Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Perfluorononan-1-oic acid [1];
(2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro nonanoic 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

Adopted

12 September 2014

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12 September 2014

CLH-O-0000004708-66-03/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemicals name: **Perfluorononan-1-oic acid [1] (2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluorononanoic acid) and its sodium [2] and ammonium [3] salts**

EC number: 206-801-3 [1];

CAS number: 375-95-1 [1];

The proposal was submitted by **Sweden** and received by the RAC on **11 December 2013.**

In this opinion, all classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS). The classification notation for 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer provided.

PROCESS FOR ADOPTION OF THE OPINION

Sweden has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at http://echa.europa.eu/harmonised-classification-and-labelling-consultation on **13 December 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **27 January 2014**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling was reached on 12 September 2014 and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **12 September 2014.**

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion that **Perfluorononanoic acid and its sodium and ammonium salts** should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	**No current Annex VI entry**										
Dossier submitters proposal	607-718-00-9	perfluorononan-1-oic acid [1] and its sodium [2] and ammonium [3] salts	206-801-3 [1]; - [2]; - [3]	375-95-1 [1]; 21049-39-8 [2]; 4149-60-4 [3]	Carc. 2 Repr. 1B STOT RE 1 Acute Tox. 4 Acute Tox. 4 Eye Dam. 1 Lact.	H351 H360D H372 (liver) H302 H332 H318 H362	GHS05 GSH07 GSH08 Dgr	H351 H360D H372 (liver) H302 H332 H318 H362			
RAC opinion	607-718-00-9	perfluorononan-1-oic acid [1] and its sodium [2] and ammonium [3] salts	206-801-3 [1]; - [2]; - [3]	375-95-1 [1]; 21049-39-8 [2]; 4149-60-4 [3]	Carc. 2 Repr. 1B STOT RE 1 Acute Tox. 4 Acute Tox. 4 Eye Dam. 1 Lact.	H351 H360Df H372 (liver, thymus, spleen) H302 H332 H318 H362	GHS05 GSH07 GSH08 Dgr	H351 H360Df H372 (liver, thymus, spleen) H302 H332 H318 H362			

Resulting Annex VI entry if agreed by COM	607-718-00-9	perfluorononan-1-oic acid [1] and its sodium [2] and ammonium [3] salts	206-801-3 [1]; - [2]; - [3]	375-95-1 [1]; 21049-39-8 [2]; 4149-60-4 [3]	Carc. 2 Repr. 1B STOT RE 1 Acute Tox. 4 Acute Tox. 4 Eye Dam. 1 Lact.	H351 H360Df H372 (liver, thymus, spleen) H302 H332 H318 H362	GHS05 GSH07 GSH08 Dgr	H351 H360Df H372 (liver, thymus, spleen) H302 H332 H318 H362			

SCIENTIFIC GROUNDS FOR THE OPINION

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 $-CF_2-$ group in the fluorine substituted aliphatic chain.

HUMAN HEALTH HAZARD ASSESSMENT

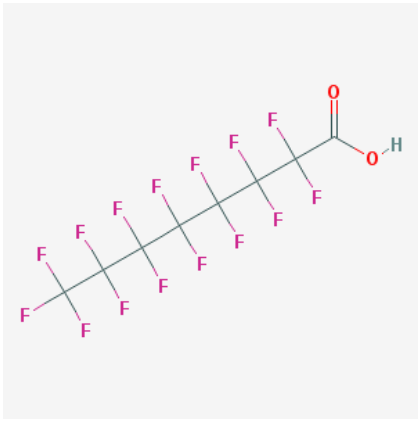
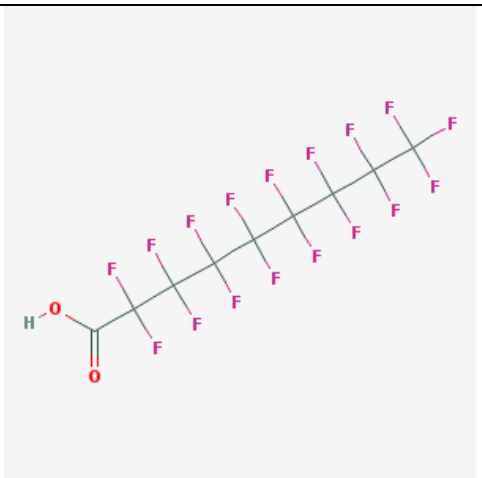
RAC evaluation of acute toxicity

Summary of the Dossier submitter's (DS) proposal
The Dossier Submitter proposes to classify 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Heptadecafluoro-nonanoic 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 opinion[1] [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)

	
(PFOA) 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid	(PFNA) 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Heptadecafluorononanoic acid

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 LD_{50} 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 LC_{50} of 0.98 mg/L (4 hours exposure) was established, a result which is borderline between Category 3 and Category 4. Another LC_{50} 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 LC_{50} 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 LD_{50} and LC_{50} 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 LD_{50} for APFO from two rabbit studies and one rat study were above 2000 mg/kg.

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 then 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 perfluorated 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.*"

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 PNF-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 perifluorinated 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 EC_{50} 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 perifluorinated 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).

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 (Schecter *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 (C_6-C_{13}), known as S-III-S-WB, was administered orally to Crl: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 ß-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 ß-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 ß-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 (C_8), PFNA (C_9), PFUDA (perfluoroundecanoic acid,C_{11}), and PFTDA (perfluorotridecanoic acid (C_{13}) 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 C_6 acid (PFHA) (males) and C_9 (PFNA) (females), and via the faeces for acid with longer chain C_{11} (PFUDA) and C_{13} (PFTDA).

The no-observed-effect level (NOEL) for a mixture of perfluoro fatty acid ammonium salts (C_6-C_{13}) 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 ß-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 IkBα and IkBα 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, IFNα, and H_2O_2, but decreased levels of IFNγ 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.

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 PNFA, 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).

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.

Assessment and comparison with the classification criteria

<u>Fertility</u>

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

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

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

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

Oral APFO-treatment (0, 1 and 5 mg/kg bw/day) of mPPARα, *Pparα*-null and hPPARα□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 mPPARα mice (1.4- and 1.5 fold in comparison with frequency of sperm morphology abnormalities in respective control mice (ca. 7%) and in hPPARα mice (1.3- and 2.6 fold in comparison with frequency of sperm morphology abnormalities in respective control mice (ca. 7%). The types of abnormalities observed were not described.

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

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

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

Cholesterol transport

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

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

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

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

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

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

In the mechanistic study of Feng *et al.* (2009), male SD rats were exposed by gavage to PFNA at doses of 0, 1, 3 and 5 mg/kg bw/d for 14 days. In this study, a dose-dependent increase in the number of apoptotic cells was observed. No sperm cell counts were done in this study. In the histological examination of testes from rats exposed to 5 mg/kg bw PFNA, the spermatogenic cells exhibited apoptotic features, namely crescent chromatin condensation and chromatin margination. To evaluate the impact of PFNA on germ cell survival, testes sections were examined for DNA fragmentation indicative of cell death using the TUNEL staining (terminal deoxynucleotide transferase mediated dUTP-biotin nick end labeling). Seminiferous tubules of control animals had very few TUNEL-positive cells, indicating very low level germ cell attrition in normal testes. In the 1 mg PFNA/kg/d group, only a few TUNEL-positive cells were observed, but this staining was more pronounced and the TUNEL-positive cells were increased in testes of animals receiving 3 and 5 mg PFNA/kg/d. The TUNEL-positive germ cells were mainly spermatocytes and spermatogonia, and these cells seemed to be initially more susceptible to PFNA toxicity. No quantitative data on numbers of observed TUNEL-positive cells were provided.

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

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

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

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

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

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

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

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

In the oral 2-generation reproductive toxicity study using S-111-S-WB in rats (Stump *et al.*, 2008) no effect on fertility was observed. S-111-S-WB (fatty acids C6–C18, perfluoro, ammonium salts, CAS No. 72968-38-8) is a mixture of perfluorinated fatty acid ammonium salts of different carbon chain lengths that is used a surfactant in polymer manufacturing. The major component of S-111-S-WB is PFNA, although detailed information on content of various constituents was not provided. S-111-S-WB was administered daily via oral gavage to 30 Crl:CD(SD) rats/sex/group at doses of 0.025, 0.125 and 0.6 mg/kg/d over two generations to assess potential reproductive toxicity.

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

Sperm concentration (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 histopatological examinations of testes of F0 and F1 male rats.

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

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

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

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

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

The proposal for classification of PFNA, PFN-S and PFN-A as Repr. 2, H361f (Suspected of damaging fertility) is further supported by preliminary human data. In the study of Nordström Joensen *et al.* (2009), a group of 105 young adult men reporting for military draft in Denmark was examined to discover the possible association between the levels in serum of perfluoroalkyl acids (PFAA) and testicular function. The serum level of 10 different PFAA with carbon chain length from C6 to C13 was examined. Out of all examined PFAAs, the highest concentrations were found for perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), PFOA and PFNA (medians of 24.5, 6.6, 4.9, and 0.8 ng/mL, respectively). The high serum concentrations of PFAAs were significantly associated with reduced numbers of normal spermatozoa. In addition, sperm concentration, total sperm count, and sperm motility showed some tendency toward lower levels in men with high PFAA levels, although not at statistically significant levels. The authors noted that the results from this preliminary study should be corroborated in larger studies.

Taking into account

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

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

In the opinion of RAC, the existing evidence is not sufficient to classify PFNA, PFN-S and PFN-A as Repr.1B, H360F (May damage fertility), because the effect on the sperm count was observed only in the F0 generation, but not in F1 males exposed to a mixture of perfluorinated fatty acid ammonium salts of different carbon chain lengths in a 2-generation study (Stump *et al.*, 2008) and the epididymal sperm count was not affected in wild-type, *Pparα*-null and PPARα-humanized mice exposed orally to APFO for 6 weeks. The fact that PFOA and APFO, were not classified for sexual function and fertility (due to negative results of a 2-generation study with APFO; York, 2002, Butenhoff *et al.*, 2004; and the lack of supporting evidence from repeated dose toxicity

studies, which gave no indication of disturbances of fertility) in the RAC opinion (2 December 2011) was also considered.

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/SvlmJ wild-type (WT) and PPARα knockout (KO) mice were given PFNA by oral gavage once daily on GD 1−18 at 0, 0.83, 1.1, 1.5 and 2 mg/kg/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 PPARα is an essential mediator of PFNA-induced developmental toxicity in the mouse.

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

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

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

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

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.**

Additional references

Additional references not included in the CLH report

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ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and rapporteurs' comments (excl. confidential information).