1995
New Zealand white rabbits (5)	4300	Four groups of rabbits were treated with 1500, 3000, 5000 and 7500 mg APFO/kg bw. Dosing sites were wrapped. The contact time was 24 hours at which time the application sites were washed with water and rabbits were observed for clinical signs of response for a 14-day recovery/observation period. LD ₅₀ values were calculated from the mortality data.	Kennedy, 1985
CrI:CD Rat (5/sex/group)	7000 (male) Greater than 7500 (female)	Three groups of male and two groups of female rats were treated with 1500, 3000, 5000 and 7500 mg APFO/kg bw. Dosing sites were wrapped. The contact time was 24 hours at which time the application sites were washed with water and rats were observed for clinical signs of response for a 14-day recovery/observation period. LD ₅₀ values were calculated from the mortality data.	Kennedy, 1985

4.2.2 Human information

4.2.3 Summary on acute toxicity

Below, the outcome of the RAC assessment has been copied from the Opinion Document for APFO (ECHA Opinion, 2011a):

Oral

“In the study of Glaza (1977) the lowest LD₅₀ was reported to be between 250 and 500 mg/kg for female rats. Minor clinical signs such as colored feces and wet urogenital area were reported in females at 250 mg/kg, but no other signs of toxicity or mortalities were reported. Moribundity was reported for animals at 500 mg/kg. Details on the used test guideline and on whether mortalities occurred at all are unknown.

Other limited studies give indications on LD₅₀ in the range of 200-250 mg/kg, also these studies are of limited validity due to lack of information. An LD₅₀ at approximately 250 mg/kg was derived in newborn rats (Du Pont, 1983a). In Guinea pigs the LD₅₀ was below 200 mg/kg (Du Pont, 1981f).

In the most reliable study of Glaza no definitive mortalities below 300 mg/kg, the borderline dosage between category 3 and category 4 (CLP), have been identified and other studies have neither

characterized substance identity nor were conducted according to guideline protocols, RAC decided to propose Acute Tox. 4. Thus the original proposal of the dossier submitter on Acute Tox. 3 was not supported.

Based on the guidance value of 200 mg/kg a classification as harmful with Xn; R22 (Harmful if swallowed) is proposed along the Directive 67/548/EEC criteria.”

Inhalation

“Following inhalation exposure to APFO an LC50 of 0.98 mg/l (4 hour exposure) was identified at the borderline between category 3 and category 4. Another LC50 was > 18.6 mg/l after 1 hour inhalation, which corresponds to 4.6 mg/l for 4 hours and supports category 4 as more appropriate. Beyond the evidence from acute testing, data from repeated dose study could be taken into consideration. Mortalities observed on day 3 and during the fourth exposure in the repeated inhalation study on rats (Kennedy et al., 1986) are more relevant for acute toxicity than for chronic toxicity and support argumentation that Acute Tox. 3 (H331) could remain as proposed by the dossier submitter. 84 mg/m³ caused mortality after third day (6 h/day) (84 mg/m³ x 18 h/4 h = 378 mg/m³ (0.378 mg/l). A value in this range can also be derived for the second death during the fourth exposure.

However, RAC gave more weight to the supporting evidence from 1 hour testing than from mortalities after 18 hours of (interrupted) treatment. Although the exact value of 1 mg/l is the upper limit for category 3, RAC came to the overall conclusion was that LC50 is considered to be 1 mg/l and above.

With respect to the CLP criteria RAC decided to propose classification as Acute Tox. 4 (H332), since relevant LC50 values were considered to be in the range of 1.0 mg/l <ATE ≤ 5.0 mg/l. According to Directive 67/548/EEC RAC agreed with the dossier submitter who proposed classification as harmful with Xn; R20 (Harmful by inhalation) as agreed at TC C&L.”

Dermal

“RAC agrees that no classification should be proposed.”

4.2.4 Conclusions on classification and labeling

Based on read across to APFO, PFNA should be classified as Acute Tox. 4 (H332, H302).

RAC general comment

In the opinion of RAC, due to their high structural similarity and chemical analogy:

- the 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Heptadecafluorononanoic acid (PFNA) with its sodium (PFN-S) and ammonium (PFN-A) salts and
- the 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid (PFOA) with its ammonium salt - (Ammoniumpentadecafluorooctanoate (APFO)

fulfill the criteria for a read-across approach to be applied, as defined in Section 1.5 of Annex XI of the REACH Regulation (underlining added): “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).” At least two of the following three similarities listed in the REACH Regulation and upon which the

read-across approach is based on, were met:

- 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.

As it was assumed that PFOA and APFO form the corresponding anion (PFO) in the gastro-intestinal system or lung fluid, also PFNA, PFN-S and PFN-A were anticipated to become available to cells at physiological pH in the form of their corresponding anion (PFN), thus exerting the same toxic effects, although their potency may differ. For systemic effects such as those following oral or inhalation exposure, the read-across is in fact between two anions: PFO and PFN, which are analogous chemical groups and differ only by one $-CF_2-$ group in the fluorine substituted aliphatic chain.

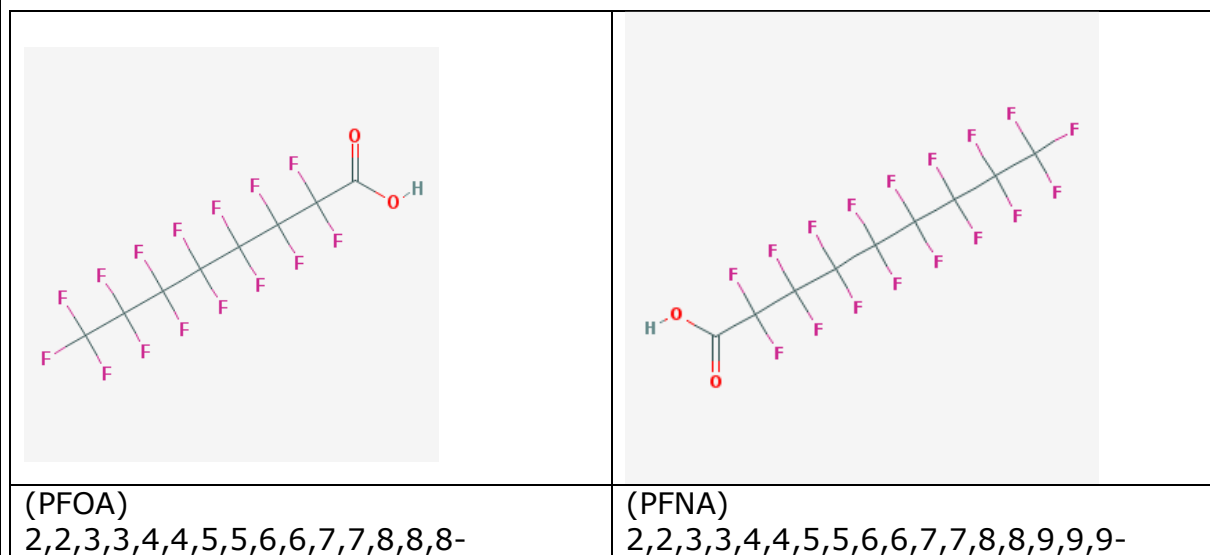
RAC evaluation of acute toxicity

Summary of the Dossier submitter's (DS) proposal

The DS proposes to classify 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Heptadecafluorononanoic acid (PFNA) and its sodium (PFN-S) and ammonium (PFN-A) salts as Acute Tox. 4 with hazard statements H302 (Harmful if swallowed) and H332 (Harmful if inhaled).

There is no information available on acute toxicity for PFNA, PFN-S and PFN-A; therefore the classification is based on read-across from data for ammonium pentadecafluorooctanoate (APFO), which was used to read-across to Perfluorooctanoic acid (PFOA; 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid) in a previous opinion¹ [Ref].

PFNA (heptadecafluorononanoic acid) is an analogue of PFOA and it contains in its structure one atom of carbon and two atoms of fluorine more than PFOA.



¹ [RAC Opinion proposing harmonised classification and labelling at Community level of Perfluorooctanoic acid \(PFOA\)](#)

Oral toxicity

As noted in the RAC opinion adopted on 2 December 2011, the studies on human health hazards with PFOA were not available. The PFOA proposal (2011) exclusively referred to the classification proposal for its salt APFO, which has been extensively tested. In the most reliable study by Glaza (1997), the lowest LD₅₀ for APFO was between 250 and 500 mg/kg bw, with no mortalities identified below 300 mg/kg (the upper limit for Acute toxicity Category 3 and lower limit for Category 4; CLP). Other studies have neither characterised the substance identity nor were conducted according to test guideline protocols. Based on this, RAC therefore decided to adopt an opinion for classification as Acute Tox. 4 for APFO and PFOA.

Inhalation Toxicity

Following inhalation exposure to APFO, an LC₅₀ of 0.98 mg/L (4 hours exposure) was established, a result which is borderline between Category 3 and Category 4. Another LC₅₀ was > 18.6 mg/L after 1 hour inhalation exposure, which corresponds to 4.65 mg/L for 4 hours exposure when using a conversion factor of 4 (3.1.2.1, Annex I, CLP), and this supported classification in Category 4. RAC therefore decided to adopt an opinion for classification as Acute Tox. 4 (H332) for APFO and PFOA, since the relevant LC₅₀ values were considered to be in the range of 1.0 mg/L < ATE ≤ 5.0 mg/L.

Dermal toxicity

No classification was proposed by the DS.

Comments received during public consultation

One MSCA disagreed with the proposed classifications as Acute Tox. 4 by the oral route (H302) and Acute Tox. 4 by inhalation (H332) because of the lack of sufficient data for an adequate read-across (particularly physical/chemical properties establishing similar potencies) with APFO. As a result, in their view the LD₅₀ and LC₅₀ for PFNA and its salts could not be reliably estimated for classification purposes.

Assessment and comparison with the classification criteria

Taking into account the considerations noted under "RAC General Comments" (above) and applying a read-across between PFO and PFN anions, RAC agreed with the DS proposal and proposed to classify PFNA and its sodium (PFN-S) and ammonium (PFN-A) salts as Acute Tox. 4 with hazard statements H302 (Harmful if swallowed) and H332 (Harmful if inhaled) based on the results of the acute toxicity assessment of APFO.

No classification for dermal toxicity was proposed by the DS for PFNA, PFN-S and PFN-A since the dermal LD₅₀ for APFO from two rabbit studies and one rat study were above 2000 mg/kg.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated in this dossier.

4.4 Irritation

There is no available information on irritation for PFNA. PFNA and APFO/PFOA have very similar physico-chemical as well as toxicokinetic properties. This justifies that the classification for PFNA is based on read-across from data for APFO/PFOA. To aid the reader of this CLH report we have therefore included tables from the Background Document for APFO (ECHA Background

document, 2011) as well as text from the Opinion Document for APFO (ECHA Opinion, 2011a) that was produced during the classification process of APFO and PFOA by the Committee for Risk Assessment (RAC) at ECHA.

Table 13: Irritation-skin (Table 5 in Background Document for APFO)

Species and No of animals	Conc.	Exposure time (h/day)	Dressing: occlusive, semi-occlusive, open	Observations and Remarks	Reference
Rabbit female (3/exposure period)	0.5 g	3 min, 1 and 4 h	occluded	APFO produced irreversible tissue damage following a 3-minute, 1- and 4-hour contact period. Moderate erythema and edema, as well as chemical burn, eschar, and necrosis were produced following all three contact periods. Inadequate information was presented in the report to evaluate the quality of the study and validity of the conclusions.	Markoe, 1983
Rabbit (6)	0.5 g	24 h	occluded	APFO as powder was applied to dry and moistened abraded skin. No information regarding washing of the test site was given. The skin test sites were scored according to the Draize method after 24 hours and 48 hours. No irritation was observed. The primary skin irritation score was 0.	Griffith and Long, 1980
Rabbit male (6)	0.5 g	24 h	occluded	APFO was applied to shaved intact skin as an aqueous paste for 24 hours. Observation for dermal irritation was performed after removal of patches and after 24 hours (48 hours after dose application). APFO caused mild erythema (color deep pink) in 3 rabbits and moderate erythema (redness deepened, dose-site outline sharp) in 3 rabbits. Of 6 rabbits 4 had evidence of oedema (1 mild and 3 slight) at 24 hours. At 48 hours the reactions were still present although the degree and number of affected animals were reduced (erythema - 2 moderate, 3 mild and 1 slight; oedema – 1 mild, 2 slight and 3 not present).	Kennedy, 1985; Hazleton, 1990

Table 14: Irritation-eye (Table 6 in Background document for APFO)

Species and number of animals	Conc.	Exposure time (h/day)	Observations and Remarks	Reference
Rabbit (6)	0.1g	Single dose	The eyes were examined 1,24, 48 and 72 hours and 5 and 7 days after installation. Installation of APFO caused moderate corneal opacity, iritis, and conjunctivitis. The effect was most pronounced at the	Griffith and Long, 1980

			<p>one hour reading (mean score 14, highest possible score 110). Scoring was made by the method: Illustrated Guide for Grading Eye Irritation by Hazardous Substances.</p> <p>Corneal opacity and area=4, Iris=2, Conjunctival redness=2, Conjunctival chemosis=4 and Conjunctival discharge=3.</p> <p>The irritation was persistent but by day 7 the mean score was 2. A subsequent wash out study with 6 albino rabbits was performed. After installation of 0.1 g APFO the eyes of 3 rabbits were washed with 200 ml water after 5 seconds and the 3 other rabbits were washed similarly after 30 seconds. The eyes were examined and scored the same way as the eyes that were not washed. In the wash-out study the ocular effects were limited to conjunctival irritation. Those eyes washed after 5 seconds had a maximum score of 5.3 noted at 72 hours and after 5 and 7 days. The mild conjunctival effects were immediate and persistent.</p>	
Rat (6/sex/group)	0.81mg/l	4	In rats exposed to APFO particulate (0.81mg/l) during a 4 hours inhalation period (head only) exhibited corneal opacity and ulceration, which were microscopically evident 42 post-exposure.	Kennedy et al., 1986

4.4.1 Summary and discussion of irritation

Below, the outcome of the RAC assessment has been copied from the Opinion Document for APFO (ECHA Opinion, 2011a)

Skin

“Differences in the applied form of the test sample do not enable to explain the different outcome of the studies. Griffith and Long applied the test substance as dry and as moistened samples, while Kennedy (1995) applied an aqueous paste that resulted in mild to moderate erythema. The negative study of Griffith and Long as well as the mean values from Kennedy do not justify classification. In contrast, the study of Markoe (1983) revealed skin irritant effects including necrosis from 3 minutes of exposure that would require classification as corrosive. No more details are available (no access to the study report). RAC followed the argumentation that data are inconclusive. At present no proposal for classification was given.”

Eye

“RAC discussed the adequacy of the category 2 classification (CLP) and decided to deviate from the proposal of the dossier submitter due to consistent evidence from two studies. Although these studies were not compliant to the test guideline, corneal opacity (grade 4) and iris effects (grade 2) (observed in rabbits of the Griffith study) are lead effects that in combination with observed corneal ulceration (acute inhalation study, Kennedy et al., 1986) justify Eye Dam. 1 (CLP) and for the DSD Xi; R41 accordingly.”

4.4.2 Conclusions on classification and labeling

Based on read across to data for APFO, PFNA should be classified as Eye Dam 1 (H318).

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

The DS proposed to classify PFNA and PFN-S and PFN-A as Eye Dam. 1, H318 (Causes serious eye damage).

There was no information available on eye damage/irritation for PFNA. PFNA and APFO/PFOA have very similar physico-chemical as well as toxicokinetic properties. This justifies that the classification for PFNA is based on read-across from data for APFO/PFOA.

Comments received during public consultation

One MSCA disagreed with the proposal to classify as Eye Dam. 1 (H318) because according to the CLP Regulation (Annex I: 3.3.1.1.) "*Serious eye damage means the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application.*", and in relation to this, "*given the lack of sufficient data for an adequate read-across from APFO (particularly physical/chemical properties such as pH are important in establishing similar potencies), classification for eye damage/irritation cannot be reliably assessed for PFNA and its salts.*" It was further argued by the MSCA that "*for local effects requiring potency-based classification such as irritation and dermal toxicity, the actual form may affect the irritation (a salt may have a different effect than an acid), and also affect the dermal absorption. Therefore, read-across needs additional justification for local endpoints. Given the lack of pH information of PFNA and its salts, it is difficult to make comparisons with the other two substances, PFOA and APFO.*"

Another MSCA observed that "*Uncertainties on the relevance of the read-across are probably more important for local effects considering that the mechanism of irritation is not understood or discussed. Information on other members of the family of perfluorinated acids would be useful to see if it is a common property in the family and whether there is a trend in the local effect related to the number of carbons.*"

Additional key elements

When considering the pH values for the read-across of the eye corrosion property from APFO to PFNA, PFN-S and PFN-A, it is noted that there is no information on measured pH values for PFNA and its salts. However, the calculations of pH using estimated pKa values provided by the DS (see Tables 2A and 2B attached to the Response to Comments document (RCOM)) indicate that at equimolar concentrations, the pH of a PFNA solution will be low (estimated pH=3.0) and the same as that of a PFOA solution (estimated pH=3.0), while the pH of an APFO solution (estimated pH=5.9) will also be the same as that of an equimolar solution of the ammonium salt of PFNA (estimated pH=5.9).

According to the DS, both acids (PFNA and PFOA), based on their calculated very low PKa values (Table 2A, RCOM), are extremely strong acids and are virtually completely dissociated in water, i.e. the pH will only depend on the concentration of the acid. However, the estimated pKa values of PFOA and PFNA do not easily allow such a conclusion as to whether they are strong or weak acids, because these values differ considerably depending on which software has been used for the estimation (Table 2, RCOM).

The experimental determination of the the pKa values of perfluorinated compounds is difficult since the chemical structure of these compounds renders them hydrophobic as well as surface active; sorption to interfaces such as the water surface or the walls of glass vessels may occur to an extent that is unknown for "ordinary" molecules (Goss, 2008).

Goss (2008) investigated, by using two different pKa prediction softwares (SPARC and COSMO-RS), how the pKa value was influenced by the chain length of the perfluorinated carboxylic acids (C₄ vs. C₁₂). As shown in the table below, the chain length seems to have a minimal effect on the pKa values even though there is a slight difference in the predicted values between the two software packages used. However, the pKa values estimated above indicate that the level of dissociation in water of perfluorinated alkyl

Compound	pKa (SPARC)	pKa (COSMO-RS)
F(CF ₂) ₃ COOH	0.4	0.7
PFOA - F(CF ₂) ₇ COOH	-0.1	0.7
PFDoDA - F(CF ₂) ₁₁ COOH	-0.2	0.8

acids is rather low.

Assessment and comparison with the classification criteria

APFO caused a persistent inflammatory reaction in the eye of rabbits after instillation to the conjunctival sac (Griffith and Long, 1980) and corneal opacity and ulceration, seen even 42 days after a 4 hour exposure to APFO particulate material (Kennedy *et al.*, 1986). PFOA and PFNA, PFN-S and PFN-A were not tested for eye irritancy, but their eye irritancy/corrosive properties were assumed to be comparable to that of APFO based on a read-across approach.

In their opinions from 2011 on APFO and on PFOA, RAC considered that the corneal opacity (grade 4) and iris effects (grade 2) (observed in rabbits in Griffith and Long, 1980) were main effects that in combination with observed corneal ulceration (acute inhalation study, Kennedy *et al.*, 1986) justify classification of APFO and of PFOA as Eye Dam. 1, H318 (Causes serious eye damage).

The mechanism of the eye damage caused by APFO is not known, but it did not seem to be related to the high or low pH of solutions of APFO in water. The pH of a water-solution of APFO is around 5-6 (see attached tables 1 and 2A), and comparable to the pH of PFN-A.

Thus, from the perspective of possible differences in pH, read-across for local effects from APFO to PFN-A and PFN-S would be justified, since their estimated pH values were similar (Table 2A, RCOM). These measured or estimated pH values for APFO and estimated pH values for PFN-A and PFN-S were too high to be responsible for local irritation or corrosive effects. It is highly probable that these effects may also be caused by corresponding perfluorinated carboxylate anions of PFOA and PFNA. PFOA and PFNA have the same pH value (equal to 3.0), estimated with both the predictive software platforms used (Tables 2A and 2B, RCOM).

There were additional *in vitro* data indicating similar cytotoxicity of PFOA and PFNA (Kleszczynski *et al.*, 2007). The EC₅₀ for reducing the number of viable cells by 50% in a cell proliferation assay utilizing the human colon carcinoma (HCT116) cell lines were similar for PFOA and PFNA. The study also demonstrated that the penetration of perfluorinated fatty acids through the cell membrane to the cytoplasm may be considerable (15% after 2 hours, 60% after 72 hours) based on experiments with perfluorodecanoic acid (PFDA). In general, the cytotoxicity of perfluorinated carboxylic acids (PFAs) was assessed as low, indicating, however, that they can trigger cell apoptosis, which can lead to toxic effects.

The measured values for the water solubility of PFOA (3.4 – 9.5 g/L, dependent on the temperature; the critical micelle concentration = 3.7 g/L for the PFO anion) and PFNA (2

g/L at 60°C; critical micelle concentration = 1.3 g/L) were in the same range. The APFO is much more soluble than PFOA and PFNA (Table 1, RCOM). The predicted values for water solubility of PFNA and PFOA (Table 3, RCOM) are much lower than the measured values (in the mg/L or µg/L range, depending upon prediction model, and the predicted solubility differs 10- to 20-fold between PFOA and PFNA (no information on the salt). There were 2 orders of magnitude difference in solubility of both acids depending upon which prediction software was used (Table 2, RCOM). However, overall the solubility of PFNA and PFOA seemed not to differ extensively if one compares data that originates from the same method of measurement or prediction model. Thus from a solubility perspective, read-across seems overall to be justified at least between PFOA and PFNA.

Taking into account the above considerations, RAC is of the opinion that read-across of eye corrosive properties from APFO to PFNA and PFN-S and PFN-A based on similarities between their structure and physicochemical properties is justified and that these substances should be classified as Eye Dam. 1, H318 (Causes serious eye damage).

4.5 Corrosivity

Skin corrosivity was not evaluated in this dossier, for eye damage see previous section.

4.6 Sensitization

Not evaluated in this dossier

4.7 Repeated dose toxicity

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

There are very few studies from which some information regarding repeated toxicity of PFNA can be extracted; the results from these studies are presented in Table 15. It should be noted that the majority of the studies are of a mechanistic nature that did not cause toxicity as revealed by effects on body weights or clear clinical signs. The classification for repeated dose toxicity of PFNA is therefor based on read-across to available data for APFO/PFOA.

Table 15: Summary table of relevant repeated dose toxicity studies for PFNA

Species	Dose (mg/kg bw)	Duration of treatment	Observations and Remarks	Reference
Female (non pregnant) 129S1/SvIm J mice wild-type (WT) and PPAR α knockout (KO)	0,0.83,1.1,1.5 and 2.0 oral gavage	18 days	<p>There was no difference in body weight between control groups and both WT and KO mice treated with PFNA.</p> <p>A significant dose dependent ($p < 0.01$) increase in absolute liver weight was seen for all WT PFNA exposed mice and also for KO mice exposed to PFNA at doses 1.5 and 2mg/kg bw.</p> <p>A significant dose dependent ($p < 0.01$) increase in relative liver weight was seen for all WT PFNA exposed mice and also for KO mice exposed to PFNA at doses 1.1, 1.5, and 2mg/kg bw.</p>	Wolf et al. 2010
Male BALB/c mice	0,1,3 and 5 oral gavage	14 days	<p>Significant body weight reduction in mice exposed to PFNA at 3mg/kg bw (12.6 % compared to controls $p < 0.01$) and 5mg/kg bw (13.6% compared to controls $p < 0.01$).</p> <p>PFNA exposure also significantly reduced ($p < 0.01$) the relative thymus weights at 3 and 5mg/kg bw. At 5 mg/kg bw PFNA ($p < 0.001$) caused an increase in apoptotic cells in the thymus.</p> <p>PFNA also reduced ($p < 0.05$) relative spleen weight at 5 mg/kg bw and significantly ($p < 0.01$) increased serum levels of ACTH and cortisol at this dose. There were no changes in the spleen red pulp or white pulp however the spleen capsule was crinkled.</p>	Fang et al. 2008
Male Sprague-Dawley (SD) rats	0,1,3 and 5 oral gavage	14 days	<p>There was a dose-dependent decrease in absolute spleen weight for all rats exposed to 1, 3 and 5mg/kg bw PFNA by 22.2%, 28.7% and 57.9% ($p < 0.01$) compared to control rats. However, the ratio of spleen weight to body weight was only significantly decreased in PFNA rats exposed at the dose of 5mg/kg bw (91.5% of the controls, $p < 0.01$).</p> <p>Exposure to PFNA caused an increase in apoptotic lymphoid cells in the spleen at a dose of 3 and 5 mg/kg bw. The dose 5 mg/kg bw also increased levels of pro-inflammatory IL-1, IL-6, IFNα, and H$_2$O$_2$ but decreased levels of IFNγ and IL-10. SOD activity was decreased, and increased the mRNA expression of both PPARα and PPARγ, in rats exposed to both 3 and 5 mg/kg bw PFNA.</p> <p>Body weight, food consumption, and signs of clinical effects were not reported.</p>	Fang et al. 2010

Male Sprague-Dawley (SD) rats	0,1,3 and 5 oral gavage	14 days	<p>There was a dose-dependent increase in the number of apoptotic cells. Spermatogenic cells from rats exposed to 5 mg/kg bw PFNA exhibited apoptotic features: crescent chromatin condensation and chromatin margination.</p> <p>The serum estradiol levels were increased in the rats exposed to 5mg/kg bw PFNA by 1 times higher compared to the control rats. Testosterone levels were significantly increased in the 1 mg/kg bw PFNA rats by 1.87 times higher than the control rats but significantly decreased in the 5 mg/kg bw PFNA rats compared to the control rats.</p> <p>The expression of Fas and Bax mRNA levels were significantly up regulated in rats exposed to 5 mg/kg bw, while Bcl-2 mRNA was down regulated in both the 3 and 5 mg/kg bw PFNA rats. A dose-dependent increase in active caspase-8 but no changes in active caspase-9 were observed.</p> <p>Body weight, food consumption, and signs of clinical effects were not reported.</p>	Feng et al. 2009
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Analogue data:

Table 16: Summary table of repeated dose toxicity studies (oral) for APFO/PFOA (Table 8 in APFO Background document)

Species	Dose (diet, ppm; mg/kg bw /day)	Duration of treatment	Observations and Remarks	Reference
ChR-CD mice (5/sex/group)	0, 30, 100, 300, 1000, 3000, 10 000 and 30 0000 ppm APFO, corresponding to approximately 1.5 to 1500 mg/kg bw/day	28 days	<p>All animals in the 1000 ppm group and higher died before the end of day 9. All animals in the 300 ppm group died within 26 days except one male. One animal in each of the 30 and 100 ppm groups died prematurely. Clinical signs were reported in mice exposed to 100 ppm and higher. After four days, rough hair coat and muscular weakness were evident in animals fed 3000 ppm or more APFO. Similar reactions and cyanosis were present in the 1000 ppm group after six days and in the 300 ppm group after nine days. Some 100 ppm animals had slight cyanosis on days 10 and 11 but appeared normal thereafter. There was a statistically significant dose-related reduction in mean body weight in all treated groups from 30 ppm. Relative and absolute liver weights were statistically significantly increased in mice fed 30 ppm and more. Treatment related changes were reported in the livers among all treated animals including enlargement and/or</p>	Christopher and Marisa, 1977; Griffith and Long, 1980

			discoloration of 1 or more liver lobes. Histopathologic examination of all surviving treated mice revealed diffuse cytoplasmic enlargement of hepatocytes throughout the liver accompanied by focal to multifocal cytoplasmic lipid vacuoles of variable size which were random in distribution from 30 ppm. The LOAEL was 30 ppm based on hepatocellular hypertrophy, hepatocellular degeneration and/or necrosis; cytoplasmic vacuoles; increased absolute and relative liver weight; body weight loss.	
ChR-CD rats (5/sex/goup)	0, 30, 100, 300, 1000, 3000, 10 000 and 30 000 ppm APFO corresponding to approximately 1.5 to 1500 mg/kg bw/day	28 days	All animals in the 10 000 and 30 000 ppm groups died before the end of the first week. There were no premature deaths or unusual behaviour reactions in the other groups. Body weight gain was reduced as the dose increased. The reduction in body weight gain was statistically significant for males from 1000 ppm and females from 3000 ppm. Absolute liver weights were increased in males from 30 ppm and in females from 300 ppm. Treatment-related morphological changes were reported in the livers of all test animals. These lesions consisted of focal to multifocal cytoplasmic enlargement (hypertrophy) of hepatocytes in animals in the control, 30 and 100 ppm dose groups, and multifocal to diffuse enlargement of hepatocytes among animals exposed to 300, 1000 and 3000 ppm APFO. The severity and degree of tissue involvement were more pronounced in males than in females. LOAEL 30 ppm based on increased liver weight and hepatocyte hypertrophy.	Metrick and Marisa, 1977; Griffith and Long, 1980
ChR-CD rats (5/sex/group)	0, 10, 30, 100, 300 and 1000 ppm APFO corresponding to 0, 0.056, 1.72, 5.64, 17.9 and 63.5 mg/kg bw/day in males and 0, 0.74, 2.3, 7.7, 22.36, 76.47 mg/kg bw/day in females	90 days	One female in the 100 and 300 ppm group died, however, this was not considered to be treatment related. No treatment-related changes in behaviour or appearance were reported. In males a statistically significant decrease in body weight was reported at 1000 ppm. The relative kidney weights were significantly increased in males from 100 ppm. However, absolute kidney weights were comparable among groups, and there were no histopathological lesions. Absolute liver weights were significantly increased in males from 30 ppm and in females at 1000 ppm. Relative liver weights were significantly increased in males from 300 ppm and in females at 1000 ppm. Hepatocellular hypertrophy (focal to multifocal in the centrilobular to midzonal regions) was reported in 4/5, 5/5 and 5/5 males in the 100, 300 and 1000 ppm groups, respectively. Hepatocyte necrosis was reported in 2/5, 2/5, 1/5 and 2/5 males in the 30, 100, 300 and 1000	Goldenthal, 1978; Griffith and Long, 1980

			ppm groups, respectively. ppm groups, respectively.	
ChR-CD male rats (45-55 per group)	0, 1, 10, 30 and 100 ppm APFO corresponding to 0, 0.06, 0.64, 1.94 and 6.50 mg/kg bw/day. Two control groups (a non-pair fed group and a pair-fed group to the 100 ppm dose group). Following 13 weeks exposure, 10 rats/group were fed control diet for a 8-week recovery period	13 weeks. 15 Animals per group were sacrificed following 4, 7 and 13 weeks of treatment. 10 animals per group were sacrificed after 13 weeks of treatment and after a 8 weeks recovery period.	When analysing the data, animals exposed to 1, 10, 30 and 100 ppm were compared to the control animals in the non-pair fed group, while data from the pair-fed control group were compared to animals exposed to 100 ppm. No treatment clinical signs were reported. At 100 ppm a significant reduction in bw was reported compared to the pair-fed control group during week 1 and the non pair-fed control group during weeks 1-13. Bw data in the other dosed-groups were comparable to controls. At 100 ppm mean body weight gains were significantly higher than the pair-fed control group during week 1 and significantly lower than the non pair-fed control group during weeks 1-13. At 10 and 30 ppm, mean body weight gains were significantly lower than the non-pair-fed control group at week 2. These differences in body weight and body weight gains were not reported during the recovery period. A significant increase in absolute and relative liver weights and hepatocellular hypertrophy were reported at weeks 4, 7 and 13 in the 10, 30 and 100 ppm groups. There was no evidence of any degenerative changes or abnormalities associated with the hypertrophy. Hepatic palmitoyl CoA oxidase activity (indicating peroxisome proliferation) was significantly increased at weeks 4, 7, and 13 in the 30 and 100 ppm groups. At 10 ppm, hepatic palmitoyl CoA oxidase activity was significantly increased at week 4 only. During the recovery period none of the liver effects were reported, indicating that these treatment-related liver effects were reversible.	Palazzolo, 1993
Rhesus monkeys (2/sex/group)	0, 3, 10, 30 and 100 mg APFO/kg bw/day by gavage.	90 days	All monkeys in the 100 mg/kg bw/day, and 3 monkeys in the 30 mg/kg bw/day group died during the study. Clinical signs (anorexia, pale and swollen face, black stools, marked diarrhoea) were reported in the 3 and 10 mg/kg bw/day. No changes in bw at 3 and 10 mg/kg bw/day, however, significant reduction in bw in the one male left in the 30 mg/kg bw/day group. Absolute and relative organ weight changes were reported in the heart (from 10 mg/kg bw/day in females, brain (from 10 mg/kg bw/day in females) and pituitary (from 3 mg/kg bw/day in males), however, no morphological changes were reported in the organs. The male from the 30 mg/kg bw/day group that survived had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. No treatment related	Goldenthal, 1978b; Griffith and Long, 1980

			lesions were reported in the organs of animals in the 3 and 10 mg/kg bw/day dose groups.	
Cynomolgus male monkeys (4-6 animals/group)	0 (6), 3 (4), 10 (6) and 30 (6) mg/kg bw/day APFO by oral capsule.	26 weeks	Dosing of animals in the 30 mg/kg bw/day group was stopped on day 11-21 due to severe toxicity. From day 22 these animals received 20 mg/kg bw/day, and this group was called the 30/20 mg/kg bw/day dose group. At the end of the 26 weeks treatment period, 2 animals in the control group and 10 mg/kg bw/day groups were observed for a 13-week recovery period. One male from the 30/20 and 3 mg/kg bw/day dose groups were sacrificed in moribund conditions during the study. The cause of the deaths was not determined, but APFO treatment could not be excluded. Of the 5 remaining animals in the highest dose group only 2 animals tolerated this dose level for the rest of the study. In 3 animals from the highest dose group the treatment was halted on day 43, 66 and 81, respectively. Clinical signs in these animals included low or no food consumption and weight loss. The animals appeared to recover from compound-related effects within 3 weeks after cessation of treatment. At terminal sacrifice at 26 weeks a significant increase in mean absolute liver weights and liver-to-body weight percentages in all dose groups, considered to be treatment-related, and due, in part to hepatocellular hypertrophy. However, there was no evidence of peroxisome proliferators-activated receptor alpha activity (PPAR α). At recovery sacrifice, no treatment-related effects on terminal body weights or on absolute or relative organ weight were reported, indicating that these effects were reversible over time	Thomford, 2001b; Butenhoff et al., 2002

4.7.1.2 Repeated dose toxicity: inhalation

There is no data on PFNA.

Analogue data:

Table 17: Summary table of repeated dose toxicity, inhalation studies, for APFO/PFOA (Table 9 in APFO Background document)

Species	Concentration (mg/l or mg/m ³)	Exposure time (h/day) and duration of treatment	Observations and Remarks	Reference
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<p>CrI:CD rats 24 males</p>	<p>0, 1, 8, 84 mg/m³ APFO (head only exposure)</p>	<p>6 h/day 5 days per week, for 2 Weeks followed by 28 – 84 day recovery.</p>	<p>Mortality (2) was reported in the highest dose group. One rat was killed after the third day of exposure due to severe weight loss, respiratory distress and lethargy. The other rat died during the fourth exposure. A statistically significant reduction in body weight was reported on test day 5 that recovered by day 16. A statistically significant increase in absolute and relative liver weight and serum alkaline phosphatase was reported from 8 mg/m³ that persisted through 28 days of recovery. Hepatocellular atrophy, and necrosis was reported from 8 mg/m³. These included panlobular and centrilobular hepatocellular hypertrophy and necrosis. Panlobular hepatocellular hypertrophy was reported only in rats killed immediately after the last exposure; the affected livers contained entire lobules with uniformly enlarged hepatocytes. This change was limited to the centrilobular hepatocytes following a 14- or 28-day recovery period and was absent after either 42 or 84 days. Focal or multifocal hepatocellular necrosis was seen in 2/5 rats from the high-dose group (one killed on day 0 and one of day 14 of recovery), in 3/5 rats from the mid-dose group (one each on day 0, 42 and 84 of recovery), and in 1/5 control rats (on recovery day 28). (Five rats from each group were given a complete histopathologic examination). The authors of the study considered the hepatocellular necrosis to be treatment related since hepatocellular necrosis rarely is encountered as a spontaneous lesion in young male rats.</p>	<p>Kennedy et al., 1986</p>
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4.7.1.3 Repeated dose toxicity: dermal

There is no data for PFNA

Analogue data:

Table 18: Summary table of repeated dose toxicity (dermal route) studies for APFO/PFOA (Table 10 in APFO Background document)

Species	Dose (mg/kg bw)	Exposure (h/day) and Duration of treatment	Observations and Remarks	Reference
CrI:CD Rat (15 males)	20, 200, 2000 mg/kg APFO, 10 applications dermal (6	6 hours/day 2 weeks, 5 days/week	Skin irritation and reversible reduction in bw at doses from 200 mg/kg. Increased liver weight was seen in all groups at the end of treatment, in the two higher groups after 14 day recovery period and at the top	Kennedy, 1985

	hours/day, 5 days/week) 5 rats/group killed at the end of treatment, on day 14 and on day 42 of recovery		dose at 42 days of recovery. Increased AST and ALT, as well as hepatocellular hypertrophy and necrosis from 20 mg/kg. Affected livers contained one or more foci of coagulative necrosis. The Kupffer cells within the foci of hepatocellular necrosis contained large vesicular nuclei and were markedly increased in number. Inflammatory cells were occasionally present within and at the periphery of the necrotizing lesions. All of the treatment-related toxicity findings of clinical pathology resolved during a 42- day recovery period. After 10 th treatment of 20, 200 and 2000 mg/kg incidences of rats with liver lesions were 2, 3 and 3 out of 5 rats per group. No data on severity, multifocal appearance or extension of lesions in the liver were reported. The number of animals with liver lesions as reported above decreased during recovery, but was still present in 1 of 5 rats at 20 and 2000 mg/kg. Blood organofluoride concentrations were increased in all test groups with the concentrations decreasing during recovery. 52 ppm was obtained after 10th treatment in rats at 20 mg/kd bw/d APFO. This value is higher than values observed for comparable oral doses (300 ppm) in feed (corresponding to 17.9 mg/kg in this dossier) for 90 days resulted in 38 ppm blood concentration in the oral study of Griffith and Long (1980). *	
Rabbit (10 males/ females)	100 mg/kg, 10 applications dermal and 14 days recovery.	5 hours/day 2 weeks, 5 days/week	Reversible reduction in body weight. The only information regarding the identity of the test substance was T-2618.	Riker, 1981

4.7.1.4 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE

Even though the data for PFNA are not sufficient on its own for classification, the available information indicate that the target organ for PFNA (liver and immune system (PFOA; Dewitt et al., 2012)) is similar to the identified target organ for APFO/PFOA. The classification for repeated dose toxicity of PFNA thus relies on read-across to available data for APFO/PFOA.

Below, the outcome of the RAC assessment has been copied in from the Opinion Document on APFO (ECHA Opinion, 2011):

“With respect to the CLP Regulation, RAC agreed to propose classification as STOT RE1 and hazard statement H372 to be phrased: ‘Causes damage to organs (liver)through prolonged or repeated exposure’.”

Adverse effects that are of relevance for the oral route are mortalities, reduced body weight gain, cyanosis and liver cell degeneration and necrosis. Effects that are expected to be related to peroxisome proliferation such as liver weight increase, liver cell hypertrophy were not regarded

and would not, if occurring alone, justify classification (see CLP guidance, 3.9.2.5.3). Remaining effects that justify classification are: Delayed mortalities at ≥ 300 ppm (15 mg/kg/d), reduced body weight gain liver cell degeneration and necrosis at ≥ 30 ppm (1.5 mg/kg/d) and dose-related onset of cyanosis (≥ 100 ppm (5 mg/kg/d) in mice (28-day study (Christopher and Marisa, 1977); reduced body weight gain in rats at 1000 ppm (50 mg/kg/d) (28-day study, Metrick and Marisa, 1977); reduced body weight gain in rats at 100 ppm (6.5 mg/kg/d) (13 week study, Palazzolo, 1993); mortalities, bad general health state and immunosuppression in Rhesus monkeys at ≥ 30 mg/kg/d (90-day study, Goldenthal 1978b), general toxicity and increased liver weight at 30 mg/kg/d in Cynomolgus monkeys (where PPAR α should not be active). Liver cell necrosis was also observed in rats exposed to APFO for 90 days (Goldenthal, 1978a). However, no clear dose response (only five animals/sex/group!) was seen for this effect. Comparisons with the guidance values of the classification criteria reveal that some of the observed effects may be considered to justify T; R48/25, however, lacking of data on severity and incidences from the documentation of this report do allow only rough evaluation.

According to the CLP criteria the final classification shall be the most severe classification of the three routes. This also covers that oral toxicity from repeated dose studies was also a borderline case for STOT RE 1.

The criteria say that if it is shown that classification for this endpoint is not required for a specific route, then this can be included in the hazard statement. With respect to the dermal route data are insufficient to prove that the dermal route could be excluded. The available dermal study (Kennedy, 1985) indicated that liver cell necrosis was observed from 20 mg/kg bw/d onwards after 2 weeks of treatment and remained up to 42 days of recovery. This is far below the guidance values for the dermal route which are 100 mg/kg/d (DSD) (corresponding values for 28 days: 321 mg/kg and for 14 days 643 mg/kg bw/d) respectively 200 mg/kg/d (CLP) for a 90 day-study.

Target organ and toxic effects in the dermal rat study are consistent to those seen in repeated dose tests using oral and inhalation routes. Although the study is limited (mainly due to its shortness of 14 day treatment period and lack of details on grading histopathological findings), liver findings are supporting the conclusion that all routes are effective. External dosages of about 20 mg/kg bw/d resulted in comparable organofluoride concentrations after 90 days of oral exposure to that after 10 dermal applications. This fact and the observations of liver toxicity after repeated dermal exposure give evidence on the dermal route as of relevance.

Thus there is no reason to include information on the dermal route to be excluded in the hazard statement according to CLP. On the other hand toxicity by the dermal route is already covered by STOT RE 1.

Moreover RAC decided to propose R48/21 based on the observation of liver toxicity from 20 mg/kg bw/d in a dermal 14 day study in rats. The LOAEL for liver toxicity of 20 mg/kg (which is much lower than the corresponding dermal guidance values (for category 1) of 60 mg/kg for a 28 day study) might also argue for a higher classification. However, taking the limits of the dermal repeated dose study into account (mainly due to limited information on severity of liver lesions) the proposal of R48/21 is thought to be adequate.”

4.7.1.5 Conclusions on classification and labeling of repeated dose toxicity findings relevant for classification as STOT RE

Even though the data for PFNA are not sufficient on its own for classification, the available information indicate that the target organ for PFNA (liver and immune system (PFOA; Dewitt et al.,

2012)) is similar to the identified target organ for APFO/PFOA. The classification for repeated dose toxicity of PFNA thus relies on read-across to available data for APFO/PFOA.

The resulting classification for PFNA is STOT RE 1 (Liver), H372.

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

Summary of the Dossier submitter's proposal

The DS proposed to classify PFNA and its sodium and ammonium salts as STOT RE 1, H373 (Causes damage to liver through prolonged or repeated exposure) based on read-across of this toxic property from APFO/PFOA.

Comments received during public consultation

Two MSCAs supported classification of PFNA and its sodium and ammonium salts as STOT RE 1 (liver), H 372.

One MSCA agreed with the proposed classification STOT RE 1 (affected organs: liver) (H372) and proposed consideration of the immune system as an additional target organ.

Another MSCA in principle supported the DS proposal but suggested to consider data on toxicokinetics of PFNA and PFOA in the justification of the read-across approach.

Assessment and comparison with the classification criteria

PFNA and APFO/PFOA have similar toxicokinetics in mice, rats and humans, although toxicokinetics in mice resemble that in humans more than that in rats.

There was a large gender difference in the elimination half-life values of PFNA, as well as PFOA, in rats. Ohmori *et al.* (2003) reported a PFNA elimination half-life of 29.6 days in male and 2.3 days in female Wistar rats after a single intra-venous dose of 48.64 mmol/kg bw PFNA. Major sex differences in the rate of elimination were observed in Sprague-Dawley (SD) rats (estimated half-life of 30.6 days for males and 1.4 days for females) (Tatum-Gibbs *et al.*, 2011). In mice, this gender difference was much smaller. The estimated serum half-life was from 25.8 days (at 1 mg/kg bw) to 68.4 days (at 10 mg/kg bw) in female mice as compared to 34.3 days (at 1 mg/kg bw) to 68.9 days (at 10 mg/kg bw) in male mice. For both rats and mice, PFNA was preferentially stored in the liver but not the kidneys (Tatum-Gibbs *et al.*, 2011). In a study by Benskin *et al.* (2009) in SD rats, the highest concentrations of PFNA were found in the liver followed by kidneys, lungs, heart, spleen, testes, muscle, fat, intestines and brain.

Median human PFNA and PFOA serum concentrations in children were found to be very similar for girls and boys (Schechter *et al.*, 2012). Average serum concentrations ranged from 2-3 ng/mL for PFOA and 0.6-1.4 ng/mL for PFNA from birth to 12 years of age. The PFOA serum elimination half-life was estimated to be 3.8 years (the range was 1.5 – 9.1 years) in 26 retired workers (24 men and 2 women) (Olsen *et al.*, 2007 and Harada *et al.*, 2005).

The existing data indicate that toxicokinetics of PFNA and PFOA are similar in rats, mice and in humans.

Taking into account the very close chemical analogy of their structures, a common functional group and similar toxicokinetics of PNFA, PFN-S and PFN-A with those of PFOA/APFO, already classified as STOT RE 1, H372 (Causes damage to organs (liver) through prolonged or repeated exposure) in Annex VI to the CLP Regulation, RAC was of the opinion that PNFA, PFN-S and PFN-A should also be classified as STOT RE 1, H372, based on a read-across approach.

There were also studies, listed below, which supported and in some cases, even justified extending the hazard statement to other organs.

PFNA has been found to be very toxic following repeated oral exposure in mice. Half of the mice died during repeated oral exposure for 14 days when given a dose of 10 mg PFNA/kg/day (Fang *et al.*, 2008). This seemed to fulfil the guidance value set for 90-day rat studies (table 3.9.2 in the CLP Regulation) for the level of exposure inducing a clearly adverse effect (mortality) for classification as STOT RE 1. It may be expected that longer repeated oral exposure of mice to a lower dose would also result in high mortality.

In the study of Mertens *et al.* (2010), submitted by industry, a mixture of perfluoro fatty acid ammonium salts (C₆-C₁₃), known as S-III-S-WB, was administered orally to CrI:CD (SD)IGS-BR rats for 90 consecutive days at doses of 0.125, 0.25 and 0.6 mg/kg/day. S-III-S-WB-related higher liver weights were present at study week 13 in the 0.125 mg/kg/d males and 0.6 mg/kg/d males and females. In the females, the liver effect was less pronounced and was reflected only in the relative to final body weight values.

S-III-S-WB-related microscopic findings were seen in male rats, but not in female rats, and consisted of hepatocellular hypertrophy and eosinophilic foci in the 0.125 mg/kg/d and 0.6 mg/kg/d males. The males given 0.6 mg/kg/d developed hepatocellular degeneration and necrosis. Higher hepatic β -oxidation was found in the 0.125 mg/kg/d group males and in the 0.6 mg/kg/d males and females exposed orally by gavage once daily, indicating that S-III-S-WB is a mild peroxisome proliferator. After 10-days of oral exposure, hepatic β -oxidation was 1.7-fold higher in the 0.6 mg/kg/d males than in control rats. At the primary toxicology necropsy after 90-day exposure, hepatic β -oxidation was 2-fold and 3.1-fold higher in the 0.125 and 0.6 mg/kg/d group males, respectively, and 1.5-fold higher in the 0.6 mg/kg/d group females than in control rats. Analysis at the end of the recovery period showed partial recovery in the males and complete recovery in the females.

Lower serum protein and higher bilirubin and BUN were seen in the 0.6 mg/kg/d males and lower globulin and higher alkaline phosphatase in the 0.125 mg/kg/d males and 0.6 mg/kg/d animals.

After 2 weeks, serum concentrations of PFOA (C₈), PFNA (C₉), PFUDA (perfluoroundecanoic acid, C₁₁), and PFTDA (perfluorotridecanoic acid (C₁₃) were constant for at least 8 hours. After 90 days, only PFNA in the 0.025 mg/kg/d females had reached steady state. Serum PFOA and PFNA concentrations in the males were 10-fold higher than in the females, whereas PFUDA and PFTDA were similar for both genders. The main elimination was via the urine for C₆ acid (PFHA) (males) and C₉ (PFNA) (females), and via the faeces for acid with longer chain C₁₁ (PFUDA) and C₁₃ (PFTDA).

The no-observed-effect level (NOEL) for a mixture of perfluoro fatty acid ammonium salts (C₆-C₁₃) was 0.025 mg/kg/d for males and 0.125 mg/kg/d for females, due to the serum chemistry differences and the higher hepatic β -oxidation and liver weights present in the 0.125 mg/kg/d males and 0.6 mg/kg/d females and the hepatocellular hypertrophy and eosinophilic foci present in the 0.125 mg/kg/d males. Thus, the lowest-observed-effect level (LOEL) in the current study was 0.125 mg/kg/d for males and 0.6 mg/kg/d for females.

During the public consultation, the immune system was proposed as additional target organ in the specific target organ toxicity – repeated exposure (STOT RE) classification. The DS agreed that the immune system should be considered as a target organ and left this question up to RAC for further discussion, although no formal proposal for such a classification containing a comparison with the classification criteria had been provided by the DS.

However, in their response to this issue, the DS provided additional data indicating that the serum IgM antibody titres were significantly reduced in a dose-dependent manner in female mice exposed to APFO via drinking water at doses of 3.75, 7.5, 15 and 30 mg/kg/day for 15 days with reduction of absolute and relative weight of the spleen

thymus at doses of 7.5 mg/kg and above (DeWitt *et al.*, 2008). This study indicated that the synthesis of IgM is affected at dose levels of APFO where no effect on bodyweights was observed (DeWitt *et al.*, 2008). In another mechanistic study (Dewitt *et al.*, 2009), summarised by the DS at public consultation, it was demonstrated that suppression of antibody synthesis to a T-dependent antigen due to 5-day exposure to PFOA in drinking water at doses of 7.5 and 15 mg/kg/day was not the result of liver toxicity or stress-related corticosterone production.

In the 14-day study in which mice were given 1, 3 and 5 mg/kg bw of PFNA by gavage, the relative thymus weights at 3 and 5 mg/kg bw were significantly reduced. At 5 mg/kg bw, PFNA caused an increase in apoptotic cells in the thymus (Fang *et al.*, 2008). Exposure to PFNA led to a decrease in body weight and in the weight of the lymphoid organs. Cell cycle arrest and apoptosis were observed in the spleen and thymus following PFNA exposure. In the thymus, PFNA mostly modulated CD4⁺CD8⁺ thymocytes, whereas the F4/80⁺, CD11c⁺, and CD49b⁺ cells were major targets in the spleen. Although concanavalin A-induced T-lymphocyte blastogenesis was not altered by PFNA, production of interleukin (IL)-4 and interferon-gamma by splenic lymphocytes was markedly impaired. The levels of cortisol and adrenocorticotrophic hormone in sera were increased; however, the expression of glucocorticoid receptors in the thymus was unchanged. In addition, expression of the peroxisome proliferator-activated receptors (PPAR- α and PPAR- γ) and IL-1b were upregulated significantly in the thymus at a dose of 1 mg PFNA/kg/day. No significant changes in expression of the inhibitory protein I κ B α and I κ B α kinase were observed. Together, these results suggest that PFNA exerts toxic effects on lymphoid organs and T-cell and innate immune cell homeostasis in mice and that these effects may result from the activation of PPAR- α , PPAR- γ , and the hypothalamic-pituitary-adrenal axis.

In the 14-day study in male SD rats there was a dose-dependent decrease in absolute spleen weight for all rats exposed to PFNA at 1, 3 and 5 mg/kg bw by 22.2%, 28.7% and 57.9% ($p < 0.01$), respectively, compared to control rats. However, the ratio of spleen weight to body weight was only significantly decreased in PFNA exposed rats at 5 mg/kg bw (91.5% of the controls, $p < 0.01$). Exposure to PFNA caused an increase in apoptotic lymphoid cells in the spleen at doses of 3 and 5 mg/kg bw. The 5 mg/kg bw dose also increased levels of pro-inflammatory IL-1, IL-6, IFN α , and H₂O₂, but decreased levels of IFN γ and IL-10.

The above studies demonstrated that PFNA and its structural analog APFO induce adverse effects on the immune system at the relatively low dose of 3-5 mg/kg bw already after 14 days oral exposure. It is reasonable to assume, in accordance with Haber's law, that several fold lower doses would induce similar effects in the immune system in the event that the exposure to PFNA or APFO would last 90 days.

The data reviewed above fulfil the requirement set in section 3.9.2.7.3 of the CLP Regulation and provide evidence of significant functional changes in the immune system at doses equal to or below respective guidance values (Table 3.9.2-3) which reveal hazards that may not be life-threatening, but indicate functional impairment.

Thus, in the opinion of RAC, classification of PFNA and its sodium and ammonium salts as STOT RE 1 (for effects on the thymus and spleen) is warranted, because significant immunological toxic effects were observed in experimental animals below the guidance values of 10 mg/kg bw/d even after oral exposures shorter than 90 days.

In conclusion, taking into account the read-across approach of specific target organ toxicity after repeated exposure to APFO/PFOA to PFNA and its salts and the adverse effects of PFNA in the immune system (thymus, spleen), RAC is of the opinion that PFNA and its sodium and ammonium salts warrant classification as STOT RE 1, H372 - Causes damage to organs (liver, thymus, spleen) through prolonged or repeated exposure.

4.8 Germ cell mutagenicity (Mutagenicity)

Not evaluated in this dossier

4.9 Carcinogenicity

There is no available information on carcinogenicity for PFNA. PFNA and APFO/PFOA have very similar physico-chemical as well as toxicokinetic properties. This justifies that the classification for PFNA is based on read-across from data for APFO/PFOA. To aid the reader of this CLH report we have therefore included a table from the Background Document for APFO (ECHA Background document, 2011) as well as text from the Opinion Document for APFO (ECHA Opinion, 2011) that was produced during the classification process of APFO and PFOA by the Committee for Risk Assessment (RAC) at ECHA.

Table 19: Summary table of carcinogenicity studies (oral route) for PFOA (Table 13 in the Background document for APFO)

Species	Dose (mg/kg bw)	Duration of treatment	Observations and Remarks	Reference
Sprague-Dawley rats 50/sex/group Groups of 15 additionally rats/sex were fed 0 or 300 ppm and evaluated after 1 year	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	Sibinski, 1987;

		<p>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.</p> <p>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 6/50 high dosed and 1/50 low-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 statistical 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. 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).</p> <p>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</p>	
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			<p>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.</p>	
<p>Sprague-Dawley male rats, 76 rats in the treatment group and 80 rats in the control group</p>	<p>300 ppm APFO</p>	<p>2 years</p>	<p>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 (P450-19A1¹, 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 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.</p> <p>In the study by Sibinski, 1987, no increase in the incidence of PACT was reported (0/33,</p>	<p>Cook et al., 1994; Biegel et al., 2001 Liu et al, 1996</p>

			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.	
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4.9.1 Summary and discussion of carcinogenicity

Below, the outcome of the RAC assessment has been included from the Opinion Document for APFO (ECHA Opinion, 2011):

“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			Cook et al., 1994, Biegel et al., 2001		Historical control values for S-D rats#
	50 rats/sex/group 2 year 15 rats/sex/group 1 year			76 males at 300 ppm, 80 control males		
Ppm Mg/kg bw/d	0	30 1.3	300 14.2	0	300	
<i>Liver</i>						
<i>2 year study</i>						
<i>Liver cell adenomas</i>				2.5% (2/80)	13% (10/76)	
<i>Hyperplastic nodules</i>	0% / 0%		6% / 0%			
<i>Liver cell megalocytosis</i>	0% / 0% ^s	12% / 2%	80% / 16%			
<i>Cystoid degeneration</i>	8% / 0%	14% / 0%	56% / 0%			
<i>1 year</i>						
<i>Liver cell megalocytosis</i>	0% / 0%*		80% / %			
<i>Portal mononuclear cell infiltration</i>	47% / 0%		80% / 0%			
<i>Hepatocellular necrosis</i>						

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 adenoma	- / 12%	- / 16%	- / 17%			
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)	

^SPercentages 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 PPAR α -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 PPAR α , 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 PPAR α -activity in a 6-month cynomolgus monkey study (Butenhoff et al., 2002).

Evidence from PPAR α -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 PPAR γ coactivator-1 α (Pgc-1 α) 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-1 α pathway is consistent with PPAR γ 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).

PPAR γ transactivation by APFO were also concluded from dose-related increase in PPAR γ mRNA in PPAR α -null mice, while only slightly in hPPAR α -mice was observed (Nakagawa et al. 2011) In conclusion, much of the response to APFO can be attributed to PPAR α and induction of PPAR α regulated genes. The impact of activation of PPAR γ -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 PPAR α 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 PPAR α . 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)."

4.9.2 Conclusions on classification and labeling

Based on read across to APFO/PFOA, the resulting classification of carcinogenicity for PFNA is Carc. 2; H351.

RAC general comment

In the opinion of RAC, due to their high structural similarity and chemical analogy:

- the 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Heptadecafluorononanoic acid (PFNA) with its sodium (PFN-S) and ammonium (PFN-A) salts and
- the 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid (PFOA) with its ammonium salt - (Ammoniumpentadecafluorooctanoate (APFO))

fulfill the criteria for a read-across approach to be applied, as defined in Section 1.5 of Annex XI of the REACH Regulation (underlining added): "*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).*" At least two of the following three similarities listed in the REACH Regulation and upon which the read-across approach is based on, were met:

- 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.

As it was assumed that PFOA and APFO form the corresponding anion (PFO) in the gastro-intestinal system or lung fluid, also PFNA, PFN-S and PFN-A were anticipated to become available to cells at physiological pH in the form of their corresponding anion (PFN), thus exerting the same toxic effects, although their potency may differ. For systemic effects such as those following oral or inhalation exposure, the read-across is in fact between two anions: PFO and PFN, which are analogous chemical groups and differ only by one -CF₂- group in the fluorine substituted aliphatic chain.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The DS proposed to classify PFNA and its sodium and ammonium salts as Carc. 2, H351 (Suspected of causing cancer) based on read-across from APFO/PFOA.

Comments received during public consultation

Three MSCAs supported classification of PFNA and its sodium and ammonium salts as

Carc. 2, H351 (Suspected of causing cancer) based on read-across from APFO/PFOA. No objection to this proposal was made during public consultation.

Assessment and comparison with the classification criteria

APFO and PFOA, used as reference substances in read-across approach for PFNA, PFN-S and PFN-A, have been classified as Carc. 2, H351, in Annex VI to the CLP Regulation.

In the opinion of RAC, due to high similarity of the structure of PFNA and its sodium and ammonium salts with the structure of PFOA and APFO, they can be grouped and used for read-across of toxic properties.

Taking into account the considerations noted under "RAC General Comments" (above) and applying a read-across between PFO and PFN anions, RAC agrees with the DS and proposes to classify PFNA and its sodium (PFN-S) and ammonium (PFN-A) salts as Carc. 2, H351 (Suspected of causing cancer).

4.10 Toxicity for reproduction

4.10.1 Effects on fertility

4.10.1.1 Non-human information

There is very limited data for PFNA and its effects on fertility. The only information available is from one in vivo study and one in vitro study. In a repeated dose toxicity study by Feng et al. (2009; described in section 4.7.1.1), male rats (six animals per dose) were dosed orally with PFNA (0, 1, 3, 5 mg/kg) for 14 consecutive days. This mechanistic study showed that PFNA (similar to what is known for other polyfluoroalkyl acids (Bookstaff et al., 1990; Shi et al., 2007; Wei et al., 2007)) affected the sex steroid hormone biosynthesis. In comparison to the control animals, the serum testosterone levels were increased at 1 mg/kg and decreased at 5 mg/kg while the serum levels of estradiol were increased in high dose animals. Histopathological examination of the testis showed an increase in the number of apoptotic spermatogenic cells in the high dose animals. In some seminiferous tubules, the germ cells exhibited a loss of adhesion to the Sertoli cells and were found in the lumen of seminiferous tubules.

In summary, there is not sufficient information on PFNA for classification for adverse effects on sexual function and fertility.

Analogue data:

Below, the outcome of the RAC assessment has been included from the Opinion Document for APFO (ECHA Opinion, 2011):

“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 PPAR α 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 hPPAR α 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 PPAR α mice treated with the highest concentration was seen (Li et al., 2011). The authors reported inconsistencies of PPAR α -expressed in interstitial Leydig cells or seminiferous tubule cells of testis in m PPAR α -mice, but not in testis of hPPAR α -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.”

4.10.1.2 Human information

No data available.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

Table 20: Summary table of relevant reproductive toxicity studies

Species Dosing period Endpoints	Dose mg/kg bw	Observations and Remarks					Reference
		Maternal tox and liver effects	Implantation sites Pup viability at birth and pup survival	Pup weight	Eye opening	Serum concentration (µg/ml) of PFNA on PND 21 (i.e. 3 weeks after last dose)	
<p>Species 129S1/SvImJ mice wild- type (WT)</p> <p>Dosing period GD1- 18</p> <p>Endpoints *Maternal Bodyweight during gestation and on PND 21 *Pup viability (monitored twice per day) *Pup weight: PND 0, 1, 2, 3, 7, 10, 14 and 21 *Time of eye opening *Necropsy of dams and pups on PND 21 *Number of implantations *Serum from dams and pooled from pups for PFNA analysis</p>	<p>0, 0.83, 1.1, 1.5 and 2.0 mg/kg bw PFNA via oral gavage</p> <p>Group size 12, 11, 12, 14 and 17 in 0, 0.83, 1.1, 1.5 and 2 mg/kg bw PFNA, respectively</p>	<p>No effect on body weight gain during gestation or on maternal weight at necropsy (PND 21) at any dose level.</p> <p>Dose dependent increase in the relative liver weight of both dams and pups.</p>	<p>Implantation No effect</p> <p>Pups <u>1.1 mg/kg</u> Significant (p< 0.05) reduction in the number of live pups/litter at birth and reduced pup survival until PND 21.</p> <p><u>1.5 mg/kg:</u> Reduced (not stat. significant) number of live pups/litter at birth and reduced survival rate up until PND 21.</p> <p><u>2 mg/kg:</u> reduced (p< 0.001), number of live pups/litter at birth and reduced pup survival up until PND 21.</p>	<p>No effect on pup weight at birth at any dose level in either male or females.</p> <p>2 mg/kg Reduced male pup weight on PND 7, 10 and 14 p<0.001/ 0.01). Female pup weight was reduced on PND 7, 10, 14 and 21 (p<0.001 – 0.05).</p>	<p>2 mg/kg: ~2 days delayed eye opening (p<0.01)</p>	<p>Dose- dependent increase in both pups (~10 at lowest dose and ~25 at highest dose) and dams (~9 at lowest dose and ~35 at highest dose).</p> <p>Adult females[#] had higher serum concentra- tions of PFNA as compared to those seen in pups and dams (~28 at lowest dose and ~64 at highest dose)</p>	<p>Wolf et al., 2010 (WT info)</p>

<p>Species 129S1/SvImJ mice PPARα knockout (KO)</p> <p>Dosing period and endpoints Same as those for WT mice</p>	<p>Group size 18, 13, 14, 9 and 16 in 0, 0.83, 1.1, 1.5 and 2 mg/kg PFNA, respectively</p>	<p>No effect on maternal body weight gain during gestation or on maternal weight at necropsy (PND 21) at any dose level.</p> <p>No effects on relative liver weights in dams with nursing pups.</p> <p>Increased relative liver weight in pups at the 2 mg/kg dose level.</p>	<p>No effect on number of implantations, on pup viability at birth or on pup survival during lactation</p>	<p>No effect</p>	<p>No effect</p>	<p>Dose dependent increase in both pups (~15 at lowest dose and ~38 at highest dose) and dams (~3 at lowest dose and ~23 at highest dose).</p> <p>Adult females[#] had higher serum concentrations of PFNA compared to those seen for pups and dams (~38 at lowest dose and ~83 at highest dose)</p>	<p>Wolf et al., 2010 (KO info)</p>
<p>Species CD-1 mice,</p> <p>Dosing period: GD 1-16.</p> <p>At GD 17 each group subdivided (ratio not specified). Some dams were sacrificed for maternal and fetal examinations on GD 17 (c-section cohort) and other mice were allowed to give birth (littering cohort). Postnatal survival, growth and development</p>	<p>1, 3 and 5mg/kg (also 10 mg/kg bw but very little information provided) PFNA via gavage</p>	<p>No effect on body weight gain during gestation up to 5 mg/kg. At 10 mg/kg slightly decreased gain.</p> <p>Dose dependent increase of relative liver weight in dams.</p> <p>Pups: dose dependent increase of relative liver weight.</p>	<p><u>C-section cohort</u> No effect on numbers of implantations, live fetuses or no of dams with FLR (full litter resorption) up to 5 mg/kg bw</p> <p><u>Littering cohort:</u> 5 mg/kg: somewhat lower pup viability at birth, gradual decrease in pup viability over time, and on PND 10 only 20% of the pups were still viable.</p>	<p>No effect at birth</p> <p>Dose dependent deficits in growth (very marked at 5 mg/kg) from PND 3 and onwards. (no stat. analysis)</p> <p>Growth reduction persisted until last time of recording at PND 300.</p>	<p>Dose dependent delays of eye opening and onset of puberty (vaginal opening and preputial separation) stat. significance at 3 or 5 mg/kg</p>	<p>Effective placental transfer since [PFNA]_{serum} of newborns matched those of the dams.</p> <p>PFNA remained detectable in pups past weaning.</p>	<p>Lau et al., SOT Poster 2009</p>

of offspring were recorded.							
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Adult females = non pregnant females and dams with litter loss

In a developmental toxicity study in mouse by Wolf et al. (2010), pregnant 129S1/SvImJ wild-type (WT) and PPAR α knockout (KO) mice were dosed orally at 0, 0.83, 1.1, 1.5 or 2 mg/kg PFNA on gestational days (GD) 1-18. No effect on body weight gain during gestation, on maternal body weight at PND 21, on number of implantations or on pup weight at birth were recorded at any dose levels in the KO and WT mice. A dose dependent increase in the relative weight of the liver was recorded in WT dams and pups but no effect was observed in the KO dams, and for KO pups an increase in the relative liver weight was only seen at the highest dose level.

In the WT mice, the number of live pups per litter was clearly reduced at the first observation after birth. The viability was further reduced during the postnatal period so that at weaning 36% (1.1 mg/kg bw) and 31% (2 mg/kg bw) of the viable pups at birth were alive. Decreased number of pups shortly after birth and a reduced pup survival up until weaning were also seen at the 1.5 mg/kg dose level but the values did not reach statistical significance. A closer analysis of the presented data for the WT strain in the Wolf et al. paper indicates that the lower number of live pups per litter shortly after birth was, at least in the highest dose, partly due to full litter resorption (i.e. uterine implants present but no pups) and/or whole litter loss (i.e. only dead pups). Consequently a somewhat higher percentage of litter loss was recorded at the high dose group (35% as compared to 14.3% in the controls). The paper specifies that in the high dose group 4 dams had full litter resorption (the authors of the paper argue that embryo died during early pregnancy) and 2 dams out of 17 had whole litter loss. However it is not clear from the paper if the litter resorption in the control group was due to full litter resorption and/or whole litter loss. One can only conclude that there is at least a doubling in the number of dams with full litter resorption (i.e. dams with early intrauterine death) in the high dose group as compared to the controls. No effect on pup viability at birth or on pup survival during lactation was recorded at any dose level in the KO mice. However, as compared to the control KO mice, there was a decreased pregnancy rate ($p < 0.001$) in KO mice at all dose levels (pregnancy rate was 75, 65, 58, 21 and 43% in the 0, 0.83, 1.1, 1.5 and 2 mg/kg dose groups) which suggest that PFNA may have interfered with implantation when PPAR α was not functional.

Although there was no effect on pup weight at birth, significantly lower pup body weight was recorded on PND 7, 10 and 14 in male pups and at all observation occasions between PND 7 and 21 for female pups at the high dose level. The body weight gain for female pups was ~25% lower as compared to the gain in the controls for the period between PND 7 and 21. No effect on pup weight or gain was recorded for the KO pups up until PND 21. The mean day of eye opening in the controls was PND 13.7 ± 0.3 in WT and PND 13.9 ± 0.2 in KO. The day of eye opening was significantly ($p < 0.01$) delayed in the 2 mg/kg dose group to PND 15.8 ± 0.2 in the WT pups, whereas no effect was seen in KO pups.

At all dose levels examined in both KO and WT dams, the serum concentration of PFNA was significantly higher at weaning (21 days after end of exposure to the dams) in adult females (non-pregnant females and dams with litter loss) when compared to those recorded in dams that had live pups. The PFNA levels in the pups were similar or even higher than those recorded in nursing dams. The PFNA levels in all dams with nursing pups were lower in KO compared to WT ($p < 0.001$) and the PFNA levels in the pups were higher in KO compared to WT ($p < 0.0001$).

In a poster by Lau et al. (2009) (presented at SOT, a manuscript is being prepared), CD-1 mice were dosed orally GD1-17 with PFNA at 0, 1, 3 or 5 mg/kg (10 mg/kg was also used but this dose level

was dropped due to severe maternal toxicity including mortality (not more specified)). 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 (GD1-17), 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. However decreased pup viability was observed already at the first examination after birth in the 5 mg/kg 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 (FLR). So, like APFO, PFNA at a high enough dose will cause full litter resorption.”*

Pups displayed a (dose dependent) reduction in body weight from shortly after birth until PND 300 and delayed eye opening and delayed onset of puberty (vaginal opening and preputial separation) was recorded at the 3 and 5 mg/kg dose level. The serum levels of PFNA in newborn pups were found to be similar to those of the dams and thus one can conclude that PFNA can efficiently cross the placenta.

All together, 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 will cause full litter resorption if the dose is high enough. These effects are very similar to the effects reported for APFO/PFOA (see table 21).

Table 21: Data matrix for the analogue read-across: Physicochemical properties and mammalian toxicity

CAS number	375-95-1		335-67-1			
Chemical name	PFNA		PFOA			
Chemical formula	C ₉ HF ₁₇ O ₂		C ₈ HF ₁₅ O ₂			
Physicochemical properties						
Molecular weight	464.08		414.07			
Physical state	Solid		Solid			
Melting point	65-68°C		52-54°C			
Boiling point	218°C at 740 mm Hg		189-192°C at 736 mm Hg			
Density	1.753g/cm ³		1.792g/cm ³			
pKa	Estimated -0.15		2.8			
Mammalian toxicity						
Developmental toxicity – APFO						
Dose, dosing period, strain of mice	Pup weight	Relative liver weight in dams and pups	Intrauterine data	Pup viability at birth and pup survival during lactation	Delay in eye opening and effects on onset of puberty	Serum concentration of PFOA @ weaning
0, 1, 3, 5, 10, 20 and 40 mg/kg GD 1-17, CD-1 Oral gavage Lau et al., 2006	↓ LOAEL= 3 mg/kg bw	↑ LOAEL=1 mg/kg bw (absolute liver weight in dams). No data for pups	LOAEL _{Full litter resorption (FLR)} = 5 mg/kg bw	↓ no of pups at birth, LOAEL =5mg/kg bw LOAEL _{Pup survival up until PND 22:} =5 mg/kg bw	LOAEL _{Eye opening} =5 mg/kg bw LOAEL _{accelerated preputial separation} =1 mg/kg bw	Not investigated

0, 3, 5 mg/kg GD 1-17, Cross-fostering CD-1 Oral gavage Wolf et al., 2007	↓ LOAEL= 3 mg/kg bw (pups exposed in utero (U) and lactationally (L)) and 5 mg/kg bw if exposed only in utero	↑ LOAEL=3 mg/kg bw (dams and pups)		↑No of dams with WLL (dams with implants but no live pups on PND 1), LOAEL = 5 mg/kg Pup survival: ↓ LOAEL =5 mg/kg bw, and only in pups exposed both in utero and lactationally.	LOAEL _{Eye opening} = 3 mg/kg bw (for U+L exposure) and 5 mg/kg bw for U exposure) Effect on onset of puberty was not examined	At 5 mg/kg: ~ 22 – 25 µg/ml in pups (U+L exposure) and ~37 µg/ml in corresponding dams
0, 0.1, 0.3, 0.6, 1, 3, 5, 10, and 20 mg/kg bw 129S1/SvImJ Oral gavage Abbott et al.,2007	WT					
	↓ LOAEL= 1mg/kg bw	↑ LOAEL _{Dams} = 1mg/kg bw ↑ LOAEL _{Pups} = 0.1mg/kg bw	LOAEL _{FLR} = tendency at 1 clear at 5mg/kg bw	↓Pup survival LOAEL= 0.6mg/kg bw	LOAEL _{Eye opening} = 1mg/kg bw Effect on onset of puberty was not examined	At 1 mg/kg: ~10 µg/ml in pups, ~9 µg/ml in dams and ~26 µg/ml in adult females with no pups
	PPAR α KO					
	NOAEL > 3mg/kg bw	↑ LOAEL _{pups and dams} = 3mg/kg bw	LOAEL _{FLR} = 5mg/kg	NOAEL > 3 mg/kg	NOAEL _{Eye opening} > 3 mg/kg bw Effect on onset of puberty was not examined	At 1 mg/kg: ~8µg/ml in pups and dams and ~25 µg/ml in adult females with no pups.
0 and 3mg/kg bw	WT					

GD 1-17 Oral gavage 129S1/SvlmJ	NOAEL >3 mg/kg	LOAEL _{pups and dams} =3mg/kg bw	NOAEL > 3 mg/kg	Slight decrease (the litter size was 3.7 on PND 20 and 5.3 at birth)	NOAEL _{Eye} opening > 3 mg/kg Effect on onset of puberty was not examined	~ 7µg/mL in dams
Palkar et al., 2012	PPARα KO					
	NOAEL > 3 mg/kg	LOAEL _{pups and dams} =3mg/kg bw	NOAEL > 3 mg/kg	NOAEL > 3 mg/kg	NOAEL _{Eye} opening > 3 mg/kg Effect on onset of puberty was not examined	~5 µg/ml in dams
	hPPARα KI					
	NOAEL > 3 mg/kg	LOAEL _{pups and dams} =3mg/kg bw	NOAEL > 3 mg/kg	NOAEL > 3 mg/kg	NOAEL _{Eye} opening > 3 mg/kg Effect on onset of puberty was not examined	~2µg/ml in pups
Mammalian toxicity						
Developmental toxicity – PFNA						
Dose and Study	Pup weight	Relative liver weight in dams and pups	Intrauterine data	Pup viability at birth and pup survival during lactation	Delay in eye opening and effects on onset of puberty	Serum concentration of PFNA
0, 0.83, 1.1, 1.5 and 2 mg/kg bw GD 1-18 Oral gavage	WT					
	↓ LOAEL= 2 mg/kg bw	↑ LOAEL=0.83 mg/kg bw in both pups and	An indication of an effect on early intrauterine	↓ pup viability at birth and ↓ pup survival during lactation	LOAEL _{Eye} opening = 2 mg/kg bw	PND 22: 25 µg/ml in pups, 35 µg/ml in

129S1/SvlmJ Wolf et al., 2010		dams	survival at 2 mg/kg (see text for more details)	LOAEL for both endpoints= 1.1mg/kg bw	Onset of puberty was not assessed	dams and 64 µg/ml in adult females with no pups (at the dose 2 mg/kg bw)
	PPAR α KO					
	NOAEL > 2 mg/kg bw	↑ LOAEL= 1.1 mg/kg bw in non pregnant females LOAEL = 2 mg/kg bw in pups	Reduced pregnancy rate in all PFNA exposed groups (at doses 0, 0.8, 1.1 1.5 and 2.0 mg/kg bw the pregnancy rates were 75, 65, 58, 21, and 43% respectively)	NOAEL > 2 mg/kg bw	NOAEL > 2 mg/kg bw	38 µg/ml in pups, 23 µg/ml in dams and 83µg/ml in adult females with no pups (at the dose 2 mg/kg bw)
0, 1, 3, 5, and 10 mg/kg bw GD 1-16 CD-1 Oral gavage Lau et al., 2009 (poster)	↓ LOAEL=5 mg/kg bw	↑ LOAEL = 1 mg/kg bw in both pups and dams	LOAEL _{FLR} = 10	LOAEL = 5 mg/kg bw	LOAEL _{Eye opening} = 3 mg/kg bw LOAEL _{delayed pre-putial separation} =3 mg/kg bw	Not investigated

Analogue data:

A study from Lau et al. (2006) showed that APFO administered by oral gavage (0, 1, 3, 5, 10, 20, and 40 mg/kg) on GD 1-17 produced dose dependent full litter resorption (from 5 to 40 mg/kg). APFO also reduced postnatal survival (≥ 5 mg/kg), delayed general growth (≥ 3 mg/kg), delayed eye opening (≥ 5 mg/kg), and early onset of separable prepuce (≥ 1 mg/kg) indicating an earlier onset of male puberty. A cross-foster study (Wolf et al., 2007) showed that pup survival from birth to weaning was only affected if the pups had been exposed in utero and via lactation, whereas dosing of the dams during gestation was sufficient to produce postnatal body weight deficits and developmental delay in the pups.

A study by Abbott et al. (2007) looked into the influence of PPAR α on APFO-induced developmental toxicity. They reported that 129S1/SvImJ wild-type (WT) and PPAR α knockout (KO) mice dosed with PFOA via oral gavage (0, 0.1, 0.3, 0.6, 1, 3, 5, 10 and 20 mg/kg) during GD 1-17 resulted in full litter resorption (≥ 5 mg/kg) in both WT and KO, reduced neonatal survival (≥ 0.6 mg/kg) in WT and delayed eye opening (1 mg/kg, this endpoint was only studied up to 1mg/kg in WT) in WT mice. Absolute liver weight was increased in WT adult females (≥ 1 mg/kg) and in KO adult females (≥ 3 mg/kg). In both WT and KO there was an increase in the serum level of PFOA in females without pups compared with dams with pups. Several of the developmental effects in mice seemed to be attributed to PPAR α (postnatal lethality, delayed eye opening and decrease in postnatal weight gain) although other mechanisms may contribute. However, full litter resorptions appeared to be independent of the PPAR α expression. Interestingly Wolf et al. (2007) showed that full litter resorption is only induced if the exposure window included early pregnancy (i.e. GD 1-7).

In addition to the effects mentioned previously in this section, APFO also effects 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 (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 GD1-17 or GD 10-17.

4.10.2.2 Human information

No data available.

4.10.3 Other relevant information

4.10.4 Summary and discussion of reproductive toxicity

Developmental Toxicity

The study by Wolf et al. (2010) showed that gestational exposure to PFNA in 129S/SvImJ reduced the number of live pups at birth as well as the survival of the offspring during the post natal period (LOAEL=1.1 mg/kg bw, NOAEL=0.83 mg/kg bw). Delay in the onset of eye opening (LOAEL=2.0 mg/kg bw) was also seen but this could partly be a consequence of the general delayed pup

development as revealed by the reduced pup weight at this dose level. All these effects were observed at dose levels that did not cause maternal toxicity as revealed by effects on maternal body weight. Similar findings (that also were observed at dose levels that did not affect maternal body weights) were seen for mice exposed to APFO; delayed eye opening (LOAEL=1 mg/kg bw for both CD-1 and 129S/SvImJ mice), and reduction in pup survival (LOAEL=0.6 mg/kg bw for 129S/SvImJ and 5 mg/kg bw for CD-1 mice). In addition, APFO also induced full litter resorptions (LOAEL=1 mg/kg bw SvImJ and 5 mg/kg bw in CD-1 mice) (Abbott et al., 2007, Lau et al., 2006 and Wolf et al., 2007)). According to the authors (Wolf et al., 2010) PFNA does not cause full litter resorption in the SvImJ strain up to 2 mg/kg. However, as indicated in section 4.10.2.1, a closer analysis of the presented data shows that there is a weak signal (although not statistically significant) of full litter resorption at the 2 mg/kg dose level. The data from CD-1 mice do indicate that PFNA can induce full litter resorption if the dose levels are high enough. Thus, the lack of a clear signal for full litter resorption in the SvImJ strain could very well be due to the dose levels used in that study. The high dose was 2 mg/kg and at this dose levels an increase in the relative liver weight was recorded, but no effect on maternal weight or even on maternal body weight gain was observed. This indicates that the potential of PFNA to induce full litter resorption was not fully explored in the Wolf study.

No effects were seen on pup survival, on pup weights or on eye opening in studies where PPAR α KO mice were exposed to either PFNA or APFO (during gestation). However, full litter resorption was still found in the PPAR α KO mice. These studies indicate that the activation of PPAR α could be the MoA for some but not all of these effects. In addition, other PPAR α agonists are known to induce full litter resorptions at high dose levels. Palkar et al. 2010 reported that gestational exposure to PPAR α agonist WY-14 643 (0.005% in the diet) did not induce any developmental toxicity. However Yang et al., 2006 reported in another study that gestational exposure of WY-14 643 in the diet at a much higher level (0.1%) indeed produced 100% early embryo lethality. Interestingly they also highlighted the fact that embryo lethality was not seen if dosing first started on GD 7.5. This is similar to what has been reported for APFO where embryo lethality was not observed when dosing commenced at GD 10.

There is limited information concerning the expression of PPAR α during embryonic development. PPAR α protein was detected immunohistochemically in the mouse embryo on GD 5, and on GD 11 it was found in the liver, heart, digestive tract, tongue, and vertebrae (Keller et al., 2000). A study by Abbott et al. (2010) investigated the expression of PPAR α mRNA and protein during human fetal development. The study showed that PPAR α is highly expressed in the human fetal liver. In a study by Palkar et al. (2012) no effects were detected following APFO exposure (3 mg/kg bw, GD 1-17) in wild type, in PPAR α KO or in mice where the mouse PPAR α gene had been replaced with the human PPAR α gene. They only found a slight effect on pup viability in the WT mice (~75% of the pups were viable at PND 20), which is far less as compared to the effect (45% viability) that was observed at 1 mg/kg in the same strain (Abbott et al., 2007). However, upon examination of the serum levels of PFOA in the dams on PND 20 in the study by Palkar, they were found comparable to the serum levels of PFOA in dams that had been dosed with 0.3 mg/kg bw in the Abbott study. Thus, the lack of effects in the hPPAR α knock-in (as well as the recorded lesser response in the WT mice) could partly be due to an unexpected low exposure in the study by Palkar. The role and to what extent PPAR α is mediating developmental effects in humans is uncertain; nevertheless, the fact is that it cannot be regarded as irrelevant for humans. PPAR α is present during embryonic development, and is both present and functional in humans. Humans are less sensitive to PPAR α related effects than rodents, with approximately 10-fold lower expression of PPAR α in liver compared to mice (Tilton et al., 2008). However, it is important to remember that the half-life of PFNA in human serum is estimated to be very long (years).

PFOA (and thus most likely PFNA) can also activate other members of the PPAR family. Van de Heuvel and coworkers (2006) reported that PFOA activated both human and murine PPAR γ but at much higher concentration as compared to those needed to activate PPAR α . It has also been reported that PFNA as well as APFO can up-regulate the mRNA expression of PPAR γ (Feng et al., 2008; Takacs and Abbott, 2006). PPAR γ is expressed in a very phase-specific manner during embryo-fetal development. The expression of PPAR γ is increased during fertilization and it then declines during implantation which suggests that it has an important role during early pregnancy (Nishimura et al., 2011). It has also been shown that PPAR γ null embryos die by embryonic day 10 due to placenta alteration, malformed vascular labyrinth and embryonic myocardial thinning (Yang et al., 2008; Barak et al., 1999). One interesting hypothesis is therefore that the full litter resorption observed after APFO and PFNA dosing possibly could be mediated via PPAR γ . This would fit with the early expression pattern of PPAR γ and the observation that full litter loss was only induced if the exposure window was started before implantation.

In conclusion, PFNA caused developmental toxicity; the reported effects are very similar as those reported for APFO/PFOA. In addition PFNA does cross the placenta and has been detected in cord blood as well as in human breast milk. Thus classification of PFNA regarding developmental toxicity in Cat 1B is justified.

Analogue data:

To help the reader, below, the outcome of the RAC assessment has been copied from the Opinion Document for APFO (ECHA Opinion, 2011):

“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 PPAR α -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 PPAR α -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.”

Lactation effects

There is no available information on lactation effects for PFNA. However PFNA has been detected in human breast milk. PFNA and APFO/PFOA have very similar physico-chemical as well as toxicokinetic properties and this justifies that the classification for PFNA is based on read-across from data for APFO/PFOA. To aid the reader of this CLH report we have therefor copied text from the Opinion Document for APFO (ECHA Opinion, 2011) that was produced during the classification process of APFO and PFOA by the Committee for Risk Assessment (RAC) at ECHA.

“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 (H 362: May cause harm to breast-fed children and R64 May cause harm to breastfed babies).”

4.10.5 Conclusions on classification and labeling

No classification of PFNA for fertility is proposed.

The resulting classification for developmental toxicity for PFNA is Repr. 1B (H360D)

The resulting classification of lactation effects for PFNA is H362.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter’s proposal

The DS proposed to classify PFNA and its sodium and ammonium salts as Repr. 1B, H360D (May damage the unborn child) and Lact., H362 (May cause harm to breast-fed children) based on some data on reproductive toxicity of PNFA as well as on read-across from APFO/PFOA.

Comments received during public consultation

Four MSCAs supported classification of PFNA and its sodium and ammonium salts as Repr. 1B, H360D and Lact., H362 as proposed by the DS. No objection to this proposal was made during public consultation.

Additional key elements

NONE

Assessment and comparison with the classification criteria

Fertility

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 PPAR α 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 (mPPAR α), *Ppara*-null and PPAR α -humanized (hPPAR α) for 6 weeks. Both low- and high-dose APFO exposure significantly

reduced plasma testosterone concentrations in mPPAR α and hPPAR α , 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 mPPAR α , *Ppara*-null and hPPAR α mice 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 mPPAR α mice (1.4- and 1.5 fold in comparison with frequency of sperm morphology abnormalities in respective control mice (ca. 7%) and in hPPAR α 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 mPPAR α and hPPAR α mice. Necrotic cells were also observed in the testes of mPPAR α mice after 5 mg/kg APFO exposure. However, no obvious effects of APFO treatment were morphologically observed in the testes of *Ppara*-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 hPPAR α mice than mPPAR α and *Ppara*-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 mPPAR α mice, and at the low and high dose in the testis of hPPAR α mice. PBR mRNA level was not affected by APFO treatment, except in hPPAR α 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 hPPAR α mice than in mPPAR α and *Ppara*-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 PPAR α 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 mPPAR α and hPPAR α mice. However, these findings were not observed in *Ppara*-null mice. It shows that activation of peroxisome proliferator-activated receptor alpha (PPAR α) by APFO is an essential step in induction of toxicity in testes.

PPAR α is expressed in interstitial Leydig cells or seminiferous tubule cells of testis in mPPAR α mice, but not in the testis in hPPAR α , similarly to *Ppara*-null mice (Cheung *et al.*,

2004).

Nevertheless, APFO caused reproductive impairment in hPPAR α mice similar to that seen in mPPAR α mice, suggesting that some toxic molecule(s) such as reactive oxidative species (ROS) molecules due to activation of hepatic PPAR α may be produced in the liver and circulated in the body, because a common point between mPPAR α and hPPAR α mice was that both had PPAR α 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 PPAR α , 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 ($10^6/g$) 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 ($10^6/g$) in the left epididymis was not affected by S-111-S-WB treatment. No pathological changes were observed in histopathological 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 histopathological 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 (mPPAR α) and hPPAR α 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 *Ppara*-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, *Ppara*-null and PPAR α -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.

Developmental toxicity

In Annex VI to the CLP Regulation, APFO and PFOA, used as reference substances in a 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 on gestation day (GD) 1-17 with PFNA at 0, 1, 3 or 5 mg/kg/d. 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 caesarean-section at dose levels up to and including 5 mg/kg/d. However, decreased pup viability was already observed at the first examination after birth in the 5 mg/kg/d group. Over the course of the first 12 days after birth there was a continuous loss of pups, and at post natal day (PND) 12, ~80 % of the pups had died. In written communication with Dr. Lau (study author), the DS was 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/SvImJ wild-type (WT) and PPAR α 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/d. 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 PPAR α 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 PPAR α (Wolf *et al.*, 2010). PPAR α is a nuclear receptor that plays a role in regulating lipid and glucose homeostasis, cell proliferation and differentiation, and inflammation. However, the role of PPAR α 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.

Lactation

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 Lact. H362. PFNA and APFO/PFOA have very similar structure, physico-chemical as well as toxicokinetic properties and this justifies that the classification for PFNA can also be based on read-across from data for APFO/PFOA.

There are however also studies indicating that PFNA, similarly to its structural analogs PFOA and APFO, can induce effects on or via lactation.

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.**

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

6 OTHER INFORMATION

Not applicable

7 REFERENCES

Abbott, B., Wolf, C., Schmid, J., Das, K., Zehr, R., Helfant, L., Nakayama, S., Lindstrom, A., Strynar, M., and Lau, C., 2007. Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha. *Toxicol. Sci.*, 98(2), 571-581.

Abbott, B., Wood, C., Watkins, A., Das, K., and Lau, C., 2010. Peroxisome proliferator-activated receptors alpha, beta and gamma mRNA and protein expression in human fetal tissues. *PPAR Res.*, vol. 2010, art ID 690907, 19pg; Doi: 10.1155/2010/690907.

Barak, Y., Nelson, M., Ong, E., Jones, Y., Ruiz-Lozano, P., Chien, K., Koder, A., and Evans, R., 1999. PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol. Cell.*, 4(4), 585-595.

Benskin, J., De Silva, A., Martin, L., Arsenault, G., McCrindle, R., Riddell, N., Mabury, S., and Matin, J., 2009. Disposition of perfluorinated acid isomers in Sprague-dawley rats; part1: single dose. *Environ. Toxicol. Chem.*, 28(3), 542-554.

Bookstaff, R., Moore, R., Ingall, G., and Peterson, R., 1990. Androgenic deficiency in male rats treated with perfluorodecanoic acid. *Toxicol. Appl. Pharmacol.* 104, 322-333.

Chen, M., Ha, E.H., Wen, T.W., Su, Y.N., Lien, G.W., Chen, C.Y., Chen, P.C., and Hsieh, W.S., 2012. Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. *PLoS ONE* 7(8): e42474. Doi:10.1371/journal.pone.0042474.

Dewitt, J., Peden-Adams, M., Keller, J., and Germolec, D., 2012. Immunotoxicity of perfluorinated compounds: recent developments, *Toxicol. Pathol.*, 40(2), 300-311.

ECHA Background, 2011. Committee for Risk Assessment RAC, Annex 1 Background document to the Opinion proposing harmonized classification and labeling at Community level of Ammoniumpentadecafluorooctanoate (APFO), ECHA/RAC/ CLH-O-0000002225-82-01/A1. Helsinki: European Chemicals Agency.

ECHA Opinion, 2011. Committee for Risk Assessment RAC, Opinion proposing harmonized classification and labeling at Community level of Ammoniumpentadecafluorooctanoate (APFO), ECHA/RAC/DOC No CLH-O-0000002225-82-01/F. Helsinki: European Chemicals Agency. Available at <http://echa.europa.eu/documents/10162/4d9637d4-a066-4bdb-a014-9ee5fcb0b676>

ECHA Opinion, 2011. Committee for Risk Assessment RAC, Opinion proposing harmonized classification and labeling at Community level of pentadecafluorooctanoate (PFO), ECHA/RAC/DOC No CLH-O-0000002227-78-01/F. Helsinki: European Chemicals Agency. Available at <http://echa.europa.eu/documents/10162/e7f15a22-ba28-4ad6-918a-6280392fa5ae>

Fang, X., Zhang, L., Feng, Y., Zhao, Y., and Dai, J., 2008. Immunotoxic effects of perfluorononanoic acid on BALB/c mice. *Toxicol. Sci.*, 105(2) 312-321.

Fang, X., Feng, Y., Wang, J., and Dai, J., 2010. Perfluorononanoic acid-induced apoptosis in rat spleen involves oxidative stress and the activation of caspase-independent death pathway. *Toxicol.*, 267, 54-59.

Feng, Y., Zhimin, S., Xuemei, F., Xu, M., and Dai, J., 2009. Perfluorononanoic acid induces apoptosis involving the Fas death receptor signaling pathway in rat testis. *Toxicol. Let.* 190(2), 224-230.

Griffith, FD., and Long, JE., 1980. Animal toxicity studies with ammonium perfluorooctanoate. *AM. Ind. Hyg. Assoc. J.*, 41, 576-583.

Harada, K., Inoue, K., Morikawa, A., Yoshinaga, T., Saito, N., and Koizumi, A., 2005. Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. *Environ. Res.*, 99, 253-261.

Heuvel, J., Thompson, J., Frame, S., and Gillies, P., 2006. Differential activation of nuclear receptors by perfluorinated fatty acids analogs and natural fatty acids: a comparison of human, mouse, and rat peroxisome proliferator-activated receptor- α , - β , and - γ , liver X receptor- β , and retinoid X receptor- α . *Toxicol. Sci.*, 92(2), 476-489.

Keller, J., Collet, P., Bianchi, A., Huin, C., Bouillaud-Kremarik, P., Becuwe, P., Schohn, H., Domenjoud, L., and Dauca, M., 2000. Implications of peroxisome proliferator-activated receptors (PPAR) in development, cell life status and disease. *Int. J. Biol.*, 44, 429-442.

Kärman, A., Ericson, I., van Bavel, B., Darnerud, PO., Aune, M., Glynn, A., Lignell, S., and Lindström, G., 2007. Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996-2004, in Sweden. *Environ. Health Perspect.*, 115(2), 226-230.

Lau, C., Thibodeaux, J., Hansin, R., Narotsky, M., Rogers, J., Lindstrom, A., and Strynar, M., 2006. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicol. Sci.*, 90(2), 510-518.

- Lau, C., Das, K., Tatum, K., Zehr, D., Wood, C., and Rosen, M., 2009. Developmental toxicity of perfluorononanoic acid in the mouse. *Toxicologist*, 108, 417.
- Liu, J., Li, J., Liu, Y., Chan, H.M., Zhao, Y., Cai, Z., and Wu, Y., 2011. Comparison on gestation and lactation exposure of perfluorinated compounds for newborns. *Environ. Intern.*, 37, 1206-1212.
- Lou, I., Wambaugh, J., Lau, C., Hanson, R., Lindstrom, A., Strynar, M., Zehr, R., Setzer, R., and Barton, H., 2008. Modeling single and repeated dose pharmacokinetics of PFOA in mice. *Toxicol. Sci.*, 107(2), 331-341.
- Macon, M., Villanueva, L., Tatum-Gibbs, K., Zehr, R., Strynar, M., Stanko, J., White, S., Helfant, L., and Fenton, S., 2010. Prenatal perfluorooctanoic acid exposure in CD-1 mice: low dose developmental effects and internal dosimetry. *Toxicol. Sci.*, 122(1), 134-145.
- Nishimura, K., Yamauchi, N., Chowdhury, V., Torii, M., Hattori, M., and Kaneto, M., 2011. Expression of peroxisome proliferator-activated receptors isoforms in the rat uterus during early pregnancy. *Cell Tissue Res.*, 345(2), 275-284.
- Ohmori, K., Kudo, N., Katayama, K., and Kawashima, Y., 2003. Comparison of the toxicokinetics between perfluorocarboxylic acids with different carbon chain length. *Toxicol.*, 184, 135-140.
- Olsen, G., Burris, J., Ehresman, D., Froehlich, J., Seacat, A., Butenhoff, J., and Zobel, L., 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ. Health Perspect.*, 115, 1298-1305.
- Palkar, P., Anderson, C., Ferry, C., Gonzalez, F., and Peters, J., 2010. Effect of prenatal peroxisome proliferator-activated receptor α (PPAR α) agonism on postnatal development. *Toxicol.*, 276, 79-84.
- Palkar, P., Torsell, N., Krishnan, P., Ehresman, D., Frame, S., Chang, S., Butenhoff, J., Kennedy, G., Gonzalez, F., and Peters, J., 2012. A species difference in the peroxisome proliferator-activated receptor α (PPAR α)-dependent response to the developmental effects of perfluorooctanoic acid. *Toxicol. Sci.*, advance access, <http://toxsci.oxfordjournals.org>
- Prevedouros, K., Cousins, I., Buck, R., Korzeniowski, S., 2006. Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol.*, 40, 32-44.
- Schechter, A., Malik-Bass, N., Calafat, A., Kato, K., Colacino, J., Gent, T., Hynan, L., Harris, T., Malla, S., and Birnbaum, L., 2012. Polyfluoroalkyl compounds in Texas children from birth through 12 years of age. *Environ. Health Perspec.*, 120(4), 590-594.
- Shi, Z., Zhang, H., Lui, Y., Xu, M., and Dai, J., 2007. Alterations in gene expression and testosterone synthesis in the testes of male rats exposed to perfluorododecanoic acid. *Toxicol. Sci.*, 98, 206-215.
- Swerea IVF Project report 09/46, 2009. Survey of the extent of use and occurrence of PFNA (perfluorononanoic acid) in Norway. Mölndal Sweden: Swerea IVF, TA-2562/2009.
- Tao, L., Kannan, K., Wong, C., Arcaro, K., and Butenhoff, J., 2008. Perfluorinated compounds in human milk from Massachusetts, USA. *Environ. Sci. Technol.*, 42, 3096-101.
- Tatum-Gibbs, K., Wambaugh, J., Das, K., Zehr, R., Strynar, M., Lindstrom, A., Delinsky, A., and Lau, C., 2011. Comparative pharmacokinetics of perfluorononanoic acid in rat and mouse. *Toxicol.*, 281, 48-55.

Tilton, S., Orner, G., Benninghoff, A., Carpenter, H., Hendricks, J., Pereira, C., and Williams, D., 2008. Genomic profiling reveals an alternate mechanism for hepatic tumor promotion by perfluorooctanoic acid in rainbow trout. *Environ. Health Perspect.*, 116, 1047-1055.

Van den Henderson, W.M., and Smith, M.A., 2006. Perfluorooctanoic acid and perfluorononanoic acid in fetal and neonatal mice following in utero exposure to 8-2 fluorotelomer alcohol. *Toxicol. Sci.*, 95(2), 452-461.

Wei, Y., Dai, J., Lui, M., Wang, J., Xu, M., Zha, J., and Wang, Z., 2007. Estrogen-like properties of perfluorooctanoic acid as revealed by expressing hepatic estrogen-responsive genes in rare minnows (*Gobiocypris rarus*). *Environ. Toxicol. Chem.*, 26, 2440-2447.

White, S., Calafat, A., Kuklennyik, Z., Villanueva, L., Zehr, R., Helfant, L., Strynar, M., Lindstrom, A., Thibodeaux, J., Wood, C., and Fenton, S., 2007. Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. *Toxicol. Sci.*, 96(1), 133-144.

White, S., Kato, K., Jia, L., Basden, B., Calafat, A., Hines, E., Stanko, J., Wolf, C., Abbott, B., and Fenton, S., 2009. Effects of perfluorooctanoic acid on mouse mammary gland development and differentiation resulting from cross-foster and restricted gestational exposures. *Reprod. Toxicol.*, 27, 289-298.

Wolf, C., Fenton, S., Schmid, J., Calafat, A., Kuklennyik, Z., Bryant, X., Thibodeaux, J., Das, K., White, S., Lau, C., and Abbott, B., 2007. Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. *Toxicol. Sci.*, 95(2), 462-473.

Wolf, C., Takacs, M., Schmid, J., Lau, C., and Abbott, B., 2008. Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. *Toxicol. Sci.*, 106(1), 162-171.

Wolf, C., Zehr, R., Schmid, J., Lau, C., and Abbott, B., 2010. Developmental effects of perfluorononanoic acid in the mouse are dependent on peroxisome proliferator-activated receptor-alpha. *PPAR Res.*, doi:10.1155/2010/282896.

Yang, J., Chen, L., Zhang, X., Zhou, Y., Zhang, D., Huo, M., and Guan, Y., 2008. PPARs and female reproduction: evidence from genetically manipulated mice. *PPAR Res.*, doi: 10.1155/2008/723243.

Yang, Q., Kurotani, R., Yamada, A., Kimura, S., and Gonzalez, F., 2006. Peroxisome proliferator-activated receptor α activation during pregnancy severely impairs mammary lobuloalveolar development in mice. *Endocrin.*, 147(10), 4772-4780.

Additional references

Additional references not included in the CLH report

DeWitt JC, Copeland CB, Strynar MJ, Luebke RW (2008). *Environmental Health Perspective* 116: 644- 650.

Dewitt JC, Copeland CB, Luebke RW (2009). *Toxicol Sci* 109:106-112.

Goss K-U (2008). *Environ Sci Technol*, 42: 456 – 458.

Kleszczynski K, Gardzielewski P, Mulkiwicz E, Stepnowski P, Skladanowski AC (2007). Analysis of structure–cytotoxicity in vitro relationship (SAR) for perfluorinated carboxylic acids. *Toxicol. In Vitro* 21, 1206–1211.

Nordström Joensen U, Bossi R, Leffers H, Jensen A, Skakkebaek N (2009). Do perfluoroalkyl compounds impair human semen quality? *Environ. Health Perspectives* 117/6: 923-927.

Purchase IHF (1994). *Human & Experimental Toxicology*, 13, Suppl.2 S47-S48