Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)
to the 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-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

Adopted
30 September 2014

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON HEPTADECAFLUORONONANOIC ACID AND ITS SODIUM AND AMMONIUM SALTS

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

ECHA accepts no responsibility or liability for the content of this table.

Substance name: heptadecafluorononanoic acid and its sodium and ammonium salts
CAS number: 375-95-1,21049-39-8,4149-60-4
EC number: 206-801-3
Dossier submitter: Sweden

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
23.01.2014	Netherlands		MemberState	1
Comment received				
The classification of PFNA and its salts was based on a read-across from perfluorooctanoic acid (PFOA) and its ammonium salt, ammoniumpentadecafluorooctnoate (APFO). PFOA is an analogue to PFNA, which contains one less carbon and two less fluorines. The RAC has recently adopted the proposed classification of PFOA and APFO as Carc. 2 (H351), Repr. 1B (H360D), Lact. (H362), STOT RE 1 (liver, H372), Acute Tox. 4 inhalation (H332), Acute Tox. 4 oral (H302), and Eye Dam. 1 (H318) (ECHA, 2011a, b). We suggest inclusion of some more specific approaches for read-across depending on whether the effects are systemic requiring evidence-based classification (CMR) or local or systemic requiring potency-based classification (STOT, acute and irritation). Here, we suggest applying a similar justification as was made for using data on APFO for a read-across and classification of PFOA (ECHA, 2011b). For systemic effects requiring evidence-based classification, it is assumed that the different forms (salts and acids) will form the same ion after reaching the stomach or the lung fluid. This is in line with the justification applied when using APFO data as a read-across for classification of PFOA in the PFOA CLH report (p.6) (ECHA, 2011b): 'For systemic effects it might be assumed that both substances (APFO and PFOA) are mainly available to cells with its physiological pH in form of the corresponding anion (PFO). That might be the central justification for read-across for systemic effects. For hazard classes with criteria based on the level of evidence such as CMR, small differences in molecular weight and physical/chemical properties may result in some differences in absorption and the number of molecules becoming available for interaction with the molecular target, but these normally may not be important because they affect only the potency and not the effect. However, 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, and the argument that: "For local effects available literature indicates that PFOA and APFO in water yield acidic pH values. The differences in the pH values are considered small and therefore read-across for				

local effects is considered relevant." (PFOA CLH report (p.6) (ECHA, 2011b)) is not applicable to PFNA and its salts.
Therefore, for potency-driven hazard classes, a correction for the difference in molecular weight could be considered, as well as differences in kinetics may be important with regard to uptake and distribution. The different pKa's (PFNA -0.15 and PFOA 2.8) could also be relevant. Altogether, these factors should be taken into account when applying a read-across approach for the classification of PFNA and its salts. We agree with the use of read-across for evidence-based systemic effects (CMR) but based on the currently provided data, not for all the hazard classes.

For PFNA, a clear distinction between local and systemic effects should be made in conjunction with criteria outlined in the OECD guidelines for grouping chemicals (OECD, 2007). The following should be considered:
• Given the limited physical/chemical properties data provided (p. 12 CHL Report), caution should be taken when making conclusions on similarities in physical/chemical properties (p. 7 and 16, CHL Report)? In Table 9, only data on state of substance at 200C and 101.3 kPa, melting/freezing point, and boiling point are provided. The density is presented in Table 21 (p. 44, CLH Report) but not in Table 9 (p.12, CLH Report). Please comment on the implications of having different pKa's (PFNA -0.15 and PFOA 2.8).
• Please provide pH comparisons due to their relevance to local effects.
• Read-across should be applied and justified for each endpoint individually, starting with the substance for which there is actual data and providing a suitable argument for its applicability in the read-across for PFNA and its salts individually.

Dossier Submitter's Response

Read-across –

Thank you for your comments. We agree that we in our justification should have been more specific with argumentation for why read across is justified for both systemic and local effects and we realize that the data for physical chemical properties should have been presented more clearly and in a similar way at the two locations in the dossier.

The majority of the comments received agree that read across for systemic effects are justified but question the read across for local effects. We agree that read across for local effects depends on many factors and welcome a discussion at RAC on what data is required to justify a read across. For PFNA and its salts there is only very limited information available on measured physicochemical properties and therefore we provide tables (see end of this RCOM document) that summarize the available information from some reports from governmental agencies and the CLH dossiers on PFOA and APFO (the ammonium salt of PFOA). In addition, predicted values on selected endpoints (using the EPI suite, ChemID plus and in some cases also the the SPARC software) are also included to help in the discussion.

However, we think that some of the reservations on the read across proposal for local effects was partly due the fact that it was not clear in the PFNA dossier that the stated pKa value for PFOA (2.8) was a measured value (in 50/50 alcohol/water mixture), whereas the pKa value for PFNA was a calculated value (due to spelling error the pKa was stated as being -0.15 in the current dossier but should have been -0.17. This value originates from the Agency for Toxic Substances and Disease Registry Draft report "Toxicological Profile for Perfluoroalkyls " and was predicted using the SPARC software). We have not been able to find a measured pKa value for PFNA. However, if one compares the calculated pKa values for PFNA (-0.21) and PFOA (-0.11) using the same software (SPARC) they are very similar. Goss (Goss, K-U., Environ Sci Technol, 42: 456 – 458, 2008.) investigated, by using two different pKa prediction soft wares (SPARC and COSMO-RS), how the pKa value was

influenced by the chain length of the perfluorinated carboxylic acids (C_4 vs C_{12}). As can be seen 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 used softwares .

Compound	**pKa (SPARC)**	**pKa (COSMO-RS)**
$F(CF_2)_3COOH$	0.4	0.7
PFOA - $F(CF_2)_7COOH$	-0.1	0.7
PFDoDA - $F(CF_2)_{11}COOH$	-0.2	0.8

The conclusion would therefore be that the pKa value for PFOA and PFNA (C_9) most likely will be very similar. It should be mentioned that estimated pKa values using the ChemId plus software are somewhat different from the values predicted by the SPARC software (i.e pKa of PFOA and PFNA are -4.2 and -6.51, respectively). However both these acids are extremely strong acids and are virtualy completely protolyzed in water, i.e. the pH will only depend on the concentration of the acid. In addition it is worth mentioning that the experimental determination of the the pKa values of perifluorinated compounds is difficult since the chemical structure of these compounds renders them both lipid and hydrorepellent and sorption to interfaces such as the watersurface or the walls of glass vessels may occur to an extent that is unknown for "ordinary" molecules (Goss, 2008).
Both PFNA and PFOA are very strong acids, as indicated by their very low pKa values, and both substances will therefore be available to tissues in form of the corresponding carboxylate anion.
There is no measured pH value available for the ammonium salt of PFNA. In water the pH values for the ammonium salts will be determined by the ammonium ion and thus it seems reasonable to assume that the pH value of the ammonium salt of PFNA will be the same as the one for the ammonium salt of PFOA (see Table 2A) . Thus, from a perspective of possible differences in pH, read across for local effects from the ammonium salt of PFOA (APFO) to the ammonium salt of PFNA would be justified as well as the following read across to PFNA. 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) are in the same range and the ammonium salt and sodium salt should be more soluble. The predicted values for water solubility of PFNA and PFOA (see Table 3, page 15 of this document) are much lower (in the mg/l or µg/l range, depending on prediction model. Thus the solubilty differs 10 to 20-fold between PFOA and PFNA (no information on the salt). The reason for the discrepancies between the measured values for PFNA and PFOA and the estimated values are most likely due to the nature of these compounds.
However, overall the solubility of PFNA and PFOA seems not to differ extensively if one compares data that originates from the same method of measurement/ prediction model. Thus also from a solubility perspective read across seems to be overall justified at least between PFOA and PFNA.

RAC's response

In the response the DS provided sufficient arguments to justify the read-across from local effects of PFOA/APFO to PFNA and its sodium (PFN-S) and ammonium (PFN-A) salts.
There is no information on measured pH for PFNA and its salts. However, the calculations of pH using estimated pKa values provided by the DS (see Table 2A and 2B attached to this document 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, and that the pH of a APFO solution will also be the same as that of an equimolar solution of the ammonium salt of PFNA.
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. The solubility of PFNA and PFOA seems not to differ extensively if one compares data that originates from the same method of measurement/prediction model. Thus, from a solubility perspective, read across also seems to be overall justified at least between PFOA and PFNA. Taking the above considerations into account, RAC is of the opinion that read-across of the eye corrosive property from APFO to PFNA and its sodium and ammonium salts is justified based on similarity of their structure and physicochemical properties and that these substances should be classified as Eye Dam. 1, H318 (Causes serious eye damage).

Date	Country	Organisation	Type of Organisation	Comment number
23.01.2014	Norway		MemberState	2
Comment received				
Norway would like to thank Sweden for the proposal for harmonised classification and labeling of PFNA and its sodium and ammonium salts, CAS- no. 375-95-1, 21049-39-8 and 4149-60-4. We support the proposal to classify PFNA and its sodium and ammonium salts based on a read across to PFOA/APFO. PFOA and PFNA are both acids that structurally only differentiate in an added carbon and two fluorines, both have a long half-time life in the human body and very similar toxicokinetics in exposed animals. Further, the very limited data on PFNA shows the same effects as those observed for PFOA/APFO. The similarities between PFNA and PFOA/APFO should be sufficient to justify a read across approach.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Support and justification of read-across from PFOA/APFO to PNFA and its sodium and ammonium salts is noted. It is in line with the justification of RAC.				

Date	Country	Organisation	Type of Organisation	Comment number
27.01.2014	France		MemberState	3
Comment received				
PFNA and PFOA present a very close chemical structure. Available toxicokinetic data supports similarity of the in vivo behaviour of both substances with comparable sex-species differences in the rat, preferential distribution in the liver and similar capacity of crossing placenta and excretion in milk. The half-life measured in mice are longer with PFNA than with PFOA, which tend to show that the capacity to accumulate with repeated exposure may be higher for PFNA whereas this value in humans for PFOA is already very long (3.8 years). Related to systemic effects of PFNA and PFOA, the few studies available with PFNA support similarity of toxic systemic effects: - Target organs in rats and mice are liver and organs of the immune system and effects occur at the same range of dose. - Liver effects are in part mediated through activation of the PPARα Some evidence show that PPARγ may also to play a role and other mechanisms of action are not excluded. - In WT mice, similar developmental effects are observed and include effect on postnatal viability, pup weight and delay in eye opening and alteration in puberty onset (that cannot				

be fully explained by body weight reduction in the case of PFOA, analysis not available for PFNA).
It is noted that in PFNA studies there is some but no clear evidence that this substance induces resorptions, in particular in PPARα knock-out- mice, whereas this effect is observed both in WT and KO mice at higher doses with PFOA. However, the highest PFNA doses in Wolf (2010) using WT and KO mice do not explore the full relevant dose range. Besides, the role of PPARα in mediating developmental effects in humans is not excluded and the overall developmental profile of PFNA supports its classification as Repr 1B – H360D supported by analogy with PFOA.

Overall, the available data on PFNA supports the similarity of toxicological systemic properties with PFOA and FR supports the read-across of systemic effects of PFOA to PFNA. In line with the conclusion of RAC for PFOA, FR therefore agrees with classification STOT RE 1 (liver) – H372, Carc 2 – H351, Repr 1B – H360D, Lact - H362.

The CLH report also proposes to read-across classification for local effects such as eye irritation as well as for acute toxicity that may be linked to both systemic and local effects of substances.
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.

FR supports the read-across for systemic effects but is therefore requesting more read-across data for justifying the read across for local effects.

Dossier Submitter's Response

Thank you for your support for the read across for systemic effects. Please see dossier submitter's response under comment number 1 for response regarding read across for local effects.

RAC's response

Your support for read across for systemic effects is noted. It is in line with the RAC opinion. Regarding read-across for local effects, the DS provided additional data which also substantiate the read-across of eye corrosive property from APFO to PFNA and its sodium (PFN-S) and ammonium (PFN-A) salts. For more details, see response to comment no. 1.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2014	Germany		MemberState	4

Comment received

Read across:
PFNA does not appear to be registered as well as the sodium and ammonium salts. The dossier submitter chose a read-across approach based on structural similarity of PFNA (and the salts) with PFOA (and its salts). Though the German CA generally agrees with the approach, we feel that the justification or the presentation of the read-across applicability could be further improved. It would be helpful to have a statement on the read across approach in the beginning of the human health section, best with the phys/chem data in the beginning as it is referred to the similarity in every chapter. A short comparison of the toxicological endpoints for which data with the target and the source substance are available would be appreciated. For the acceptance of the read across approach comparison of toxicokinetics (TK) of target and source substance is important, more so as for PFNA data on toxicological endpoints are scarce. Toxicokinetics information for PFOA/APFO is available in various species. Due to differences in species and sex shown for TK of PFOA/APFO it

would be appreciated if this was reflected when compared to TK of PFNA.

Moreover, it should be discussed if the read-across to APFO/PFOA can be applied on endpoints where classification depends on differences in potency, like acute toxicity.

Labelling:
1.3: Concerning the proposed labelling (CLP) the pictogram "GHS05" is missing. It is necessary because of classification as "Eye Dam. 1; H318 (cf. corrected RAC-Opinion on PFOA). Furthermore, the name of the target organ (liver) should be placed straight after H372 (cf. Corrected RAC-Opinion on PFOA).

Substance ID:
For the sake of completeness, CAS and IUPAC names should be added to table 5 both for the sodium and ammonium salt as the dossier concerns these two substances alongside PFNA.
Furthermore, it would be better for clarification, when the IUPAC name of these substances could also be stated in the dossier title.

In the IUCLID Section 1.1, a reference substance for perfluorononanoic acid is given. Additionally, "mono constituent substance" is stated as type of substance. Therefore, only the dossier name in IUCLID indicates that the dossier concerns a group of substances. Thus it would aid comprehensibility, if a reference substance for PFNA and its sodium and ammonium salts was linked in the IUCLID dossier and the group entry were indicated by type of substance.

Dossier Submitter's Response

Thank you for your valuable comments.

<u>Read across – general comment</u>
We agree that our justification for read across should have been expanded to more specifically include separate justifications for read across for local and systemic effects. There are not that many CLH reports that have used a read across approach so it is a learning process for us all on how to present this data in the best way.

<u>Read across local effects</u>
Please see dossier submitter's response under comment number 1.

<u>TK-information</u>
In the CLH report, text from the APFO Background Document was included to provide data on APFO/PFOA. This text did however not contain any information on TK data for mouse and thus unfortunately the CLH report lack specific information on this. Lou and colleagues (Lou, I. et al., 2009. Modelling single and repeated dose pharmacokinetics of PFOA in mice. Toxicological Sciences 107:331-341) reported a serum elimination half-life of 15.6 days (female) and 21.7 days (male) after a single oral gavage dose of 1 or 10 mg/kg. Thus as indicated in section 4.1 of the CLH report, all together the available TK data indicate that there is a major sex difference in the serum elimination half-life in rats for PFNA (30.6 days for males and 1.4 days for females) and for PFOA (Female: 2.8 – 16 hrs, depending on the dose and for males 138- 202 hrs depending on the dose). In contrast only a minimal sex difference was recorded in the mouse for both PFNA (half-life varied dose dependently between 25.8 – 68.4 days for females and between 34.3 – 68.9 days for males) and PFOA (21.7 days for males and 15.6 days for females). Thus TK data for mice and rats support similar TK of PFOA and PFNA.

Labelling We agree with your comments. Substance ID and IUCLID This was discussed and agreed during the Accordance Check process with ECHA but maybe it could have been done even better.
RAC's response
1. Regarding toxicokinetics, the analysis of the existing data presented under the STOT RE section of the opinion indicate that toxicokinetics of PFNA and PFOA are similar in rats, mice and in humans, although it is different in rats and in mice depending on gender of the animal. Thus, the toxicokinetic data support read-across. 2. Justification of read-across is presented in the opinion separately for each endpoint/hazard class. 3. Labelling suggestions are included in the opinion. 4. Substance ID: Noted

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
23.01.2014	Netherlands		MemberState	5
Comment received				
The Netherlands agrees with the proposed classification for carcinogenicity in Cat 2 (H351) based on the increase in pancreatic tumors for PFOA. Read across is justified because this is a systemic effect which is classified based on weight of evidence.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your support.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
23.01.2014	Netherlands		MemberState	6
Comment received				
The Netherlands agrees with the classification Repr. 1B (H360D) based on the data presented in p. 40-43 (CHL Report), and supported by the read-across data. The data provided show 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. In addition, studies in KO mice indicate that the mechanism of toxicity is related to peroxisome proliferator-activated receptor α (PPARα) given that the KO pups showed no developmental effects. Even though it is not known whether adverse developmental effects induced PPARα activation by PFNA are relevant to humans, these adverse effects cannot be excluded. The Netherlands agrees with the classification Lact. (H362). The presence of PFNA in lactating pups and in human breast milk (p. 15, CLH Report) without evidence of adverse effects or evidence that adverse effects can be expected is insufficient for classification.				

However, based on read-across from PFOA classification, there is agreement that there is a systemic effect which is classified based on the level of evidence provided.
Dossier Submitter's Response
Thank you for your support.
RAC's response
Thank you for your support.

Date	Country	Organisation	Type of Organisation	Comment number
24.01.2014	Germany		MemberState	7
Comment received				
The observed effects of PFNA in the developmental toxicity study in mice are qualitatively and quantitatively similar to PFOA (reduced pup viability, full litter resorption and delay in the onset of eye opening) at tested doses up to 2 mg/kg bw/d (Wolf et al., 2010). The poster by Lau et al. (2009) provides additional information about similarities occurring at higher doses. There is no information on lactation effects for PFNA available. However PFNA has been detected in human breast milk like PFOA.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your support.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
23.01.2014	Netherlands		MemberState	8
Comment received				
The Netherlands disagrees with the classification Acute Tox. 4 oral (H302) and Acute Tox.4 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, the LD50 and LC50 for PFNA and its salts cannot be reliably estimated for classification.				
Dossier Submitter's Response				
Please see dossier submitter's response under comment number 1. The compounds seems to have similair physical-chemical properties. yet potencies could differ and we welcome a discussion in RAC.				
RAC's response				
In the opinion of RAC, due to the 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 the read-across approach to be applied, as defined in Section 1.5 of Annex XI of the REACH Regulation: "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 criteria for read across approach are met based on:

1) a common functional group;
2) the common precursors and/or the likelihood of common breakdown products via physical and biological processes, which result in structurally similar chemicals; or
3) a constant pattern in the changing of the potency of the properties across the category.

As was assumed for PFOA and APFO, which in stomach or lung fluid form the corresponding anion (PFO), also PFNA, PFN-S and PFN-A will be 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 oral or inhalation toxicity, the read-across is in fact between two anions, PFO and PFN, these being analogue chemical groups and differing only by one $-CF_2-$ group in the fluorine substituted aliphatic chain.

Taking into account the above considerations and adopting a read-across approach between PFO and PNF anions, RAC agrees with the DS's proposal and proposes 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 acute toxicity assessment of APFO.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
23.01.2014	Netherlands		MemberState	9

Comment received

The Netherlands disagrees with the classification Eye Dam. 1 (H318) because according to CLP Guidance: '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.' Given the lack of sufficient data for an adequate read-across (particularly physical/chemical properties such as pH are important in establishing similar potencies) with APFO, classification for eye damage/irritation cannot be reliably assessed for PFNA and its salts.

Dossier Submitter's Response

Please see dossier submitter's response under comment number 1. There is no information on measured pH for PFNA and its salts. However, calculations (see Table 2A and 2B on page 14 – 15 of this document) indicate that at equimolar concentration, the pH of a PFNA solution will be the same as that of a PFOA solution, and the pH of a APFO solution will also be same as that of an equimolar solution of the ammoniumsalt of PFNA.

RAC's response

Considering the read-across of the eye corrosive property from APFO to PFNA and its sodium (PFN-S) and ammonium (PFN-A) salts, it is noted that there is no information on measured pH for PFNA and its salts. However, the calculations of pH using estimated pKa values provided by the DS (see Table 2A and 2B attached to this document) 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, and the pH of a APFO solution will also be same as that of an equimolar solution of the ammonium salt of PFNA.

Both acids (PFNA and PFOA), based on their calculated very low PKa values, are extremely

strong acids and are virtually completely protolyzed in water, i.e. the pH will only depend on the concentration of the acid. The experimental determination of the the pKa values of perifluorinated compounds is difficult since the chemical structure of these compounds renders them both lipid and hydro-repellent and 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). Therefore it is concluded that PFNA and PFOA are very strong acids, as indicated by their very low pKa values, and both substances will therefore be available to tissues in the form of the corresponding carboxylate anion.

Goss (2008) investigated, by using two different pKa prediction software packages (SPARC and COSMO-RS), how the pKa value was influenced by the chain length of the perfluorinated carboxylic acids (C_4 vs C_{12}). As can be seen 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.

Compound	**pKa (SPARC)**	**pKa (COSMO-RS)**
$F(CF_2)_3COOH$	0.4	0.7
PFOA - $F(CF_2)_7COOH$	-0.1	0.7
PFDoDA - $F(CF_2)_{11}COOH$	-0.2	0.8

There is no measured pH value available for the ammonium salt of PFNA. In water the pH values for the ammonium salts will be determined by the ammonium ion and thus it seems reasonable to assume that the pH value of the ammonium salt of PFNA (estimated pH =5.9) will be the same as the one for the ammonium salt of PFOA (see Table 2A). Thus, from the perspective of possible differences in pH, read across for local effects from the ammonium salt of PFOA (APFO) to the ammonium salt of PFNA would be justified as well as the read across to PFNA.

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) are in the same range and the ammonium salt and sodium salt should be more soluble. The predicted values for water solubility of PFNA and PFOA (see Table 3 attached to this document) are much lower (in the mg/L or µg/L range, depending on prediction model) than the measured values. Thus the predicted solubility differs 10- to 20-fold between PFOA and PFNA (no information on the salt). The reason for the discrepancies between the measured values for PFNA and PFOA and the estimated values are most likely due to the physicochemical nature of these compounds. However, overall the solubility of PFNA and PFOA seems not to differ extensively if one compares data that originates from the same method of measurement/ prediction model. Thus also from a solubility perspective, read across seems to be overall justified at least between PFOA and PFNA.

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

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON HEPTADECAFLUORONONANOIC ACID AND ITS SODIUM AND AMMONIUM SALTS

23.01.2014	Netherlands		MemberState	10

Comment received

Given that it is difficult to assess the severity of the liver and immune effects observed in repeated dose-toxicity studies (increase in liver and thymus weight, induction of apoptosis in the thymus and spleen, increases in pro-inflammatory molecules (IL-1, IL-6, IFNα, and H2O2) in the spleen and increases in serum stress-response molecules (adrenocorticotropic hormone and cortisol) which can reduce immune function in rodents) (Table 15, p. 23, CHL Report), we suggest to check whether any range-finding studies with PFNA are available from the study authors because the tested dose level in these short assays are low and suggest that higher dose levels might induce more severe effects. For example, Fang et al. (2008) reported that a dose of 10 mg/kg bw/day induced 50% mortality. This type of effect is obviously severe enough for classification. A dose of 10 mg/kg was also used in the study by Lau et al. (2009, p. 42-42 CLH Report) and was dropped because of severe maternal toxicity including mortality. The dose level of 10 mg/kg bw/day in a 14 day study should be compared to a cut-off for STOT RE 2 of 500 mg/kg bw/day (= 100 mg/kg bw/day * 14 days / 90 days) and for STOT RE 1 of 50 mg/kg bw/day. Classification with STOT RE 1 would be clearly justified based on data with the substance PFNA unless it is shown that PPARα is involved in the mortality. Therefore, it would be interesting to know whether there are comparable range-finding information from KO mice. Read-across to the salts is agreed as the same ions will form. The proposed classification only includes liver as a target organ (STOT RE 1 (liver)) while the immune system is also a target organ in the studies provided (Table 15, p. 23, p.29-30, CHL Report) and mentioned in p. 29 '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' (CHL Report). Immunosuppression was observed in Rhesus Monkeys at ≥ 30 mg/kg bw/day of APFO (p. 30, CHL Report). Therefore, the immune system should be evaluated as a target organ via read-across with APFO. Finally, as data for PFNA are only available from oral exposure studies and data from other routes of exposure are not available, a route should not be specified.

Overall, the Netherlands agrees with the proposed classification STOT RE 1 (affected organs: liver) (H372) and proposes consideration of immune system as an additional target organ.

Dossier Submitter's Response

Thank you for your support.
We agree that the observed mortality reported in the study by Feng can be taken as a support for a STOT RE1 classification. We also agree that the immune system should be considered as a target organ as well and leave this question to RAC for further discussion.

There are some papers examining the immuntoxicity of PFOA that was not taken into account in the PFOA/APFO CLH reports and since they add information that could be of importance when evaluating whether the immunsysteme should be considered as a target organ we have added some information from 3 papers below. For a general review on this subject we refer to the reviews by Dewitt, J.C., et al., (Toxicology Pathology 40: 300-311, 2011; Critical Reviews in Toxicology 39;76-94, 2009)

The study by DeWitt and collaborators (DeWitt, JC., et al., Environmental Health Perspective 116: 644- 650, 2008 – http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2367677/pdf/ehp0116-000644.pdf) examined the effect of PFOA on the immune system. C57BL/6N female mice were dosed with APFO via the drinking water (0, 0.94, 1.88, 3.75, 7.5, 15 and 30 mg/kg/day) for 15 days. On day 11 of dosing the animals were immunized with sheep red blodd cells and on the day after end of dosing organs were collected and sera was collected for IgM

measurements. Statistical significant decrease in body weight was seen at 30 mg/kg (from day 8 of dosing (-6%, until end of experiment, -15%) and at 15 mg/kg/day (6% less as compared to controls, only at day 1 post dosing, i.e. when the IgM production was measured) whereas no effect was seen at the lower dose levels. Reduced relative and absolute weights of the spleen and thymus was recorded at the 7.5, 15 and 30 mg/kg/day dose levels, but were only statistically significant at the 15 and 30 mg/kg/day dose levels. No histopathological examination was conducted. Interestingly a dose-dependent and statistically significant decrease in the serum IgM antibody titres was observed at all dose levels down to 3.75 mg/kg (at 3.75 mg/kg ~11% less as compared to controls, as compared to an 29% decrease at the 30 mg/kg dose level). The plasma concentration of PFOA at the LOAEL (3.75 mg/kg/day was 7.5×10^4 ng/mL. This study indicates that the synthesis of IgM is affected at dose levels where no effect on bodyweights was observed. It has been claimed (Loveless, S.E. et al., Toxicol Sci 105:86-96, 2008) that the observed effects on the immunsystem is secondary to maternal toxicity. The study was performed using higher dose levels, 10 or 30 mg/kg, although using male mice of another mice strain (CD-1).

In another study Dewitt and colleagues (Dewitt, J.C., Toxicol Sci 109:106-112, 2009) investigated the hypothesis that the observed immunosuppression is secondary to elevated serum corticosterone levels by assessing immunfunction in adrenalectomized (adx) or sham-operated C57BL/6N female mice exposed to 0, 7.5, or 15 mg/kg of PFOA/kg bw in drinking water for 5 days. Bodyweights, primary antibody response to a T-dependent antigen, clinical serum chemistries related to liver health and serum corticosterone levels were evaluated. Exposure to 15 mg/kg decreased bodyweight by ~10% after 8 days of dosing until 2 days postdosing in both adx and sham animals; body weight of adx animals were still reduced 5 days after end of dosing when IgM levels were measured. IgM antibody titers were statistically significantly reduced by 15% in sham animals and by 18 % in adx animals exposed to 15 mg/kg and by 11.8% in adx animals exposed to 7.5 mg/kg. Corticosterone concentrations were elevated by 157% in dosed sham animals relative to controls and were reduced by 27% in adx animals relative to control animals (neither changes were statistically significant) Clinical serum chemistry related to liver health were not statistically altered by either dose or adrenalectomy. The failure of adrenalectomy to protect mice from immunosuppressive effects of PFOA indicates that suppression of antibody synthesis is not the result of liver toxicity or stress-related corticosterone production.

RAC's response

Regarding the consideration of the immune system in the STOT RE classification, the existing studies reviewed in the opinion 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/d 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 be for 90 days.

The data reviewed in the opinion 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 the 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 at doses below the guidance values of 10 mg/kg bw/d even after oral exposure shorter than 90 days.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON HEPTADECAFLUORONONANOIC ACID AND ITS SODIUM AND AMMONIUM SALTS

In conclusion, RAC agreed with the DS and with comments from the Netherlands that PFNA and its sodium and ammonium salts be classified as STOT RE 1, H372 for effects on the liver but also considered that effects on the immune system (thymus, spleen) should be included in the classification.

Table .1 Physical chemical properties of APFO, PFOA and PFNA. Data collected from CLH reports and governmental agencies.

Compound	**APFO**	**PFOA**		**PFNA**		
Source	***CLH Back-ground Document***	***CLH Background Document***	***ATSD* (Draft Report)***	***Environment Canada** (Draft Report)***	***CLH Proposal***	***Hazard Substance Data Bank******
Physical state (@ 20 °C and 101.3KPa)	Solid[a]	Solid	Solid	No information	Solid	No information
Melting/freezing point	57-165 °C (decomposition starts above 105 °C)[b]	• 52-54 °C[a] • 54.3 °C[b]	54.3 °C[a]	59.3 – 77	65 – 68 °C[a]	No information
Boiling point	Decomposition	189-192 °C @736 mm Hg[c]	188 °C[a]	203.4 °C, calculated[a]	218 °C @ 740 mm Hg [a]	No information
Relative density	0,6-0,7 g/ml @ 20 °C[c]	Density/specific gravity: 1.792 g/ml @20 °C[a]	1.8 g /cm3[b]	No information	1.753 g/cm^3	No information
Vapor pressure	0.0081 Pa @ 20 °C, calculated from measured data[d]	• 4.2 Pa @ 25 °C, extrapo-lated from measured data[d,e]. • 2.3 Pa @ 20 °C, extrapo-lated from measured data[e]	• 0,017 mm Hg @ 20 °C, extrapolated[c] • 0,962 mm Hg @ 59.2 °C, measured[c]	0.10 Pa @ 25 °C, measured[b]	No information	0,083 mm Hg @ 25 °C, estimated[a]
Water solubility	Conc at saturation > 500 g/l @ 20 °C[e]	• 3.4 g/l @ 20°C[f] • 9.5 g/l @25 °C[g]	• 9.5 g/l @ 25 °C[d] • Critical micelle concentration =of 3.7 g/L for PFO anion[e]	• <2 g/l @ 60 °C, measured[c,d] • Critical micelle concentration = 1.3 g/L, measured[d,e]	No information	6.25X10^{-2} mg/L at 25 °C, estimated[a]

Log K_{ow}	No data	No data	The log Kow is not measurable since these substances are expected to form multiple layers in an octanol-water mixture[f].	K_{ow} is a problematic parameter for surfactants because they tend to aggre-gate at the interface of a liquid-liquid system and therefore cannot be measured directly.	No information	5.48, estimated[a]
pKa **Measured**	2.80, measured in 50% aqueous ethanol[f]	2.8, measured in 50% aqueous ethanol[h]	2.8[g]	No data	No data	No data
Estimated	No data	No data	No data	< 0.8, calculated[f].	- 0.15, estimated[b] [transfer error of data from source, should have been -0. 17]	-0.21, estimated[b]
pH value	~5 in H_2O @ 23 ^oC[g]	2.6 @ 1g/l @ 20 ^oC[i]	No information	No information	No information	No information
References/ notes	a) Kirk-Othmer, 1994. b) Lines and Sutcliff, 1984. c) Griffith and Long, 1980. d) Washburn 2005. e) 3M, 1987. f) Brace 1962. g) 3M, reliability not assignable.	a) Kirk Othmer 1994. b) Lide 2003. c) Boit 1975. d) Kaiser 2005 e) Washburn 2005. f) Merck, undated. g) Kauck and Diesslin 1951. h) Brace 1962. i) Merck 2005, reliability not	a) Lide 2005. b) Kroschwitz and Howe-Grant 1994. c) Washbum 2005.hitta d) Kauck and Diesslin 1951. e) Prevedouros et al., 2006. f) 3M 1999, 2008; EPA 2005. g) Kissa 2001.	a) Kaiser et al., 2005. b) Arp et al., 2006. c) Fontell and Lindman 1983. d) These solubility values refer to an aqueous phase containing a mixture of protonated acid and perfluoro-carboxylate anion,	a) Oxford University Chemical Safety Data sheet. b) Data from SPARC 2008, as cited in ATSDR report.	a) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1 b) SPARC, Ver 3.

		assignable.		at an "autogenous" pH. If the pH is reduced by addition of, for example, a mineral acid, the proportion of protonated acid will increase and the overall solubility will decrease. e) Kunieda and Shinoda 1976. f) Goss 2008.		

*****Draft Ecological Screening Assessment Report, Long-Chain (C9 - C20) Pefluorocarboxylic Acids, their Salts and their Precursors. Environment Canada, 2010. (https://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&n=CB279C36-1&printfullpage=true).

**Draft Toxicological Profile for Perfluoroalkyls, Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, 2009. (Available at http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=1117&tid=237).

***Available at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB

Table 2. Calculation of pH using estimated pKa values from either the ChemID Plus software(table segment A) or the SPARC software (table segment B), with the assumption that the concentration is 1mM.

A.

Name	**Perfluoro-heptanoic acid**	**Perfluoroocta-noic acid**	**Perfluoro-nonanoic acid**	**Perfluoro-decanoic acid**	**Perfluoro-heptanoic acid, am-monium salt**	**Perfluoro-octanoic acid, am-monium salt**	**Perfluoro-nonanoic acid, am-monium salt**	**Perfluoro-decanoic acid, am-monium salt**
Cas No	375-85-9	335-67-1	375-95-1	335-76-2	6130-43-4	3825-26-1	4149-60-4	3108-42-7
Molecular formula	$C_7HF_{13}O_2$	$C_8HF_{15}O_2$	$C_9HF_{17}O_2$	$C_{10}HF_{19}O_2$	$C_7H_4F_{13}NO_2$	$C_8H_4F_{15}NO_2$	$C_9H_4F_{17}NO_2$	$C_{10}H_4F_{19}NO_2$
Molecular weight (g/mol)	364.057	414.064	464.071	514.078	381.088	431.095	481.102	531.109
pK_a (ChemID Plus Advanced*)	-2.29	-4.2	-6.51	-5.2	8.86 (NH_4^+)	8.86 (NH_4^+)	8.86 (NH_4^+)	8.86 (NH_4^+)
Conc of HA or of the salt(M)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
pH (=-log [H+])	3.0	3.0	3.0	3.0	5.9	5.9	5.9	5.9

*Available at http://chem.sis.nlm.nih.gov/chemidplus/ProxyServlet?objectHandle=Search&actionHandle=transferStructure&nextPage=chemidheavy.jsp&responseHandle=JSP&superlistid=0000064197&MOLFILE_REFERENCE=&QF10=&TRANSFER=true

B.

Name	**Perfluoroheptanoic acid**	**Perfluorooctanoic acid**	**Perfluorononanoic acid**	**Perfluorodecanoic acid**
Molecular formula	$C_7HF_{13}O_2$	$C_8HF_{15}O_2$	$C_9HF_{17}O_2$	$C_{10}HF_{19}O_2$
pKa (SPARC*)	-0.2	-0.21	-0.21	-0.22
Conc of HA (M)	0,001	0,001	0,001	0,001
pH (=-log [H+])	3.0	3.0	3.0	3.0

.* SPARC; pKa/property server. Available at http://archemcalc.com/sparc/

The pK_a values for the acids indicate that they are very strong acids and thus they will be completely protolysed in water. Consequently, the pH will only depend on the concentration of the acids (which in this case was assumed to be 1 mM). For the ammonium salts, the pH will mainly depend on the concentration and the pKa of the ammonium ion. The concentration of $H^+$was calculated using the mathematical approximation (se page 109, Stumm and Morgan,. Aquatic Chemistry 3rd edition, John Wiley & Sons, 1996).: $[H^+] = (-K_a + (K_a^2 + 4xK_a x[compound])^{1/2})/2$. The concentration of the compounds was assumed to be 1mM.
The two prediction softwares estimate slightly different pKa values for the carboxylic acids. However, these acids are very strong and the calculated pH values will be the same for all the acids. Their corresponding ammonium salts will have the same pH values . Although they will be slightly higher as compared to the acids, since the pH will depend on the ammonium ion (the SPARC software could not predict the pKa value for the ammonium ion). If a lower concentration is used in the pH calculations (1 mg/ml resulting in concentrations of 2.7 to 1.9 µM, for the different acids) this will result in a slightly higher pH for the acids (~5.6 –5.7) as well as for their corresponding ammonium salts (7.2 -7.3), data not shown.

Table 3. Water solubility and log K_{ow} as predicted by the EPISuite* software

Name	**Perfluoro-heptanoic acid**	**Perfluoro-octanoic acid**	**Perfluoro-nonanoic acid**	**Perfluoro-decanoic acid**	**Perfluoro-heptanoic acid, ammonium salt**	**Perfluoro-octanoic acid, ammonium salt**	**Perfluoro-nonanoic acid, ammonium salt**	**Perfluoro-decanoic acid, ammonium salt**
Molecular formula	$C_7HF_{13}O_2$	$C_8HF_{15}O_2$	$C_9HF_{17}O_2$	$C_{10}HF_{19}O_2$	$C_7H_4F_{13}NO_2$	$C_8H_4F_{15}NO_2$	$C_9H_4F_{17}NO_2$	$C_{10}H_4F_{19}NO_2$
Water solubility (mg/L)								
WSKOW program, v1.42 (water sol from Kow)	3.647	0.4813	0.06258	0.008043	330.2	43.34	No data returned	No data returned
WATERNT Program, v1.01 (from fragments)	0.042398	0.0020683	$9.942x10^{-5}$	$4.7238x10^{-6}$	0.0082956	0.00040249	No data returned	No data returned
log Kow								
EPISuite: KOWWIN Program (v1.68)	4,15	4,81	5,48	6,15	1,27	1,94	No data returned	No data returned

* US EPA; Estimation Program Interface (EPI) Suite available from http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm

Please note that the estimated values for water solubility differ depending on the prediction method used for the assessments. The predicted values also are much lower than the measured values. The difference towards the measured values could partly be due to the nature of these compounds.