Substance names:	**Perfluorononan-1-oic-acid [1] and its sodium [2] and ammonium salts [3]**
EC numbers:	**206-801-3 [1], Not applicable [2], Not applicable [3]**
CAS numbers:	**375-95-1 [1], 21049-39-8 [2], 4149-60-4 [3]**

MEMBER STATE COMMITTEE

SUPPORT DOCUMENT

FOR IDENTIFICATION OF

PERFLUORONONAN-1-OIC ACID AND ITS SODIUM AND AMMONIUM SALTS

AS SUBSTANCES OF VERY HIGH CONCERN

BECAUSE OF THEIR TOXIC FOR REPRODUCTION AND PBT[1] PROPERTIES

Adopted on 30 November 2015

[1] Persistent, bioaccumulative and toxic.

CONTENTS

TABLES

ABBREVIATIONS

APFO	Ammonium perfluorooctanoate
B	Bioaccumulative
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BMF	Biomagnification factor
BSAF	Biota-sediment accumulation factor
CL_{renal}	Renal clearance
CL_{total}	Total clearance
CLP	Classification, Labelling and Packaging
CMR	Carcinogenic, Mutagenic and toxic for Reproduction
EC	European Commission
EU	European Union
L-FABP	Liver fatty acid binding protein
P	Persistent
PBT	Persistent, bioaccumulative and toxic
PFAA	Per- and polyfluoralkylated acids
PFAS	Per- and polyfluoroalkylated substances
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutane sulfonate
PFC	Fluorocarbons, perfluorocarbons
PFCA	Perfluorinated carboxylic acid
PFDA	Perfluorodecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxS	Perfluorohexane sulfonate
PFN	Perfluorononanoate
PFN-A	Ammonium salt of perfluorononanoic acid, ammonium perfluorononanoate
PFN-S	Sodium salt of perfluorononanoic acid, sodium perfluorononanoate
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFSA	Perfluorinated sulfonic acid
PFUnA	Perfluoroundecanoic acid
RAC	Committee for Risk Assessment
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SVHC	Substances of Very High Concern
TMF	Trophic magnification factor
vB	Very bioaccumulative
vP	Very persistent
vPvB	Very persistent, very bioaccumulative

IDENTIFICATION OF A SUBSTANCE OF VERY HIGH CONCERN ON THE BASIS OF THE CRITERIA SET OUT IN REACH ARTICLE 57

Substance Names: Perfluorononan-1-oic-acid (PFNA) [1] and its sodium [2] and ammonium salts [3]

EC Numbers: 206-801-3 [1], Not applicable [2], Not applicable [3]

CAS numbers: 375-95-1 [1], 21049-39-8 [2], 4149-60-4 [3]

***Perfluorononan-1-oic-acid and its sodium and ammonium salts* are identified as substances of very high concern because:**

- **they meet the criteria of Article 57 (c) of Regulation (EC) 1907/2006 (REACH)** [2] **as toxic for reproduction 1B, and**
- **they meet the criteria of Article 57 (d) of REACH as substances which are persistent, bioaccumulative and toxic, in accordance with the criteria and provisions set out in Annex XIII of Regulation (EC) 1907/2006 (REACH).**

Summary of how the substances meet the criteria set out in Article 57 (c) and 57 (d) of REACH.

As justified in Section 1, in the following the abbreviation PFNA refers to the acid (PFNA) as well as to its ammonium and sodium salts PFN-A and PFN-S, where these are not specified.

PFNA belongs to the chemical group of long-chained perfluorinated carboxylic acids (PFCAs). The substances in this group have a highly similar chemical structure: a perfluorinated carbon chain and a carboxylic acid group. They differ only in the number of CF_2-groups whereas all other fragments are the same within the group. As a result of comparing the experimental and estimated data of other PFCAs with experimental and estimated data on PFNA, it can be assumed that with increasing chain length water solubility decreases and the sorption potential increases (See Annex 1). It can be stated with sufficient reliability that the behaviour of the PFCAs follows a regular pattern.

Six entries of long-chained PFCAs have already been included into the Candidate List:

EC number	CAS number	Substance name	Length of the carbon chain	Details on SVHC-identification	Reference
223-320-4	3825-26-1	Ammonium pentadecafluorooctanoate (APFO)	8	Toxic for reproduction (Article 57 c); PBT (Article 57 d)	European Chemicals Agency (2013a)

[2] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

206-397-9	335-67-1	Pentadecafluorooctanoic acid (PFOA)	8	Toxic for reproduction (Article 57 c); PBT (Article 57 d)	European Chemicals Agency (2013b)
218-165-4	2058-94-8	Henicosafluoroundecanoic acid	11	vPvB (Article 57 e)	European Chemicals Agency (2012a)
206-203-2	307-55-1	Tricosafluorododecanoic acid	12	vPvB (Article 57 e)	European Chemicals Agency (2012b)
276-745-2	72629-94-8	Pentacosafluorotridecanoic acid	13	vPvB (Article 57 e)	European Chemicals Agency (2012c)
206-803-4	376-06-7	Heptacosafluorotetradecanoic acid	14	vPvB (Article 57 e)	European Chemicals Agency (2012d)

Toxicity for reproduction:
In its opinion of September 2014 on the proposal for harmonised classification and labelling at EU level of *Perfluorononan-1-oic acid (2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluorononanoic acid) (PFNA) and its sodium and ammonium salts*[3], ECHA's Risk Assessment Committee (RAC) concluded that the evidence is sufficiently convincing to classify PFNA for developmental effects as Repr. 1B, H360Df ("May damage the unborn child. Suspected of damaging fertility") in accordance with the CLP criteria (Regulation (EC) 1272/2008).

Therefore, even though PFNA is not yet listed in Annex VI of CLP (Regulation (EC) 1272/2008), there is evidence based on the RAC opinion on PFNA that *PFNA and its sodium and ammonium salts* meet the criteria for classification as toxic for reproduction in accordance with Article 57 (c) of REACH.

PBT

A weight-of-evidence determination according to the provisions of Annex XIII of REACH was used to identify the substance as P and B. All available relevant information was considered together in a weight-of-evidence approach.

Persistence

PFNA is, based on its stabile structure, not expected to undergo abiotic degradation under relevant environmental conditions. A standard screening study on PFNA supporting this understanding is available.

In general, the persistence of PFCAs can be explained by the shielding effect of the fluorine atoms, blocking e.g. nucleophilic attacks to the carbon chain. High electronegativity, low

[3] 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 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 12 September 2014. Available at: http://echa.europa.eu/documents/10162/0b290fee-19b7-4d7e-8365-312df5d1ae37

polarizability and high bond energies make highly fluorinated alkanes the most stable organic compounds. It is not expected that the carboxylic group in PFCAs alters the persistence of these chemicals. The persistence of five PFCAs (PFOA and C_{11}-C_{14}-PFCAs) (P and vP) was already confirmed by the Member State Committee (see table above).

Therefore, based on the read-across approach with PFOA, it is concluded that PFNA is not degraded in the environment and thus fulfils the P- and vP- criteria in accordance with the criteria and provisions set out in Annex XIII of REACH.

Bioaccumulation

There are no experimental BCF values available for PFNA. The numeric criterion as suggested in REACH Annex XIII (sections 1.1.2 and 3.2.2(a)) for a bioaccumulative substance in aquatic species is not expected to be fulfilled for PFNA based on read across. Due to its expected notable water solubility, PFNA is, like the other PFCAs, expected to quickly be excreted via gill permeation. Furthermore, PFNA is present mainly in protein rich tissues like blood and liver (OECD, 2006; Kelly et al. 2009). Hence, bioconcentration in gill breathing organisms and the accumulation in lipids is not the most relevant endpoint to consider. Field studies show that air-breathing organisms are more likely to bioaccumulate PFNA and other PFCAs compared to water breathing organisms. Therefore, the numerical bioaccumulation (B) criterion defined in the REACH regulation Annex XIII (sections 1.1.2 and 3.2.2(a)) is not suitable to assess the bioaccumulation potential of PFNA.

Annex XIII (section 3.2.2) defines information which shall be taken into account in the assessment and can be used to draw conclusions on the assessment even when the numerical criterion is not applicable. Such data are, for example, data on the bioaccumulation potential in terrestrial species, such as elevated levels in endangered species. PFNA was found in terrestrial species as well as in endangered species as shown for the polar bear and in beluga whale. These findings indicate a bioaccumulation potential and are of high concern.

Furthermore, Annex XIII (section 3.2.2 (b)) requires to consider data from human body fluids or tissues and to take the toxicokinetic behavior of the substance assessed into account. For PFNA, gestational and lactational exposure in humans has been shown, which is of special concern as the foetus and newborn babies are highly vulnerable to exposure by toxic substances. On top of that, data from human body fluids clearly provide quantitative proof of the bioaccumulation of PFNA: Elimination half-lives in humans are > 1 year. In addition, recent studies, taking into account relevant confounding factors, show that PFNA blood concentrations in humans increase with increasing age.

Finally, Annex XIII (section 3.2.2 (c)) foresees that the potential for biomagnification in food chains of a substance is assessed. The available field data provide evidence that bioaccumulation and trophic magnification do occur in certain food webs in the environment. For PFNA field studies provide trophic magnification factors (TMFs) or biomagnification factors (BMFs) for PFNA for aquatic and terrestrial food chains. When air breathing organisms are the top predators in these food chains biomagnification could be demonstrated by calculation of TMFs and BMFs > 1 in several food chains, for example for wolves and beluga whales.

The data summarised above is in high accordance with the bioaccumulation data on the other PFCAs. Altogether these show a regular pattern of bioaccumulation which depends on the chain-length of the perfluorinated alkyl chain.

Conclusion:

1. PFNA accumulates in humans
    a. PFNA is present in human blood of the general population
    b. Elimination half-lives are > 1.7 years.

c. Human elimination half-lives seem to be the longest amongst the available mammalian data, whereas the elimination half-lives in laboratory mammals vary highly depending on the study conditions.
d. PFNA levels increase with age after adjusting for relevant confounding factors.

2. There is evidence that PFNA preferentially bioaccumulates in air-breathing mammals, including endangered species and humans
a. BMFs range from 1.4 – 24 based on estimated whole body values
b. TMFs range from 2.9 to 9.88 referring to either whole body measurements or estimated whole body values

3. PFNA does not seem to consistently accumulate in water breathing animals
a. No experimental BCFs are available for PFNA. For the closest structural analogues BCFs range from 4.0 to 27 (PFOA) and from 450 to 2700 (PFDA)
b. Whole body BAFs range from 0 to 3981
c. Whole body BMFs range from 0.13 to 5.3 whereas most of the data are below 1
d. Whole body TMFs range from 0.33 to 1.22 in aquatic piscivorous food webs

4. The bioaccumulation data on PFNA in environmental species, in laboratory mammals and in humans is consistent with the data on other long-chain perfluorinated carboxylic acids, such as PFOA.
a. Recent models to explain the substantial bioaccumulation of PFCAs take into account the observed pattern of animal tissue distribution, the relationship between chain length and bioaccumulation and the species and gender-specific variation in elimination half-life.

To conclude, taken all available information together in a weight-of-evidence approach, the elimination half-lives from humans and other mammals show that PFNA bioaccumulates. The available field data also indicate that bioaccumulation and trophic magnification occur in certain food webs in the environment. The data on PFNA are in line with the expected regular pattern of fate properties of the already assessed PFOA and C_{11}-C_{14}-PFCAs. Therefore it is considered that the B criterion of REACH Annex XIII is fulfilled. Whether the vB criterion is fulfiled has not been assessed.

Toxicity

There is evidence based on the RAC opinion on PFNA and its sodium and ammonium salts that these substances meet the criteria for classification as toxic for reproduction in accordance with Article 57 (c) of the REACH Regulation. As a consequence the toxicity criterion of REACH Annex XIII is fulfilled.

Conclusion on PBT

In conclusion, PFNA and its sodium and ammonium salts meet the criteria for a PBT substance according to Article 57 (d) of the REACH Regulation.

Registration dossiers submitted for the substance: No

PART I

Justification

1. Identity of the substance and physical and chemical properties

The free perfluorononanoic acid (PFNA) stays in equilibrium with perfluorononanoate (PFN), the conjugate base, in aqueous media in the environment and in organisms, as well as in the laboratory. The physico-chemical properties of PFNA and PFN are different. Therefore, the expected environmental fate depends on the environmental conditions, which influence the equilibrium between base and acid (pH and pKa).

The ammonium (PFN-A) and sodium (PFN-S) salts are very soluble in water. In aqueous solution, they will be present as anion PFN and the ammonium or the sodium cation. The dissolved anion PFN will stay in equilibrium with the corresponding acid (PFNA) in aqueous media.

It is not possible with currently available analytical methods to distinguish between PFN and PFNA in samples. In the literature, the concentrations reported in environmental and human monitoring studies will always include both species (PFN and PFNA).

PFNA will refer in the following to both the acid (PFNA) and to its conjugate base PFN. It will only be clearly indicated which of the acid PFNA or the conjugate base PFN that is meant where it is important to distinguish between both species and when species-specific knowledge is available.

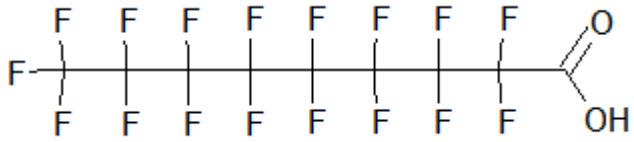
For simplicity, in the discussions and conclusions in this document, PFNA is usually referred to. Based on the reasoning above, the conclusions are, however, considered valid for PFN-A and PFN-S as well.

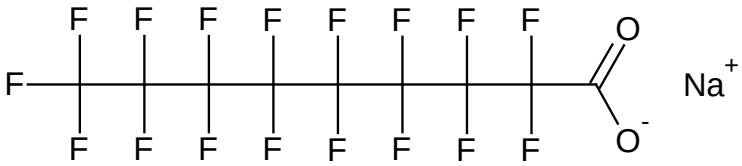
1.1. Name and other identifiers of the substance

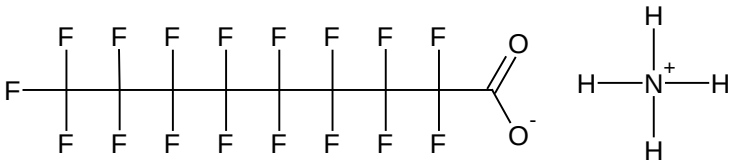
Table 1: Substance identity

EC number:	206-801-3 [1], Not applicable [2], Not applicable [3]
EC name:	Perfluorononan-1-oic-acid [1], Not applicable [2], Not applicable [3]
CAS number (in the EC inventory):	375-95-1 [1], 21049-39-8 [2], 4149-60-4 [3]
CAS name:	Nonanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro- [1], Nonanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-, sodium salt (1:1) [2], Nonanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-, ammonium salt (1:1) [3].
IUPAC name:	heptadecafluorononanoic acid [1], sodium heptadecafluorononanoate [2], ammonium heptadecafluorononanoate [3].
Index number in Annex VI of the CLP Regulation	Not yet included
Molecular formula:	$C_9HF_{17}O$ [1]
Molecular weight range:	464.08 [1]
Synonyms:	C9-PFA heptadecafluorononanoic acid Nonanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro- Nonanoic acid, heptadecafluoro- perfluoro-n-nonanoic acid perfluoro-n-nonanoic acid, ammonium salt perfluoro-n-nonanoic acid, sodium salt Perfluorononanoic acid Perfluoroonanoic acid (PFNA) PFNA PFNonanoic acid Serum Perfluorononanoic Acid

Structural formulas:







The following substances belong to the same substance category of long-chained perfluorinated carboxylic acids (PFCAs). Please, see Annex I for the read across justification.

EC number	CAS number	Substance name	Length of the carbon chain
223-320-4	3825-26-1	Ammonium pentadecafluorooctanoate (APFO)	8
206-397-9	335-67-1	Pentadecafluorooctanoic acid (PFOA)	8
206-400-3	335-76-2	Nonadecafluorodecanoic acid (PFDA)	10
218-165-4	2058-94-8	Henicosafluoroundecanoic acid	11
206-203-2	307-55-1	Tricosafluorododecanoic acid	12
276-745-2	72629-94-8	Pentacosafluorotridecanoic acid	13
206-803-4	376-06-7	Heptacosafluorotetradecanoic acid	14

1.2. Composition of the substance

Name: Perfluorononanoic acid (PFNA)

Description: Mono-constituent substance

Degree of purity: 80-100 %

Perfluorononanoic acid, as well as its sodium and ammonium salts, are mono constituent substances. The identification as SVHC is based on the properties of the main constituent only. Therefore, in this case, other possible constituents or impurities are not relevant for the identification as SVHC.

1.3. Physicochemical properties

Table 2: Overview of physicochemical properties (PFNA)

Property	PFNA	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101.3 kPa	The substance is a solid	Yaws 2008	
Melting point	68 °C (at 1 atm)	Yaws 2008	
Boiling point	218 °C (at 1 atm)	Yaws 2008	
Vapour pressure	No data		
Density	No data		
Water solubility	No data		
Partition coefficient n-octanol/water (log value)	5.9	Wang et al. (2011)	Estimated using COSMOtherm PFNA has surface active properties
Dissociation constant	<1.6 0.82 (COSMOtherm)	Vierke et al., 2013 Wang et al., 2011)	Estimated values. Dissociation behaviour is discussed in Annex 1.

Physical-chemical properties of the other C_8- to C_{14}-PFCAs are provided in Table A.2 in Annex 1.

2. Harmonised classification and labelling

The RAC has adopted an opinion[4] at RAC-30 that Perfluorononan-1-oic acid (2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluorononanoic acid) (PFNA) and its sodium and ammonium salts meet the following criteria for classification and labelling (European Chemicals Agency – Committee for Risk Assessment (2014)):

[4] 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 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 12 September 2014. Available at: http://echa.europa.eu/documents/10162/0b290fee-19b7-4d7e-8365-312df5d1ae37

Table 3: The RAC opinion on classification and labelling in accordance with Regulation (EC) No 1272/2008 (CLP)

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Spec. Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement code(s)	Pictogram, Signal Word Code(s)	Hazard statement code(s)	Suppl. Hazard statement code(s)		
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 GHS07 GHS08	H351 H360Df H372 (liver, thymus, spleen) H302 H332 H318 H362			

H360Df: May damage the unborn child. Suspected of damaging fertility.

3. Environmental fate properties

3.1. Degradation

3.1.1. Abiotic degradation

3.1.1.1. Hydrolysis

Hori et al. (2008) investigated the decomposition of PFNA in hot water (80°C) at a constant reaction time of six hours. In absence of persulfate ion ($S_2O_8^{2-}$) 97% of the initial amounts of PFNA remained in the aqueous phase. After addition of $S_2O_8^{2-}$ to the reaction system efficient decomposition of PFCAs has been observed. After six hours, PFNA was decomposed completely. The reaction products were mainly F^- and CO_2 at a yield of 88.9 % ((moles of F- formed)/(moles of fluorine content in initial PFNA)) and 75.2 % ((moles of CO_2 formed)/(moles of carbon content in initial PFNA)). Short chain PFCAs were minor reaction products.

The analogue substance PFOA is hydrolytically stable under environmental conditions with a hydrolytic half-life greater than 92 years (European Chemicals Agency, 2013b).

Hence, based on the available study on PFNA and the read across to PFOA (Annex 1), PFNA is considered to be hydrolytically stable under environmental conditions.

3.1.1.2. Phototransformation/photolysis

3.1.1.2.1. Phototransformation in air

There are no studies on phototransformation in air for PFNA available. However, the atmospheric lifetime of PFOA has been predicted to be 130 days (conclusion by analogy from short-chain perfluorinated acids) (European Chemicals Agency, 2013b).

3.1.1.2.2. Phototransformation in water

The photochemical decomposition of long-chain PFCAs in water by use of persulfate ion ($S_2O_8^{2-}$) in water and in an aqueous/liquid CO_2 biphasic system was examined by Hori et al. (2005a; 2005b) (Reliability = 2). In water and in the absence of $S_2O_8^{2-}$ (direct photolysis)

PFNA decomposition of 64.5 % was determined after 12 hours. In the presence of $S_2O_8^{2-}$ the decomposition increased to 100% (Hori et al., 2005a). The reaction products were mainly F^- and CO_2. Short chain PFCAs ($C_nF_{2n+1}COOH$; n=1-5) were minor reaction products. The decompositions after 12 hours in the biphasic system were 100% for PFNA (Hori et al., 2005b). The reaction product was mainly F^- (66.2 %, of (moles of F- formed)/(moles of fluorine content in PFNA)) and the minor reaction products were shorter chain PFCAs ($C_nF_{2n+1}COOH$; n=1-7). Since the conditions in these studies are not relevant for an aqueous environment (wave length used for irradiation <300 nm), the studies were not described in detail.

PFOA does not undergo direct photodegradation in natural waters. The estimated half-life for indirect photolysis (Fe_2O_3) is greater than 349 days (European Chemicals Agency, 2013b).

3.1.1.3. Summary on abiotic degradation

In general, the perfluorinated carboxylic acids are extremely stable. As there are no degradation studies under relevant environmental conditions available for PFNA, data from similar substances need to be considered and discussed. Based on the data given in Annex 1, results of studies of structurally similar substances of the same chemical group are used to evaluate the abiotic degradation of PFNA.

The data on PFOA indicate that abiotic degradation in the atmosphere is expected to be slow (atmospheric lifetime = 130 days; conclusion by analogy from short-chain perfluorinated acids). Under relevant environmental conditions PFOA is hydrolytically stable (estimated DT50 > 92 years) and do not undergo direct photodegradation in natural waters. The estimated half-life for indirect photolysis (Fe_2O_3) is greater than 349 days.

Based on the read-across rationale described in Annex 1, data on PFOA are used as evidence for PFNA to conclude that it is stable under environmental conditions and abiotic degradation is expected to be as low as for the chemically similar substance PFOA.

3.1.2. Biodegradation

3.1.2.1. Biodegradation in water

3.1.2.1.1. Estimated data

For PFNA a half-life in water of 2477 days and a half-life in soil of 4954 days were estimated (Lambert et al., 2011). Nevertheless, these estimates are assumed to be low because the bond between carbon and fluorine is one of the most stable ones in organic chemistry and not subject to degradation by microorganisms occurring in the environment.

3.1.2.1.2. Screening tests

In a ready biodegradability test (OECD 301 F) using 50mg/L PFNA (28.4 mg/l ThOD), 30 mg/L activated sludge and 10mg/L allythiourea (to prevent nitrification) no biodegradation was observed after 28 days (Stasinakis et al., 2008).

Based on the available screening test PFNA is not readily biodegradable.

3.1.2.1.3. Simulation tests (water and sediments)

For PFNA no experimental degradation test is available.

3.1.2.2. Biodegradation in soil

For PFNA no experimental degradation test is available.

3.1.2.3. Summary and discussion on biodegradation

A screening study indicates that PFNA is not readily biodegradable. No experimental simulation tests are available.

Results from non-standard degradation studies of the chemically similar compound PFOA, used for read-across approach as described in Annex 1, indicate that PFNA is absolutely not biodegradable. The results on PFOA provide good evidence that no biodegradation in water, soil and sediment occurs. The persistence of PFOA was already confirmed by the Member State Committee that identified the substance as SVHC based on its PBT properties (European Chemicals Agency, 2013b). Since the stability of PFCAs is in general mainly based on the stability of the fluorinated carbon chain, it can be concluded that also for PFNA no biodegradation in water, soil and sediment can be expected. Thus, it can be assumed that PFNA is not biodegradable.

3.1.3. Summary and discussion of degradation

For PFNA there is no abiotic degradation study under relevant environmental conditions available. Regarding biodegradation there is only one study available showing that the substance is not readily biodegradable. Therefore, data from a chemically similar compound are considered in a read-across approach (please see Annex 1 for further details). Generally, it is known that the bond between carbon and fluorine is one of the most stable ones in organic chemistry and not subject to degradation by microorganisms occurring in the environment.

A number of studies for the shorter chain homologue PFOA show that this substance is extremely persistent and does not undergo abiotic or biotic degradation at all under environmental conditions (European Chemicals Agency, 2013b). The persistence of PFOA was already confirmed by the Member State Committee that identified the substance as SVHC i.a. based on its PBT properties (European Chemicals Agency, 2013b).

PFCAs are synthetic compounds which contain a common structural feature: a perfluorinated carbon chain combined with a carboxylic group. The chemical structure of these compounds differs only in the number of perfluorinated carbons in the carbon chain.

The stability of organic fluorine compounds has been described in detail by Siegemund et al. (2000). When all valences of a carbon chain are satisfied by fluorine, the zig-zag-shaped carbon skeleton is twisted out of its plane in the form of a helix. This situation allows the electronegative fluorine substituents to envelope the carbon skeleton completely and shield it from chemical attack. Several other properties of the carbon-fluorine bond contribute to the fact that highly fluorinated alkanes are the most stable organic compounds. These include polarizability and high bond energies, which increase with increasing substitution by fluorine. The influence of fluorine is greatest in highly fluorinated and perfluorinated compounds. Properties that are exploited commercially include high thermal and chemical stability.

Based on their molecular properties it is, thus, clear, that researchers could not measure degradation of the intensively studied PFOA or its salts. Considering the organic chemistry of this substance group it seems to be very likely that a carbon chain being one CF_2-group longer is as persistent as a shorter chain. We therefore conclude that PFNA is as resistant to degradation as it has been shown for PFOA.

In summary, using the described read-across approach, we conclude that PFNA is a very persistent synthetic compound which is resistant to abiotic and biotic degradation.

3.2. Environmental distribution

Based on information in the Table entitled “Basic substance information and physical chemical properties relevant to justify read across in the PBT assessment” in the support documents for PFCAs, the distribution of PFCAs is influenced by the pH of the environment (e.g. European Chemicals Agency, 2012a). The water solubility, the adsorption potential and hence the distribution in the environment express a regular pattern depending on the alkyl chain length of the PFCA.

3.3. Bioaccumulation

3.3.1. General remarks

According to to section 3.2.2 (b) and (c) of Annex XIII not only the numerical bioaccumulation (B) criterion based on bioconcentration factors can be used to assess the bioaccumulation potential of a substance, but also other information can be used in a weight-of-evidence approach. This additional information, which includes measured elevated levels in biota, information on the ability of the substance to biomagnify in the food chain, data from analysis of human body fluids or tissues and assessment of toxicokinetic behaviour of the substance, should also be considered for the assessment using a weight-of-evidence approach.

Information on the bioaccumulation potential of PFNA in humans as well as data from analysis of human body fluids are described in section 4.1.

3.3.2. Bioaccumulation in aquatic organisms

3.3.2.1. Bioconcentration factor BCF

Bioconcentration is the process by which a chemical enters an organism and/or is adsorbed on to it as a result of exposure to the chemical in water – it often refers to a condition usually achieved under laboratory and steady state conditions. The BCF is typically measured as the ratio of the chemical concentrations in the organism and the water once a steady state has been achieved:

$$BCF = \frac{c_{Biota}}{c_{Water}}$$

or alternatively, can be determined kinetically by using the uptake rate k_1 and the depuration rate k_2:

$$BCF = \frac{k_1}{k_2}$$

There is no study available which determined the BCFs of PFNA. A BCF study for two chemically similar substances, PFOA and PFDA is available. This study is described below:

In this study rainbow trouts were exposed in a flow-through system for 12 days followed by a depuration time of 33 days in fresh water to determine tissue distribution and bioconcentration (Martin et al., 2003a). For determination of bioconcentration, juvenile fish (5-10 g) were exposed simultaneously to PFCAs of varying chain length. No adverse effects

were observable based on fish mortality, growth and liver somatic index. The exposure concentration of each PFCA was analytically checked. PFCA concentrations were stable throughout the uptake phase. For PFDA the measured concentration was 0.71 µg/L with a relative standard deviation of 24% and for PFOA the measured concentration was 1.5 µg/L with a relative standard deviation of 13%. There was an initial decrease between 0.25 h and 24 h which is considered to be caused by the rapid uptake of the PFCAs. At seven occasions during the uptake period and nine occasions during the depuration phase, three fishes from the exposure tank and one fish from the control tank were removed to determine the kinetics of uptake and depuration.

The BCFs (in carcass, blood and liver) were determined on the basis of the uptake and depuration kinetics and results are given in Table 4. All tissue concentrations were corrected for growth dilution. Additionally, for the tissue distribution study, four immature trouts (30 – 48 g) were exposed in separate tanks but under the same uptake conditions (Martin et al., 2003a). The BCFs reported from laboratory experiments are summarized in Table 4. This tissue distribution study showed that unlike lipophilic organic compounds PFCAs did not preferentially accumulate in adipose tissue. Hence a lipid-normalisation of the BCFs would not be reasonable. PFCA concentrations were highest in blood, kidney, liver and gall bladder and low in the gonads, adipose and muscle tissue. Within the blood, the plasma contained between 94 – 99 % of PFCA, with only a minor fraction detectable in the cellular fraction. Recovery from hearts and spleen was low (<10%). Based on high blood, liver and gall bladder concentrations and slow depuration the authors assume that PFCA enter the enterohepatic recirculation in fish. That means the compounds are continuously transferred between the different organs (Martin et al., 2003a).

Table 4: Measured growth corrected bioconcentration factors (BCF) of PFOA and PFDA

	Location	Species (tissue)	BCF	Reliability	Reference
PFOA	Laboratory	Rainbow trout (Carcass)	4.0 ± 0.6	2	Martin et al., 2003a
	Laboratory	Rainbow trout (Blood)	27 ± 9.7		
	Laboratory	Rainbow trout (Liver)	8.0 ± 0.59		
PFDA	Laboratory	Rainbow trout (Carcass)	450 ± 62		
	Laboratory	Rainbow trout (Blood)	2700 ± 350		
	Laboratory	Rainbow trout (Liver)	1100 ± 180		

Conclusion:

BCFs for PFOA are below 2000, indicating no bioconcentration in aquatic organisms due to uptake from the aqueous phase by diffusion via the gills. The high water solubility of PFOA may enable fish to quickly excrete this substance via gill permeation, facilitated by the high water throughput (Kelly et al., 2004; Martin et al., 2003a; Martin et al., 2003b). Unlike lipophilic organic compounds PFCAs do not preferentially accumulate in adipose tissue but in protein rich tissues. Based on the BCF for blood PFDA may be regarded as bioaccumulative. Conclusions on bioaccumulation are normally based on whole body BCF values and in this case, carcass is seen as a good approximation for whole body by the authors. Based on the BCF of the carcass it has to be concluded that PFDA is less bioaccumulative.

Due to the structural similarity and the regular pattern of physico-chemical properties within this group of PFCAs it can be assumed that also PFNA has a BCF smaller than 2000. However, bioconcentration in fish may not be the most relevant endpoint to consider. Other mechanisms of bioaccumulation might be of relevance for protein binding substances.

3.3.2.2. Bioaccumulation factors (BAFs)

In field studies on bioaccumulation of chemicals bioaccumulation factors (BAF) are measured. The BAF is typically measured in the field as the ratio of the chemical concentrations in the organism and the surrounding medium (e.g. water in natural ecosystems). In contrast to the BCF, the uptake is not only limited to exposure via water but all routes including diet contribute to the concentration in organisms:

$$BAF = \frac{C_{Biota}}{C_{Water}}$$

where chemicals concentration in the organism (c_{biota}) is usually expressed in units of gram of chemical per kilogram of organism. The weight of the organism can be expressed on a wet weight basis or appropriately normalized, if needed, (e.g. lipid- or protein-normalized) (Conder et al., 2012). BCFs are measured under controlled laboratory conditions, whereas the BAF is a field measurement and therefore different from BCF.

Although some authors describe BCF values in their field studies, BAFs would be more appropriate, because it cannot be excluded that the tested organisms did not take up PFNA via the diet. BAFs are summerised in Table 5.

Loi et al. (2011) investigated a subtropical pelagic food web in a nature reserve including phytoplankton (n=1), zooplankton (n=2), gastropod (n=3), worm (n=2-3), shrimp (n=2-3), fish (n=2-6), and water bird (n=3). Samples were collected between 2008 and 2010. Surface water (n=12) and sediment samples (n=6) were collected concurrently with the biota samples. Livers samples from water birds were all collected in 2003. A BAF for the phytoplankton for PFNA of 1680 was derived (Loi et al., 2011).

Labadie et al. (2011) investigated the partitioning of various PFCAs in the Orge River, an urban tributary of the Seine River. Bioaccumulation and tissue distribution were studied in European chub, a common cyprinid in European freshwater and a benthopelagic fish. Five adult fish were collected in April 2010. The sex of each individual fish was analysed according to gonad morphology. Whole liver, gills and gonads were taken along with portions of muscle. Water and sediment samples were taken as triplicates at the same site. Large inter-individual variations, not sex-related, were observed. In agreement with the findings made by Martin et al. (2003a) tissue distribution shows that PFNA is especially accumulated in blood and liver. The results of this study are summarized in Table 5. All values are below 2000, although this trigger value relates to whole body BCFs.

In a study conducted by Furdiu et al. (2007) individual whole body homogenates of 4 year old lake trout (*Salvelinus namaycush*) sampled and collected in 2001 from Lake Superior (n= 10), Lake Michigan (n = 10), Lake Huron (n = 10), Lake Erie (n = 6) and Lake Ontario (n = 10) were analyzed for PFCAs. The samples from all five lakes showed similar concentrations of PFCAs. Whole body BAFs were calculated by dividing the average concentration of PFCs in lake trout by the average concentration in water from each lake.

Furthermore, BAFs were calculated from water and biota concentrations reported in the studies of Loi et al (2011) and Houde et al (2006) (see Table 5). The study of Houde is described in detail in section 3.3.2.4. Variations in calculated BAFs originate from variations in measured concentrations in fish.

All studies are field studies and were neither growth corrected nor normalised to a lipid content. BAFs were calculated based on wet weight.

Table 5: Examples of measured bioaccumulation factors (BAF) of PFNA

Location	Species (tissue)	BAF	Reliability	Reference
Mai Po Marshes Nature Reserve	Phytoplankton(whole)	1680	2	Loi et al., 2011
all of the Great Lakes	lake trout/water concentration from each Great Lake (whole)	3981	2	Furdui et al. 2007
Orge river	european chub(plasma)	630	2	Labdie and Chevreuil 2011
	european chub(liver)	79		
	european chub(gills)	125		
	european chub(gonads)	79		
	european chub(muscle)	39		
South Carolina, Charleston	striped mullet(whole)	705	2	Houde et al. 2006
	Pinfish(whole)	1118		
	red drum(whole)	2529		
	atlantic croaker(whole)	Not detected		
	Spotfish(whole)	1300		
	spotted seatrout (whole)	2800		
Mai Po Marshes Nature Reserve	grey mullet (whole)	105	2	Loi et al. 2011
	mozambique tilpia(whole)	125		
	smalll snakehead(whole)	197		
	Ladyfish(whole)	85		
	flag-tailed glass perchlet(whole)	59		

Conclusion: Except for the BAF values for red drum, spotted seatrout and lake trout which support that PFNA is bioaccumulative, the BAFs for PFNA indicate less bioaccumulation in aquatic organisms. Again, the expected[5] high water solubility of PFNA may enable fish and mussels to quickly excrete this substance via gill permeation, facilitated by the high water throughput (Kelly et al., 2004; Martin et al., 2003a; Martin et al., 2003b).

[5] Based on the data of the analogue PFOA, see Annex I.

3.3.2.3. Biota-sediment accumulation factors (BSAFs)

For evaluating the bioaccumulation potential of chemicals also biota-sediment accumulation factors (BSAFs) can be used. BSAFs are field-based measurements for the chemical concentration in the organism and the sediments calculated according to the following equation:

$$BSAF = \frac{C_{Biota}}{C_{Sediment}}$$

Where C_{Biota} is the chemical concentration in the organism at steady-state, and $C_{Sediment}$ is the sediment chemical concentration at steady-state (Conder et al., 2012).

For assessing the bioaccumulation from fresh water sediments (n=3) a study using oligochaete *Lumbriculus variegatus* was commenced (Higgins et al., 2007). This benthic-dwelling worm species is a deposit feeder and serves as an entry point for sediment-bound contaminants into food webs. During the screening one uncontaminated field sediment, laboratory-spiked with PFNA, and two contaminated field sediments were applied, respectively. Steady-state (56 days) lipid and organic carbon-normalized BSAF values, as well as non-lipid and carbon-normalised BSAF were determined. Lipid-normalization was based on lipid analysis in one worm for each jar. However, lipid-normalisation is not straight forward in the case of PFNA as this substance is ‚proteinophilic' (Kelly et al., 2009).

Table 6: Biota-sediment accumulation factors (BSAF) analyzed with Lumbriculus variegatus

Location	**Sediment**	**BSAF**		**Reliability**	**Reference**
		Lipid normalized	non lipid-normalized	2	
Downstream from two WWTP, California	*Sediment 1 (CA1 (56 days*	83 ± 13	0.64 ± 0.5		Higgins et al., 2007
	Sediment 2 (CA2 (56 days	149 ± 25	0.83 ± 0.12		
Laboratory	estimated steady-state values	55 ± 25	1.60 ± 0.40		

Conclusion: One study is available providing BSAFs for PFNA. The results of this study indicate a higher concentration in the benthic-dwelling worm than in the surrounding environment if data are lipid-normalised. However, this approach is not straight-forward because PFNA does not enrich in lipids. Non-lipid normalised BSAF do not show an increased concentration in the worms. However, the data should be used with caution. BSAF are influenced by sorption characteristics. These are usually assessed by the K_{OC} which is calculated on the basis of the K_{ow}. However, as discussed K_{ow} is not appropriate for PFNA.

3.3.2.4. Biomagnification factors (BMFs)

Besides bioconcentration also biomagnification describes the potential of a chemical to bioaccumulate. Biomagnification factors (BMFs) can be measured in the laboratory in a fashion similar to that used in the OECD and US-EPA bioconcentration test protocols.

Organisms are exposed to a chemical primarily via diet. The BMF test typically includes an uptake phase, where levels of chemicals are followed over time, ideally until the chemical concentration in the organism no longer changes with time (i.e., reaching the steady-state). If a steady-state cannot be reached in the experiment, the uptake phase is followed by a depuration phase where organisms are exposed to uncontaminated food. The rate of decline in chemical concentration over time measured in the depuration phase can then be used to derive the chemical uptake rate from which a hypothetical steady-state concentration can be estimated (Conder et al., 2012).

The laboratory-derived BMF is calculated using the ratio of the chemical concentrations in the test animals at steady-state and their diet:

$$BMF_{(diet)} = \frac{C_{biota}}{C_{diet}}$$

where chemical concentration in the organism (C_{biota}) and its diet (C_{diet}) are appropriately normalized, if needed, (e.g., lipid- or protein-normalized) (Conder et al., 2012).

BMF values based on field studies are based on the ratio of the concentration in the predator and the prey:

$$BMF_{(field)} = \frac{C_{predator}}{C_{prey}}$$

In case of laboratory dietary studies it is certain based on the test design that the diet is the sole source of exposure whereas in field studies this is not necessarily the case. It is therefore crucial to differentiate between a $BMF_{(diet)}$ and a $BMF_{(field)}$.

There are several uncertainties concerning field based BMFs similar to field based trophic magnifacation factors with regard to food webs. There are biological, ecological factors which can influence the outcome of a BMF. Dividing the concentration of a substance in a predator by that in a prey implies that this prey is the sole food source. However, the food sources may be diverse. Additionally, there is no standard procedure so far how to conduct such field studies, and different study designs may therefore have an influence. The uncertainties of field studies have been addressed and discussed by Borga et al. (2012). As the authors actually refer to field based trophic magnifacation factors a summary of the discussion has been included in chapter 3.3.2.5 Trophic magnification factors. The report of ECETOC on a weight of evidence PBT/vPvB assessment has given in the chapter on bioaccumulation a review on various issues concerning field studies (ECETOC 2014).

Problems arise with increasing body size of predators because analysis is based on tissue or serum samples. This is especially true for organisms at the higher trophic levels (e.g., polar bear), while it is feasible to measure the whole-body on smaller species at lower trophic levels. Whole-body analysis is not feasible for ethical reasons, i.e. a whole whale would be needed, and due to the challenging logistics with respect to sampling and laboratory constraints. Therefore, some of the derived BMF-values are restricted to certain tissue samples rather than whole body samples. Whole body values may be estimated if the tissue mass fraction is known for the organism regarded. There may however be some uncertainties due to inter individual and geographical differences but these uncertainties cannot be quantified (Houde et al., 2006). BMF values based on liver samples may be overestimated. From a toxicological perspective, concentrations in individual organs, such as the liver, may be more relevant when the potential for direct organ-specific toxicity (i.e., liver toxicity) is predicted. As shown by Kudo et al. (2000) PFCAs cause hepatomegaly in rodents which is an indicator for hepatotoxicity. This study investigated PFCAs with 7–10 carbon chain lengths. Upham et al (1998) showed in their study that PFOA can inhibit gap

junctional intercellulular communication in a dose dependent manner. This mode-of-action has been linked to the tumor-promoting properties of many carcinogens. Furthermore PFNA has been classified as STOT RE 1 for liver.

At present no internationally accepted trigger value for BMF exists. The question whether only enrichment of a substance in predator proofs biomagnification or whether transfer from prey to predator already may be sufficient is still up for discussion. In a scientific context a BMF or TMF above 1 suggest biomagnification (Conder et al. 2012). However, also a BMF or TMF below 1 may be of concern as this indicates that a substance is taken up into the organism and the uptake may cause an adverse effect. A high accumulation in representatives at lower trophic levels directly causing adverse effects may cause reduced prey supply. In case of a reduced supply chain, this may rather affect predators than the trophic magnification of pollutants. Thus, no observable trophic magnification or an observed trophic dilution as a single fact do not necessarily imply that there is no potential risk (Ehrlich et al. 2011). Experiences with revision or development of test guidelines show that even substances known to be bioaccumulative may show BMF < 1 in laboratory test systems (Inoue et al. 2012). However, keeping this in mind a BMF ≥ 1 will be used here as trigger value for the sake of decision-making. BMFs for PFNA are summarized in Table 7.

A study by Goeritz et al.(2013) investigated the biomagnification potential as well as the substance and tissue-specific distribution of perfluoroalkyl substances in market-size rainbow trout (*Onchorhynchus mykiss*). Laboratory-derived biomagnification factors (BMFs) for all test compounds were determined based on a kinetic approach. Distribution factors were calculated for each test compound to illustrate the disposition of PFASs in rainbow trout after 28 d of exposure. Rainbow trout with an average body weight of 314 ±21 g (n = 35) were fed a test diet supplemented with 5 different PFASs for 28 d. The nominal concentration of the spiked food was comparable to the PFAS concentration in feed applied in the dietary accumulation study by Martin et al. (2003b). The test diet was prepared by spiking commercial, extruded trout feed of 4-mm pellet size (Milkivit, F-2P B40; Skretting) with the 5 test substances (PFBS, PFHxS, PFOS, PFOA, and PFNA) at a nominal concentration of 500 mg/kg dry weight each. Biomagnification factors were determined according to the OECD document 305. A BMF value of 0.23 was reported for PFNA. The estimated biomagnification factors were not corrected for the lipid content of the experimental animals due to the low potential of the test items to accumulate in lipids (see also Table 7). The growth corrected depuration rate constant (k_{2g}) was estimated to be 0.058 d^{-1}.

The results of this study indicate that a dietary exposure will not result in biomagnification in trout. However, also for other substances which are already known to have bioaccumulative properties BMFs do not indicate bioaccumulation (BMFs < 1 for $C_{11,12}$-PFCAs and perfluorooctanoic sulfonicacid PFOS). A published comparison of BCFs and biomagnification factors (BMFs) investigated nine substances in a laboratory fish feeding study with carp (Inoue et al. 2012). Five substances showed BCFs larger than 5000 but only two of these substances were likely to biomagnify. Hence, for laboratory based dietary studies on fish showing BMFs below 1 it cannot be concluded that the substance is not bioaccumulative. Furthermore, gill breathing organisms are investigated, which might not be the most relevant to be considered as explained above.

Besides this laboratory study, BMFs were estimated from field studies. Studies are described below and results are shown in Table 7.

Transfer of PFNA was elucidated in Lake Ontario (Martin et al., 2004) including one 4-membered pelagic food chain. Whole body samples were collected. The sampled organisms included a top predator fish, lake trout (*Salvelinus namaycush*), three forage fish species including rainbow smelt (*Osmerus mordax*), slimy sculpin (*Cottus cognatus*), and alewife (*Alosa pseudoharengus*), and two invertebrates Diporeia (*Diporeia hoyi*) and Mysis (*Mysis relicta*), which were considered as primary prey. Trouts were sampled in 2001. Forage fish

species, including sculpin, smelt, and alewife, and intervebrate samples were collected on October 9th 2002 at an offshore site near Niagara-on-the-Lake, Lake Ontario. Due to the inherent uncertainties correlated with constitution of diet, four individual combinations of rainbow trout and its prey were regarded. In all examples, BMF for PFNA ranged between 0.13 and 5.3 (Table 7). A striking finding of this study was the high content of PFNA in both macro invertebrates occupying the lowest trophic level. Concentrations in *Diporeia* were as high as 57 ng/g and the mechanism leading to this exceptional accumulation still needs to be unravelled. As a consequence, sculpin as *Diporeia´*s consecutive predator still shows significant levels of PFNA (33 ng/g). Given that *Diporeia* is a benthic invertebrate species, and sculpin feed mainly in the benthic environment, this contamination may be considered a benthic contamination source.

Tomy et al. (2009) investigated beluga whale, ringed seal, fish pelagic amphipod and arctic copepod of the Western Canadian Arctic. The animals selected were from the sample archived repository at Fisheries and Oceans, Canada. Blubber and liver of beluga (n = 10, all males) from Hendrickson Island and ringed seal (n = 10, all males) from Holman Island were collected in 2007 and 2004, respectively. Fish species collected in 2004 and 2005 included the marine pelagic Arctic cod (n = 10) from the Amundsen Gulf, the marine coastal Pacific herring (n = 10) from the Mackenzie Shelf and the anadromous Arctic Cisco (n = 9) from the Mackenzie estuary. The marine pelagic amphipod *Themisto libellula* (pooled samples, n = 2) and the marine Arctic copepod *Calanus hyperboreus* (pooled samples, n = 5) were collected in 2004 from the eastern Beaufort Sea and Amundsen Gulf region. The authors state that differences in sampling years may influence the interpretation of the food web transfer. Again, some of the derived BMF-values are restricted to the liver and the resulting BMFs may be overestimates. The BMF-values reported range from 0.3 for Arctic cod (liver)/ marine pelagic amphipod (whole body) to 12.9 for Beluga whale (liver)/ arctic cod (liver). Uncertainties coming from the fact of samples from different years are expected to be minimal, because of minimal concentration changes in remote regions. Except for the arctic cod and either amphipod or copepod relationships, BMFs >1 suggest bioaccumulation of PFNA. BMFs are based on organ specific concentrations and might therefore be overestimarted. Anyhow, it is not possible to quantify this overestimation and due to target organ toxicity accumulation of PFNA in liver is of special concern.

Houde et al. (2006) investigated the biomagnification of PFNA in the food web of bottlenose dolphins. In the course of the study PFNA concentrations in bottlenose dolphins were examined at two different habitats, whereby BMFs and TMFs were calculated for only one of these habitates (Charleston Harbor and its tributaries (i.e., the Cooper, Ashley, and Wando rivers) and the Stono River estuary, South Carolina) because for the other habitate concentrations in fish were below the detection limit. Marine water (n=18), surface sediment (n=17), Atlantic croaker (n=3), pinfish (n=4), red drum (n=8), spotfish (n=10), spotted seatrout (n=11), striped mullet (n=8), and bottlenose dolphin samples (n=24) were collected around the Charleston Harbor area. Dolphin plasma, skin, and teeth were collected from both locations and, additionally, dolphin tissue samples (i.e., liver, kidney, muscle, lungs, heart, thyroid, and thymus) were collected from a recently deceased bottlenose dolphin (Charleston, female, 708.4 kg). Houde et al. (2006) claim that utilization of serum or liver concentrations of dolphins will overestimate the BMF by a factor of 10-30. Therefore they extrapolated tissue specific concentrations to whole body burdens based on the total body weight, the organ weights and the blood volumes. Samples were collected between 2002 and 2004, thus entailing some uncertainty when assessing BMF through the food chain. It may be assumed that media and biota were continuously exposed to PFNA in this area throughout the years. BMFs ranging from 1.4 to 24 for individual dolphin/prey relationships were stated using recalculated PFNA whole body burdens for dolphin (Houde et al., 2006). Wastewater treatment plant discharges in the Charleston area may have resulted in non–steady state concentrations in the food web. Even if these results come with uncertainties (samples from different years, whole body estimation) they clearly indicate bioaccumulation of PFNA.

Butt et al. (2008) conducted a study in the Canadian Arctic. Ringed seal liver samples (n=10 per site) were provided by local hunters from 11 different locations in the Canadian Arctic. Sample collection years for ringed seal populations varied from 2002 to 2005. However, for this remote region concentration variation in different years are expected to be minimal. The age of the animals was determined via tooth aging and for a few samples the age was estimated using length-age correlations. Stable isotope analysis was done with ^{15}N to ^{14}N and ^{13}C to ^{12}C. Based on liver samples from polar bears obtained from Smithwick et al. (2006) and ringed seal data measured in this study BMFs were calculated. The polar bear sample sites were associated with ringed seal populations. In four different regions these factors ranged from 35 to 111 with a mean of 56, clearly indicating biomagnification even if the factors might be overestimated due to tissue specific concentrations (Butt et al., 2008).

BMFs are summarized in Table 7. None of the studies were lipid- or protein-normalized.

Table 7: Biomagnification factors (BMF) for PFNA

Location	Species (tissue)	BMF	Reliability	Reference
Laboratory	Rainbow trout (Carcass)	0.23	2	Goeritz et al. 2013
Lake Ontario	Lake trout(whole)/alewife(whole)	5.3	2	Martin et al., 2004
	Lake trout(whole)/smelt(whole)	0.62		
	Lake trout(whole)/sculpin(whole)	0.13		
	Lake trout(whole)/prey (weighted)	2.3		
US, South Carolina	Seatrout(whole)/pinfish(whole)	1.5	3	Houde et al., 2006
	Dolphin (whole, estimated)/ striped mullet(whole)	5.0		
	Dolphin (whole, estimated)/ pinfish(whole)	3.2		
	Dolphin (whole, estimated)/red drum(whole)	1.4		
	Dolphin (whole, estimated)/ atlantic croaker(whole)	24		
	Dolphin (whole, estimated)/ spotfish(whole)	4.6		
	Dolphin (whole, estimated)/ seatrout(whole)	2.1		
Canadian Arctic	Polar bear (liver)/ ringed seal (liver)	35-111	2	Butt et al., 2008
Western Canadian Arctic	Ringed seal (liver)/ arctic cod (liver)	1.2	2	Tomy et al., 2009
	Beluga whale (liver)/ arctic cod (liver)	12.9		
	Beluga whale (liver)/ Pacific herring (liver)	5.8		
	Beluga whale (liver)/ arctic cisco (liver)	2.9		
	Arctic cod (liver)/ marine arctic copepod (whole)	0.7		
	Arctic cod (liver)/ marine pelagic amphipod (whole)	0.3		

Conclusion: The biomagnification potential of PFNA was investigated in several field studies. Gill breathing organisms like fish as predators show BMFs from 0.13 to 5.3. The expected[6] high water solubility of PFNA may enable fish and mussels to quickly excrete this substance via gill permeation, facilitated by the high water throughput (Kelly et al., 2004; Kelly et al. 2009; Martin et al., 2003a; Martin et al., 2003b). However, air-breathing homeotherms are unable to efficiently eliminate PFNA into water via body surfaces such as gills. The study on an Arctic marine food web conducted by Kelly et al (2009) showed these differences

[6] Based on the data of the analogue PFOA, see Annex I.

between piscovorous and marine mammalian food webs. For predator prey relationships, including seals, beluga whales, dolphins and polar bears, studies provide data showing bioaccumulation (BMFs 1.4-111). Estimated whole body BMFs values have been provided by the study conducted by Houde and co-workers. BMFs range between 1.4-24. These data support the conclusion that PFNA is bioaccumulative. Overall, these findings provide further indication that different accumulation mechanisms are going on for gill and air breathing organisms and that gill breathing organisms are not the most relevant organisms to be considered, whereas for air breathing organisms bioaccumulation occurs. These different accumulation mechanisms may be due to the partioning to protein-rich compartments which may leed to different toxicokinetics as Kelly et al. (2009) postulated.

3.3.2.5. Trophic magnification factors (TMFs)

The trophic magnification factor (TMF) is a measure to evaluate biomagnification occurring in food webs. In the ECHA Guidance Document on Information Requirements, Chapter R.7.10.1.1, TMF is defined as the concentration increase in organisms with an increase of one trophic level. According to Conder et al. (2012), TMFs represent some of the most conclusive evidence of the biomagnification behaviour of a chemical substance in food webs. Again, a TMF greater than one indicates accumulation within the food chain.

There are several uncertainties concerning TMFs. These have been addressed and summarized by Borga et al. (2012). Additionally, the report of ECETOC on a weight of evidence PBT/vPvB assessment gives (in the chapter on bioaccumulation) a review on various issues concerning field studies (ECETOC 2014). These include biological factors such as the differences between poikilotherms and homeotherms, sex, different energy requirements, different abilities to metabolize chemicals and slow or fast growing organisms.

Steady state between a consumer and its diet is assumed. However, as opportunistic feeders wild animals vary their diet over seasons or with life stage and point sources may influence observed TMFs. Additionally, apart from the diet there is always the possibility of a direct uptake of the substance under scrutiny and the relative importance of food versus e.g. water exposure can influence the magnitude of the TMF.

The position in the food web is quantified using relative abundances of naturally occurring stable isotopes of N ($^{15}N/^{14}N$, referred to as $\delta^{15}N$). However the relative abundance of these isotopes and thus the determination of the trophic level and TMF is influenced by the physiology of the organism and its life trait history. Rapid growth with a higher protein demand for new tissue leads to lower enrichment factors than those with slower growth rates. Insufficient food supply and fasting and starvation leads to catabolism of body proteins and an increase of ^{15}N in organisms relative to those organisms with adequate food supply.

There is no standard procedure for the conductance of TMF field studies. Hence, the conductance and sampling may vary between different studies. Disproportionate sampling of the food web or unbalanced replication of samples may significantly influence the TMF. As pointed out by Borga et al. (2012) an appropriate sample sizes is needed to achieve sufficient statistical power to evaluate TMF. The required sample sizes are affected by the design of the trophic transfer study, which improves with an advanced ecological understanding of trophic relationships.

Particular problems with averaging the TMF may occur if food webs comprise both poikilotherms and homeotherms. An investigation of an Arctic food web revealed the unequal magnification behaviour of POPs within both thermal groups (Hop et al. 2002). These results may be explained by a higher food intake, caused by a higher energy demand, and a longer life span of birds and mammals. Intrinsic differences in gastrointestinal absorption mechanisms have also been suggested as an explanation for

these differences between homeotherms and aquatic poikilotherms (Drouillard and Norstrom 2000). Therefore, when the trophic magnification potential of a substance is determined via a single regression for the overall food web, the magnification in poikilotherms may be overestimated and the magnification in homeotherms, in particular apex predators, may be underestimated (Fisk et al. 2001).

Additionally, as already discussed in the BMF section, sample collection is often restricted to tissue or serum samples in large predators due to ethical reasons and due to the challenging logistics with respect to sampling and laboratory constraints.

Martin et al. (2004) examined PFNA contents in the food web from Lake Ontario (Canada). Adult lake trouts (top predator) were collected at various locations in Lake Ontario in 2001. Samples of prey fish (sculpins, smelts and alewifes) and macroinvertebrates (*Mysis* sp., *Diporeia* sp.) were collected at one location in October 2002. Lake trout samples analyzed represented individual whole fish homogenates. The other species were processed as composites of whole individuals. The authors note that *Diporeia* sp. is a benthic invertebrate species, and sculpins feed mainly in the benthic environment. Benthic contamination may therefore be the source of contamination of this food web.

Houde et al. (2006) investigated the food web of bottlenose dolphins. The results are summarised in Table 8. The authors sampled different biota, i.e. Atlantic croaker (n=3), pinfish (n=4), spotfish (n=10), spotted seatrout (n=11), striped mullet (n=8) and plasma samples from livecaptured bottlenose dolphins (n=24), as well as water (n=18, samples analyzed in duplicate) and surface sediment (n=17, samples analyzed in triplicate). Sample collection was conducted between 2002 and 2004. Based on stable isotope (^{15}N) analysis the trophic level of each biota sample was determined. PFNA was additionally analysed in plasma, liver, lung, kidney, heart, thymus, thyroid and muscle of two freshly dead dolphins and afterwards a whole body burden was calculated. The extrapolation of tissue specific concentrations to whole body burdens is based on the total body weight, the organ weights and the blood volumes. For prey whole body homogenates were analysed for PFNA. TMFs indicate bioaccumulation of PFNA when dolphin plasma concentrations were taken into account, as well as when whole body burdens for dolphins were considered. Wastewater treatment plant discharges in the Charleston area may have resulted in non–steady state concentrations of perfluorinated compounds in the food web.

Kelly et al. (2009) measured PFOA in the Canadian Arctic marine food web. Concentrations in sediment (n=9) and in different organisms (lichens, macroalgae (n=6), bivalves, fish (n=3-6)) and tissues and organs (stomach contents, liver, muscle, blubber and/or milk) of common eider ducks (n=5), seaducks (n=4), and marine mammals beluga whales and ringed seals were used to calculate TMFs (Table 8). Sample collection was conducted between 1999 and 2003 along the eastern Hudson Bay coastline in close proximity to the Inuit village Umiujaq. PFNA was measured in different tissues/fluids of the beluga whale including blood (n=18), muscle (n=18), liver (n=22), milk (n=6) and also in foetuses (n=2). The authors showed that PFNA especially accumulates in protein rich compartments such as blood and liver and that the TMFs of perfluorinated compounds such as PFNA correlate with the partitioning behaviour between protein and water and protein and air. Wet weight PFCA concentrations were expressed on a protein weight basis (ng·g-1 protein wt), using total protein content (P_{TOTAL}) values of biological tissues/fluids of fish, birds and mammals. P_{TOTAL} values of 2% for macroalgae, 25% for muscle, 25% for bird and mammalian liver tissue (25%), 7% for beluga blood and 11% for beluga milk were used. A pharmacokinetic model for beluga wales was used (Hickkie et al. 1999) to estimate the whole body burden. The basis for the model development were PCBs. Because PFCAs are primarily retained in protein-rich compartments (blood and liver), organism- and compartment-specific protein turnover rates may influence the toxicokinetics of these compounds. Comparisons of different food webs show that the TMF is below one in the case of piscivorous food webs if air breathing organisms are excluded but becomes larger than one if air breathing organisms are taken into account. TMFs for the food web of the beluga

whale are >1, indicating bioaccumulation, when they are normalized to protein contents as well as without that normalization (Kelly et al. 2009).

Xu et al. (2014) investigated the bioaccumulation of perfluorinated compounds in a food web in Taihu Lake in China. As the study by Martin et al. (2004) this is a study for fresh water food web. As stated by Loi et al. (2011) bioaccumulation patterns depend on salinity. Taihu Lake, is the second largest lake in China, and serves as drinking water supply, irrigation water, aquaculture farm as well as for recreational attractions. From the late 1980s, water and soil pollution from industry, agriculture, and urban wastes has been increasing significantly in the Taihu Lake region. Surface water (n =30), surficial sediment (n = 30), phytoplankton (mainly include *Chlorophyta, Bacillariophyta* and *Cyanophyta*), zooplankton (mainly include *Copepoda, Cladocera*, and *Rotifers*), two zoobenthos species (*Bellamya* sp. (snail) (n = 9) and *Corbiculidae* (bivalve) (n = 8)), white shrimp (*Exopalaemon modestus Heller*) (n =18), fish samples of nine different species, i.e. *Hypophthalmichthys molitrix* (n = 10), *Protosalanx hyalocranius* (n = 6), *Hemiculter leucisculus* (n = 7), *Aristichthys nobilis* (n = 4), *Hyporhamphus intermedius* (n = 6), *Pelteobagrus fulvidraco* (n =4), *Erythroculter ilishaefor* (n = 8), *Cyprinus carpio* (n = 7), *Coilia ectenes* (n = 22), and two egret bird species (Egrets and Night Herons) as prey animals were collected from Taihu Lake in May 2010. In order to investigate the diet relationship in this food web stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were determined. Following groups were formed: zooplankton and zoobenthos (*Bellamya* sp. and Corbiculidae) (11-12 ‰), herbivorous fish (14-16 ‰), omnivorous fish (17-18 ‰), and carnivorous fish and egrets (19-21 ‰).

The 12 analyzed PFCAs (C8-C12) were detected in all biological samples. In water, PFCAs with six to nine carbons were regularly detected. The long-chain PFCAs (C11, C12) were only detectable in sediments. PFNA concentrations were ranged in the different biological samples as follows: 1.02 (phytoplankton), 0.086 (zooplankton), 0.15 (zoobenthos), 0.15 (white shrimp), 0.42-2.7 (fish), 2.86 (egrets) ng/g, ww. PFCAs with nine to twelve carbons were significantly biomagnified, with a TMF value of 2.1 for PFNA.

TMFs are summarised in Table 8 below. With the exception of the study conducted by Kelly et al. (2009) none of the studies were protein-normalised. None of the studies were lipid normalized.

Table 8: Trophic Magnification Factors (TMF) of PFNA

Location	Species (tissue)	TMF	Reliability	Reference
Lake Ontario	Diporeia/slimy sculpin (all whole)	1	2	Martin et al., 2004
	Mysis/alewife/rainbow smelt/lake trout (all whole)	1		
US, South Carolina	Dolphin plasma/croaker, pinfish, spotfish, spotted seatrout (fish all whole)	4.7 ± 8.4	3	Houde et al., 2006
	Whole dolphin body burden	2.4 ± 3.1		
Hudson Bay (north-eastern Canada	**Marine mammalian food web:** Sediment/ macroalgae(whole)/ bivalves(whole)/ fish(muscle)/ seaduck (liver)/ ringed seal (liver) beluga whale(estimated whole body)	4.99-9.88 2.9-6.19 (protein corrected)	2	Kelly et al., 2009
	Piscivorous food web: Sediment/ macroalgae(whole)/ bivalves(whole)/ fish(muscle)	0.33-1.22 (protein corrected)		
Taihu Lake/ China	zooplankton and zoobenthos, herbivorous fish, omnivorous fish, and carnivorous fish and egrets (all whole)	2.1	2	Xu et al., 2014

Conclusion: A number of field studies are available which analyzed the trophic magnification potential of PFNA. In the same manner as BMFs, also TMFs are lower when gill breathing organisms are top predators of the investigated food chain either showing no significant association with the trophic level or values near 1 (TMF 0.33 – 1.22). When air-breathing animals are top predators in the food chains, all studies indicate trophic magnification of PFNA (2.1 – 9.88). All TMF values refer to either whole body measurements or estimated whole body values. Several studies have shown that PFCAs especially accumulate in protein rich compartments such as blood. Equivalent to lipid-normalisation Kelly and co-workers have thus conducted a protein-normalisation. Protein corrected TMFs range between 2.9-6.19.

3.3.3. Terrestrial bioaccumulation (soil dwelling organisms, vertebrates)

3.3.3.1. Bioaccumulation in soil dwelling organisms

There are two studies available which investigated the bioaccumulation of PFCs in earthworms.

In the first study earthworms were exposed to artificially contaminated soils with ten PFCs with different chain length. A soil without detectable PFASs was collected and spiked with one mL of a mixed solution of 10 PFASs. The soil concentration of each PFAS was 100, 200, and 500 ng/g respectively. The soil was then incubated in the dark for four days at room temperature. Mature earthworms (*Eisenia fetida*) were exposed for up to 30 days. Samples were taken at day 2, 4, 6, 8, 12, 16, 20, 24, 28 and 30. All the 10 PFASs were detected in the earthworms already after an exposure for 2 d, indicating that they can be taken up by earthworms from soil quickly. The estimated time to steady state was 23.2 days. An important finding is that the uptake increased with increasing chain length. The bioaccumulation was calculated as a biota to soil accumulation factor (BSAF) which was normalised for the organic carbon content in the soil. The BSAF values ranged from 0.044-0.08 for PFNA (Zhao et al., 2013). The BSAF Values for PFOA were 0.131 and ranged between 0.542-3.408 for the longer chained PFDA, PFUnA and PFDoA.

In a second study bioaccumulation was investigated exposing *Eisenia fetida* to contaminated soil. As the authors state, spiked soil as used in the study by Zhao et al. (2013) may not reflect typical field conditions as spiked soils are generally not representative of field conditions of bioavailability. Therefore in this study contaminated soil was collected; one soil that had received long-term field application of municipal biosolids, an industrial impacted soil and two soils from a former fire fighting training area either adjacent to the source zone or 180 m from the source zone. Five adult earthworms per container were exposed for 28 days to achieve steady state values. For the determination of kinetic values additional sets of triplicate containers were prepared for sampling on day 1, 3, 5, 7, 9, 12, 16 and 21. Biota to soil accumulation factors not normalised to the carbon content in the soil (BAFs) as well as BSAFs normalised to the different carbon contents were reported (Table 9). The BSAF values ranged from 0.034-0.038 for PFNA and 0.019-0.02 for PFOA and ranged between 0.033-0.105 for the longer chained PFDA, PFUnA and PFDoA (Rich et al., 2015).

Table 9: Bioaccumulation in soil of PFNA

Location	**species**		**BSAF[g_{oc}/g_{ww}] /BAF[$g_{dw,soil}/g_{dw,worm}$]**	**Reliability**	**Reference**
Laboratory	earthworm (*eisenia fetida*)	BSAF	0.8±0.004 (100 ng/g) kinetic 0.046±0.001 (200 ng/g) kinetic 0.044±0.006 (500ng/g) kinetic	2	Zhao et al., 2013
laborartory field soils	earthworm (*eisenia fetida*)	BAF	4.08±1.79 (estimated steady state) 3.64±0.59 (measured)	2	Rich et al., 2015
		BSAF	0.038±0.017 (estimated steady state) 0.034±0,0055 (measured)		

Conclusion:
According to the REACH PBT guidance it is not possible to give any threshold values for BAF and BSAF in soil, as there are not enough scientific data available at the present time. A case-by-case assessment based on expert judgement of the reliability and relevance of the available information is required in order to be able to give BAF and BSAF values an appropriate weight in the B / vB assessment. It can however be seen that the BSAF values for PFNA are in the same order of magnitude as the longer chained PFCAs and PFOA which

have been identified as PBT and /or vPvB.

3.3.3.2. Bioaccumulation in vertebrates

Müller et al. (2011) conducted a terrestrial food web study consisting of lichen and plants, caribou, and wolves from two remote northern areas in Canada. This food web is considered as a relatively well documented example (Kelly and Gobas, 2003) and in particular caribous have been studied intensively due to their economic and social importance for indigenous people in the Canadian Arctic. Furthermore, the food web is relatively simple, as caribous feed mostly on lichen (in summer the diet also consists of willow, sedges and grasses) and wolves living near barren-ground caribou herds almost exclusively feed on them. Liver, muscle, and kidney samples (n=7 for Porcupine herd food web and n=10 for the Bathurst food web) from two caribou herds were collected; from the Porcupine herd in northern Yukon Territory and the Bathurst herd in the Northwest Territories (NWT)/western Nunavut. Wolf (n=6 for Porcupine herd food web and n=10 for the Bathurst food web), lichen, and plant samples were collected in the same region as the caribou. Plant samples included cottongrass, aquatic sedge, willow, moss, and mushrooms. Liver and muscle samples were collected from the sampled wolves. Lichen, moss and mushrooms were collected as a whole, grass and willow without roots. Plant samples are from the same season (summer 2008 in Porcupine and summer 2009 in Bathurst) whereas wolf and caribou samples are from different years (2007 and 2010 in Porcupine and 2008 and 2007 in Bathurst). As variations in concentrations in remote regions are expected to be low the influence of samples from different years is expected to be low, as well. Whole body concentrations were calculated for each individual caribou and wolf based on the concentration in the specific tissue and the mass fraction of this tissue. If one tissue has not been measured in this study, the concentration was estimated based on data in the literature, i.e. concentration in blood and lungs were assumed to be half of that of liver and the carcass was assumed to have half the concentration found in muscle tissue. Bones were excluded from the whole body calculation because per- and polyfluorinated chemicals are assumed to not enrich in this media and bones are not part of the diet of wolves. As the authors state: it is very complex and laborious to obtain all information needed to calculate whole body concentrations for larger animals. Concentrations or body composition need to be estimated. If not all information is available, uncertainties are introduced. For Caribou PFNA concentrations in liver were 2.2 ± 0.2 and 3.2 ± 0.4 ng/g ww for the Porcupine and Bathurst herds, respectively. For Wolves PFNA concentrations in liver were 4.7 ± 0.9 and 7.4 ± 1.3 ng/g ww for the Porcupine and Bathurst herds, respectively. Mean PFC concentrations were higher in the Bathurst samples, this difference was statistically significant ($p < 0.05$) for PFNA. The authors assume that this might be due to differences in distance from source regions in North America. PFCA concentrations in muscle and kidney were 10 to 20 times lower. For Caribou PFNA concentrations in muscle were 0.064 ± 0.008 and 0.093 ± 0.009 ng/g ww for the Porcupine and Bathurst herds, respectively. For Wolves PFNA concentrations in muscle were 0.44 ± 0.06 and 1.1 ± 0.1 ng/g ww for the Porcupine and Bathurst herds, respectively. The study illustrates a considerable carry over between plants and caribou. Caribou is a major human food source in numerous arctic communities. This food-chain may also be considered comparable to the pasture-cow food-chain in temperate regions. The results of the study, BMFs as well as TMFs, are shown in Table 10 and Table 11. Due to the large difference between lichen and caribou compared to usually assumed trophic enrichment, the TMF values may be less reliable.Tissue concentrations and whole body concentrations were used for calculations. Tissue based BMFs differ considerably. Therefore, it is concluded that BMFs based on whole body concentrations are more appropriate (Müller et al., 2011). Overall, although there are uncertainties, the results indicate bioaccumulation of PFNA within this food-chain.

In order to understand how PFCAs are accumulated and transferred through agricultural food chains the bioaccumulation of PFCAs in dairy cows receiving naturally contaminated feed and drinking water was investigated by conducting a mass balance of PFCAs for a herd of dairy cows in a barn on a typical Swedish dairy farm (Vestergren et al., 2013). Unlike the

other field studies there is no uncertainty concerning opportunistic feeding as this study has a clear defined diet. The farm was selected to represent a background contaminated agricultural area with no known point sources of PFCAs in the proximity. Silage, barley, and feed supplements were collected monthly from November 2010 to April 2011 (n=6) from the daily feed portion just before being distributed to the animals. The average individual intake of silage (38.5 kg per day), barley (8.8 kg per day), and supplements (8.6 kg per day) was derived from the total annual consumption on the farm divided by the average number of cows present in the barn. Drinking water was sampled from the farm water supply. The cow's drinking water intake was estimated to be 50 L per day. Milk samples were collected from a milk tank, where milk from the entire farm is stored after milking. Muscle, liver, and whole blood samples were obtained from five individual cows from the slaughterhouse on two different occasions (April and June 2011). Tissue-specific BMFs were calculated for liver, blood, and muscle. Biomagnification factors (BMFs) were highly tissue and homologue specific. The highest concentrations of PFCAs in cow tissues were observed in liver samples and similar to those observed in caribou from the Canadian Arctic (Müller et al. 2011). Consumption of silage was identified as the dominant intake pathway for all PFCAs (75-81%). Drinking water intake was neglible for the total intake. The results suggest that long-chain PFCAs have a relatively high potential for transfer to milk and beef from the diet of dairy cows. Tissue specific BMFs are >1 for blood and liver and near 1 for muscle.

Table 10: BMFs for PFNA in a terrestrial food chain

Location	Species (tissue)	BMF	Reliability	Reference
Porcupine in northern Yukon Territory and Bathurst in the Northwest Territories of Canada	Caribou (muscle)/lichen	1.2 ± 0.3 (Porcupine) 0.9 ± 0.2 (Bathurst)	2	Müller et al., 2011
	Caribou (liver)/lichen	40 ± 12 (Porcupine) 32 ± 7.0 (Bathurst)		
	Wolf (muscle)/caribou muscle	6.9 ± 1.2 (Porcupine) 12.4 ± 1.7 (Bathurst)		
	Wolf (liver)/caribou liver	1.0 ± 0.1 (Porcupine) 1.4 ± 0.1 (Bathurst)		
	Caribou (whole)/lichen	2.8 ± 0.7 (Porcupine) 2.7 ± 0.6 (Bathurst)		
	Caribou (whole)/vegetation	8.5 ± 2.6 (Porcupine) 5.3 ± 1.0 (Bathurst)		
	Wolf (whole)/caribou (whole)	3.8 ± 0.6 (Porcupine) 5.4 ± 0.8 (Bathurst)		
Swedish dairy cattle farm, Backa Gård in Kårsta	silage, barley/sweedish red (bos taurus)muscle	1.4	2	Vestergren et al., 2013
	silage, barley/sweedish red (bos taurus)liver	4.9		
	silage, barley/sweedish red (bos taurus)blood	4.5		

Table 11: TMFs for PFNA in a remote terrestrial food chain (from two different locations)

Location	Species (tissue)	TMF	Reliability	Reference
Porcupine in northern Yukon Territory and Bathurst in the Northwest Territories of Canada	Wolf (liver) /caribou (liver)/lichen	6.7 ± 0.5 (porcupine) 4.5 ± 0.2 (Bathurst)	3	Müller et al., 2011
	Wolf (whole)/caribou (whole)/lichen	2.7 ± 0.2 (porcupine) 2.2 ± 0.1 (Bathurst)		
	Wolf (whole)/caribou (whole)/vegetation	2.0 ± 0.2 (porcupine) 1.9 ± 0.1 (Bathurst)		

Conclusion: The terrestrial BMF and TMF of PFNA are greater than one for the remote Arctic food chain lichen – caribou – wolf, indicating trophic biomagnification. BMF and TMF values based on estimated whole body values range between 2.7-8.5 for caribou and lichen or vegetation and 3.8-5.4 for the predator-prey relationship between wolf and caribou. TMF values range between 1.9-2.7. This food-chain may also be considered comparable to the pasture-cow food-chain in temperate regions. The study conducted with cattle from a Swedish dairy cattle farm confirms these findings. Additionaly, the results suggest that long-chain PFCAs have a relatively high potential for transfer to milk and beef from the diet of dairy cows.

3.3.4. Summary and discussion of bioaccumulation

There are no experimentally determined BCF values available for PFNA. Due to the structural similarity and the regular pattern of physico-chemical properties within this group of PFCAs it can be assumed that also PFNA has a BCF smaller than 2000 based on the shorter or longer chained homologues PFOA and PFDA. BCFs range from 1.8 to 8.0 for PFOA and from 450 to 2700 for PFDA. Conclusions on bioaccumulation should be based on whole body values and carcass is seen as a good approximation for whole body. Based on the BCFs of the carcass for PFOA and PFDA it is concluded that PFNA does not bioaccumulate.

BAFs vary considerably for PFNA. Whole body BAF values from water breathing animals range between 0-3981. BAF values for red drum, spotted seatrout and lake trout range between 2529-3981 and support that PFNA is bioaccumulative.

Whole body BMF values for gill breathing organisms like fish as predators range from 0.13 to 5.3. The expected[7] high water solubility of PFNA may enable fish and mussels to quickly excrete this substance via gill permeation, facilitated by the high water throughput. For instance, the study of Goeritz et al. (2013) shows a considerable growth corrected depuration rate constant (k_{2g}) of 0.058 d^{-1}. Air-breathing homeotherms are unable to efficiently eliminate PFNA to avoid accumulation. For predator-prey relationships, including seals, beluga whales, dolphins and polar bears studies, available data are indicating bioaccumulation (BMFs 1.2-111). BMFs range between 1.4 and 24 based on estimated whole body values for dolphin and beluga whale. These data support the conclusion that PFNA is bioaccumulative.

In the same manner as BMFs also TMFs are lower when gill breathing organisms are top

[7] Based on the data of the analogue PFOA, see Annex I.

predators of the investigated food chain (TMF 1 – 2.1). When air-breathing animals are top predators in the food chains all studies indicate trophic magnification of PFNA (2.9 – 9.88). All TMF values refer to either whole body measurements or estimated whole body values. One study additionally conducted a protein normalization. Protein corrected TMFs range between 2.9-6.19.

The terrestrial BMF and TMF of PFNA is greater than one for the remote Arctic food chain lichen – caribou – wolf as well as for cattle from a Swedish dairy cattle farm. TMFs range between 1.9 and 6.7. BMFs range between 1.0 and 40. Terrestrial BMFs and TMFs range between 2.7-8.5 and 1.9-2.7, respectively, based on estimated whole body values. The results from the Swedish dairy cattle farm additionaly suggest that long-chain PFCAs have a relatively high potential for transfer to milk and beef from the diet of dairy cows. Caribou are a major human food source in numerous arctic communities. This food-chain may also be considered comparable to the pasture-cow food-chain in temperate regions.

Many field analyses are based on tissue or serum samples. Problems with respect to sampling and laboratory constraints increase with increasing body size of predators at the top of the food chain. If the mass fraction is known whole body values may be estimated for the organism. This has been conducted in some of the studies especially concerning mammalian top predators. TMFs and BMFs based on whole body values should be preferred, as utilization of serum or organ specific concentrations may be overestimative. The extrapolation to whole body burdens may however include some unquantifiable uncertainties (Houde et al., 2006). It is very complex and laborious to obtain all information needed to calculate whole body concentrations for larger animals. If not all information is available, uncertainties are introduced. Nevertheless, all BMFs and TMFs including air-breathing animals and based on whole body estimations are well above 1 and thus indicate biomagnification and trophic magnification.

With the exception of the study by Xu et al. (2014) and Vestergren et al. (2013) sampling was conducted in different years all around the millenial. The time laps were three to four years. Some uncertainty may therefore exist due to varying environmental concentrations in the different years. Many of the studies have been conducted in remote regions were the variation of the environmental concentration may be expected to be lower than in urban areas with possible point sorces of emissions. In polar bears, PFNA concentrations increased gradually from 1984 to 2006 with a doubling time of 11.2 years and an annual increase of 6.2% in east Greenland (Rigét et al. 2013). In the same study ringed seals showed an annual increase of 1.7% (doubling time 41.2 years) and 3.7% (doubling time 18.6 years) in west and east Greenland respectively. A similar annual increase of 6.1% in Polar bears in east Greenland was reported by Dietz et al. (2008). Butt et al. (2007) reported a doubling time of 10.0 to 7.7 years for arctic ring seals sampled between 1993-2005. Smithwick et al (2006) reported a doubling time of 3.6 and 5.6 between 1972 and 2002. Between 1997 and 2008 Lake Ontario Lake Trout showed an annual decrease of 0.03% (Gewurtz et al., 2012). Considering the time laps of the sampling years and comparing these with the time trends reported for PFNA the variation due to sampling in different years is probably less pronounced than the variation between individual animals.

In contrast to many other chemicals PFCAs do not accumulate in storage lipids. Therefore, the customary approach to normalise all values to a certain lipid portion is not straightforward. A protein-normalisation has been conducted in one study. This seems reasonable as it has been observed that PFCAs accumulate especially in protein rich tissue. A protein normalisation of all B- values in this dossier is however not feasible as the fraction of protein are unknown for the organisms that were investigated with regard to accumulation of PFCAs. Additionally, the fraction of protein content may vary considerably between different organisms. Therefore, a generalised approach with respect to protein-normalisation does not seem reasonable. Furthermore, PFCAs do not only tend to bind to proteins but also to membrane phospholipids (Ng and Hungerbühler, 2014). Depending on the individual PFCA and its physico-chemical properties, PFCAs do not exclusively bind to

proteins alone.

The data summarised above is in high accordance with the bioaccumulation data on the other PFCAs (for details, see the Support Documents of PFOA and the C_{11}-C_{14}-PFCAs (European Chemicals Agency 2013b, 2012a, 2012b, 2012c, 2012d) and Figure 1-3 in Annex 1). Altogether these data show a regular pattern of bioaccumulation which depends on the chain-length of the perfluorinated alkyl chain.

In addition to the information on bioaccumulation on environmental species, data on laboratory mammals and humans provide evidence on the bioaccumulative behavior (see Section 4 for further details).

4. Human health hazard assessment

4.1. Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

4.1.1.1. Absorption

No data for PFNA has been found, but based on other toxicokinetic data for PFNA (Ohmori et al. 2003, Tatum-Gibbs et al., 2011), and data for other PFCAs, such as PFOA and APFO (US EPA 2005), it can be assumed that PFNA is well absorbed in laboratory animals following oral and inhalation exposure, and to a lesser extent following dermal exposure.

4.1.1.2. Metabolism

Carbon-fluoride bonds are among the strongest in organic chemistry, and there are, to our knowledge, no indications that PFNA is metabolized.

4.1.1.3. Distribution

Several animal studies in rats, mice, rainbow trouts, seals, whales and gulls demonstrated that perfluoroalkylacids accumulate preferentially in the blood and liver, while in-vitro studies have shown that they are able to strongly bind proteins such as serum albumin and liver fatty acid protein (L-FABP) (Ng and Hungerbühler, 2014).

In particular, PFNA accumulates preferentially in the liver compartment in both rat (Tatum-Gibbs et al., 2011) and mouse (Tatum-Gibbs et al., 2011; Das et al, 2015), with a mouse liver-to-serum ration of 5-8:1 after gavage administration (Das et al, 2015).

In a study by Benskin et al. (2009) in Sprague-Dawley rats, the highest concentrations of PFNA were found in the liver followed by kidneys, lungs, heart, spleen, testes, muscle, fat, intestines and brain.

Similar to PFOA and PFOS, PFNA was demonstrated to cross the placental barrier readily, and to be transferred via lactation in mice, with both serum and liver concentrations in non-pregnant mice approximatively twice as high as those in pregnant mice and post-weaning dams (Das et al, 2015). In a developmental study on mice, where dams were exposed to PFNA during gestational days 1-18, PFNA serum levels in pups at weaning were comparable to that of their mothers (Wolf et al., 2010).

While liver and blood are primary sites of accumulation for PFAAs, some variability exists

among different species and different PFAA derivatives (Ng and Hungerbühler, 2014) with the kidneys and bladder being also important distribution sites. However, unlike neutral hydrophobic organic chemicals, PFAAs do not accumulate in fat tissues (storage lipids) owing to their water, oil and grease repellent properties.

Recent models to explain the substantial bioaccumulation of PFCAs include association to phospholipids as well as interaction with proteins within the organism, such as serum albumin in blood, L-FABP in both liver and kidney, and renal organic anionic transport proteins (Ng and Hungerbühler, 2014).

4.1.1.4. Elimination

Urine is the primary route of excretion of PFNA. There are large sex and species differences in the elimination half-lives of PFNA, as well as of PFOA (cf. Table 13). 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 µmol/kg bw PFNA. Major sex differences in the rate of elimination were also 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 is 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 (Tatum-Gibbs et al., 2011).

A reason for the differences in elimination rates is likely that PFNA is a substrate for renal organic anion transporters, regulating active renal reabsorption, and these transporters are differentially expressed between species and sex (Han et al., 2012).

The serum and hepatic half-lives of PFNA are generally longer than those of PFOA (Tatum-Gibbs et al., 2011).

The following behavior of PFNA and other PFCAs can be observed based on the toxicokinetic studies.

- Comparing C7 to C10 PFCAs (Ohmori et al., 2003), there is a trend between carbon chain legth and clearance from the plasma in both male and female rats (cf. Table 13).

- PFNA, as well as PFOA, plasma clearance was faster in female rats compared with male rats (cf. Table 13).

- Concerning the high gender- and species-specific variability in elimination half-lives:

    - Renal elimination of PFCAs (and in particular, species and gender difference): It has been recognized that organic anion transport proteins play a key role in PFCA renal tubular reabsorption, a process that is sex-, species-, and chain length-dependent (Han et al., 2012).

    - Renal excretion in female rats is substantially higher than in male rats: (approximately 50 h vs approximately 1000 h for PFNA in female and male rats, respectively). This was linked to the male-dominant gender expression of the organic anion transporting polypeptide (Oatp) a1 in rats (Han et al., 2012).

- PFCAs vary in their affinities to serum albumins, which is an important factor in

determining the renal clearance of PFCAs (Han et al., 2012).

RAC, in their opinion on the harmonised classification of PFNA and its sodium (PFN-S) and ammonium (PFN-A) salts, concluded that *"existing data indicate that toxicokinetics of PFNA and PFOA are similar in rats, mice and in humans"* (European Chemicals Agency 2014).

4.1.2. Human information (including bioaccumulation in humans)

4.1.2.1. Absorption

There are no studies on absorption of PFNA in humans. However, based on animal studies of PFNA and other PFCAs, as well as on abundant findings of PFNA and other PFCAs in human blood, it can be assumed that PFNA is well absorbed after oral and inhalation exposure and to a lesser extent following dermal exposure.

4.1.2.2. Levels of PFNA in human body fluids

Exposure

There are two important sources of exposure of per- and polyfluoroalkyl substances (PFASs) such as PFNA to the general population, namely via food and drink intake (Haug et al., 2010a and 2010b) and through exposure to house dust (Huber et al., 2011, Ericson Jogsten et al., 2012). Food intake is assumed to be the main source of exposure. PFNA has been detected in among others fish, cereals, milk and dairy products, meat and meat products (Haug et al., 2010a and 2010b). If the drinking water is contaminated with PFNA, this may be an important source of PFNA exposure and result in increased serum levels (DWQI 2015).

Workers may be exposed to higher levels, for example when using high amounts of fluorinated ski waxes (Nilsson et al. 2010, Freberg et al., 2010).

General population

PFNA has been detected in various body fluids, such as serum, cord blood and human breast milk (Chen et al., 2012, Haug et al., 2010b, Kärrman et al., 2007, Tao et al., 2008, Schecter et al., 2012, Falandysz et al. 2006). Several studies in the Swedish health related biomonitoring program (Swedish EPA) have generated data on PFNA in the general population. The median serum level in more recent samples is around 1 ng/ml.

In a study with blood sampled 2011-2012 from 270 adults in Sweden (age 18-80 years), the median serum concentration of PFNA was 0.80 ng/ml (P5-P95 range 0.35-1.66) (Bjermo et al., 2013). Men had statistically higher serum levels than women (adjusted mean 0.73 vs 0.62 ng/ml) , which may be due at least in part to elimination of this and other PFAAs during breastfeeding (Bjermo et al., 2013). Age was positively correlated to the serum levels of PFNA, suggesting an ongoing bioaccumulation process. The mean level among 61-80 years old was 59 % higher compared to the age group 18-40 years. The study also showed a positive correlation between PFNA levels and fish intake.

Similar levels have been found in Norway; the mean serum level of PFNA in 175 samples was 1.1 ng/ml (range 0.27-4.3) (Haug et al., 2010b). Consumption of fish and shrimps was significantly correlated to the serum levels of PFNA.

Median human PFNA and PFOA serum concentrations in children were found to be very similar for girls and boys (Schecter et al., 2012). This study collected and analyzed serum concentrations of PFNA and PFOA in children from Texas of zero to 12 years of age. 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. There were also no differences in the serum levels between young (approx. 18 years) males and females in a Swedish investigation (Jönsson et al., 2014).

A temporal trend analysis from 1996 to 2010 in primiparous women in Sweden three weeks after delivery showed increasing levels of PFNA in serum with 4.3 % increase per year (Glynn et al., 2012). Also data from the USA indicate increasing levels of PFNA. Median serum levels in women of child-bearing age increased from 0.5 ng/ml in 1999-2000 to 1.2 ng/ml in 2007-2008, the increasing trend being statistically significant (US EPA). This is in contrast to PFOA and PFOS, for which there has been a general downward trend since year 2000 (see also DeWitt, 2015).

Professional workers

A study on occupational exposure of professional ski waxers (n=8) showed blood concentrations of PFNA much higher (up to 100-fold) than in the general population, the highest value was 163 ng/ml in whole blood, corresponding to approx. 326 ng/ml in serum (Nilsson et al. 2010). Monthly blood samples were collected before the ski season, i.e., preseason, then at four FIS World Cup competitions in cross country skiing, and finally during an unexposed 5-month post-season period. The PFNA levels in technicians with lower initial levels of PFNA increased from pre-season to post-season, whereas no increases in the blood levels were observed for the technicians with higher (> 20 ng/ml) initial levels. There was a significant association between number of years in the profession and blood levels of PFNA, indicating bioaccumulation.

In a Norwegian study, serum samples from 13 professional male ski waxers were collected at three occasions (Freberg et al., 2010). The first blood sample was drawn at the end of season I (spring), the second at the beginning of season II (autumn) and the third at the end of season II (spring). The median serum concentrations of PFNA were similar at all samplings; 13 ng/ml (range; 3.6-37 ng/ml); 13 ng/ml (range; 3.3-38 ng/ml) and 12 ng/ml (range; 3.9-33 ng/ml), respectively. There was thus no decrease in serum levels during the 8 months between end of season I and start of season II, suggesting a long elimination half-life for PFNA. Also in this study a statistically significant positive association between years exposed as a ski waxer and concentration of PFNA in serum was observed.

4.1.2.3. Gestational and lactational transfer

As already demonstrated in the studies summarised in section 4.1.1.3 Distribution, PFNA can be expected to be transferred also from mothers to infants.

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).

In a study from Norway including 123 pairs of maternal and cord plasma samples, the median PFNA concentration in cord plasma (0.12 ng/ml, range 0.04-0.97) was 35 % of the corresponding concentration in maternal plasma (0.34 ng/ml, range 0.04-2.18) (Gützkow et al., 2012). The concentrations of PFNA in cord blood correlated significantly with the concentrations in maternal serum at the time of delivery, which confirms the placental transfer of PFNA. Breast-feeding can also transfer PFNA to infants. In a Norwegian study, breastfeeding for more than 4 months was significantly associated with lower maternal serum levels of PFNA (Haug et al, 2010).

Parity has been shown to decrease serum levels of PFNA (Brantsaeter et al., 2013). When compared to nulliparous women, parous women had 62 % lower concentrations of PFNA. The levels of PFNA increased significantly with time from the most recent delivery. The total breastfeeding duration was negatively associated with serum levels of PFNA.

The levels of PFNA in breast milk are low compared to the serum levels. In a Swedish study, where PFNA was analyzed in 50 matched milk and serum samples from primipara women, collected 2004-2011, PFNA was detected in 30 % of the milk samples at levels between 0.028 and 0.079 ng/ml, compared to serum levels between 0.20 and 1.67 ng/ml (Kärrman et al., 2013).

Fromme et al. (2010) measured the concentration of a number of perfluorinated compounds, including PFNA, in maternal blood during pregnancy (at two time points; n = 40 and 38) and six months after delivery (n = 47), in cord blood (n = 33) and in blood of infants six months (n = 40) and nineteen months (n =40) after birth in 53 mothers and their breast-fed infants in Munich, Germany. The median concentrations in maternal serum (during pregnancy, at delivery and six months after delivery) and fetus/infant (cord blood, at 6 and 19 months after birth) are presented in Table 12 below. Even though the concentration of PFNA in blood serum of children were less than in maternal serum at birth, the levels increased at 6 months so that they were higher than in maternal serum, which is expected to be a consequence of breast-feeding. At 19 months of age, which is a time point when breast-feeding had stopped or decreased, the child blood serum levels had decreased to levels close to maternal levels, which is expected to be a consequence of decreased exposure on a body weight basis combined with dilution due to rapid growth.

Table 12: Concentrations of PFNA (ng/ml) in blood serum from mothers and infants in Munich, Germany

	Mother			Fetus/infant		
	Pregnancy	At delivery	6 months post delivery	Cord blood	6 months post birth	19 months post birth
Median (% > LOQ)	0.6 (86 %)	0.6 (83 %)	0.5 (83 %)	0.4 (30 %)	1.0 (90 %)	0.6 (83 %)

Data from (Fromme et al. 2010)

In conclusion, PFNA can be transferred to the foetus through the placenta. Further, PFNA has been detected in human breast milk and breast-feeding is thus one exposure route for PFNA in infants.

4.1.2.4. Distribution in the human body

No data for PFNA has been found, but it may be assumed that PFNA is distributed in a similar way as PFOA, i.e. with highest concentrations in liver, blood, lung and kidney (European Chemicals Agency 2013b).

4.1.2.5. Elimination

Zhang *et al.* (2013) collected paired blood and urine samples (n = 86) from Chinese adults and measured the concentrations of a number of perfluorinated compounds, including PFNA and other PFAAs. The participants were first divided into four groups; young females (age ≤ 50 years, n = 20), older females (> 50 years, n = 19), young males (≤ 50 years, n = 32), and older males (> 50 years, n = 15). The group of young females had significantly lower levels of PFAAs than the other groups and therefore the three other groups were combined. A reason for the lower levels in younger women is that menstrual bleeding, pregnancy and lactation are important elimination routes in addition to the major elimination via urine.

For most PFAAs, very good correlations (i.e., $p < 0.05$) were observed between the blood and urine concentrations. The weakest correlations were observed for PFUnA ($p > 0.05$), which is the longest chain-length PFCA that could be routinely detected in urine (detection frequency 98%), and for the branched isomer 3m-PFOA, which was only detected in 23% of samples (versus >75% for all other PFOA isomers). Among all urine samples, the

predominant PFCs (median values were reported and normalized by urinary volume) were PFOS (25 ng/L), followed by PFOA (19 ng/L), PFNA (1.7 ng/L), PFHxS (1.1 ng/L), PFHpA (0.82 ng/L), PFUnA (0.30 ng/L), and PFDA (0.22 ng/L).

Based on literature data and strong associations between urinary and blood concentrations (except PFUnA), renal clearance was assumed to be the major pathway for human elimination of the PFAAs, thus, CL_{total} was set equal to CL_{renal} (mL/day/kg). However, considering that menstrual clearance is an important clearance pathway for PFAAs in young females, menstrual clearance was estimated and added to renal clearance for calculation of CL_{total} in young females, using the same rate previously estimated for Japanese women (0.029 mL/day/kg). This estimated menstrual clearance is lower than CL_{renal} for most PFAAs and was considered a reasonable estimate because it is in the range of CL_{renal} for PFHxS and PFOS, which are two PFCs that were significantly lower in younger females. Although other clearance mechanisms are acknowledged, they are believed to be minor and were not accounted for experimentally (albeit, fecal elimination might be important for some longer chain (> C10) PFAAs).

The estimated geometric mean elimination half-lives for the young female group and the combined male and older female group for PFNA were 1.7 and 3.2 years, respectively. Corresponding values for PFHpA, PFOA, PFDA and PFUnDA are included in Table 13 in section 4.1.3. The authors stated that these estimated half-lives should be viewed as upper limits due to the possibility that there might be other elimination routes than via the urine and menstrual bleeding.

In conclusion, renal clearance is the major elimination pathway in humans for PFNA. In younger women, menstrual clearance is another important clearance pathway. Estimated geometric mean elimination half-lives for PFNA in young females and in the group of males and older females are 1.7 and 3.2 years, respectively. Corresponding values for the shorter (PFOA) and longer (PFDA) homologues are 1.5 y/1.2 y and 4.0 y/7.1 y. The values for PFUnDA (4.0 y/7.4 y) are similar to those of PFDA.

4.1.2.6. Bioaccumulation in humans

Taken together, there is strong evidence that PFNA bioaccumulates in humans.

- PFNA is present in quantifiable amounts in blood serum from the general population (eg. present in all samples from Sweden).
- Humans have very long elimination half-lives for PFNA, i. e. > 1 year.
    - Estimated average half-lives in the general population are between 1.7 and 3.2 years, depending on sex and age.
- In professional ski waxers, with comparatively high exposure to PFNA during the winter season, serum levels of PFNA did not decrease during 8 months between end of season and start of next season indicating slow elimination.
- Positive associations between years exposed as a ski waxer and concentration of PFNA in serum has been observed.
- In the general population, age is positively associated with serum levels of PFNA.
- Men have higher serum levels of PFNA than women, which may be due at least in part to elimination of this and other PFAAs during pregnancy and breastfeeding.
- High protein binding to albumin, L-FABP and renal anionic transporters, as well as partitioning to membrane phospholipids, are plausable mechanisms for the slow elimination of PFNA, as for other PFCAs.

4.1.3. Conclusion on toxicokinetics and bioaccumulation in humans

The toxicokinetics of PFNA have been observed in available studies to be very similar to PFOA. This was also stated in the RAC opinion on the harmonised classification of PFNA and its sodium (PFN-S) and ammonium (PFN-A) salts (*"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."*). It may therefore be assumed that PFNA is well absorbed following oral and inhalation exposure, and to a lesser extent following dermal exposure. PFNA is present in human blood of the general population and elevated concentrations are seen following specific exposures such as in professional ski waxers, which remain high over a very long timespan although the high exposure is ceased. In experimental animals (rats and mice) PFNA accumulates preferentially in the liver. As for PFOA, PFNA may also distribute to lung and kidney. There are no indications that PFNA is metabolised.

PFNA can be transferred to the foetus through the placenta. The concentration in cord blood has been shown to be approximately one third of the concentration in mother's blood. Further, parity has been shown to decrease serum levels of PFNA.

PFNA is present in human breast milk. The concentration is lower than in serum (5-10 % of serum concentrations), but breast-feeding is nevertheless one source of PFNA in infants. Gestational and lactational exposure is of special concern as the foetus and newborn babies may be more vulnerable to exposure to toxic substances.

Urine is the primary route of elimination, but in younger females menstrual bleeding is another important elimination pathway. Humans show a considerably slower elimination of PFNA, in particular compared to rats, with average half-lives around 2-4 years. The reason for this phenomenon is not fully understood, but might be attributed to e.g. different expression and amounts of renal anionic transporter proteins. Albumin, fatty acid binding protein and renal anionic tranporters, proteins that have been shown in animal studies to affect the distribution and elimination of PFNA and other PFCAs, are found in humans as well.

Available half-lives of PFCAs in humans and in rat, mice, pig and monkey are presented in Table 13 below. These data show that the potential bioaccumulation differs significantly across different species and gender. Variation within species/sex groups may be due to test conditions, such as dosing scheme (exposure route, single versus repeated dose). The half-lives of PFCAs generally increase with increasing chain-length. The half-life of PFNA in human serum is above 1.7 years, wheras the same parameter in rats and mice varies between 2 and 69 days.

It may also be valuable to compare data for PFNA with those for the longer and shorter homologous PFCAs, in particular when coming from the same study where identical methods have been used. In the study by Zhang *et al* (2013), estimated geometric mean elimination half-lives for PFNA in young females and in the group of males and older females are 1.7 and 3.2 years, respectively. Corresponding values for the shorter (PFOA) and longer (PFDA) homologues are 1.5 y/1.2 y and 4.0 y/7.1 y. The values for PFUnDA (4.0 y/7.4 y) are similar to those of PFDA.

Table 13: Half-lives of PFCAs in humans and other species.

Number of C/F-atoms	Name	Species half-lives						
		Rat	Mice	Pig	Monkey	Human (Arithm. mean = AM, Geom. Mean = GM, Median = M)		
						Retired and non-retired occupational workers	Young females	Males and older females
4/7	PFBA	M: 9.2h, F: 1.8h [a] M: 6.4h, F: 1.0h [b]	M: 13h, F: 2.9h [c] M: 16h, F: 3.1h [d] M: 5.2h, F: 2.8h [e]		M: 1.7d, F: 1.7d [f]	M: 3.0d AM, 2.7d GM [g] F: 3.6d AM, 3.4d GM [g]		
6/11	PFHxA	M: 1.0h, F: 0.42h [h] M: 2.2h, F: 2.7h [i] M: 2.7h, F: 2.4h [j] M: 2.8h, F: 2.3h [k] M: 1.7h, F: 0.5h [l] M: 1.5h, F: 0.7h [m]		M, F: 4.1d [n]	M: 5.3h, F: 2.4h [h]			
7/13	PFHpA	M: 2.4h, F: 1.2h [q]		M, F: 74d [o]			1.5y AM, 1.0y GM, 1.6y M [F]	1.2y AM, 0.82y GM, 0.79y M [F]
8/15	PFOA	M: 5.6d, F: 0.08d [q] M: 13d [r] M: 9.1d [s]	M: 22d, F: 16d [u]	M, F: 236d [p]	M: 21d, F: 33d [B] M: 20d [C] M: 21d [D]	M, F: 1.4y AM, 1.3y GM, 1.3y M [E]	2.3y AM, 1.7y GM, 2.0y M [F]	2.8y AM, 1.2y GM, 1.8y M [F]
9/17	PFNA	M: 30d, F: 2.4d [q] M: 41d [r] M: 47d, F: 2.1d [t] M: 42d [v] M: 24d, F: 32d [x] M: 28d [y]	M: 34d, F: 26d [z] M: 228d, F: 69d [A]				2.5y AM, 1.7y GM, 1.5y M [F]	4.3y AM, 3.2y GM, 3.5y M [F]
10/19	PFDA	M: 40d, F: 59d [q]					4.5y AM, 4.0y GM, 4.2y M [F]	12y AM, 7.1y GM, 9.2y M [F]
11/21	PFUnDA						4.5y AM, 4.0y GM, 4.4y M [F]	12y AM, 7.4y GM, 9.2y M [F]

[a] Data from Chang et al., 2008. Mean β-phase of one compartment model with first-order elimination, single oral dose of 30 mg/kg
[b] Data from Chang et al., 2008. Mean β-phase of one compartment model with first-order elimination, single IV dose of 30 mg/kg
[c] Data from Chang et al., 2008. Mean β-phase of one compartment model with first-order elimination, single oral dose of 10 mg/kg
[d]Data from Chang et al., 2008. Mean β-phase of one compartment model with first-order elimination, single oral dose of 30 mg/kg
[e] Data from Chang et al., 2008. Mean β-phase of one compartment model with first-order elimination, single oral dose of 100 mg/kg
[f] Data from Chang et al., 2008. Mean β-phase of two compartment model with first-order elimination, single IV dose of 10 mg/kg
[g] Data from Chang et al., 2008. β-phase estimate of occupational exposure to PFBA precursors with half-life calculated from two blood samples 7-11 d apart

[h] Data from Chengelis et al., 2009. Mean β-phase of two compartment model with first-order elimination, single IV dose of 10 mg/kg
[i] Data from Chengelis et al., 2009. Mean β-phase of two compartment model with first-order elimination, repeated oral dose of 50 mg/kg, day 25
[j] Data from Chengelis et al., 2009. Mean β-phase of two compartment model with first-order elimination, repeated oral dose of 150 mg/kg, day 25
[k] Data from Chengelis et al., 2009. Mean β-phase of two compartment model with first-order elimination, repeated oral dose of 300 mg/kg, day 25
[l] Data from Gannon et al., 2011. Mean β-phase of one compartment model with first-order elimination, single oral dose of 2 mg/kg
[m] Data from Gannon et al., 2011. Mean β-phase of one compartment model with first-order elimination, single oral dose of 100 mg/kg
[n] Data from Numata et al., 2014. Mean β-phase of two compartment model with first-order elimination, 21d exposure to 48 µg/kg dw in diet
[o] Data from Numata et al., 2014. Mean β-phase of two compartment model with first-order elimination, 21d exposure to 10.2 µg/kg dw in diet
[p] Data from Numata et al., 2014. Mean β-phase of two compartment model with first-order elimination, 21d exposure to 22.4 µg/kg dw in diet
[q] Data from Ohmori et al., 2003. Mean β-phase of two compartment model with first-order elimination, single IV dose of 48.64 mmol/kg bw
[r] Data from Benskin et al., 2009. β-phase elimination rate, single oral dose, 0.5 mg/kg
[s] Data from De Silva et al., 2009. β-phase elimination rate, repeated dose, 12 week exposure to 0.40 mg/kg in diet
[t] Data from De Silva et al., 2009. β-phase elimination rate, repeated dose, 12 week exposure to 0.54 mg/kg in diet
[u] Data from Lou et al., 2009. Mean β-phase of one compartment model with first-order elimination, single oral dose of 1 or 10 mg/kg
[v] Data from Tatum-Gibbs et al., 2011. Mean β-phase of two compartment model with first-order elimination, single oral dose of 1 mg/kg
[x] Data from Tatum-Gibbs et al., 2011. Mean β-phase of two compartment model with first-order elimination, single oral dose of 3 mg/kg
[y] Data from Tatum-Gibbs et al., 2011. Mean β-phase of two compartment model with first-order elimination, single oral dose of 10 mg/kg
[z] Data from Tatum-Gibbs et al., 2011. Mean β-phase of two compartment model with first-order elimination, single oral dose of 1 mg/kg
[A] Data from Tatum-Gibbs et al., 2011. Mean β-phase of two compartment model with first-order elimination, single oral dose of 10 mg/kg
[B] Data from Butenhoff et al., 2004. Mean β-phase of non-compartment model with first-order elimination, single IV dose of 10 mg/kg
[C] Data from Butenhoff et al., 2004. Mean β-phase of non-compartment model with first-order elimination, repeated dose, six month oral dosing of 10 mg/kg
[D] Data from Butenhoff et al., 2004. Mean β-phase of non-compartment model with first-order elimination, repeated dose, six month oral dosing of 20 mg/kg
[E] Data from Olsen et al., 2007. Mean β-phase of non-compartment model with first-order elimination, periodic blood samples collected over 5 years
[F] Data from Zhang et al., 2013. β-phase estimate based on one compartment modelling of urine and blood samples. Should according to the authors be considered as upper limit estimates.

Taken together, there is strong evidence that PFNA bioaccumulates in humans.

- PFNA is present in quantifiable amounts in blood serum from the general population (eg. present in all samples from Sweden).
- Humans have very long elimination half-lives for PFNA, i. e. > 1 year.
  - Estimated average half-lives in the general population are between 1.7 and 3.2 years, depending on sex and age.
- In professional ski waxers, with comparatively high exposure to PFNA during the winter season, serum levels of PFNA did not decrease during 8 months between end of season and start of next season indicating slow elimination.
- Positive associations between years exposed as a ski waxer and concentration of PFNA in serum has been observed.
- In the general population, age is positively associated with serum levels of PFNA.
- Men have higher serum levels of PFNA than women, which may be due at least in part to elimination of this and other PFAAs during pregnancy and breastfeeding.
- High protein binding to albumin, L-FABP and renal anionic transporters, as well as partitioning to membrane phospholipids, are plausable mechanisms for the slow elimination of PFNA, as for other PFCAs.

5. Environmental hazard assessment

Not relevant for the identification of the substance as SVHC in accordance with REACH Article 57 (c) or (d), in this case.

6. Conclusions on the SVHC Properties

6.1. CMR assessment

In its opinion of September 2014 on the proposal for harmonised classification and labelling at EU level of Perfluorononan-1-oic acid (2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluorononanoic acid) (PFNA) and its sodium and ammonium salts, ECHA's Risk Assessment Committee (RAC) concluded that the evidence is sufficiently convincing to classify PFNA for developmental effects as Repr. 1B, H360Df ("May damage the unborn child. Suspected of damaging fertility") in accordance with the CLP criteria (Regulation (EC) 1272/2008).

Therefore, even though PFNA is not yet listed in Annex VI of CLP (Regulation (EC) 1272/2008) there is evidence based on the RAC opinion on PFNA that PFNA and its sodium and ammonium salts meet the criteria for classification as toxic for reproduction in accordance with Article 57 (c) of REACH

6.2. PBT and vPvB assessment

6.2.1. Assessment of PBT / vPvB properties

A weight-of-evidence determination according to the provisions of Annex XIII of REACH is used to identify the substance as PBT. All available information (such as the results of standard tests, monitoring and modelling, information from the application of the category and analogue approach (grouping, read-across) and (Q)SAR results) is considered together in a weight-of-evidence approach.

PFNA belongs to the chemical group of long-chained perfluorinated carboxylic acids

(PFCAs). The substances in this group have a highly similar chemical structure: a perfluorinated carbon chain and a carboxylic acid group. They differ only in the number of CF_2-groups whereas all other fragments are the same within the group. As a result of comparing the experimental and estimated data of other PFCAs with experimental and estimated data on PFNA, it can be assumed that with increasing chain length water solubility decreases and the sorption potential increases (See Annex 1). It can be stated with sufficient reliability that the behaviour of the PFCAs follows a regular pattern.

6.2.1.1. Persistence

PFNA is based on its stabile structure not expected to undergo abiotic degradation under relevant environmental conditions. A standard screening study on PFNA supporting this understanding is available.

In general, the persistence of PFCAs can be explained by the shielding effect of the fluorine atoms, blocking e.g. nucleophilic attacks to the carbon chain. High electronegativity, low polarizability and high bond energies make highly fluorinated alkanes the most stable organic compounds. It is not expected that the carboxylic group in PFCAs alters the persistence of these chemicals. The persistence of five PFCAs (PFOA and C_{11}-C_{14}-PFCAs) (P and vP) was already confirmed by the Member State Committee (European Chemicals Agency, 2012a-d, 2013a-b).

Therefore, based on the information summarized above it is concluded that PFNA is not degraded in the environment and thus fulfils the P- and vP- criteria in accordance with the criteria and provisions set out in Annex XIII of REACH.

6.2.1.2. Bioaccumulation

There are no experimental BCF values available for PFNA. The numeric criterion as suggested in REACH Annex XIII (sections 1.1.2 and 3.2.2(a)) for a bioaccumulative substance in aquatic species is not expected to be fulfilled for PFNA based on read across. Due to its expected notable water solubility, PFNA is, like the other PFCAs expected to quickly be excreted via gill permeation. Furthermore, PFNA is present mainly in protein rich tissues like blood and liver (OECD, 2006; Kelly et al. 2009). Hence, bioconcentration in gill breathing organisms and the accumulation in lipids is not the most relevant endpoint to consider. Field studies show that air-breathing organisms are more likely to bioaccumulate PFNA and other PFCAs compared to water breathing organisms. Therefore, the numerical bioaccumulation (B) criterion defined in the REACH regulation Annex XIII (sections 1.1.2 and 3.2.2(a)) is not suitable to assess the bioaccumulation potential of PFNA.

Annex XIII (section 3.2.2) defines information which shall be taken into account in the assessment and can be used to draw conclusions on the assessment even when the numerical criterion is not applicable. Such data are, for example, data on the bioaccumulation potential in terrestrial species, such as elevated levels in endangered species. PFNA was found in terrestrial species as well as in endangered species as shown for the polar bear and beluga whale. These findings indicate a bioaccumulation potential and are of high concern.

Furthermore, Annex XIII (section 3.2.2 (b)) requires to consider data from human body fluids or tissues and to take the toxicokinetic behavior of the substance assessed into account. For PFNA gestational and lactational exposure in humans has been shown, which is of special concern as the foetus and newborn babies are highly vulnerable to exposure by toxic substances. On top of that, data from human body fluids clearly provide quantitative proof of the bioaccumulation of PFNA: Elimination half-lives in humans are > 1 year . In addition, recent studies, taking into account relevant confounding factors, show that PFNA blood concentrations in humans increase with increasing age.

Finally, Annex XIII (section 3.2.2 (c)) foresees that the potential for biomagnification in food chains of a substance is assessed. The available field data provide evidence that bioaccumulation and trophic magnification do occur in certain food webs in the environment. For PFNA, field studies provide trophic magnification factors (TMFs) or biomagnification factors (BMFs) for PFNA for aquatic and terrestrial food chains. When air breathing organisms are the top predators in these food chains biomagnification could be demonstrated by calculation of TMFs and BMFs > 1 in several food chains, for example for wolves and beluga whales.

The data summarized above is in high accordance with the bioaccumulation data on the other PFCAs. Altogether these show a regular pattern of bioaccumulation which depends on the chain-length of the perfluorinated alkyl chain.

Conclusion:

1. PFNA accumulates in humans
    a. PFNA is present in human blood of the general population
    b. Elimination half-lives are > 1.7 years.
    c. Human elimination half-lives seem to be the longest amongst the available mammalian data, whereas the elimination half-lives in laboratory mammals vary highly depending on the study conditions.
    d. PFNA levels increase with age after adjusting for relevant confounding factors.
2. There is evidence that PFNA preferentially bioaccumulates in air-breathing mammals, including endangered species and humans
    a. BMFs range from 1.4 and 24 based on whole body values
    b. TMFs range from 2.9 to 9.88 referring to either whole body measurements or estimated whole body values.
    c. Protein corrected TMFs range between 2.9 to 6.19.

3. PFNA does not seem to consistently accumulate in water breathing animals.
    a. No experimental BCFs are available for PFNA. For the closest structural analogues BCFs range from 4.0 to 27 (PFOA) and from 450 to 2700 (PFDA)
    b. Whole body BAFs range from 0 to 3981
    c. Whole body BMFs range from 0.13 to 5.3 whereas most of the data are below 1
    d. Whole body TMFs range from 1 to 2.1 in aquatic piscivorous food webs

4. The bioaccumulation data on PFNA in environmental species, in laboratory mammals and in humans is consistent with the data on other long-chain perfluorinated carboxylic acids, such as PFOA.
    a. Recent models to explain the substantial bioaccumulation of PFCAs take into account the observed pattern of animal tissue distribution, the relationship between chain length and bioaccumulation and the species and gender-specific variation in elimination half-life.

To conclude, taken all available information together in a weight-of-evidence approach, the elimination half-lives from humans and other mammals show that PFNA bioaccumulates. The available field data also indicate that bioaccumulation and trophic magnification occur in certain food webs in the environment. The data on PFNA are in line with the expected regular pattern of fate properties of the already assessed PFOA and C11-C14-PFCAs. Therefore it is considered that the B criterion of REACH Annex XIII is fulfilled. Whether the vB criterion is fulfiled has not been assessed.

6.2.1.3. Toxicity

There is evidence based on the RAC opinion on PFNA and its sodium and ammonium salts that these substances meet the criteria for classification as toxic for reproduction in accordance with Article 57 (c) of the REACH Regulation. As a consequence the toxicity criterion of REACH Annex XIII is fulfilled.

In conclusion, PFNA and its sodium and ammonium salts meet the criteria for PBT substances according to Article 57 (d) of the REACH Regulation.

6.2.2. Summary and overall conclusions on the PBT and vPvB properties

In conclusion, PFNA and its sodium and ammonium salts are identified as PBT substances according to Art. 57(d) of REACH by comparing all relevant and available information listed in Annex XIII of REACH with the criteria set out in the same Annex, in a weight-of-evidence determination.

Table 14: Comparison with Annex XIII criteria

	Annex XIII	PFNA	Conclusion
P/vP	P Half-life: a) in marine water > 60 days, or b) in fresh- or estuarine water > 40 days, or c) in marine sediment > 180 days, or d) in fresh- or estuarine water sediment > 120 days, or e) in soil > 120 days vP Half-life: a) in marine, fresh- or estuarine water > 60 days, or b) in marine, fresh- or estuarine water sediment > 180 days, or c) in soil > 180 days	No simulation test available => read-across to PFOA: All studies of PFOA demonstrate the extremely high persistence of the compound. No environmental half-life could be determined during duration of the studies => Persistence (vP) of PFOA and C_{11}-C_{14}-PFCAs was confirmed by Member State Committee.	P/vP
B	Assessment of B or vB properties Results from a bioconcentration or bioaccumulation study in aquatic species; ▪ A substance fulfils the bioaccumulation criterion (B) when the bioconcentration factor in aquatic species is higher than 2 000. ▪ A substance fulfils the 'very bioaccumulative' criterion (vB) when the bioconcentration factor in aquatic species is higher than 5 000. (b) Other information on the bioaccumulation potential provided that its suitability and reliability can be reasonably demonstrated, such as: ▪ Results from a bioaccumulation study in terrestrial species; ▪ Data from scientific analysis of human body fluids or tissues, such as blood, milk, or fat; ▪ Detection of elevated levels in biota, in particular in endangered species or in vulnerable populations, compared to levels in their surrounding environment; ▪ Results from a chronic toxicity study on animals; ▪ Assessment of the toxicokinetic behaviour of the substance; (c) Information on the ability of the substance to biomagnify in the food chain, where possible expressed by biomagnification factors or trophic magnification factors.	• Based on the BCF values of PFOA and PFDA PFNA does not bioconcentrate. • Whole body BAF values from water breathing animals range between 0-3981 • Whole body BMF values for gill breathing organisms range between 0.13-5.3. • BMFs including air breathing animals range between 1.4 - 24 based on estimated whole body values. • TMF values for gill breathing organisms range between 1 - 2.1 based on whole body values. • When air breathing animals are top predators TMFs range between 2.9 – 9.88 referring to either whole body measurements or estimated whole body values. • Protein corrected TMFs range between 2.9-6.19. • Terrestrial BMFs and TMFs range between 2.7-8.5 and 1.9-2.7, respectively, based on estimated whole body values. • PFCAs have a high potential for transfer to milk and beef from the diet of dairy cows.	B (vB not assessed)

		• PFNA is present in quantifiable amounts in blood serum from the general population. • Humans have long elimination half-lives for PFNA, i. e. > 1.7 year in blood serum. • Estimated average half-lives in the general population are 1.7-3.2 years depending on sex and age. • In professional ski waxers, with comparatively high exposure to PFNA during the winter season, serum levels of PFNA did not decrease during 8 months between end of season and start of next season. • Positive associations between years exposed as a ski waxer and concentration of PFNA in serum have been observed. • Age is positively associated with serum levels of PFNA.	
T	a) NOEC < 0.01 mg/L, or b) Classified as carcinogenig (cat. 1or 2), mutagenig (cat. 1 or 2), or toxic for reproduction (cat. 1, 2 or 3), or c) Classified as STOT RE cat. 1 or 2	RAC opinion: Repr. 1 B STOT RE 1	T

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Annex I - Read-across approach

In general, the read-across approach can be applied if substances whose physicochemical and/or toxicological and/or ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity. Those substances may be considered as a group or a category of substances. According to ECHA`s practical guide 6 “How to report readacross and categories” similarities may be due to a common functional group, common precursor or breakdown products, constant pattern in changing potency or common constituents or chemical class.

Structural similarities of C_{8-10}-PFCAs

In Table A.1 the chemicals structures of the PFOA, PFNA and PFDA are displayed. Both contain a carboxylic acids group and a perfluorinated carbon chain. The compounds differ only in the number of carbon atoms within the fluorinated carbon chain. Thus, we conclude, that C_{8-10}-PFCAs belong to the same chemical class and contain not only a common functional group but are highly similar according to their chemical structure.

Table A.1: CAS-Numbers and similarity of chemical structures of C8-10-PFCAs.

Name	Abbreviation	CAS-No	IUPAC Name	Chemical structure
PFOA	C_8-PFCA	335-67-1	Octanoic acid, pentadecafluoro-	$CF_3(CF_2)_6$-COOH
PFNA	C_9-PFCA	375-95-1	Nonanoic acid, heptadecafluoro-	$CF_3(CF_2)_7$-COOH
PFDA	C_{10}-PFCA	335-76-2	Decapnoic acid, nonadecalfuoro	$CF_3(CF_2)_8$-COOH

Dissociation of C8-14-PFCAs and its salts in aqueous media

Under environmental conditions in aqueous media the free perfluorinated carboxylic acids (PFCAs) stay in equilibrium with their conjugate bases, the perfluorinated carboxylates. The fraction of each species depends on the acid dissociation constant (pKa) and the pH of the environmental compartment. Salts of PFCAs, which are sometimes used in laboratory experiments, will be in equilibrium with the corresponding acid in aqueous phases as well. Currently used techniques for analysis and quantification of PFCAs in i.e. environmental samples are not able to distinguish between both of the species. Therefore, reported concentrations always include the acids as well as the bases. If reported concentrations are used for the determination of bioaccumulation factors or for experiments determining the persistency, aqueous phase concentrations include both species. Experimental determination of pKa is difficult for PFCAs, i.e. because of the surface active properties. Calculated values should be taken with care, because for most of the models it is unclear whether PFCAs are within their applicability domain. For assessing the intrinsic properties of the PFCA within this dossier the exact knowledge of the fraction of each species is not required, because both of the species will be available independently from the starting conditions.

Physicochemical properties and partition coefficients of C8-10-PFCAs and some salts

The experimental determination of partition coefficients is difficult for example because of the surface active properties of the ionic PFCAs. The presence of ionic PFCAs depends on the dissociation of PFCAs in aqueous media. Nevertheless, there are models available, i.e. COSMOtherm calculating partitioning coefficients of neutral PFCAs. COSMOtherm is a quantum chemistry-based method that requires no specific calibration. This calibration

would be difficult because of missing measured data of PFCAs. Therefore COSMOtherm is expected to be able to estimate properties for PFCAs. Studies have shown that properties estimated with COSMOtherm showed good agreement with the experimental data for a number of per- and polyfluorinated chemicals, eg. C_8-PFCA (Arp et al., 2006; Wang et al., 2011)). Again, whether neutral PFCAs are present in aqueous media depends in the dissociation of the acids. Air-water as well as octanol-water partition coefficients are of course different for PFCAs with 8 to 10 carbon atoms but they show a clear increasing, trend with chain length (see Table A.2 below, (Wang et al., 2011)). This can be explained by the increasing molecular volume with each additional CF2-unit. The trend of the fate of PFCAs with chain length is supported by information on sorption of PFCAs on sediment. Sorption increases with increasing chain length (Higgins and Luthy, 2006) also under environmental conditions (Ahrens et al., 2010) (Table A.2)

Table A.2: Basic substance information and physical chemical properties relevant to justify read across in the PBT assessment

Abbreviation	**C_8-PFCA**			**C_9-PFCA**	**C_{10}-PFCA**	**C_{11}-PFCA**	**C_{12}-PFCA**	**C_{13}-PFCA**	**C_{14}-PFCA**
Acronym	PFOA	APFO	NaPFO	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
IUPAC Name	**Octanoic acid, pentadecafluoro-**	**ammonium pentadeca-fluoro-octanoate**	**pentadeca octanoic acid sodium salt**	**Nonanoic acid, heptadeca-fluoro-**	**Decanoic acid, nonadeca-fluoro-**	**Undecanoic acid, heneicosa-fluoro-**	**Dodecanoic acid, tricosafluoro-**	**Tridecanoic acid, pentacosa-fluoro-**	**Tetradecanoic acid, heptacosa-fluoro-**
Chemical Structure	CF3(CF2)6-COOH	CF3(CF2)6-COO-$NH4^+$	CF3(CF2)6-COO-Na^+	CF3(CF2)7-COOH	CF3(CF2)8-COOH	CF3(CF2)9-COOH	CF3(CF2)10-COOH	CF3(CF2)11-COOH	CF3(CF2)12-COOH
CAS No	335-67-1	3825-26-1	335-95-5	375-95-1	335-76-2	2058-94-8	307-55-1	72629-94-8	376-06-7
	Physico-chemical data								
Molecular Weight g/mol	414.09	431.1		464.08	514.08	564.0909	614.0984	664.1059	714.11
Partitioning Coefficient logKow				2.3 – 2.48 (exp)	2.65 – 2.87 (exp)	3.19 – 3.41	logP 9.363±0.888 at 25°C (calc)	logP 10.093± 0.901 at 25 °C (calc)	logP 10.823± 0.914 at 25 °C (calc)
	5.30 (calc., COSMOtherm, Wang et al., 2011)			5.9 (calc., COSMOtherm, Wang et al., 2011)	6.5 (calc., COSMOtherm, Wang et al., 2011)	7.2 (calc., COSMOtherm, Wang et al., 2011)	7.8 (calc., COSMOtherm, Wang et al., 2011)	8.25 (calc., COSMOtherm, Wang et al., 2011)	8.90 (calc., COSMOtherm, Wang et al., 2011)
log K_{OA}	7.23 (calc., COSMOtherm, Wang et al., 2011)			7.50 (calc., COSMOtherm, Wang et al., 2011)	7.77 (calc., COSMOtherm, Wang et al., 2011)	8.08 (calc., COSMOtherm, Wang et al., 2011)	8.36 (calc., COSMOtherm, Wang et al., 2011)	8.63 (calc., COSMOtherm, Wang et al., 2011)	8.87 (calc., COSMOtherm, Wang et al., 2011)
log K_{AW}	-1.93 (calc., COSMOtherm, Wang et al., 2011)			-1.58 (calc., COSMOtherm, Wang et al., 2011)	-1.27 (calc., COSMOtherm, Wang et al., 2011)	-0.92 (calc., COSMOtherm, Wang et al., 2011)	-0.58 (calc., COSMOtherm, Wang et al., 2011)	-0.38 (calc., COSMOtherm, Wang et al., 2011)	0.03 (calc., COSMOtherm, Wang et al., 2011)
Dissocia	0.5			<1.6	<1.6	0.52±0.1	0.52±0.1	0.52±0.1	0.52±0.

Abbreviation	**C_8-PFCA**			**C_9-PFCA**	**C_{10}-PFCA**	**C_{11}-PFCA**	**C_{12}-PFCA**	**C_{13}-PFCA**	**C_{14}-PFCA**
Acronym	PFOA	APFO	NaPFO	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
tion constant	(Vierke et al., 2013) 2.5 (Ylinen et al., 1990) 2.8 in 50% aqueous ethanol (Brace, 1962) 1.3 (López-Fontán et al., 2005)			(Vierke et al., 2013) 0.82 (calc., COSMOtherm, Wang et al., 2011)	(Vierke et al., 2013) 2.58 (Moroi et al., 2001)	0; (calculated)	0 (calculated)	0; (calculated)	10; (calculated)
Partition coefficients log Kd (sediment and overlapping dissolved phase)	0.04 (Ahrens et al., 2010)*			0.6 (Ahrens et al., 2010) *	1.8 (Ahrens et al., 2010) *	3.0 (Ahrens et al., 2010) *			
Log Koc (sediment organic carbon-normalized distribution coefficient)	2.06 (Higgins and Luthy, 2006)# 1.09 (Ahrens et al., 2010) *			2.39 (Higgins and Luthy, 2006) # 2.4 (Ahrens et al., 2010) *	2.76 (Higgins and Luthy, 2006) # 3.6 (Ahrens et al., 2010) *	3.3 (Higgins and Luthy, 2006) # 4.8 (Ahrens et al., 2010) *			
Water solubility	9.5 g/L (25° C) 4.14 g/L (22°C)	0.033 mol/L, 14.2 g/L at 2.5 °C (Nielsen 2012)	0.036 mol/L at 8.0 °C at critical micelle concentration (Nielsen 2012)			1.2E-4 g/L; pH 1 at 25°C 9.0E-4 g/L; pH 2 at 25°C 8.5E-3 g/L; pH 3 at 25°C 0.056 g/L; pH 4 at 25°C 0.14 g/L; pH 5 at 25°C 0.16 g/L; pH 6-10 at 25°C (calculated)	2.9E-5 g/L pH 1 at 25°C 2.2E-4 g/L pH 2 at 25°C 2.0E-3 g/L pH 3 at 25°C 0.014 g/L pH 4 at 25°C 0.034 g/L pH 5 at 25°C 0.039 g/L pH 6 at 25°C 0.040 g/L pH 7 at 25°C 0.041 g/L pH 8-10 at 25°C (calculated)	7.3E-6 g/L; pH 1 at 25 °C 5.5E-5 g/L; pH 2 at 25 °C 5.1E-4 g/L; pH 3 at 25 °C 3.5E-3 g/L; pH 4 at 25 °C 8.6E-3 g/L; pH 5 at 25 °C 0.0100 g/L; pH 6-10 at 25 °C (calculated)	1.9E-6 g/L; pH 1 at 25°C 1.4E-5 g/L; pH 2 at 25°C 1.3E-4 g/L; pH 3 at 25°C 9.3E-4 g/L; pH 4 at 25°C 2.2E-3 g/L; pH 5 at 25°C 2.6E-3 g/L; pH 6-10 at 25°C (calculated)

Abbreviation	**C_8-PFCA**			**C_9-PFCA**	**C_{10}-PFCA**	**C_{11}-PFCA**	**C_{12}-PFCA**	**C_{13}-PFCA**	**C_{14}-PFCA**
Acronym	PFOA	APFO	NaPFO	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
Vapour pressure	4.2 Pa (25 °C) for PFOA extrapolated from measured data 2.3Pa (20 °C) for PFOA extrapolated from measured data 128 Pa (59.3 °C) for PFOA measured	0.0081 Pa at 20 °C, calculated from measured data <0.1 hPa at 20 °C 0.012 Pa at 25 °C 0.0028 Pa at 25 °C (Nielsen 2012)				0.6 to 99.97 kPa (112 to 237.7°C) (calculated)	9.40E-3 Torr at 25°C(calculated)	3.59E-3 Torr at 25°C (calculated)	1.37E-3 Torr at 25 °C (calculated)
	Stability								
Phototransformation in water DT50	No photodegradation detected under relevant env. conditions	No photodegradation detected under relevant env. conditions		No photodegradation tested under relevant env. conditions 100 % after 12 h by use of persulfate ion (S2O82-) in water	No photodegradation tested under relevant env. Conditions 100 % after 12 h by use of persulfate ion (S2O82-) in water	No photodegradation tested under relevant env. Conditions 77% after 12 h by use of persulfate ion (S2O82-) in water			
Hydrolysis DT50	>97 yr			No hydrolysis tested under relevant env.. conditions; 97% (absence of $S_2O_8^{2-}$) and 100% (by use of $S_2O_8^{2-}$) after 6 h in 80°C water					
Direct photolysis		No photo-degradation							

Abbreviation	**C_8-PFCA**			**C_9-PFCA**	**C_{10}-PFCA**	**C_{11}-PFCA**	**C_{12}-PFCA**	**C_{13}-PFCA**	**C_{14}-PFCA**
Acronym	PFOA	APFO	NaPFO	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
indirect photolysis		No photo-degradation (H2O2; synthethic humic water, Fe2O3) estimated half-life > 349 days (Fe2O3)							
ready biodegradability screening test	not readily biodegradable (OECD 301 C,F)	not readily biodegradable (OECD 301 B)		not readily biodegradable (OECD 301 F)			not readily biodegradable (OECD 301 C)		not readily biodegradable (OECD 301 C)
Simulation tests	No elimination by metabolic processes, mineralization or adsorption								
Biodegradation in soil, sediment	No degradation detected								

* pH of the water samples analyzed 7.1-8.3 Temp.: 15.3 – 17.7 °C

Table A.3: Information on BCF, BMF and TMF relevant to justify read across in the B assessment.

Abbreviation	**C8-PFCA**	**C9-PFCA**	**C10-PFCA**
Acronym	PFOA	PFNA	PFDA
Name	**Octanoic acid, pentadecafluoro-**	**Nonanoic acid, heptadecafluoro-**	**Decanoic acid, nonadecafluoro-**
Chemical Structure	CF3(CF2)6-COOH	CF3(CF2)7-COOH	CF3(CF2)8-COOH
CAS No	335-67-1	375-95-1	335-76-2
Physico-chemical data			
Molecular Weight g/mol	414.09	464.08	514.08
Partitioning Coefficient log K_{OW}		2.3 – 2.48 (exp)	2.65 – 2.87 (exp)
	5.30 (calc., COSMOtherm, Wang et al., 2011)	5.9 (calc., COSMOtherm, Wang et al., 2011)	6.5 (calc., COSMOtherm, Wang et al., 2011)
log K_{OA}	7.23 (calc., COSMOtherm, Wang et al., 2011)	7.50 (calc., COSMOtherm, Wang et al., 2011)	7.77 (calc., COSMOtherm, Wang et al., 2011)

log K_{AW}	-1.93 (calc., COSMOtherm, Wang et al., 2011)	-1.58 (calc., COSMOtherm, Wang et al., 2011)	-1.27 (calc., COSMOtherm, Wang et al., 2011)
BCF			
Rainbow trout (carcass)	4.5 ± 0.6	-	450 ± 6 62
Rainbow trout (blood)	27 ± 9.7	-	2700±350
Rainbow trout (liver)	8.0 ± 0.59	-	1100 ± 180
Carp (whole)	3.2-94	-	-
BAF	0.038-292	0-3981	714-158489
BMF	0.02-125	0.13-111	0.21-87
TMF	0.2-13	1-9.88	0.39-12.1

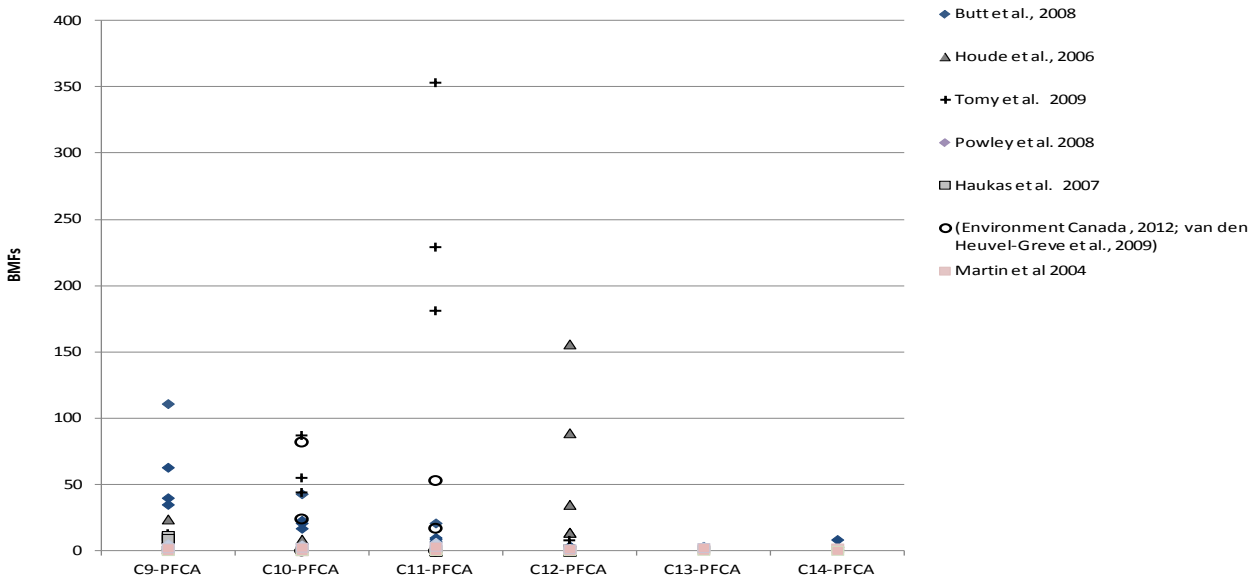

Figure 1: Biomagnification factors (BMFs) for C_{9-14}-PFCA.

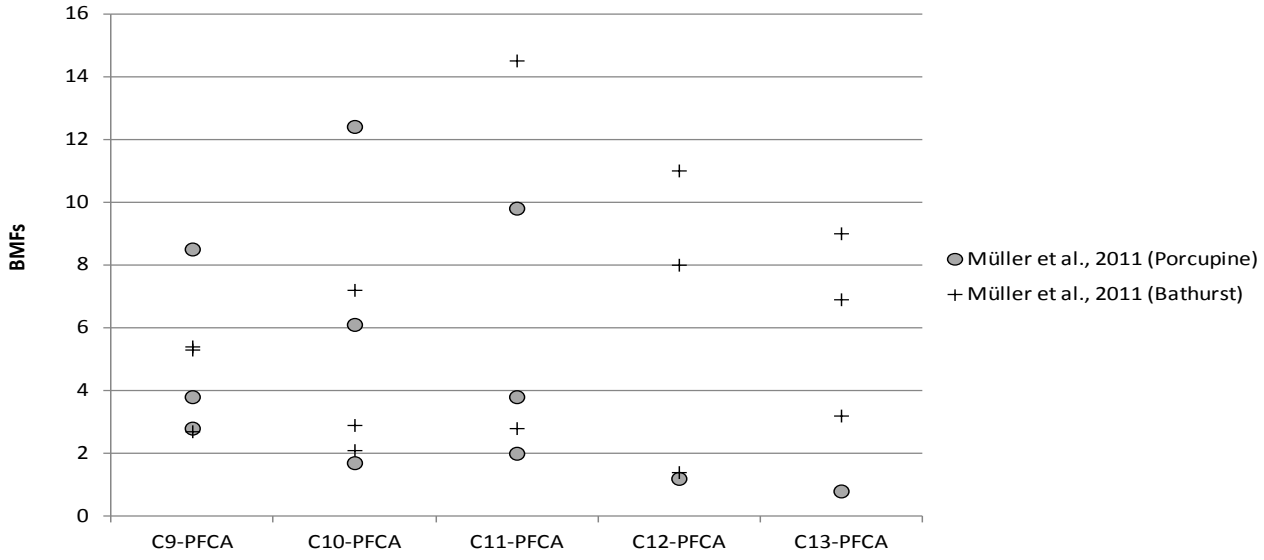

Figure 2: BMFs for C_{9-13}-PFCAs in a remote terrestrial food chain from two different locations (whole body, Müller et al., 2011). The study is reliable (reliability 2). See for further discussion on the study in the Support Document of, e.g., C11-PFCA (European Chemicals Agency, 2012)

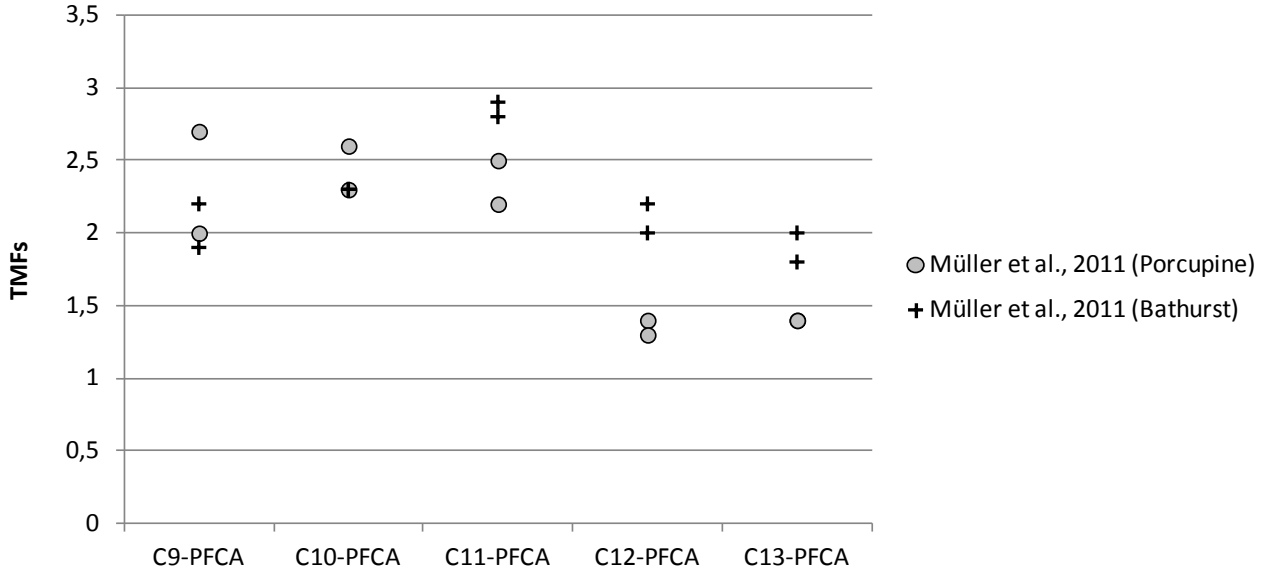

Figure 3: TMFs for C_{9-13}-PFCAs in a remote terrestrial food chain from two different locations (whole-body, Müller et al., 2011). The study is reliable (reliability 2).

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