SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Ammonium salts of mono- and bis[3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl and/or poly (substituted alkene)] phosphate

List No 700-403-8

Evaluating Member State : Belgium

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Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2013

Before concluding the substance evaluation a Decision to request further information was issued on 31 August 2015.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

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Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Ammonium salts of mono- and bis[3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl and/or poly (substituted alkene)] phosphate, hereafter referred to as 'the Substance', was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB
- Wide dispersive use
- Consumer use.

During the evaluation a new concern was identified, namely a potential for endocrine disrupting (ED) properties.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Perfluoroheptanoic acid (PFHpA) and its salts (PFHp) were added to the Candidate List of substances of very high concern for Authorisation on 17 January 2023 based on Articles 57(c)(d)(e)(f)[2]. Based on its evaluation, the eMSCA considers that PFHpA or its deprotonated form (PFHp) is a relevant, stable degradation product of the Substance. It should be noted that in water under environmental conditions and in the human body PFHpA exists in the form of perfluoroheptanoate (PFHp).

In December 2019, the German Competent Authority submitted an Annex XV dossier proposing restrictions on "undecafluorohexanoic acid (PFHxA), its salts and related substances" (EC No: -; CAS No: -). The final opinion of the Committee for Risk Assessment (RAC) and the Committee for Socio-economic Analysis (SEAC) on this restriction proposal has been published[3]. The Substance is included in the scope of this restriction.

[2] ECHA Candidate List of substances of very high concern for Authorisation. Perfluoroheptanoic acid and its salts (EC No: -; CAS no: -). https://www.echa.europa.eu/candidate-list-table

[3] Registry of restriction intentions until outcome (ECHA, 2022). Undecafluorohexanoic acid (PFHxA), its salts and related substances (EC No: -; CAS no: -). https://echa.europa.eu/nl/registry-of-restriction-intentions/-/dislist/details/0b0236e18323a25d (latest update on 18 May 2022)

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1: Conclusion of substance evaluation

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	
Identification as SVHC (authorisation)	
Restrictions	X (within the scope of restriction of PFHxA)
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

Not applicable.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not applicable.

4.1.3. Restriction

An Annex XV dossier proposing restrictions on “Undecafluorohexanoic acid (PFHxA), its salts and related substances” (EC No: -; CAS No: -) is currently being processed. The RAC and SEAC final opinion is available, however the Adopted Restriction / Commission Communication is not available yet at the time this conclusion document is submitted.

In the RAC and SEAC final opinion (RAC and SEAC, 2021), it is clearly defined which substances qualify as a related substance to PFHxA, and which substances are therefore included in the restriction for “Undecafluorohexanoic acid (PFHxA), its salts and related substances”. The restriction will include the following substances, according to the RAC and SEAC final opinion (RAC and SEAC, 2021):

"1. Undecafluorohexanoic acid (PFHxA), its salts and related substances

(a) having a linear or branched perfluoropentyl group with the formula C_5F_{11}- directly attached to another carbon atom as one of the structural elements;
(b) having a linear or branched perfluorohexyl group with the formula C_6F_{13}-.

2. The following substances shall be derogated:

(a) C_6F_{14};
(b) C_6F_{13}-C(=O)OH, C_6F_{13}-C(=O)O-X′ or C_6F_{13}-CF_2-X′ (where X′ = any group, including salts).
(c) Any substance having a perfluoroalkyl group C_6F_{13}- directly attached to a sulphur atom.
(d) Any substance having a perfluoroalkyl group C_6F_{13} -directly attached to an oxygen atom at one of the non-terminal carbons."

The Substance is defined as a related substance to PFHxA, and is therefore included in the scope of the restriction for "Undecafluorohexanoic acid (PFHxA), its salts and related substances".

4.1.4. Other EU-wide regulatory risk management measures

Not applicable.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable.

5.2. Other actions

Not applicable.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

The Substance is defined as a related substance to PFHxA, and is therefore included in the scope of the restriction for "Undecafluorohexanoic acid (PFHxA), its salts and related substances". As explained in section 4.1.3, the annex XV dossier proposing restrictions for "Undecafluorohexanoic acid (PFHxA), its salts and related substances" is currently being processed.[4]

[4] Registry of restriction intentions until outcome (ECHA, 2022). Undecafluorohexanoic acid (PFHxA), its salts and related substances (EC No: -; CAS No: -). https://echa.europa.eu/nl/registry-of-restriction-intentions/-/dislist/details/0b0236e18323a25d (latest update on 18 May 2022)

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The substance ammonium salts of mono- and bis[3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl and/or poly (substituted alkene)] phosphate (further referred to as 'the Substance'), was originally selected for substance evaluation in order to clarify concerns about:

- Suspected PBT/vPvB
- Wide dispersive use
- Consumer use.

During the evaluation a new concern was identified, namely a potential for endocrine disrupting properties.

Table 2: Evaluated endpoints

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome / conclusion**
Suspected PBT/vPvB	Concern confirmed: The eMSCA concludes that PFHp is a relevant degradation product of the Substance. Furthermore, 'Perfluoroheptanoic acid and its salts' (EC No: -; CAS No: -) have been identified as a substance of very high concern (SVHC), meeting the criteria set out in Article 57 c, d, e and f[5]. On 17 January 2023, 'Perfluoroheptanoic acid and its salts' was included as an SVHC in the Candidate List for eventual inclusion in Annex XIV (ECHA Decision, 2022). Given the inclusion in the Candidate List of PFHpA and PFHp, which is a degradation product of the Substance, the concerns regarding PBT and vPvB are also confirmed for the Substance. In Chapter R.11 (ECHA, 2017: ECHA Guidance on PBT/vPvB assessment, p. 25) it is mentioned that: *'If a registered substance contains a constituent, impurity or additive or transforms/degrades to a substance which is in the Candidate List because of meeting the PBT and/or vPvB criteria, the registrant must conclude his substance to meet the PBT or vPvB criteria accordingly.'*
Suspected ED	Concern unresolved: As a result of the evaluation of the submitted reproduction developmental toxicity screening study in mice with sodium perfluoroheptanoate, a potential for endocrine disrupting properties is recognised. Based on the available information a definitive conclusion could not be drawn, but in view of the ongoing restriction proposal for undecafluorohexanoic acid, its salts and related substances, further examination is not expected to result in additional Risk Management Measures for the Substance.

[5] ECHA Candidate List of substances of very high concern for Authorisation. Perfluoroheptanoic acid and its salts (EC No: -; CAS no: -). https://www.echa.europa.eu/candidate-list-table

	In the conclusion document for EC No 241-527-8[6] and EC No 218-407-9[7], the German CA concluded that the available data clarifies that PFHxA acts as an ED for the environment in accordance with the Endocrine Disruptor definition of the World Health Organisation (WHO). However, for the moment there is no official identification of PFHxA as ED for the environment by MSC or RAC.
Wide dispersive use	Concern confirmed: Because the use of the Substance is considered to be wide dispersive, exposure of the environment is expected to be substantial and difficult to mitigate.
Consumer use	Concern confirmed: Because the Substance is also used by consumers, exposure of the environment occurs via many point sources and is difficult to mitigate.

The focus of this substance evaluation was on the potential PBT/vPvB properties of the Substance. In this framework, not only the intrinsic properties of the parent compound were assessed, but the eMSCA focussed on the properties of degradation products that showed high persistency. The parent compound (ammonium salts of mono- and bis[3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl and/or poly (substituted alkene)] phosphate, further referred to as 'the Substance', consists of polyfluorinated constituents and non-fluorinated constituents. Based on a thorough analysis of ready biodegradation tests with the Substance and the analogous substance EC 700-161-3, it is concluded that the non-fluorinated constituents of the Substance meet the ready biodegradation criteria and consequently these non-fluorinated constituents are considered to be not PBT/vPvB substances.

The eMSCA examined the properties of the fluorinated 6:2 monoPAP and 6:2 diPAP constituents and more specifically their degradation patterns in various environmental matrices (see § 7.7.1.3.). Degradation patterns can differ depending whether the transformations are triggered by microorganisms or are initiated by abiotic chemical processes. In a first instance breakdown of these phosphates results in the formation of 6:2 fluorotelomeralcohol (6:2 FTOH). In turn, this compound degrades more or less slowly via varying patterns and dependent on specific circumstances into a series of poly- and perfluorinated chemicals. In scientific literature various degradation patterns and products relevant for field conditions are described. A common feature of the degradation patterns is that under these study conditions transformation stops at the stage of the perfluorinated carboxylic acids (PFCAs) and their anions. These acids and their salts tend to be significantly less prone to further degradation than their precursors. Among the recalcitrant degradation products, perfluorohexanoate (PFHx) is always detected, but in some studies also perfluoroheptanoate (PFHp) is found as a stable end product. Therefore the eMSCA concludes that PFHp and PFHx are the most relevant degradation products for the Substance.

[6] Substance Evaluation Conclusion Document on EC No 241-527-8. 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl acrylate. April 2023. Evaluating Member State: Germany. https://echa.europa.eu/documents/10162/5c201ae4-8b10-ca18-df24-7ae5a7f83e37

[7] Substance Evaluation Conclusion Document on EC No 218-407-9. 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl methacrylate. April 2023. Evaluating Member State: Germany. https://echa.europa.eu/documents/10162/15a5aaeb-0159-d730-c4f7-66c8dd85a8b4

7.2. Procedure

- The evaluation of the Substance was started after the official publication of the CoRAP list which took place on 20 March 2013. The initial concern was the suspected PBT character of the Substance.

- The evaluation was based in first instance on the last update of the registration dossier for the substance. There is also a registration dossier available for a similar substance (EC No 700-161-3), which contains in part the same main constituents. Data from this registration dossier were also taken into consideration.

- A meeting with representatives of the Registrant took place on 19 April 2013. The available data in the dossier were discussed in detail and further clarifications were given. However, no new studies were presented.

- In June 2013, the registration dossier was updated by the Registrant. The changes made were only of an administrative nature, and no additional information that had an impact on the evaluation was presented.

- During the evaluation process, the Belgian CA had several bilateral contacts with the Dutch CA who was evaluating the similar substance mentioned above. Both eMSCAs were of the opinion that after release to the environment the two substances undergo degradation in an analogous way leading to identical degradation products. Therefore, it was considered appropriate to request a common additional study for the two substances, as there was a concern relating to some degradation products of both parent compounds.

- On 29 April 2014, the Draft Decision requesting additional information on the degradation product PFHp was sent to the Registrant(s) who in turn delivered comments by 5 June 2014 and updated the registration dossier(s) by 23 January 2015.

- On 5 March 2015, the Belgian CA initiated the agreement seeking procedure and after reviewing the proposals for amendment the Draft Decision was amended.

- On 20 April 2015, ECHA referred the Draft Decision to the Member State Committee and a unanimous agreement was reached on 28 May 2015.

- The Decision on the Substance Evaluation was sent to the Registrant(s) on 31 August 2015.

- On 7 December 2017, the Registrant(s) submitted an update of the registration dossier(s) comprising the requested toxicity study in mice with the sodium salt of PFHpA.

- The eMSCA assessed this toxicity study in mice with the sodium salt of PFHpA and decided to finish the evaluation of the Substance without requesting further studies.

7.3. Identity of the substance

Table 3: Substance identity

SUBSTANCE IDENTITY	
Public name:	Ammonium salts of mono- and bis[3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl and/or poly (substituted alkene)] phosphate
EC number:	700-403-8
CAS number:	n.a.
Index number in Annex VI of the CLP Regulation:	n.a.
Molecular formula:	n.a., as a result of the UVCB character
Molecular weight range:	n.a., as a result of the UVCB character
Synonyms:	/

Type of substance ☐ Mono-constituent ☐ Multi-constituent ☒ UVCB

Structural formula:

In view of the UVCB character of the Substance, a structural formula cannot be presented.

Composition:

Confidential.

7.4. Physicochemical properties

Table 4: Summary of physicochemical properties

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES[8]	
Property	**Value**
Physical state at 20 °C and 101.3 kPa	Brown-coloured solid No guideline mentioned According to visual observation
Vapour pressure	EPI Suite estimation from registration dossier (MPBPVP v1.43): 0.0074 Pa at 25 °C and 5.55 x 10^{-5} mm Hg at 25 °C

[8] REACH Registration dossier (https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/21281)

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES[8]	
Property	**Value**
Water solubility	20.11 g/L at 26 °C No guideline mentioned pH not reported Determined via 'cloud point technique', solubility defined as the weight of the test substance that resulted in a non-cloudy solution
Partition coefficient n-octanol/water (log K_{ow})	Log K_{ow} (P_{ow}): -0.92 at 26 °C No guideline mentioned pH not reported Solubility of the test substance in octanol was determined via 'cloud point technique'; Equation using solubility in octanol and solubility in water
Flammability	Not flammable According to EU Method A.10
Explosive properties	Test waived. Structure of the test substance does not contain chemical groups which are associated with explosive properties.
Oxidising properties	Test waived. Structure of the test substance does not contain chemical groups which are associated with oxidising properties.
Granulometry	Test waived. Study does not need to be conducted since the test substance is marketed or used in a non-solid or non-granular form.
Melting point	36 °C at 101.3 kPa According to EU Method A.1
Boiling point	Decomposes at approximately 160 °C According to EU Method A.2; ASTM Method D1120
Relative density	1.27 g/mL at 24 °C According to OECD TG 109 Sec. 10; ASTM Method D501-03 Sec. 31
Surface tension	20.6 mN/m at 23 °C According to OECD TG 115, Paragraph 8
Auto-flammability	Test waived. Study does not need to be conducted since the test substance is a solid which has a melting point ≤160 °C.
Flash point	Test waived. Study does not need to be conducted since a result on flash point is only relevant for liquids and for solids with a low melting point.

7.5. Manufacture and uses

7.5.1. Quantities

Table 5: Quantities

AGGREGATED TONNAGE (PER YEAR)[9]				
☒ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

Table 6: Overview of uses

USES[9]	Use(s)
Uses as intermediate	n.a.
Formulation	- Formulation into mixture
Uses at industrial sites	- Water-based floor finishes, waxes and polishes o Use at industrial site leading to inclusion into/onto article - Cleaning o Use of non-reactive processing aid at industrial site (no inclusion into or onto article) - Water-based paints and inks o Use at industrial site leading to inclusion into/onto article
Uses by professional workers	- Water-based floor finishes, waxes and polishes o Widespread use leading to inclusion into/onto article (indoor) - Cleaning products o Widespread use of non-reactive processing aid (no inclusion into or onto article, indoor) - Water-based paints and inks o Widespread use leading to inclusion into/onto article (indoor)
Consumer Uses	- Water-based floor finishes, waxes and polishes o Widespread use leading to inclusion into/onto article (indoor) - Cleaning products o Widespread use of non-reactive processing aid (no inclusion into or onto article, indoor)

[9] REACH Registration dossier (https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/21281)

USES[9]	Use(s)
	- Water-based paints and inks o Widespread use leading to inclusion into/onto article (indoor)
Article service life	- Article service life o Widespread use of articles with low release (indoor) ❖ Vehicles ❖ Stone, plaster, cement, glass and ceramic articles ❖ Wood articles

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

No harmonised classification.

7.6.2. Self-classification

- In the registration(s):
  Acute Tox. 2, H330: Fatal if inhaled
  STOT SE 1, H370: Causes damage to organs (lungs and larynx via inhalation)
  Aquatic Chronic 3, H412: Harmful to aquatic life with long lasting effects.

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory[10]:
  The same as mentioned above are listed in the C&L Inventory.

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. Abiotic degradation

The constituents of the Substance are mono- and diesters of phosphoric acid and in principle such compounds can undergo hydrolysis in acid, base and neutral conditions to form the corresponding alcohol and phosphoric acid (Hilal, 2006).

However, a comprehensive study by Wolfenden *et al.* (1998) showed that compounds similar to the Substance are hydrolytically stable with experimental lifetimes in the order of several years. This observation is confirmed by a hydrolysis study on the analogous substance EC 700-161-3, which pointed out that at 25 °C its half-life is greater than 1 year at pH 4, 7 and 9. Although an experimental hydrolysis study is not available for the Substance, the eMSCA considers that it is appropriate to conclude that hydrolysis will not be a relevant breakdown process.

[10] C&L Inventory (https://echa.europa.eu/nl/information-on-chemicals/cl-inventory-database/-/discli/details/154029)

Further, there is no information available on the role of phototransformation in the air, water or soil compartment. Therefore, the constituents of the Substance are considered to be stable with regard to abiotic transformation processes.

7.7.1.2. Biodegradation

In the registration dossier two biodegradation screening tests wih the Substance are presented.

Table 7: Screening studies

SUMMARY OF SCREENING STUDIES ON BIODEGRADATION IN WATER		
Method	**Results**	**References / Remarks**
Test type: ready biodegradability According to OECD TG 301 B: CO_2 Evolution Test; Regulation (EC) No. 648/2004 on Detergents; Council of the European Communities Directive 67/548/EEC Annex V, Guideline C.4-C Test system: activated sludge, domestic (non-adapted) Oxygen: aerobic	Degradation: 75.3% degradation after 28 d (theoretical amount of CO_2) Degradation: 74.1% degradation after 28 d (DOC removal)	REACH Registration dossier: Unpublished study report, 2009a Reliability 1 (Key study) Test material: EC No 700-403-8 GLP Toxicity control: Yes Reference substance (Benzoic acid, sodium salt): > 60% degradation by day 9
Test type: ready biodegradability According to OECD TG 301 D: Closed Bottle Test; EU Method C.4-E: Closed Bottle Test; GB/T 21831-2008; SEPA. HJ/T 153-2004. Test system: sewage, domestic (non-adapted) Oxygen: aerobic	Degradation: 74.6% degradation after 28 d (O_2 consumption / BOD)	REACH Registration dossier: Unpublished study report, 2011a Reliability 2 (Supporting study) Test material: EC No 700-403-8 GLP Toxicity control: Not mentioned Reference substance (Sodium benzoate) 68.1% degradation by day 14

In a CO_2 Evolution Test (OECD TG 301 B; Unpublished study report, 2009a), 75.3% degradation was recorded after 28 days based on CO_2 production, and 74.1% degradation based on the measurement of dissolved organic carbon. In this study the pass level of 60% is thus exceeded and the validity criteria for the reference control were met.

In a Closed Bottle Test (OECD TG 301 D; Unpublished study report, 2011a), a degradation of 74.6%, measured as O_2 consumption, was observed after 28 days. The pass level of 60% is thus exceeded again and the validity criteria for the reference control were also met.

It is noted that the pass level in both ready tests is reached and so the Substance can be considered readily biodegradable. However, one should keep in mind that we are dealing with a UVCB substance in which the chemical characteristics of the various constituents differ substantially. Polyfluorinated and non-fluorinated compounds are present in this substance and it is reasonable to conclude that the observed biodegradation is only due to the non-fluorinated constituents.

This conclusion is in line with the screening biodegradation results with the analoguous substance EC 700-161-3. Indeed, Substance EC 700-161-3, which consists primarily of the same poly-fluorinated compounds as the Substance, shows considerably less biodegradation in screening tests. For instance, only 11.5% degradation, measured as O_2 consumption, was observed after 28 days in an OECD TG 301 D study for Substance EC 700-161-3. Also, only 16.3% degradation was apparent, based on biological oxygen demand, after 28 days in an inherent test according to OECD TG 302 C. Further, it is noted that the degradation rate of ca. 75% in the ready tests with the Substance matches the overall ratio between non-fluorinated and fluorinated carbon atoms in the Substance. These observations suggest that the two highly fluorinated constituents in the Substance are not readily biodegradable. In fact, they are expected to hardly degrade at all.

7.7.1.3. Degradation pattern of 6:2 PAPs

The Substance is a UVCB substance that comprises 5 main constituents. The eMSCA has concluded that in the framework of the PBT assessment the 2 constituents of concern are the highly polyfluorinated compounds diammonium 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl phosphate (6:2monoPAP) and ammonium bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl) phosphate (6:2 diPAP). Based on the data from screening tests, it is concluded that the other constituents are very probably readily biodegradable, not persistent and their further evaluation in the framework of the PBT assessment is thus deemed to be superfluous.

Degradation of 6:2 monoPAP and 6:2 diPAP

In the registration dossier the properties and the environmental fate of the 6:2 PAPs are not discussed. In contrast, several authors have published papers in open scientific literature on the degradation characteristics of 6:2 PAPs. Depending on the environmental compartment and the current conditions in the laboratory experiments various degradation patterns can be determined. Further, it is noted that the degradation of these 6:2 PAPs can proceed slowly and so an experimental investigation of the complete degradation pattern is, because of time constraints, not always feasible.

A degradation pathway for 6:2 PAPs that is deemed to be representative for field conditions is described by Lee *et al.* (2010). These authors examined the aerobic biodegradation of 6:2 mono- and diPAP (at 1 µg test item/mL) in a mixture of raw wastewater, sewage sludge and phosphate-free mineral medium for 92 days. This experiment is deemed to reliably simulate the fate of 6:2 PAPs in wastewater treatment plants which is a relevant scenario for substances that are used as surfactants (see Figure 1).

In a first step, microbial hydrolysis of the phosphate ester bonds takes place, which results in the formation of 6:2 fluorotelomer alcohol (6:2 FTOH). Further oxidation leads to the formation of an intermediate 6:2 fluorotelomer carboxylate (6:2 FTCA), a compound that represents a key branching point in the further degradation of 6:2 FTOH.

In one pathway further transformation takes place to 6:2 unsaturated fluorotelomer carboxylate (6:2 FTUCA). Subsequent degradation of this compound can occur via three mechanisms. A first mechanism consists of the consumption of 6:2 FTUCA via a β-oxidation resulting in the direct formation of perfluorohexanoic acid (PFHxA). Another transformation possibility is the transformation to 5:3 fluorotelomer carboxylate (5:3 FTCA) which in turn leads to the formation of perfluoropentanoic acid (PFPeA). Finally, 6:2 FTUCA can also lead to PFPeA in an indirect manner, i.e. via the formation of 5:2 fluorotelomer ketone and subsequent reduction to 5:2 secondary fluorotelomer alcohol (5:2 sFTOH).

In another pathway 6:2 fluorotelomer carboxylate can undergo α-oxidation resulting in the formation of perfluoroheptanoic acid (PFHpA).

Whatever the degradation pathway of the 6:2 PAPs may be, it is appropriate to conclude that a series of polyfluorinated compounds are formed at varying rates. However, many of these compounds are in turn intermediates as they are further transformed. As can be seen in Figure 1 in the end the most recalcitrant compounds turn out to be the saturated perfluorinated acids, namely PFPeA, PFHxA and PFHpA. Under the current conditions in the laboratory tests these saturated acids do not react any further.

Based on the results presented by Lee *et al.* (2010), it is only possible to put forward a semi-quantitative analysis of the relative formation of the various saturated perfluorinated acids. Formation of these acids does not follow a similar trend in time and monoPAP and diPAP yielded different amounts of PFCAs: PFHpA respectively ca. 8% and 7%, PFHxA ca. 3% and 6%, PFPeA ca. 1%. It would be wrong to conclude from these values that biodegradation of 6:2 PAPs leads to only low amounts of PFCAs. Indeed, the yields might have been much higher if the test vessels in this experiment were not continuously purged. Purging caused stripping of the volatile intermediate 6:2 FTOH from the system and thus it significantly reduced the degradation capacity to PFCAs. It should also be noted that the 6:2 mono- and diPAP concentrations in the aqueous phase were very low at the start of the experiment, corresponding to respectively 10 and 33% of the added test item. These losses are caused by several processes, e.g. adsorption to the polypropylene bottle walls, septa and caps, evaporation due to continuous purging, and binding to biosolids. The later process seems especially relevant for 6:2 monoPAP as it rapidly disappeared from the aqueous phase in the sterile controls. Therefore, only a qualitative interpretation of this study is appropriate: the 6:2 mono- and diPAP constituents are relevant precursors of PFHpA, PFHxA and PFPeA in wastewater treatment plants.

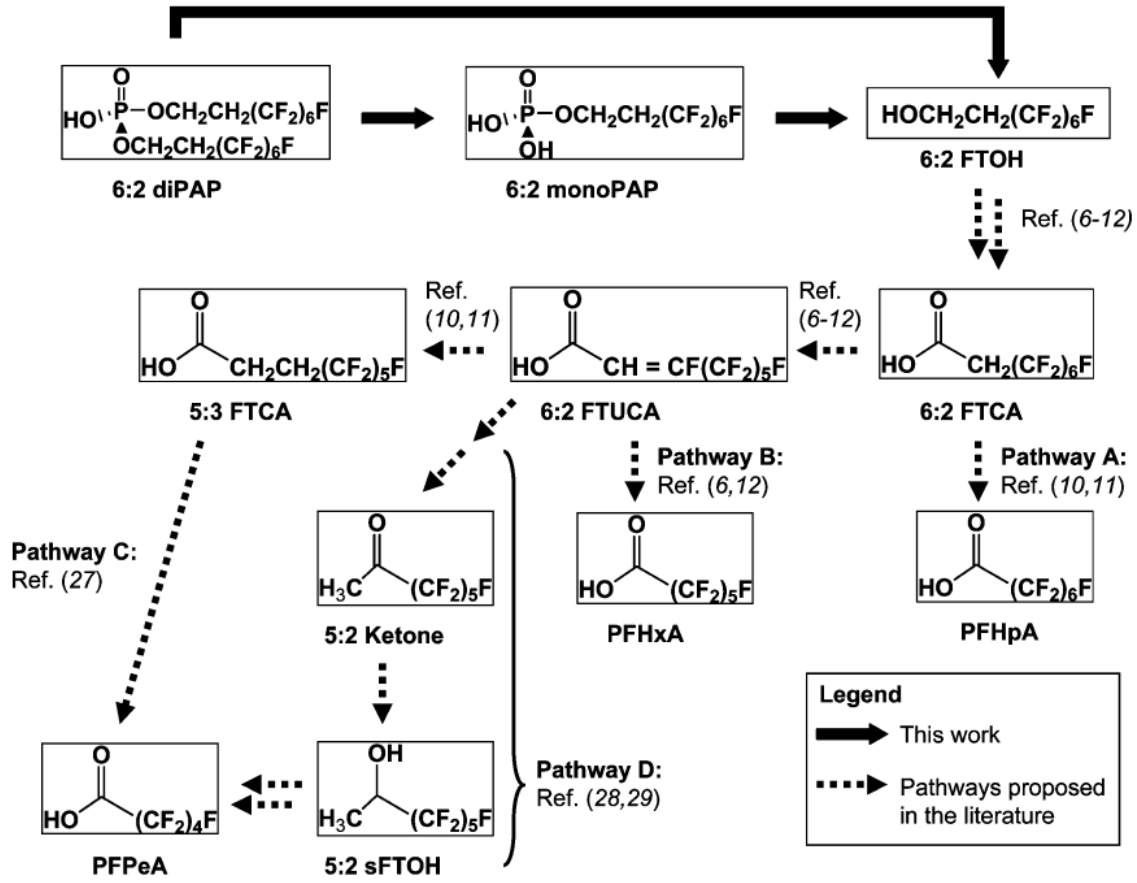

Figure 1: Degradation pathway of 6:2 diPAP and 6:2 monoPAP as proposed by Lee *et al*. (2010).

Another environmental compartment that is relevant with regard to the fate of 6:2 PAPs in field conditions is soil because soil is regarded as a major reservoir for poly- and perfluoro compounds. Liu and Liu (2016) studied the biotransformation of 8:2 diPAP and 6:2 diPAP in aerobic soil using semi-dynamics reactors during 112 days. A DT_{50} value in live soil, defined as the degradation time period leading to 50% dissipation of the initial concentration of 12 days was estimated with a double first-order in parallel (DFOP model) for 6:2 diPAP. A DT_{50} value of 15 days was derived using a single first-order kinetics (SFO model) for 6:2 diPAP. The study resulted in a mass balance for the 6:2 diPAP study (which listed the equivalent mole fractions of all quantifiable parent compounds and degradation products) containing the substances 6:2 monoPAP, 6:2 FTOH, 5:2 sFTOH, PFHxA (6.0%), PFPeA (6.4%), PFBA (0.7%) and 5:3 acid (9.3%). PFHpA is not retrieved in this study which illustrates that the degradation pattern is (also) determined by the type of microbial strains that are present.

Degradation of 6:2 FTOH

6:2 FTOH is the first transformation product that is quite readily formed in the breakdown process of 6:2 mono- and diPAPs and therefore it is informative to consider other publicly available studies that describe degradation patterns of 6:2 FTOH in various environmental matrices. In contrast to the poly- and perfluorinated acids, 6:2 FTOH is a compound that does not ionize in field conditions and so it is considerably more volatile than the fluorinated acids. Lei *et al.* (2004) calculated a Henry's Law constant for 6:2 FTOH of 1.9 $Pa.m^3/mol$ while they also point out that this value is probably still underestimated. Based on the vapour pressure and water solubility values presented in the REACH registered substance

factsheets for 6:2 FTOH (https://echa.europa.eu/nl/substance-information/-/substanceinfo/100.010.435) the Henry's Law constant would be between 349 and 854 Pa.m³/mol. Further, it is noted that the STP Fugacity Model in EPI Suite predicts that 65% of the influent 6:2 FTOH will disappear with the off gases while only 35% will reside in the primary sludge. All these observations indicate that not only degradation processes in water, sediment and soil are relevant, but also degradation of 6:2 FTOH in air should be considered.

Several authors examined the biodegradation pattern of 6:2 FTOH in condensed states. Studies conducted under aerobic conditions reported rapid 6:2 FTOH biotransformation, with most of the 6:2 FTOH disappearing from the test systems within a few days. Liu *et al*. (2010a) reported nearly equal yields of PFPeA (4.2 mol%) and PFHxA (4.5 mol%) in soil after 84 days (6:2 FTOH half-life of 1.3 days), while Liu *et al*. (2010b) detected considerably more PFPeA (30 mol%) than PFHxA (8 mol%) and PFBA (2 mol%) in soil after 180 days (6:2 FTOH half-life of less than 2 days). These differences can be due to covalent binding of transformation products, such as 5:3 FTCA, to organic matter in the first study, which limits further transformation.

6:2 FTOH aerobic biotransformation was investigated in activated sludge from two domestic wastewater treatment plants (WWTPs) in Delaware and Pennsylvania (USA) (Zhao *et al*., 2013b). The major nonvolatile, stable transformation products that resulted from 6:2 FTOH biotransformation were 5:3 FTCA and PFHxA. After 56-60 days, 5:3 FTCA accounted for 14.1 mol% of initially applied 6:2 FTOH in activated sludge from the two WWTPs. Another major transformation product was PFHxA, which was present in on average 11 mol% in the activated sludge. PFPeA was formed in a lower percentage of 4.4 mol% in the sludge. An explanation for this could be that a direct precursor for PFPeA, more specifically 5:2 sFTOH, was less bioavailable due to partitioning to the headspace. PFBA and PFHpA were not observed to be significant transformation products of 6:2 FTOH (<0.5 mol% for PFBA, mol% not mentioned for PFHpA).

The same authors (Zhao *et al*., 2013a) also investigated 6:2 FTOH biotransformation in aerobic river sediment from Brandywine Creek (USA). Based on these results, and the previous findings of Liu *et al*. (2010b) and Wang *et al*. (2012), the authors derived a biotransformation pathway for 6:2 FTOH (see Figure 2). Three enzymatic steps are required in order to transform 6:2 FTOH to 6:2 FTUCA (with 6:2 FTAL and 6:2 FTCA as intermediates). A defluorination then leads to the formation of 5:3 FTUCA and then possibly to 5:3 FTCA, or PFBA. Hydroxylation on the α-carbon can also result in α-OH 5:3 acid, which could then be transformed to 4:3 FTCA via 'one-carbon removal pathways'. Several enzymatic steps could convert 6:2 FTUCA into 5:2 ketone, which could then be further reduced to 5:2 sFTOH. It is clear that 5:2 sFTOH will finally result in the formation of the perfluorinated acids PFPeA and PFHxA, however the exact enzymatic steps that perform this conversion remain unknown. Biotransformation in the aerobic river sediment occurred rapidly, with a 6:2 FTOH half-life of 1.8 days. After 100 days, 5:3 FTCA accounted for 22.4 mol% of initially applied 6:2 FTOH; PFPeA was present in 10.4 mol%, PFHxA in 8.4 mol%, and PFBA in 1.5 mol% of initially applied 6:2 FTOH. PFHpA is not detected in this study.

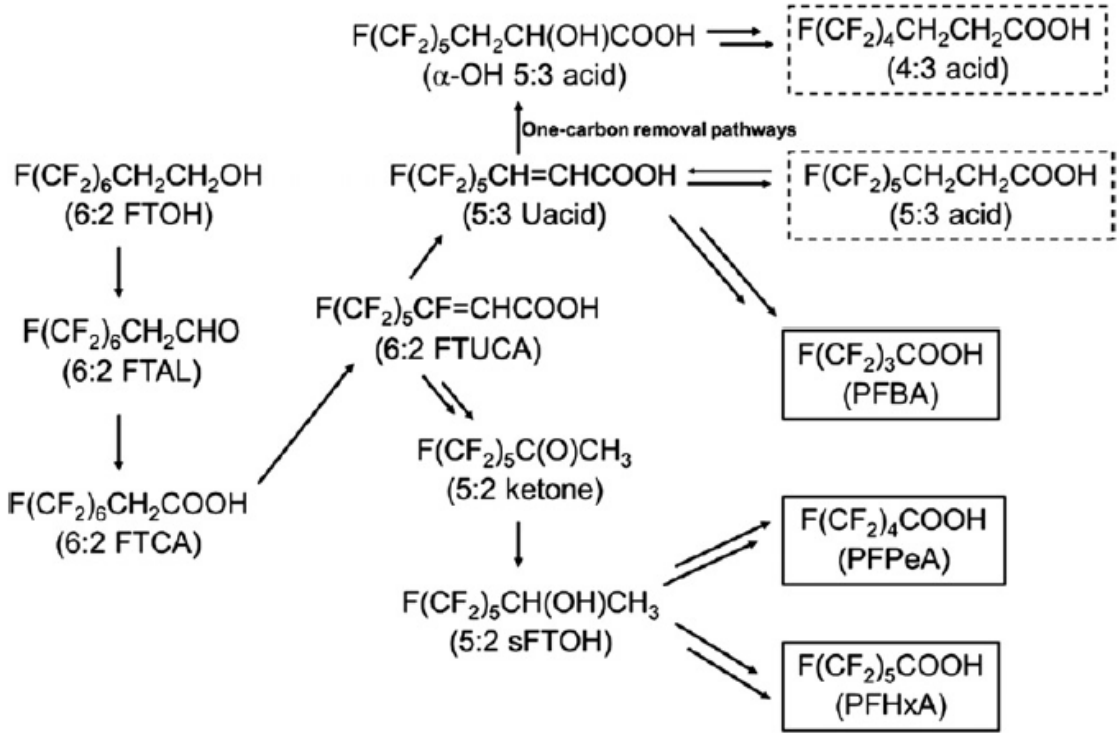

Figure 2: Degradation pathway of 6:2 FTOH as proposed by Zhao *et al*. (2013a), based on the findings of Liu *et al*. (2010b) and Wang *et al*. (2012).

A similar aerobic biotransformation pathway of 6:2 FTOH was also proposed in a recent review paper by Zhang *et al*. (2021). The pathway was based on the findings of Liu and Avendaño (2013), and Kim *et al*. (2014) (see Figure 3). Oxidation of 6:2 FTOH results in the formation of 6:2 FTAL and subsequently 6:2 FTCA. Attack on a first fluoride atom and elimination of hydrogen fluoride leads to 6:2 FTUCA, after which the pathway can further proceed in two ways. Firstly, defluorination and decarboxylation steps can subsequently lead to 5:2 ketone, 5:2 sFTOH and afterwards to PFHxA and PFPeA. Secondly, 6:2 FTUCA can be converted to 5:3 FTUCA through a defluorination step. Via this intermediate, 5:3 FTCA and PFBA could be formed. It is also a possibility that for 5:3 FTUCA, hydrolyxation on the α-carbon occurs, to produce the α-OH 5:3 acid, which is further transformed to 4:3 FTCA through 'one-carbon removal pathways'. According to this analysis PFHxA, PFPeA and PFBA are stable degradation products, but not PFHpA.

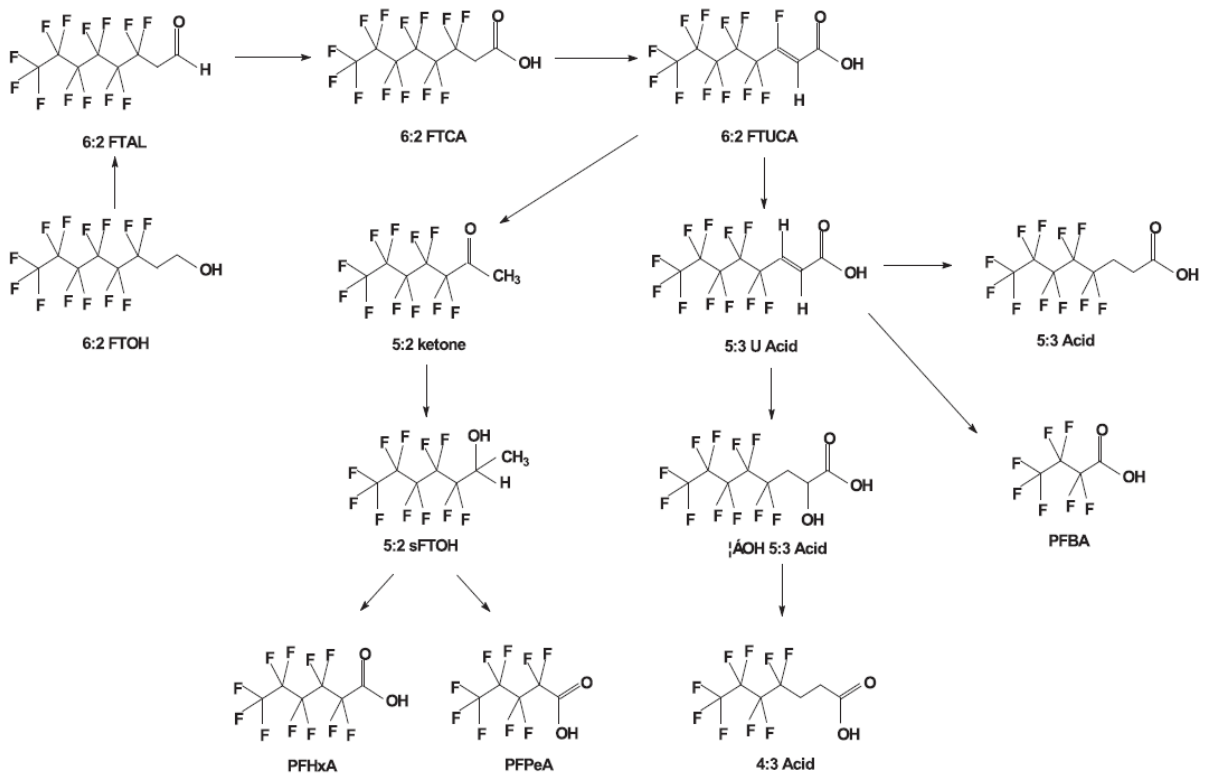

Figure 3: Degradation pathway of 6:2 FTOH as proposed by Zhang *et al*. (2021), based on the findings of Liu and Avendaño (2013) and Kim *et al*. (2014).

Thus, 6:2 FTOH is an important intermediate when following the degradation pattern of the Substance, or at least the degradation pathway of two important constituents, 6:2 monoPAP and 6:2 diPAP. Nevertheless, the most persistent degradation products at the end of the pathway are considered to be the most concerning. More specifically, these are the perfluorocarboxylic acids (PFCAs) such as PFHpA, PFHxA and PFPeA.

Because 6:2 FTOH is a rather volatile chemical, reactions in the air compartment are relevant and several studies can be found in open literature in which degradation of airborne 6:2 FTOH is described.

Ellis *et al*. (2003) investigated the persistence of 4:2, 6:2 and 8:2 FTOHs in the atmosphere. The FTOHs were incubated with OH radicals and Cl atoms in a trapped smog chamber. It was observed that the length of the $F(CF_2CF_2)_n$-group had no apparent impact on the reactivity of the molecules. It was concluded that the atmospheric lifetime of $F(CF_2CF_2)_nCH_2CH_2OH$ ($n \geq 2$) is determined by reaction with OH radicals and is approximately 20 days under atmospheric conditions. These results demonstrate that 6:2 FTOH can be distributed in the air compartment.

In a follow-up study Ellis *et al*. (2004) investigated the degradation products that are formed upon atmospheric degradation of 4:2, 6:2 and 8:2 FTOHs. The FTOHs were incubated with Cl atoms in a trapped smog chamber. Oxidation of 6:2 FTOH produced PFHpA, PFHxA, PFPeA, PFBA, PFPrA, TFA, 6:2 FTCA, 6:2 FTAL, C6 PFAL and 6:1 FTOH. Yields were only reported for the 8:2 FTOH oxidation products and amounted for the various PFCAs: PFNA (1.6%), PFOA (1.5%), PFHpA (0.32%), PFHxA (0.24%), PFPeA (0.10%), PFBA (<0.1%), PFPrA (<0.1%), TFA (<0.1%). Considering that the length of the perfluorinated chain has no meaningful impact on the reactions taking place, the eMSCA concludes that PFHpA and PFHxA are the most abundant degradation products upon oxidation of 6:2 FTOH.

Styler *et al*. (2013) investigated photooxidation of 6:2 FTOH at SiO_2, TiO_2, Fe_2O_3, Mauritanian sand and Icelandic volcanic ash. It was shown that 6:2 FTOH exhibited significant uptake to each of the surfaces under study, and that sand- and ash-catalysed heterogeneous photooxidation of 6:2 FTOH resulted in the rapid production of surface-sorbed PFCAs. At the TiO_2 surface almost equal quantities of 6:2 FTUCA, PFHpA, PFHxA, and PFPeA were found, while at the other surfaces primarily PFHxA, but also PFPeA, PFHpA, and 6:2 FTUCA were detected. Considering yields were low at the SiO_2 surface, it was suggested that transformation is catalysed by Fe and Ti contained within the samples.

It is concluded that 6:2 FTOH is volatile and can stay for a considerable time in the air. In this compartment 6:2 FTOH oxidizes to a range of PFCAs with PFHpA and PFHxA being the most abundant ones. Removal from the atmosphere can occur by binding to natural and artificial surfaces where especially at metal-rich surfaces, 6:2 FTOH is photooxidized to PFHxA and in lower amounts to PFPeA and PFHpA.

Therefore, the eMSCA considers that airborne abiotic degradation of 6:2 FTOH represents a potential relevant degradation pathway in the breakdown of 6:2 PAPs.

Overall, the eMSCA comes to the conclusion that the constituents 6:2 monoPAP and 6:2 diPAP, once released in the environment, will transform more or less slowly in various environmental compartments and via a combination of degradation patterns into a series of highly fluorinated compounds. The most recalcitrant compounds are the saturated PFCAs and one may state that these acids undergo no or extremely limited degradation under environmental conditions. Taking into account the probabilities that the various degradation mechanisms will occur in field conditions, PFHxA is considered to be the major long-lasting degradation product. Minor stable degradation products of the 6:2 PAPs are PFHpA and PFPeA.

7.7.1.4. Conclusion on persistence

Regarding persistence only the 6:2 monoPAP and 6:2 diPAP constituents of the Substance are relevant. These two PAPs are very unlikely to be persistent because in laboratory studies these two constituents readily transform into 6:2 FTOH. Depending on the environmental compartment where this intermediate compound resides, a whole series of more or less stable highly fluorinated molecules will be formed. Based on studies from open literature, the eMSCA concludes that in the long term perfluorohexanoate (PFHx) will be the major recalcitrant end product, while perfluoroheptanoate (PFHp) and perfluoropentanoate (PFPe) will be formed in lesser amounts.

In general, the stability of organic fluorine compounds has been described by Siegemund *et al*. (2012). This author concludes that '*when all valences of a carbon chain are satisfied by fluorine, the zigzag-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 C-F bond contribute to the fact that highly fluorinated compounds belong to 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.*'

On 17 January 2023, 'Perfluoroheptanoic acid and its salts' was included as an SVHC in the Candidate List[11] for eventual inclusion in Annex XIV (ECHA Decision, 2022). PFHpA and its salts were concluded to be persistent (P) and very persistent (vP).

[11] ECHA Candidate List of substances of very high concern for Authorisation. Perfluoroheptanoic acid and its salts (EC No: -; CAS no: -). https://www.echa.europa.eu/candidate-list-table

7.7.2. Environmental distribution

The Substance is a UVCB substance whose constituents show widely diverging properties. Therefore, it is not relevant to evaluate the environmental distribution of the parent compound. As a result of the persistence assessment as elaborated in paragraph 7.7.1., the eMSCA has concluded that the critical compounds are the recalcitrant substances PFHxA/PFHx and PFHpA/PFHp. Because of their highly fluorinated character, an analysis of their distribution with EPI Suite is deemed to be not sufficiently reliable.

7.7.3. Bioaccumulation

The Substance is a UVCB substance. Experimental information on the bioaccumulation potential of the constituents of the parent substance is not available and also not relevant for this PBT assessment as these constituents are very lileky not persistent. The assessment of the environmental fate of the Substance has pointed out that the most abundant recalcitrant degradation product is PFHx but also PFHp will be formed in field conditions albeit in substantial lesser amounts. Considering the bioaccumulation pattern found for the longer PFCAs, the eMSCA concluded that PFHp is expected to show the greater bioaccumulation potential of these two recalcitrant degradation products. Therefore, the further evaluation of the bioaccumulation potential is focussed here on PFHp.

7.7.3.1. Bioaccumulative properties of PFHpA/PFHp

The usual approach to assess the bioaccumulation potential, i.e. measurement of bioconcentration factor (BCF), bioaccumulation factor (BAF), biomagnification factor (BMF) or trophic magnification factor (TMF) is less relevant for perfluorinated compounds. In contrast to most substances showing substantial bioaccumulation, PFHp does not primarily accumulate in fat tissues; accumulation mainly takes place in blood and in the liver and this behaviour is triggered by binding to proteins. For the moment, there are no standardized tests to quantify this mechanism of protein binding. Neither are there agreed cutoff criteria to decide whether a substance should be classified as (very) bioaccumulative other than the BCF. Therefore assessment of the bioaccumulation potential of PFHp is based on a weight-of-evidence argumentation.

Bioaccumulation of PFHpA/PFHp in aquatic organisms

Based on a direct comparison with the bioaccumulation criterion as set out in Annex XIII of REACH, PFHp should not classified as bioaccumulative because the BCF is far below the B criterion of 2000. Also other endpoints like TMF values from aquatic food web studies do not indicate significant bioaccumulation in water-breathing organisms.

Bioaccumulation of PFHpA/PFHp in mammals

In a few toxicokinetic studies with the usual laboratory animals the fate of PFASs is examined.

In a study by Fujii *et al.* (2015) with mice, C_{6-14} PFCAs were administered intravenously (IV) or by gavage. Whole blood samples were collected from the tail veins at regular intervals up to 24 hours after IV or gavage administration. An additional collection was made at 0.5 hours for IV administration. After 24 hours, urine and feces were collected in metabolic cages. The ratio of PFCAs between whole blood and serum at 24 hours was used to convert PFCA concentrations in whole blood samples into serum PFCA concentrations.

Serum concentration data were analyzed using a two-compartment model and mouse urinary and fecal clearances were determined.

Following IV administration, for C_{7-14} PFCAs, but not for PFHxA, the serum levels were above the method detection limit. PFHpA disappeared from the serum in a time-dependent manner, while the longer PFCAs were slowly eliminated from the serum. Almost all of the PFHxA and PFHpA administered doses were recovered in the urine after 24 hours with only a small portion excreted in the feces. For PFHpA and perfluorooctanoic acid (PFOA) the average urinary clearance was 276 and 11.4 mL/d/kg respectively. From this study, it can be concluded that the clearance of PFHpA in mice is much faster than for PFOA. Urinary clearance decreases with chain length. No clearance could be determined for PFHxA as it was excreted too rapidly. These data only show that PFHpA clearance from mice is between PFHxA and PFOA.

Ohmori *et al.* (2003) studied the fate of PFCAs and other PFASs with rats. The test items were administered intravenously and urine was collected continuously. A two-compartment model was used to fit the data. The half-lives however are reported to have been fitted on the plasma concentrations in the course of time. The half-lives for PFHpA amounted to 0.05 days for female and 0.10 days for male rats. The half-life for PFOA in female rats of 0.08 days was comparable to PFHpA, but for male rats it was considerably higher (5.63 days). This difference between female and male rats was also observed for PFNA and PFDA. It can be concluded that the half-lives for PFHpA in female and male rats are quite short and certainly less than 1 day.

The eMSCA considers that a study carried out by Numata *et al.* (2014) on pigs is more informative to evaluate bioaccumulation in mammals. In this study, the pigs received food contaminated with a mixture of PFAS for a period of three weeks. The study was performed with gilts, barrows as well as with young boars. Blood samples were collected 4 days prior to exposure started, on days 4, 8, 11, 15 and 18 during feeding, and on day 22, one day after exposure stopped. The following organs were analysed: kidney, liver, fat, dorsal muscle tissues and ventral muscle tissues. Urine was collected during feeding, weighing or blood sampling of the pigs. Analysis was performed with HPLC-MS/MS.

In principle a two-compartment first-order toxicokinetic model was applied to interpret the observed data. However, the modelled parameters showed an almost instantaneous internal distribution between plasma and other body parts. Therefore, the kinetics between plasma and other organs were replaced by equilibrium partitioning, which leaves the modelling to be equivalent to a one-compartment model.

The estimated half-lives and biomagnification factors derived by the authors of the study depend on the urinary rate constant kU, and on the mass balance and distribution of the PFASs over the body of the pigs, which are associated with some uncertainties. However, the derived half-lives and the BMF values calculated by the authors are still considered reliable and instructive as they rather underestimate and not overestimate the bioaccumulation potential of PFHpA in pigs.

The elimination half-lives for PFHpA for individual pigs range from around 10 to around 500 days. For some pigs plasma concentrations start to level off and based on those individuals the lower end half-lives still seem realistic and not an artefact. For other pigs the increase in plasma concentrations of PFHpA is almost linear and no levelling off is observed during the 21 days of exposure, indicating that the half-life is considerably longer than the time span of the experiment. The geometric mean elimination half-life for PFHpA amounts to 74 days. This value falls well between the geometric mean half-lives derived for perfluorooctanoate (236 days) and perfluorohexanoate (4.1 days). The corresponding biomagnification factors for whole pigs for PFOA, PFHpA and PFHxA are, respectively 7.9, 2.7 and 0.13. Biomagnification factors based on liver (BMF = 7.0) were higher than those for whole pig, while values based on meat (BMF = 1.8) were lower than for whole pig. Overall, these findings form a reliable argument to classify PFHpA as at least bioaccumulative for pigs.

Bioaccumulation of PFHpA/PFHp in humans

In several studies the half-lives of PFHpA in humans have been examined. In these studies, the precise exposure to PFHpA is not known while the levels to which the test persons are exposed can have a substantial influence on the values that are put forward by the authors. Nevertheless, the eMSCA considers that the various half-lives that are proposed are very relevant and can be used in a weight-of-evidence approach to assess the overall bioaccumulation potential of PFHpA.

Freberg *et al.* (2010) examined the bioaccumulation potential in humans by investigating the temporal trend of a series of perfluorinated substances, including the C_{4-14} PFCAs, in the serum of 13 ski wax technicians before, during and after the skiing season. The percent reduction in serum concentration from the end of the season (March 2008) to the beginning of the new season (November 2008) was expressed graphically for those PFCAs for which the concentrations could be measured. In the 8-month period between the skiing seasons (ca 245 days) no occupational exposure to ski waxes occurred and there is no indication that significant exposure to other PFAS sources took place. Using the reductions in serum concentrations, elimination half-lives could be deduced assuming a first-order decline. The deduced half-lives for PFHxA, PFHpA, PFOA are respectively 110, 220 and 2700 days. Further it is noted that a significant positive correlation is observed for PFHpA and the longer PFCAs between the serum concentrations and the number of years working as a ski waxing technician. This implies that PFHpA builds up over the years.

Nilsson *et al.* (2010) performed a similar study on 8 professional ski waxers. Blood was sampled before (September 2007), during (from December 2007 to March 2008), and after (from April to August 2008) the exposed period. The age of the test persons ranged from 27 to 51 years, and they were active as ski waxer from 3 to 15 years. For PFHpA and longer PFCAs a significant positive correlation was found between the numbers of years in the profession and the concentration in blood. It is concluded that PFHpA and the longer PFCAs are not sufficiently eliminated between ski seasons, suggesting that these substances accumulate over the years.

In a subsequent study by Nilsson *et al.* (2013) blood was sampled from eleven ski waxers, including the 8 ski waxers sampled in the previous study, in the years 2009-2011. This study not only analysed PFCAs in blood, but also FTOHs and intermediates of FTOHs, as it was shown that ski waxers are exposed to very high concentrations of 6:2, 8:2 and 10:2 FTOHs in air during their professional activity. In theory formation of PFCAs from these intermediates could have influenced the concentration of PFCAs outside the ski season, but this influence is considered to be of subordinate importance.

No elimination half-lives were reported in the original studies. However, for six of the eleven ski waxers concentrations in at least two consecutive blood samples in between ski seasons were available from which half-lives can be estimated. A time series was only taken into account if at the first time point the data were above the Limit of Detection. For PFHpA almost all data used were above the Limit of Quantification with three exceptions.

In this way the following half-lives are deduced for the various technicians: 87 d, 10 d, 62 d, 107 d, 66 d and 124 d. This leads to a geometric mean of 60 days. The value of 10 days is probably an outlier and excluding this half-life value, one can calculate a geometric mean of 86 days and a median value of 87 days.

Finally, there is a remarkable difference between PFHpA and the shorter PFCAs. The latter were hardly detected outside the ski season, while PFHpA is similar to the longer chain PFCAs detected in almost all samples.

Zhang *et al.* (2013) examined paired blood and urine samples taken from adult individuals from the Hebei province in China. The participants were 47 females, whose age ranged from 21 to 85 years and 39 males with age ranging between 22 and 88 years. In this study the C_{7-11} PFCAs and other PFASs were analysed. Blood concentrations were converted to serum concentrations by accounting for an average haematocrit content of whole blood. From the ratio of the measured concentrations in serum and urine the renal clearance was estimated. The calculation of the renal clearance was done by assuming daily urine volumes of 1.2 and 1.4 L and body weights of 55 and 65 kg for females and males, respectively. This ratio was multiplied by the concentration ratio in urine and blood to obtain the renal clearance. In turn, this renal clearance was recalculated to a half-life by means of the volume of distribution. The assumed volume of distribution for PFCAs was 170 mL serum per kg body weight. It should be noted that in this way the calculation of the half-life could be an overestimation because it does not account for other routes of elimination than urinary excretion. The authors made the assumption that urinary excretion is the primary elimination route based on literature data for PFOA on rats and monkeys. Fecal elimination seems to become only important for PFCAs longer than PFOA.

The average renal clearance of PFHpA is assumed to be 0.61 mL/d/kg both for the group of young females and the combined group of males and older females. For the group of younger females an average half-life of 1.5 ± 0.3 year with a range of 0.11 to 3.3 year, a geometric mean of 1.0 year and a median value of 1.6 year is calculated. For the combined group of males and older females an average half-life of 1.2 ± 0.2 year with a range of 0.12 to 5.1 year, a geometric mean of 0.82 year and a median value of 0.79 year. It should be noted that the geometric mean and the median value for the half-lives can be recalculated rather precisely, while this is not the case for the mean values.

Overall, the derived half-lives for PFHpA should be considered reliable estimates. The minimum value in the total of 43 people for which both urine and serum concentration could be determined is 40 days. However, geometric and median values were much higher with values around 300 days and higher. The highest value was 5.1 years. So it can be concluded that the variability between individuals is considerable.

Xu *et al.* (2020) examined employees from an airport in northern Sweden that were exposed to high concentrations of PFAS in drinking water at the airport due to firefighting trainings. Nine employees were sampled once after exposure to contaminated drinking water stopped and in addition of that seventeen employees were involved in this first blood sampling as well as in repeated blood and urine sampling (four times at monthly intervals). Next to that, a reference group containing 58 people was involved without being exposed to elevated PFASs levels. The analysed PFASs were PFHxA, PFHpA, PFOA, perfluorobutanesulfonic acid (PFBS), perfluoropentanesulfonic acid (PFPeS), perfluorohexanesulfonic acid (PFHxS), perfluoroheptanesulfonic acid (PFHpS) and perfluorooctanesulfonic acid (PFOS).

Concentrations in the drinking water were measured as well. Initial serum concentrations obtained shortly after contaminated drinking water intake stopped were higher than the concentrations in drinking water for all PFASs. For PFHpA the concentration ratio of serum to drinking water was 4.74. Of the studied compounds, the concentration ratio of urine to serum was highest for PFHpA with a median of 0.086 for the seventeen individuals and a range from 0.018 to 0.32. It should be noted that this ratio is higher than from the Zhang study (2013) in China where this ratio is on average 0.028. This indicates a rather high renal clearance in this study from Sweden.

The half-life of PFHpA was estimated from the 5 monthly serum concentrations. This was done by fitting the data for all individuals with one generic slope and by fitting the data for each of the seventeen individuals separately. For the seventeen individual employees, the half-lives varied from 32 to 281 days, with an average half-life of 90 days, a geometric mean of 74 days and a median value of 65 days. If the half-lives are calculated from the reported ratios of urine and serum in the same way as in the Zhang study, a half-life of 63 days is found with a range of 17 to 302 days. This value is very similar as the one

determined from the decay in serum concentrations. It is noted that the serum concentrations of PFHpA ranged from 0.17 to 0.53 ng/mL, which is higher than in the general population in the Hebei province in China, but still considerably lower than the values in the blood or serum from the ski waxers.

Finally, Li *et al.* (2022) reported the results of their study regarding the exposure of the inhabitants of Ronneby in Sweden to drinking water contaminated with PFAS. 114 people participated in the study, in which blood was sampled during ten rounds over 4 years. A reference group of 64 people from a nearby municipality was included too.

For PFHpA only limited data are reported. The initial serum concentrations of PFHpA from blood sampled half a year after the consumption of contaminated drinking water stopped was 0.085 ng/mL (geometric mean) and 0.095 ng/mL (median value) with a range of <0.05 to 2.4 ng/mL. In the reference group all values were <0.1 ng/mL. At the tenth round 4.4 years after the exposure via contaminated drinking water stopped, the geometric mean and the median were <0.05 ng/mL, while the maximum concentration was 0.11 ng/mL. Based on this maximum value at the end of the study, the estimated half-life could be 320 days for this individual. Because many data are below the detection limit other conclusions cannot be drawn. It is also not clear to what extent exposure from other sources during the study could have influenced the results. So, information from this study is quite limited. It only confirms the observation that for individuals the half-life of PFHpA could be as long as 320 days.

7.7.3.2. Conclusion on bioaccumulation

Experimental data regarding the bioaccumulation potential of the various parent constituents of the Substance are not available, but also not relevant, for PBT assessment as these constituents are not considered persistent. The evaluation of the fate of the Substance pointed out that the most recalcitrant degradation products are PFHxA/PFHx and PFHpA/PFHp and so their bioaccumulation potential was further investigated. Almost all studies demonstrate that the half-life of PFHpA in air-breathing organisms is constantly greater than that of PFHxA. Therefore, the bioaccumulation potential of PFHpA is examined in more detail.

PFHpA is not bioaccumulative for aquatic organisms as the BCF is far below the threshold value of 2000. In contrast, the situation is totally different for air-breathers. In order to assess the bioaccumulation potential of PFHpA in air-breathing organisms, its half-life has been determined in several studies. These studies were performed with usual laboratory animals like mice and rats, with pigs and with human populations that were unintentionally exposed to PFASs. In these studies, the level of exposure is not always known and this unknown element could influence the results as half-lives are probably function of the exposure level.

The studies show that there is considerable variation of the half-lives of PFHpA in blood and serum, not only between different populations, but also between individuals. This is also true for studies that used the same methodology and so one may conclude that this variability is not triggered by the experimental setup or the calculation method.

The half-life for both female and male rats proved to be less than 1 day and this does not indicate a potential for bioaccumulation. In contrast, for pigs a large variability was observed with a half-life range of 10 to 500 days and a geometric half-life of 74 days. Based on this result a biomagnification factor in pigs of 2.7 is calculated. In the studies on human populations the following geometric half-lives were found: 220 days (Freberg *et al*., 2010), 86 days (Nilsson *et al*., 2010), 365 days (young females) & 299 days (rest of population) (Zhang *et al*., 2013), 74 days (Xu *et al*., 2020). All these values are quite high and they indicate a substantial bioaccumulation potential.

On 17 January 2023, 'Perfluoroheptanoic acid and its salts' was included as an SVHC in the Candidate List[12] for eventual inclusion in Annex XIV (ECHA Decision, 2022). PFHpA was concluded to be bioaccumulative (B) and very bioaccumulative (vB). As explained in the Member State Committee Support Document (ECHA Member State Committee, 2022), PFHpA is considered to be very bioaccumulative because the observed half-lives in humans are greater than 30 days and such values lead to a BMF in humans considerably greater than 1.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

7.8.1.1.1. Short-term toxicity to fish

Table 8: Short-term toxicity to fish

SUMMARY OF SHORT-TERM TOXICITY STUDIES ON FISH		
Method	**Results**	**References / Remarks**
According to OECD TG 203: Fish, Acute Toxicity Test; EPA OPPTS 850.1075: Freshwater and Saltwater Fish Acute Toxicity Test *Oncorhynchus mykiss* Test type: flow-through Test medium: freshwater	96h LC_{50} > 117 mg_{solids}/L (meas. arithm. mean) based on: mortality 96h LC_{50} > 485 mg/L (meas. arithm. mean) based on: mortality	REACH Registration dossier: Unpublished study report, 2011b Reliability 1 (Key study) Test material: EC No 700-403-8 GLP
According to OECD TG 203: Fish, Acute Toxicity Test; EPA OPPTS 850.1075: Freshwater and Saltwater Fish Acute Toxicity Test; SEPA. HJ/T 153-2004 *Gobiocypris rarus*	96h LC_{50} > 150 $mg_{total\ solids}$/L (nominal) based on: mortality 96h LC_{50} > 147 $mg_{total\ solids}$/L (mean meas. conc.) based on: mortality 96h NOEC > 150 $mg_{total\ solids}$/L (nominal) based on: mortality/abnormal behaviour 96h NOEC > 147 $mg_{total\ solids}$/L	REACH Registration dossier: Unpublished study report, 2011c Reliability 2 (Supporting study) Test material: EC No 700-403-8 GLP

[12] ECHA Candidate List of substances of very high concern for Authorisation. Perfluoroheptanoic acid and its salts (EC No: -; CAS no: -). https://www.echa.europa.eu/candidate-list-table

SUMMARY OF SHORT-TERM TOXICITY STUDIES ON FISH		
Method	**Results**	**References / Remarks**
Test type: static Test medium: freshwater	(mean meas. conc.) based on: mortality/abnormal behaviour	

The key study for fish short-term toxicity on the Substance is performed with *Oncorhynchus mykiss* (previously named *Salmo gairdneri*) according to OECD TG 203 (Unpublished study report, 2011b). The test media pH ranged from 7.7 to 8.2. The nominal exposure series was 0, 31.1, 62.3, 125, 249, and 498 mg/L; and 0, 7.5, 15, 30, 60, and 120 expressed as mg_{solids}/L. Mean measured concentrations were 0, 7.41, 13.9, 30.7, 60.2, and 117 mg_{solids}/L (based on measured values of the bis-ester component). Mortality or sublethal effects were not observed within the exposure period of 96 hours for any of the tested groups. One animal died in the control group during the 96 hours time period. Considering there was no apparent mortality in the tested groups, the 96h LC_{50}-value (meas. Arithm. mean) was >485 mg/L (based on the bis-ester component).

The registration dossier also mentions a supporting study, according to the same guideline, on the Substance, performed with *Gobiocypris rarus* (Unpublished study report, 2011c). Test media pH was not reported. In this study, only two concentrations of test material (100 and 150 $mg_{total\ solids}$/L) were used. No mortality, nor abnormal behaviour was seen in any of the tested groups, nor the control group after 96 hours. As there was no mortality, 96h LC_{50}-values were >150 $mg_{total\ solids}$/L (nominal) and >147 $mg_{total\ solids}$/L (mean meas. conc. of bis-ester components). No mortality, nor abnormal behaviour translated into 96h NOEC-values of >150 $mg_{total\ solids}$/L (nominal) and >147 $mg_{total\ solids}$/L (mean meas. conc. of bis-ester components).

Conclusion: There is no indication of short-term toxicity to fish, as all 96h LC_{50}-values are above 1 mg/L.

7.8.1.1.2. Long-term toxicity to fish

No data available.

7.8.1.2. Aquatic invertebrates

7.8.1.2.1. Short-term toxicity to aquatic invertebrates

Table 9: Short-term toxicity to aquatic invertebrates

SUMMARY OF SHORT-TERM TOXICITY STUDIES ON AQUATIC INVERTEBRATES		
Method	**Results**	**References / Remarks**
According to OECD TG 202: Daphnia sp. Acute Immobilisation Test	48h EC_{50} > 120 mg/L (nominal) based on: mobility	REACH Registration dossier: Unpublished study report, 2010a

SUMMARY OF SHORT-TERM TOXICITY STUDIES ON AQUATIC INVERTEBRATES		
Method	**Results**	**References / Remarks**
Daphnia magna Test type: static Test medium: freshwater	48h EC_{50} > 24 $mg_{\sim 20\% \text{ solids content}}$/L (nominal) based on: mobility 48h EC_{50} > 113 mg/L (meas. arithm. mean) based on: mobility 48h EC_{50} > 22.6 $mg_{\sim 20\% \text{ solids content}}$/L (meas. arithm. mean) based on: mobility	Reliability 1 (Key study) Test material: EC No 700-403-8 GLP
According to OECD TG 202: Daphnia sp. Acute Immobilisation Test *Daphnia magna* Test type: static Test medium: freshwater	48h EC_{50} > 120 mg/L (nominal) based on: mobility 48h EC_{50} > 24 $mg_{\sim 20\% \text{ solids content}}$/L (nominal) based on: mobility	REACH Registration dossier: Unpublished study report, 2009b Reliability 2 (Supporting study) Test material: EC No 700-403-8 Non-GLP

The registration dossier contains an acute immobilisation test according to OECD TG 202, on the Substance, performed with *Daphnia magna* (Unpublished study report, 2010a). The test media pH ranged from 8.6 to 8.9 at the beginning of the experiment, and from 8.0 to 8.1 at the end. The nominal exposure series was 0, 7.5, 15, 30, 60, and 120 mg/L. Mean measured concentrations were 0, 6.3, 12.9, 27.9, 57.6, and 113 mg/L (based on measured values of the bis-ester component). The control group showed no immobility or sublethal effects. For the treated groups, a 48h EC_{50}-value >120 mg/L (and >24 $mg_{\sim 20\% \text{ solids content}}$/L) (nominal), and a 48h EC_{50}-value >113 mg/L (and >22.6 $mg_{\sim 20\% \text{ solids content}}$/L) (meas. arithm. mean, based on measured values of the bis-ester component), was derived, all based on mobility.

Secondly, a similar test according to OECD TG 202 is provided (Unpublished study report, 2009b). However, the study was not performed according to GLP. Test media pH was not reported. Nominal test concentrations were 0.12, 1.2, 12 and 120 mg/L. The lowest nominal concentration, which resulted in 100% immobility at the end of the test period (and the highest concentration which caused 0% immobility), was greater than 120 mg/L (48h EC_{50} >120 mg/L or >24 $mg_{\sim 20\% \text{ solids content}}$/L, nominal).

Conclusion: There is no indication of short-term toxicity to aquatic invertebrates, as all 48h EC_{50}-values are above 1 mg/L.

7.8.1.2.2. Long-term toxicity to aquatic invertebrates

No data available.

7.8.1.3. Algae and aquatic plants

Table 10: Effects (short-term and long-term) on algae and aquatic plants

SUMMARY OF TOXICITY STUDIES ON ALGAE AND AQUATIC PLANTS		
Method	**Results**	**References / Remarks**
According to OECD TG 201: Alga, Growth Inhibition Test; EPA OPPTS 850.5400: Algal Toxicity, Tiers I and II *Raphidocelis subcapitata* Test type: static Test medium: freshwater	72h EC_{50} = 59.2 mg/L (nominal) based on: cell number 72h EC_{50} = 11.8 $mg_{\sim 20\% \text{ solids content}}$/L (nominal) based on: cell number 72h EC_{50} = 58.9 mg/L (nominal) based on: cell count yield 72h EC_{50} = 11.8 $mg_{\sim 20\% \text{ solids content}}$/L (nominal) based on: cell count yield 72h EC_{50} = 112 mg/L (nominal) based on: growth rate (cell count) 72h EC_{50} = 22.4 $mg_{\sim 20\% \text{ solids content}}$/L (nominal) based on: growth rate (cell count) 72h EC_{50} = 46.3 mg/L (meas. arithm. mean) based on: cell number 72h EC_{50} = 9.3 $mg_{\sim 20\% \text{ solids content}}$/L (meas. arithm. mean) based on: cell number 72h EC_{50} = 46.1 mg/L (meas. arithm. mean) based on: cell count yield 72h EC_{50} = 9.2 $mg_{\sim 20\% \text{ solids content}}$/L (meas. arithm. mean) based on: cell count yield 72h EC_{50} = 94 mg/L (meas. arithm. mean) based on: growth rate (cell count) 72h EC_{50} = 18.8 $mg_{\sim 20\% \text{ solids content}}$/L (meas. arithm. mean) based on: growth rate (cell count) 72h NOEC = 30 mg/L (nominal) based on: cell count, cell count yield, growth rate (cell count)	REACH Registration dossier: Unpublished study report, 2010b Reliability 1 (Key study) Test material: EC No 700-403-8 GLP

SUMMARY OF TOXICITY STUDIES ON ALGAE AND AQUATIC PLANTS		
Method	**Results**	**References / Remarks**
	72h NOEC = 12 mg$_{\sim 20\% \text{ solids content}}$/L (nominal) based on: cell count, cell count yield, growth rate (cell count) 72h NOEC = 19.8 mg/L (meas. arithm. mean) based on: cell count, cell count yield, growth rate (cell count) 72h NOEC = 3.9 mg$_{\sim 20\% \text{ solids content}}$/L (meas. arithm. mean) based on: cell count, cell count yield, growth rate (cell count) 72h LOEC = 60 mg/L (nominal) based on: cell count, cell count yield, growth rate (cell count) 72h LOEC = 12 mg$_{\sim 20\% \text{ solids content}}$/L (nominal) based on: cell count, cell count yield, growth rate (cell count) 72h LOEC = 50.8 mg/L (meas. arithm. mean) based on: cell count, cell count yield, growth rate (cell count) 72h LOEC = 10.2 mg$_{\sim 20\% \text{ solids content}}$/L (meas. arithm. mean) based on: cell count, cell count yield, growth rate (cell count)	

A toxicity study on *Raphidocelis subcapitata* (previously named *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*) with the Substance, performed according to OECD TG 201 is available (Unpublished study report, 2010b). Study endpoints were cell number / cell count, cell count yield and growth rate (cell count). The test media pH ranged from 8 to 8.7. Nominal concentrations were 7.5, 15, 30, 60, and 120 mg/L, and mean measured concentrations were 4.30, 8.57, 19.8, 50.8 and 111 mg/L (based on measured values of the bis-ester component). The lowest 72h EC_{50}-values were based on cell count yields. A 72h EC_{50}-value of 58.9 mg/L (and 11.8 mg$_{\sim 20\% \text{ solids content}}$/L) (nominal), and a 72h EC_{50}-value of 46.1 mg/L (and 9.2 mg$_{\sim 20\% \text{ solids content}}$/L) (meas. arithm. mean, based on measured values of the bis-ester component) are available. NOEC- and LOEC-values were based on cell count, cell count yield and growth rate (cell count). 72h NOEC-values were 30 mg/L (and 12 mg$_{\sim 20\% \text{ solids content}}$/L) (nominal), and 19.8 mg/L (and 3.9 mg$_{\sim 20\% \text{ solids content}}$/L) (meas. arithm. mean, based on measured values of the bis-ester component). Further, 72h LOEC-values of 60 mg/L (and 12 mg$_{\sim 20\% \text{ solids content}}$/L) (nominal), and 50.8 mg/L (and 10.2 mg$_{\sim 20\% \text{ solids content}}$/L) (meas. arithm. mean, based on measured values of the bis-ester component) were reported.

Conclusion: There is no indication of short-term and long-term toxicity to algae and aquatic plants, as all 72h EC_{50} and 72h NOEC-values are above 1 mg/L.

7.8.1.4. Sediment organisms

Table 11: Effects on sediment organisms

SUMMARY OF TOXICITY STUDIES ON SEDIMENT ORGANISMS		
Method	**Results**	**References / Remarks**
According to SOP E211 - OSPAR/PARCOM Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry 2006 Part A: A sediment bioassay using an amphipod Corophium sp.) *Corophium volutator* Test type: static Test medium: saltwater Type of sediment: natural	10d LC_{50} > 12 497 mg/kg sediment dw (nominal) 10d NOEC = 6 251 mg/kg sediment dw (nominal)	REACH Registration dossier: Unpublished study report, 2011d Reliability 1 (Supporting study) Test material: EC No 700-403-8 GLP

A toxicity study on the Substance according to an OSPAR/PARCOM Protocol, using the amphipod *Corophium volutator* as test species, is available in the registration dossier (Unpublished study report, 2011d). A 10d LC_{50}-value >12 497 mg/kg sediment dw, and a 10d NOEC-value of 6 251 mg/kg sediment dw (both nominal values) were derived.

Conclusion: There is no indication of short-term and long-term toxicity to sediment organisms.

7.8.1.5. Other aquatic organisms

No data available.

7.8.2. Terrestrial compartment

7.8.2.1. Toxicity to soil macro-organisms

Table 12: Effects (short-term and long-term) on soil macro-organisms

SUMMARY OF TOXICITY STUDIES ON SOIL MACRO-ORGANISMS		
Method	**Results**	**References / Remarks**
According to OECD TG 207: Earthworm, Acute Toxicity Tests; EU Method C.8: Toxicity for Earthworms - Artificial Soil Test; SEPA. HJ/T 153-2004 *Eisenia fetida* (annelid) Substrate: artificial soil	14d LC_{50} > 1 000 $mg_{total\ solids}$/kg soil dw (nominal) (act. ingr.) based on: mortality 14d NOEC > 1 000 $mg_{total\ solids}$/kg soil dw (nominal) (act. ingr.) based on: weight and behaviour	REACH Registration dossier: Unpublished study report, 2011e Reliability 2 (Supporting study) Test material: EC No 700-403-8 GLP

An earthworm artificial soil test (OECD TG 207) was performed with the annelid *Eisenia fetida* (Unpublished study report, 2011e). The 14d LC_{50} was considered to be >1 $g_{total\ solids}$/kg soil dw (nominal, based on mortality), and the 14d NOEC was considered to be >1$g_{total\ solids}$/kg soil dw (nominal, based on weight and behaviour).

Conclusion: There is no indication of short-term and long-term toxicity to soil macroorganisms.

7.8.3. Microbiological activity in sewage treatment systems

No data available.

7.8.4. PNEC derivation and other hazard conclusions

Not performed.

7.8.5. Conclusions for classification and labelling

Based on the available data, the Substance (considering the properties of the parent compound) should not be classified for the environment.

Acute Toxicity:

All L(E)C_{50}s >1 mg/L ➔ No classification warranted for acute aquatic toxicity.

Chronic Toxicity:

Long-term toxicity studies are only available for algae and aquatic plants. Therefore, both chronic and acute toxicity values need to be assessed:

- 72h NOEC (*Raphidocelis subcapitata*) = 19.8 mg/L (measured arithmetic mean >1 mg/L) ➔ Not classified.

- 48h EC_{50} (*Daphnia magna*) >113 mg/L (measured arithmetic mean) >100 mg/L ➔ Not classified.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

Table 13: Toxicokinetics (absorption, metabolism, distribution and elimination)

SUMMARY OF STUDIES ON TOXICOKINETICS			
Method	**Remarks**	**Results**	**Reference**
EC No 700-403-8 Basic toxicokinetics study Gavage No guideline followed GLP: no Rel. 2	Mouse (Crl:CD1(ICR)) 5 males/dose Duration of exposure: 7d Conc. 0, 30, 300 and 1000 mg/kg bw/d	NOAEL = 300 mg/kg bw/day No mortality, body weight effects, adverse clinical signs 1 000 mg/kg bw/d: Minimal to mild hepatocellular degeneration/necrosis in livers of males	REACH Registration dossier: Unpublished study report, 2011f

Conclusion: No mortality, body weight effects or adverse clinical signs were observed at any of the tested concentrations. At a dose of 1000 mg/kg bw/d, minimal to mild hepatocellular degeneration/necrosis was reported in the livers of male mice. Therefore, the NOAEL was set at 300 mg/kg bw/day.

7.9.2. Acute toxicity and Corrosion / Irritation

Acute toxicity

Table 14: Acute toxicity (oral and inhalation)

SUMMARY OF STUDIES ON ACUTE TOXICITY – ORAL AND INHALATION				
Method, guideline	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**Results**	**Reference**
EC No 700-403-8 Gavage OECD TG 425 GLP Rel. 1	Rat (SD) / female 1 female/group (except at the highest dose: 3 females)	Doses: 175, 550, 1750 and 5000 mg/kg bw	LD_{50} > 5000 mg/kg bw No mortality observed No effects observed on bw or during gross pathology examination	REACH Registration dossier: Unpublished study report, 2009c
EC No 700-403-8 Gavage OECD TG 425 GLP Rel. 1	Mouse (Crl:CD1) / female 3 animals for the low dose and 1 for the high dose	Doses: 5000 and 10000 mg/kg bw	LD_{50} > 5000 mg/kg bw No mortality observed No effects observed on bw or during gross pathology examination	REACH Registration dossier: Unpublished study report, 2010c
EC No 700-403-8 Aerosol OECD TG 436 GLP Rel. 1	Rat (RccHan:WIST) / both sexes 3/sex/group	Doses: 504 and 1046 mg test material/m^3 Duration of exposure : 4h	LC_{50} > 504 - < 1046 mg/m^3 Mortality: 1 female died at the low dose and 2 males and 2 females died at the high dose BW: lower bw Gross pathology: animals which were found dead exhibited blood around their nose and dark red lungs	REACH Registration dossier: Unpublished study report, 2012a
RA (substance not specified) Aerosol OECD TG 403 GLP Rel. 2	Rat (Crl:CD(SD)) / male 5 males/group for ALC exposure and 15 males/group for pathology exposure	Doses: 20 and 47 mg/m^3 for ALC exposure and 0, 0.12, 1.0, 8.0 and 19 mg/m^3 for pathology exposure Duration of exposure: 4h	ALC: 47 mg/m^3 3 males of the high dose level were found dead NOAEL for anatomic pathology evaluation: 1 mg/m^3 Lower bw At the 2 highest doses, microscopic examination	REACH Registration dossier: Unpublished study report, 2009d

SUMMARY OF STUDIES ON ACUTE TOXICITY – ORAL AND INHALATION				
Method, guideline	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**Results**	**Reference**
			revealed changes in the ventral larynx (mucosal erosion and ulceration, inflammation and cartilage necrosis	
RA (substance not specified) Aerosol OECD TG 436 CLP: no Rel. 2	Rat (Crl:CD(SD)) / both sexes 3/sex/group	Doses: 97 and 1200 mg/m³ solids Duration of exposure: 4h	ALC: 1200 mg/m³ Mortality: at the high dose level, 1 male and 2 females were found death Lower bw Discoloration of the lungs was observed at the high dose	REACH Registration dossier: Unpublished study report, 2012b
RA (substance not specified) Aerosol No guideline followed GLP: no Rel. 2	Rat (Crl:CD(SD)) / both sexes 10/sex/dose	Doses: 0, 5, 25 and 100 mg/m³ Duration of exposure: 4h	LC_{50} > 100 mg/m³ No mortality observed Lower bw Discoloration of the lungs observed at the highest dose Microscopic changes in larynx and lungs (minimal focal necrosis of ventral larynx, haemorrhage in lungs). Lesions were reversible following a recovery period of 14d	REACH Registration dossier: Unpublished study report, 2012c

Conclusion: The registrant has concluded that

- the Substance is not acutely toxic via the oral route (LD_{50} >5000 mg/kg bw).

- the Substance is acutely toxic via the inhalation route and self-classified in the registration dossier as Acute Tox. 2, H330 (Fatal if inhaled).

Based on the available information, the eMSCA supports these conclusions.

Corrosion/irritation

Table 15: Skin irritation / corrosion and eye irritation

SUMMARY OF STUDIES ON SKIN IRRITATION / CORROSION AND EYE IRRITATION				
Method, guideline	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**Results -Observations and time point of onset -Mean scores/animal -Reversibility**	**Reference**
EC No 700-403-8 Skin irritation study Semi-occlusive OECD TG 402 GLP Rel. 1	Rabbit (NZW) 3 animals	Conc.: 0.5 ml Duration of exposure: 4h	Mean erythema score (24, 48 and 72h): 0/4 Mean edema score (24, 48 and 72h): 0/4	REACH Registration dossier: Unpublished study report, 2009e
EC No 700-403-8 Eye irritation study OECD TG 405 GLP Rel. 1	Rabbit (NZW) 3 animals	Conc.: 0.1 ml Single exposure	Mean corneal opacity score (24, 48 and 72h): 0/4 Mean iritis score (24, 48 and 72h): 0/4 Mean conjunctivae score (24, 48 and 72h): 0.33/3 for 2 animals and 0.67/3 for 1 animal Mean chemosis score (24, 48 and 72h): 0/4	REACH Registration dossier: Unpublished study report, 2009f

Conclusion: The registrant has concluded that the Substance is not irritating for the skin and the eyes, and based on the available information, the eMSCA supports this conclusion.

7.9.3. Sensitisation

Table 16: Skin sensitisation

SUMMARY OF STUDIES ON SKIN SENSITISATION				
Method, guideline	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**Results -Observations and time point of onset -Mean scores/animal -Reversibility**	**Reference**
EC No 700-403-8 LLNA OECD TG 429 GLP Rel. 1	Mouse (CBA/JHsd) / female 5 females/group	Conc.: 0, 5, 25, 50 and 100 %	SI < 3 (NA, 1.21, 1.15, 1.55, 1.38 and 7.42 respectively for 0, 5, 25, 50, 100% and positive control) EC3 not determined	REACH Registration dossier: Unpublished study report, 2009g

Conclusion: The registrant has concluded that the Substance is not skin sensitising, and based on the available information, the eMSCA supports this conclusion.

7.9.4. Repeated dose toxicity

Table 17: Repeated dose toxicity (oral)

SUMMARY OF STUDIES ON REPEATED DOSE TOXICITY - ORAL			
Method, guideline, species, strain, sex, no/group	**Route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
EC No 700-403-8 Short-term study: 28d Mouse (Crl:CD (ICR)) 15/sex/group OECD TG 407 GLP Rel. 1	Gavage Duration of exposure: 28d Recovery period (5 mice/sex/group): 1 month Conc.: 0, 30, 100 and 300 mg/kg bw/d	Mortality, clinical signs, bw, hematology and clinical biochemistry: no effects Organ weight: higher liver weight in males at the mid and high doses Microscopic examination: minimal centrilobular hepatocellular hypertrophy was observed at the highest dose level At the end of the recovery period, liver was unaffected NOAEL: 300 mg/kg bw/d	REACH Registration dossier: Unpublished study report, 2012d

SUMMARY OF STUDIES ON REPEATED DOSE TOXICITY - ORAL			
Method, guideline, species, strain, sex, no/group	**Route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
EC No 700-403-8 Reproduction / Developmental Toxicity Screening Mouse (CD-1) 20/sex/dose + 5 additional females for control and high dose levels (not mated, for gender comparison) OECD TG 421 GLP Rel. 1	Gavage Duration of exposure: - Males: 70d - Females: 14d before cohabitation until day of weaning litters (LD21) Conc.: 0, 30, 300, 750 and 1000 mg/kg bw/d	Mortality: observed but not treatment-related Clinical signs: No adverse effects reported for P1 animals Reproductive performance, litter size, survival, sex ratio: No effects Liver: Test substance related microscopic effects at >300 mg/kg bw/d in females and at >750 mg/kg bw/d (for P1 animals). More severe in females and included oval cell hyperplasia, necrosis and degeneration of hepatocytes, and increased mitotic figures. Teeth: Adverse effects at 750 mg/kg bw/d and higher doses (for P1 males) Reproductive Tract: Adverse effects at 750 mg/kg bw/d and higher doses (for P1 females) Offspring: Test substance related adverse effects (decreased body weights for pups after day 4 of lactation + microscopic changes in kidneys of F1 males) at 300 mg/kg bw/d and higher doses NOAEL: 30 mg/kg bw/day (systemic toxicity - liver)	REACH Registration dossier: Unpublished study report, 2014
PFHpA Sub-chronic study Mouse (CD-1) 20/sex/group OECD TG 408 and 422 GLP	Gavage Duration of exposure: - Males: total of 109-113d - Females: total of 130-142d Conc.: 0, 0.5, 10 and 50 mg/kg bw/d	Elaborately discussed in section 7.9.7. (Toxicity to reproduction (effects on fertility and developmental toxicity)). LOAEL: 0.5 mg/kg bw/d	REACH Registration dossier: Unpublished study report, 2017

SUMMARY OF STUDIES ON REPEATED DOSE TOXICITY - ORAL			
Method, guideline, species, strain, sex, no/group	**Route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Rel. 1			
EC No 700-403-8 Short-term study Mouse (crl:CD1(ICR)) 5 males/group No guideline followed GLP: no Rel. 2	Gavage Duration of exposure: 7d Conc.: 0, 30, 300 and 1000 mg/kg bw/d	Mortality, clinical sign, bw: no effects observed Microscopic examination: minimal to mild hepatocellular degeneration/necrosis was observed at the highest dose level NOAEL: 300 mg/kg bw/d	REACH Registration dossier: Unpublished study report, 2011f
PFHxA 90d repeated dose oral toxicity study Rat (crl:CD(SD)) 20/sex for groups 1 and 4 and 10/sex for groups 2 and 3	Gavage Duration of exposure: 90d Post-exposure period: 28d for the control and high dose levels Conc.: 0, 10, 50 and 200 mg/kg bw/d	Mortality, clinical signs, FOB: no effects BW: significantly reduced in males of the mid and high dose (trend to decrease in females) Haematological findings: slightly lower red blood cell count, hemoglobin and hematocrit at the highest dose in both sexes. These changes were reversible following the recovery period. Clinical biochemistry findings: Sign. higher ALT values (41, 46, 50 and 138* U/L in males) and ALP values (95, 93, 109 and 127* U/L in males) Organ weight: changes were observed in liver and kidneys Histopathology examination: centrilobular hepatocellular hypertrophy was noted in 7 males out of 10 exposed to the highest dose. Effect not observed at the end of the recovery period.	Chengelis *et al*. (2009)

Conclusion for EC No 700-403-8:

The registrant has concluded that the Substance is not toxic after repeated dose via oral route. Based on the available information, the eMSCA notes that liver toxicity was observed in the Reproduction/Developmental Toxicity Screening study (Unpublished study report, 2014) at 300 mg/kg bw/d, a dose level outside the range to classify as STOT RE. The dose below (30 mg/kg bw/d) is set as the NOAEL of this study.

Conclusion for PFHpA:

Perfluoroheptanoic acid (PFHpA) (EC No 206-798-9; CAS No 375-85-9) is included in Annex VI to Regulation (EC) No 1272/2008. Perfluoroheptanoic acid is included with index number 607-761-00-3, and has received the following harmonised classification:

- Repr. 1B, H360D;
- STOT RE 1, H372 (liver).

7.9.5. Mutagenicity

Table 18: Genetic toxicity: *in vitro*

SUMMARY OF STUDIES ON GENETIC TOXICITY – *IN VITRO*			
Method, guideline	**Relevant information about the study**	**Observations**	**Reference**
EC No 700-403-8 *In vitro* gene mutation study in bacteria OECD TG 471 GLP Rel. 1	*S. typh.* TA 1535, 1537, 98 and 100 + E. Coli WP2 uvr A With and without metabolic activation	Genotoxicity: negative Cytotoxicity: no	REACH Registration dossier: Unpublished study report, 2009h
EC No 700-403-8 *In vitro* cytogenicity/chromosome aberration study in mammalian cells OECD TG 473 GLP Rel. 1	Human peripheral blood lymphocytes With and without metabolic activation	Genotoxicity: negative Cytotoxicity: no	REACH Registration dossier: Unpublished study report, 2011g

Both *in vitro* studies on mutagenicity indicate that the Substance is negative for genotoxicity and that there is no occurrence of cytotoxicity.

Conclusion: The registrant has concluded that the Substance is not mutagenic, and based on the available information, the eMSCA supports this conclusion.

7.9.6. Carcinogenicity

No information available.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Table 19: Toxicity to reproduction (fertility and developmental toxicity)

SUMMARY OF STUDIES ON REPRODUCTIVE TOXICITY			
Method, guideline, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results**	**Reference**
PFHpA Combined Repeated Dose Toxicity Study with the Reproduction Developmental Toxicity Screening Test Mouse (CD-1) 20/sex/dose OECD TG 422 GLP Rel. 1	Gavage Duration of exposure: - Males: total of 109-113d - Females: total of 130-142d Conc.: 0, 0.5, 10 and 50 mg/kg bw/d	Elaborate discussion of the study below this table.	REACH Registration dossier: Unpublished study report, 2017
EC No 700-403-8 Reproduction / Developmental Toxicity Screening Mouse (CD-1) 20/sex/dose OECD TG 421 GLP Rel. 1	Gavage Duration of exposure: - Males: 70d - Females: 14d before cohabitation, and during cohabitation (also approximately 14 d) Conc.: 0, 30, 300, 750 and 1 000 mg/kg bw/d	Mortality: observed but not treatment-related Clinical signs: no adverse effects reported for P1 animals Reproductive performance, litter size, survival, sex ratio: no effects Liver: test-substance related microscopic effects at 300 mg/kg bw/d and higher doses (for P1 animals) Teeth: adverse effects at 750 mg/kg bw/d and higher doses (for P1 males) Reproductive tract: adverse effects at 750 mg/kg bw/d and higher doses (for P1 females)	REACH Registration dossier: Unpublished study report, 2014

SUMMARY OF STUDIES ON REPRODUCTIVE TOXICITY			
Method, guideline, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results**	**Reference**
		Offspring: test-substance related adverse effects (decreased body weights for pups after day 4 of lactation + microscopic changes in kidneys of F1 males) at 300 mg/kg bw/d and higher doses NOAEL: 30 mg/kg bw/day (systemic toxicity)	

Discussion of Combined Repeated Dose Toxicity Study with the Reproduction Developmental Toxicity Screening Test (OECD TG 422), performed with the relevant degradation product PFHpA.

In a combined 90-day repeated dose toxicity study with reproduction/developmental toxicity screening (Unpublished study report, 2017), groups of male and female mice were given by gavage sodium perfluoroheptanoate (purity >99.3%) at a concentration of 0, 0.5, 10 or 50 mg/kg bw/d.

Main study phase consisted of 20 animals/sex/group and 5 extra females in the control and the high dose groups were non-mated and used for gender comparison. The main study phase males were exposed for a total of 109-113d (90d prior to pairing through day 1 to scheduled euthanasia). While, the main study phase females were dosed 90d prior to pairing and until lactation day 21 for a total of 130-142d. Females that failed to deliver were exposed through the day prior to euthanasia (postmating d23) for a total of 113 doses. The 5 extra females in the control and high dose levels were exposed during 109d. For the clinical pathology phase, which consisted of 15 mice/sex/group, animals were exposed during 75d.

Regarding the F1 generation, 16-20 pups/sex/group were randomly selected and were exposed from PND 22 to PND 42. The remaining pups were euthanisied and necropsied on PND21.

Concerning clinical pathology phase, no treatment related effects on mortality, clinical signs, body weight, nor on macroscopic examination was observed. Clinical biochemistry examination revealed enzymes modifications. At the highest dose level, higher AST, ALT and ALP values were noted in both sexes, furthermore higher AST (in females) and ALT values were observed at the mid dose level (AST : 63/112, 67/215, 79/258 and 86/228 U/L in males/females respectively at 0, 0.5, 10 and 50 mg/kg bw/d; ALT : 47/36, 39/47, 109/70 and 122/98 U/L in males/females respectively at 0, 0.5, 10 and 50 mg/kg bw/d; ALP : 122/101, 78/115, 68/95 and 227/166 U/L in males/females respectively at 0, 0.5, 10 and 50 mg/kg bw/d). Organ weight and histopathology examinations were not performed in this study phase.

The F0 generation of the main study phase did not exhibit clinical signs or treatment related body weight modification (Table 20). Regarding the clinical biochemistry analysis, males exposed to the highest dose level showed significant higher value of ALP, ALT and Trig. Significant higher ALP and Trig values were also observed in non-mated females. T4 was analysed and exhibited a significant lower value in males of the mid and high dose levels (5.42, 4.67, 3.71** and 2.95** ug/dL respectively at 0, 0.5, 10 and 50 mg/kg bw/d). T4 was not evaluated in females.

Table 20: Body weight data (in g)

Dose level (in mg/kg bw/d)	0	0.5	10	50
Males				
D0	28.2 (n=20)	28.1 (n=20)	28.2 (n=20)	27.8 (n=20)
D56	35.9 (n=20)	35.4 (n=20)	37.4 (n=19)	35.4 (n=20)
D109	37.1 (n=20)	36.6 (n=19)	38.2 (n=19)	36.8 (n=20)
Females				
D0	22.6 (n=25)	22.7 (n=20)	22.4 (n=20)	22.3 (n=24)
D56	26.2 (n=25)	26.6 (n=20)	27.1 (n=20)	26.5 (n=24)
D96	28.3 (n=7)	28.6 (n=1)	31.0 (n=1)	28.4 (n=6)
D109	27.8 (n=5)	/	/	30.2 (n=5)
GD0	26.6	27.4	27.6	27.2
GD18	50.0	49.9	53.5	52.0
LD1	33.4	34.0	35.5	34.5
LD21	25.6	36.0	37.5	36.7

Examination of the reproductive parameters did not show significant changes (Table 21). The number of implantation sites was also unaffected by the treatment (11.9, 11.3, 12.8 and 11.8 respectively at 0, 0.5, 10 and 50 mg/kg bw/d). Moreover, gestation length was similar in all groups (19.0, 19.0, 18.9 and 18.9 days respectively at 0, 0.5, 10 and 50 mg/kg bw/d).

Table 21: Reproductive performance

Dose level (in mg/kg bw/d)		0	0.5	10	50	HCD Mean (range)
Mating index (%)	Male	100.0	100.0	100.0	100.0	88.8 – 100.0
	Female	100.0	100.0	100.0	100.0	95.0 – 100.0
Fertility index (%)	Male	90.0	100.0	94.7	85.0	84.0 – 100.0
	Female	90.0	100.0	95.0	85.0	88.0 – 100.0
Male copulation index (%)		90.0	100.0	94.7	85.0	86.7 – 100.0
Female conception index (%)		90.0	100.0	95.0	85.0	88.0 – 100.0
Estrous cycle length (d)		4.5	5.0	4.9	4.5	4.4 – 7.0
Pre-coital interval (d)		2.2	2.9	2.7	2.9	2.0 – 3.3

At the end of the study, animals of the F0 generation were euthanized and necropsied. Males were euthanized following completion of the mating period. Females that delivered were euthanized on lactation day 21 while females that failed to deliver were euthanized on postmating day 23. Macroscopic examinations did not revealed test-substance related changes. Liver weight was significantly increased at the mid and high dose level in males and in females. No other treatment-related organ weight changes were noted (Table 22 and Table 23).

Table 22: Organ weight values in males

Dose level (in mg/kg bw/d)		0	0.5	10	50
FBW (g)		36.9	36.2	38.2	37.2
Liver (g)	Abs	1.8253	1.8342	2.1788**	3.1472**
	Rela	4.948	5.062	5.689**	8.460**
Epididymides (g)	Abs	0.1004	0.0964	0.1049	0.0972
	Rela	0.272	0.267	0.276	0.262
Testes (g)	Abs	0.2448	0.2449	0.2501	0.2373
	Rela	0.667	0.676	0.657	0.637
Thyroid/parathyroid (g)	Abs	0.0042	0.0044	0.0041	0.0043
	Rela	0.011	0.0012	0.011	0.012

Table 23: Organ weight values in females

		Non-mated females				Females LD21			
Dose level (in mg/kg bw/d)		0	0.5	10	50	0	0.5	10	50
FBW (g)		27.8	NA	NA	29.1	35.6	36.0	37.5	36.7
Liver (g)	Abs	1.4018	NA	NA	1.8879**	2.0740	2.2033	2.4908**	3.0901**
	Rela	5.036	NA	NA	6.489**	5.799	6.113	6.639**	8.415**
Ovaries/oviducts (g)	Abs	0.0251	NA	NA	0.0281	0.0327	0.0347	0.0303	0.0287
	Rela	0.090	NA	NA	0.096	0.092	0.097	0.081	0.078
Thyroid/parathyroid (g)	Abs	0.0038	NA	NA	0.0038	0.0051	0.0042*	0.0055	0.0049
	Rela	0.013	NA	NA	0.013	0.014	0.012*	0.015	0.014
Uterus (g)	Abs	0.2131	NA	NA	0.1576	0.2390	0.3073	0.2347	0.2051
	Rela	0.769	NA	NA	0.544	0.674	0.853	0.628	0.562

In addition to the organ weight modifications, microscopic examination revealed severe liver effects. Centrilobular hypertrophy of the hepatocytes were observed in an huge number of males and females of the all dose levels. Moreover, hepatocellular necrosis was noted at the highest dose. (Table 24 and Table 25)

Table 24: Histopathological changes seen in liver in males

Dose level (in mg/kg bw/d)		0	0.5	10	50
Total number animals examined		20	19	19	20
Number of animals without findings		16	2	2	0
Centrilobular hypertrophy of hepatocytes	Incidence	0	17	17	20
	Minimal	0	8	2	0
	Mild	0	7	2	9
	Moderate	0	2	13	11
Infiltrate, mononulcear cell	Minimal	4	7	2	2
Hepatocellular necrosis	Incidence	0	1	2	20
	Minimal	0	1	2	19
	Mild	0	0	0	1

Table 25: Histopathological changes seen in liver in females

		Non-mated females				Females LD21			
Dose level (in mg/kg bw/d)		0	0.5	10	50	0	0.5	10	50
Total number animals examined		5	0	0	4	17	20	19	16
Number of animals without findings		1	NA	NA	0	16	2	0	0
Centrilobular hypertrophy of hepatocytes	Incidence	0	NA	NA	4	0	17	19	16
	Minimal	0	NA	NA	0	0	8	2	0
	Mild	0	NA	NA	4	0	8	8	6
	Moderate	0	NA	NA	0	0	1	9	10
Infiltrate, mononulcear cell	Minimal	4	NA	NA	2	1	6	6	5
Hepatocellular necrosis	Incidence	0	NA	NA	1	0	1	4	8
	Minimal	0	NA	NA	1	0	0	4	7
	Mild	0	NA	NA	0	0	1	0	1

Each litter was examined and the number of litter was unaffected by the test substance (16, 20, 18 and 16 litters respectively at 0, 0.5, 10 and 50 mg/kg bw/d). The mean litter size at birth did not change (11.2, 10.4, 11.9 and 11.0 pups respectively at 0, 0.5, 10 and 50 mg/kg bw/d). The sex ratio was also unaffected (54.1, 55.4, 47.3 and 53.8% of males respectively at 0, 0.5, 10 and 50 mg/kg bw/d). The anogenital distance did not showed significant changes (1.85, 1.85, 1.86 and 1.86 mm in males and 1.17, 1.19, 1.18 and 1.20 mm in females respectively at 0, 0.5, 10 and 50 mg/kg bw/d). However, a trend to decrease of the postnatal survival index was noted (from birth to PND 4 (pre-selection): 99.3, 99.4, 98.7 and 87.8%; from PND 4 (post-selection) to PND 21 : 99.3, 99.4, 98.7 and 87.8% respectively at 0, 0.5, 10 and 50 mg/kg bw/d). Moreover, the pup body weight was significantly decreased at the highest dose level (Table 26).

Table 26: Pup body weight data

	Males				Females			
Dose level (in mg/kg bw/d)	0	0.5	10	50	0	0.5	10	50
PND 1	1.66	1.68	1.68	1.54*	1.58	1.61	1.59	1.52
PND 4	2.63	2.74	2.61	2.02**	2.59	2.66	2.48	2.03**
PND 10	5.95	6.03	5.80	5.00**	5.85	5.95	5.64	5.04**
PND 21	11.65	11.55	10.98	9.72**	11.25	11.09	10.28	9.58**

At PND 21, serum samples were analysed. Males of the highest dose exhibited a decrease of the total T4 value (6.29, 9.53, 6.50 and 5.61 µg/dL in males respectively at 0, 0.5, 10 and 50 mg/kg bw/d and 6.31, 6.80, 6.81 and 6.47 µg/dL in females respectively at 0, 0.5, 10 and 50 mg/kg bw/d).

Necropsy was done on pups which were found dead. Cleft palate was observed in 6 (1) and 3 (2) pups (litters) respectively in the low and high dose levels (1, 8, 3 and 28 examined pups respectively at 0, 0.5, 10 and 50 mg/kg bw/d). Scheduled pups necropsies revealed that one male pup of the high dose group had an enlarged parathyroid gland. And necropsies of nonselected pups showed that only one male pup of the highest dose had an opacity of the left eye. Thyroids and parathyroids weight was recorded and showed a slight decrease (0.0021, 0.0019, 0.0018 and 0.0019 g in males and 0.0021, 0.0020, 0.0018 and 0.0018 g in females respectively at 0, 0.5, 10 and 50 mg/kg bw/d).

Some animals were randomly selected to continue the study and were exposed until PND 42. Examination of the balanopreputial separation did not show changes (30.2, 30.2, 29.5 and 31.0 PND respectively at 0, 0.5, 10 and 50 mg/kg bw/d). However, a significant higher vaginal patency was observed (29.9, 29.4, 30.1 and 33.1* PND respectively at 0, 0.5, 10 and 50 mg/kg bw/d). During the exposure period, body weight was recorded and a significant decrease was observed (Table 27).

Table 27: Body weight data (in g)

	Males				Females			
Dose level (in mg/kg bw/d)	0	0.5	10	50	0	0.5	10	50
PND 22	12.6	12.8	12.4	11.1	12.8	12.0	11.7	10.6**
PND 28	20.8	21.6	20.4	17.5**	18.3	17.8	17.0	15.0**
PND 35	26.8	27.1	27.0	24.8*	23.2	22.5	21.9	20.5**
PND 43	29.0	29.4	29.4	27.7	24.7	23.7	23.2*	22.1**

These animals were euthanized and necropsied. Macroscopic examination did not reveal changes. Adrenal glands and brain weights were significantly affected in females exposed to the highest dose level. Furthermore, liver weight was significantly increased in males of the mid dose and in both sexes of the highest dose (Table 28).

Table 28: Organ weight data (in g)

		Males				Females			
Dose level (in mg/kg bw/d)		0	0.5	10	50	0	0.5	10	50
FBW		29.0	29.6	29.4	27.7	24.7	23.7	23.2*	22.1**
Adrenal glands	Abs	0.0062	0.0072	0.0073	0.0075	0.0116	0.0098*	0.0102	0.0081**
	Rela	0.022	0.025	0.025	0.027	0.047	0.041	0.044	0.036**
Brain	Abs	0.4651	0.4752	0.4641	0.4607	0.4707	0.4610	0.4580	0.4480*
	Rela	1.618	1.608	1.590	1.675	1.912	1.951	1.987	2.036
Liver	Abs	1.8019	1.8571	2.0644*	3.1381**	1.5775	1.5133	1.5513	1.8630**
	Rela	6.213	6.292	7.013**	11.309**	6.388	6.385	6.709	8.42**
Epididymides	Abs	0.0571	0.0593	0.0606	0.0561	-	-	-	-
	Rela	0.197	0.202	0.207	0.203	-	-	-	-
Testes	Abs	0.1962	0.1994	0.1998	0.1989	-	-	-	-
	Rela	0.680	0.691	0.678	0.720	-	-	-	-
Ovaries/ovidcuts	Abs	-	-	-	-	0.0233	0.0202	0.0209	0.0174
	Rela	-	-	-	-	0.094	0.085	0.090	0.078
Uterus	Abs	-	-	-	-	0.1740	0.1447	0.1481	0.1368
	Rela	-	-	-	-	0.704	0.605	0.640	0.613

These liver changes were confirmed by the microscopic examination. As in the F0 generation, the F1 generation showed an severe increase of the incidence of the centrilobular hypertrophy of the hepatocytes in all treated dose levels. Moreover, hepatocellular necrosis was noted in the mild and high dose levels. These effects in liver were dose related (Table 29).

Table 29: Histopathological changes seen in liver

		Males				Females			
Dose level (in mg/kg bw/d)		0	0.5	10	50	0	0.5	10	50
Total number examined		17	20	18	14	17	20	18	16
Number examined without findings		10	3	1	0	10	8	6	0
Centrilobular hypertrophy of hepatocytes	Minimal	0	8	2	1	0	6	8	5
	Mild	0	8	10	5	0	1	3	9
	Moderate	0	1	5	8	0	0	0	2
Infiltrate, mononulcear cell	Minimal	7	5	1	3	7	8	5	5
	Mild	0	0	0	0	0	0	1	0
Hepatocellular necrosis	Minimal	0	0	2	7	0	0	3	8
	Mild	0	0	0	1				
	marked	0	0	0	1				

Conclusion on EC No 700-403-8:

The registrant has concluded that the Substance is not reprotoxic. Based on the study performed with the Substance, the eMSCA supports this conclusion.

Conclusion on PFHpA:

Perfluoroheptanoic acid (EC No 206-798-9; CAS No: 375-85-9) is classified and included in Annex VI to Regulation (EC) No 1272/2008. Perfluoroheptanoic acid is included with index number 607-761-00-3, and received the following harmonised classification:

- Repr. 1B, H360D;
- STOT RE 1, H372 (liver).

7.9.8. Hazard assessment of physico-chemical properties

Not assessed.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Not assessed.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

The Substance is self-classified in the registration dossier as Acute Tox. 2 H330 and as STOT SE 1 H370. Based on the available data, the eMSCA agrees with this classification, taking into account the data on the Substance itself.

7.9.11. Toxic properties of perfluoroheptanoic acid (PFHpA)

Perfluoroheptanoic acid (PFHpA) (EC No 206-798-9; CAS No 375-85-9) is included in Annex VI to Regulation (EC) No 1272/2008. PFHpA is included with index number 607-761-00-3, and has received the following harmonised classification:

- Repr. 1B, H360D;
- STOT RE 1, H372 (liver).

Therefore, PFHpA meets the toxicity criterion (T) in accordance with Annex XIII, points 1.1.3 (b) and (c), of the REACH Regulation.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

Endocrine disruption towards the environment was not assessed in particular during this Substance Evaluation.

In the conclusion document for EC No 241-527-8[13] and EC No 218-407-9[14], the German CA concluded that the available data clarifies that PFHxA acts as an ED for the environment in accordance with the Endocrine Disruptor (ED) definition of the World Health Organisation (WHO).
However, for the moment there is no official identification of PFHxA as ED for the environment by MSC or RAC.

7.10.2. Endocrine disruption – Human health

As reported above in § 7.7.1., PFHpA is a relevant degradation product of the Substance. In the combined 90 day repeated dose toxicity study with reproduction and developmental toxicity screening with the sodium salt of PFHpA (see § 7.9.7.), submitted to clarify the T-criterion for PBT concern, a concern for thyroid hormones disruption has been identified.

In the Reproduction/Developmental Toxicity Screening Test in mice on sodium perfluoroheptanoate, oral route (OECD TG 422) extended to 90 days for the pre-mating and mating period (OECD TG 408) and extended to 21 days post-weaning, exposure of F0 males to 10 and 50 mg/kg bw/day, PFHpA induces significant lower mean total T4 values. A decrease of T4 was already observed at the lowest dose of 0.5 mg/kg bw/day (5.42, 4.67, 3.71**, 2.95** µg/dL at 0, 0.5, 10 and 50 mg/kg bw/day respectively) (measurement performed at week 15). The decrease is clearly dose-dependent (registration dossier, 2017). A gender comparison is not available, as T4 was not measured in F0 females. T4 measurements in pups were done at PND 21 (while the OECD TG 422 test guideline states that the measurement has to be done at PND 13 and if relevant also at PND 4). In F1 males and females (PND 21), there were no significant changes in T4. Differences in T4 concentration cannot be excluded between PND 4 and PND 21, especially

[13] Substance Evaluation Conclusion Document on EC No 241-527-8. 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl acrylate. April 2023. Evaluating Member State: Germany. https://echa.europa.eu/documents/10162/5c201ae4-8b10-ca18-df24-7ae5a7f83e37

[14] Substance Evaluation Conclusion Document on EC No 218-407-9. 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl methacrylate. April 2023. Evaluating Member State: Germany. https://echa.europa.eu/documents/10162/15a5aaeb-0159-d730-c4f7-66c8dd85a8b4

if the dam has a reduced T4 concentration. F1 males and females were directly exposed to the substance, following weaning, from PND 22 to PND 42. The serum samples of the F0 females and the F1 culled pups PND 4 were not analysed.

Thyroid hormones (TH) affect estrous cycle regulation (Krasses *et al.*, 2010). At 50 mg/kg/day PFHpA, two (out of 20) F0 females showed abnormal oestrus cycle. The length of oestrus cycle could not be determined for those 2 animals. A copulatory plug was observed the first day of the breeding period on those females, but it appears that they were not gravid.

Furthermore, TH have a critical role in brain development. TH insufficiency during fetal and neonatal life critical phases of brain development reduces cell number, synaptogenesis and dendritic arborisation, alters cell migration patterns and decreases axonal myelination. This can result in irreversible neurological impairments (reduced IQ levels, learning, memory and motor deficiencies, attention deficit disorder…) (Gilbert *et al.*, 2012). TH insufficiency during different stage(s) of brain development can lead to different types of defects. The mother is the sole source of TH during early pregnancy (in human, this corresponds to the first half of gestation), before the onset of fetal thyroid function.

A literature search provided additional information on a thyroid mode of action for PFHpA. *In vivo* neurodevelopmental data are not available for PFHpA. Therefore, there was a concern for endocrine disrupting properties of the substance.

An overview of the literature regarding the thyroid mode of action for PFHpA is given below.

OECD CF Level 1: Existing data and non-test information

Not available.

OECD CF Level 2 : *in vitro* assays providing data about selected endocrine mechanisms/pathways

PFHpA has been shown to alter mRNA expression of thyroid hormone-responsive genes.

Vongphachan *et al*. (2011)

Vongphachan *et al*. (2011) studied the *in vitro* effects of perfluoroalkyl compounds on mRNA expression of thyroid hormone-responsive genes in avian neuronal cells of chicken (*Gallus domesticus*) and herring gull (*Larus argentatus*).

Chicken embryonic neuronal (CEN) cells

Cerebral cortices of day 11 chicken embryos were used to prepare primary cultures of CEN cells. Cells were plated and incubated after which each perfluorinated compound (PFC) was added at six concentrations: 0.01, 0.1, 1, 3, 10 and 50 µM. The natural agonist triiodothyronine (T3) was administered at four concentrations: 0.03, 0.3, 3 and 30 nM as a positive control. DMSO was used as vehicle control in both the PFC- and T3-treatment group.

Exposure to 10 µM of PFHpA resulted in a more than twofold increase in mRNA expression of D2 (type II iodothyronine 5´-deiodinase) ($p<0.05$), similar to the trend observed in the T3-treatment group. While T3 exposure resulted in a decrease of TTR (transthyretin) mRNA expression, exposure to 10 µM of PFHpA resulted in an increase of TTR mRNA expression by fourfold.

In general, shorter-chained perfluoroalkyl compounds had a greater effect on mRNA expression than longer-chained perfluoroalkyl compounds, since PFNA was the only long-chained compound able to elicit a response (a more than twofold increase in D2 mRNA expression at 10 µM, $p<0.05$).

Herring gull embryonic neuronal (HGEN) cells

Following a similar protocol, cerebral cortices of day 14 chicken embryos were used to prepare primary cultures of HGEN cells. Each perfluoroalkyl compound was administered at five concentrations: 0.01, 0.1, 1, 3 and 10 µM. T3 was added in five concentrations, namely 0.03, 0.3, 3, 30 and 300 nM. Also here DMSO was used as vehicle control.

A more than twofold upregulation of RC3 (neurogranin) was observed after exposure to 10 µM of PFHpA ($p<0.05$), similar to the trend of the T3-treated group.

Naile *et al*. (2012)

In this study, H4IIE rat hepatoma cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were transferred onto tissue culture plates and incubated for 24h. Afterwards, the medium was replaced with medium containing test-PFCs, administered in concentrations of 0.1, 1, 10 or 100 µM. A solvent control of 0.01% methanol was used. Cells were incubated for another 72 h. Total RNA was isolated, and quantitative real-time PCR (qPCR) was applied to measure changes in mRNA expression of target genes. A mean fold change was obtained by calculating changes in mRNA expression in cells submitted to PFC-treatment relative to solvent-control cells.

The C6-perfluoroalkyl acid PFHxA, was seen to induce mRNA expression of two thyroid-related genes. At an exposure of 100 µM, PFHxA was seen to induce *Pax 8*, with a mean fold change of 3.73 ± 0.28. This is quite comparable to the result for PFOA, which managed to upregulate *Pax 8* with a mean fold change of 4.19 ± 0.68 at the same concentration. The C4-perfluoroalkyl acid PFBA increased *Pax 8* to an even greater extent at 100 µM, with a mean fold change of 6.78 ± 3.30. Another gene related to thyroid development, named *Hex*, was upregulated by PFHxA with a mean fold change of no less than 23.80 ± 3.62. This was even higher than the mean fold change reported for PFOA, with regard to the *Hex*-gene, of 22.37 ± 3.51. Perfluorohexanoic acid (C6) can thus have a comparable effect on thyroid-related genes as perfluorooctanoic acid (C8). Since the C6-perfluoroalkyl acid PFHxA is capable of inducing such effects, one can expect comparable results for the C7-perfluoroalkyl acid PFHpA.

PFCs can also affect genes related to other pathways. mRNA expression of squalene synthase (*SQSYN*), a gene involved in the cholesterol synthesis pathway, was induced with a mean fold change of 14.96 ± 2.83 by PFHxA at an exposure of 100 µM, while an upregulation of only 1.84 ± 0.30 was reported for PFOA at the same concentration. The lipoprotein related gene *ApoA4* was upregulated by PFHxA with a mean fold change of 2.18 ± 0.42 and by PFOA with a mean fold change of 1.03 ± 0.25 at a concentration of 100 µM.

Du *et al*. (2013)

In this study, *in vitro* assays, were performed to test the effect of the C8-perfluoroalkyl acid (PFOA) on estrogen receptor (ER) and thyroid hormone receptor (TR)-related genes. African green monkey kidney (CV-1) cells were seeded and cultured in microtiter plates, in order to perform ER and TR reporter gene assays.

For the ER reporter gene assay, PFOA was added in concentrations of $3x10^{-9}$, $1x10^{-8}$, $3x10^{-8}$, $1x10^{-7}$ and $3x10^{-7}$ M to $1x10^{-9}$ M E2 (estradiol). The control consisted of $1x10^{-9}$ M E2 solely submitted. Luciferase activity was analyzed, relative to the control values. All administered PFOA concentrations were seen to increase ER activity when combined with $1x10^{-9}$ M E2, relative to the control ($p<0.05$).

For the TR reporter gene assay, PFOA was added in the same concentrations mentioned above to $5x10^{-9}$ M T3 (triiodothyronine). The control was represented by the lowest concentration of PFOA ($3x10^{-9}$ M) and $5x10^{-9}$ M T3. Luciferase activity was analyzed, relative to the control values. Concentrations of $1x10^{-8}$ M to $3x10^{-7}$ M PFOA when combined with $5x10^{-9}$ M T3 reduced TR activity ($p<0.05$). PFOA thus showed anti-thyroid hormone effects when combined with $5x10^{-9}$ M T3.

A steroidogenesis assay was performed using human adrenocortical carcinoma (H295R) cells, which were seeded and cultured in microtiter plates. These were exposed to PFOA-concentrations of $3x10^{-9}$, $1x10^{-8}$, $3x10^{-8}$, $1x10^{-7}$, $3x10^{-7}$ M. Gene expression levels were measured by applying quantitative real-time PCR (Q-RT-PCR), relative to DMSO solvent control. A radioimmunoassay was used to determine E2 and T (testosterone) levels. PFOA increased E2 in a concentration-dependent way after 48 hours of exposure, with significant upregulation at concentrations of $1x10^{-8}$ to $3x10^{-7}$ M, relative to the control ($p<0.05$). Such concentration-dependent curve was also observed for T, however in this case it was a decrease, with significant downregulation at concentrations of $3x10^{-8}$ to $3x10^{-7}$ M, relative to the control ($p<0.05$).

After an exposure of $3x10^{-7}$ M PFOA, mRNA expression of the steroidogenic genes 17βHSD1, CYP19, 3βHSD2 and CYP11B2 was greatly induced ($p<0.05$). HMGR, StAR and CYP21 were also significantly induced, but to a lesser extent ($p<0.05$). On the other hand, CYP17 and 17βHSD4 were downregulated by $3x10^{-7}$ M PFOA ($p<0.05$).

In the western blotting analysis, H295R cells received PFOA-treatment following the concentrations described above. After that, protein concentrations were measured colorimetrically using a bicinchoninic acid (BCA) protein assay. mRNA expression of SF-1 (steroidogenic factors 1) was reduced by PFOA treatment at both gene and protein levels, relative to DMSO solvent control.

The results above clearly show several effects of PFOA on ER-, TR- and steroidogenesis-related genes. PFOA was seen *in vitro* to have an additive mode of action in inducing estrogen activity when combined with E2, while it decreased thyroid hormone activity when combined with T3.

PFHpA shows *in vitro* competitive binding with thyroxine (T4) to the human TTR protein, and was demonstrated to be a nearly equally strong competitor as the C8-perfluoroalkyl acid PFOA. Such competition between poly- or perfluorinated compounds and T4 regarding transthyretin-binding could lead to reduced thyroid hormone levels and associated endocrine-disrupting effects.

Weiss *et al*. (2009)

A TTR radioligand-binding assay using human thyroid hormone transport protein TTR, was applied to determine whether poly- and perfluorinated compounds compete with T4 in order to bind to human TTR. The study included twenty-four poly- and perfluorinated compounds in total and six natural fatty acids with a structural similarity.

TTR binding potency seemed to vary with chain length for perfluorinated alkyl acids, with a maximal potency observed for PFOA. PFHpA managed to reduce T4-TTR binding to 7% at maximum concentration (10μM), with a 50% inhibitory concentration (IC_{50}) of 1565 nM. Since PFOA was seen to reduce T4-TTR binding to 4% at maximum concentration, with a IC_{50} of 949 nM, PFHpA can be seen as a nearly equally strong competitor of T4 towards TTR binding.

Thyroid hormones are dependent of association with transport proteins such as TTR, which serves as a T4-carrier in serum and cerebrospinal fluid. This means competition between T4 and poly- or perfluorinated compounds, together with other environmental pollutants, could contribute to adverse effects on the thyroid hormone system.

Kar *et al*. (2017)

Kar *et al*. (2017) performed a modelling study, using the experimental *in vitro* data of Weiss *et al*. (2009) regarding TTR binding potency, described above. Two QSAR models were developed, one classification-based and one regression-based. These were applied to the dataset of twenty-four poly- and perfluorinated compounds described above. A docking study was performed in order to determine interactions between the protein (TTR) and the ligand (in this case, a poly- or perfluorinated compound).

The classification-based QSAR model indicated PFHpA to be an active compound. Regression-based QSAR analysis predicted a pIC_{50} (negative logarithmic function applied to the 50% inhibitory concentration of T4) of 2.62 mM for PFHpA. In the docking study, a binding energy of -2.83 kcal/mol was derived.

The structurally similar 7H-PFHpA was seen to fit exactly in the active protein sites, interacting with Leu17, Ala108, Ala109, Leu110, Thr118, Thr119 and Lys15. This occurred via hydrogen bonding of the negatively charged oxygen atom of the acid group with the positively charged Lys15. This compound was also classified as an active compound, with a QSAR-predicted *pIC50* of 2.56 mM and a binding energy of -3.81 kcal/mol.

Ren *et al*. (2016)

This study also performed a competitive binding assay to determine binding affinities of PFCs with TH transport proteins. For this purpose a fluorescence probe F-T4 was mixed with TH transport proteins and possible ligands (PFCs). The TH transport proteins used in the test were TTR (wild-type or mutant) and TBG (thyroxine-binding globulin, wild-type or mutant).

First, dissociation constants of F-T4 ($K_{d,probe}$ values) were calculated by nonlinear regression curve fitting of the binding data, which could then be used in further calculations. IC_{50} values of test ligands were derived by sigmoidal modelling. Relative potency (RP)-values were calculated by dividing the IC_{50}-value of T4 by that of the chemical. Dissociation constants of the test ligands ($K_{d,ligand}$) could then be obtained by using an equation containing $K_{d,probe}$ values, IC_{50} values and F-T4 concentrations. A molecular docking study was also performed, to evaluate the binding mode of PFCs to wild-type TTR and TBG.

PFHpA exhibited a relatively strong binding affinity to TTR with a RP of 0.028, a K_d of 180 ± 22 nM and a IC_{50} of 1128 ± 139 nM. PFOA exhibited the highest affinity to TTR of all perfluoroalkyl carboxylic acids included in the study, with a RP of 0.083, a IC_{50} of 378 ± 45 nM and a K_d of 60 ± 7 nM. These results are comparable with T4-REP values (relative potency of test compounds compared to T4) obtained by Weiss *et al*. (2009), namely a T4-REP for PFHpA of 0.039 and for PFOA of 0.064.

PFCs exhibited much weaker binding affinity to the mutant-form of TTR (TTRmutK15G), similar to the pattern observed for T4. Only the perfluoroalkyl acids with the longest carbon chain-lengths, PFTA (perfluorotridecanoic acid) and PFTdA (perfluorotetradecanoic acid), managed to bind to TBG. For the mutant-forms of TBG (TBGmutR378G and TBGmutR381G) binding affinities were much weaker.

Molecular docking analysis showed that PFOA and PFOS could nearly fill the TTR ligand-binding pocket, similar to the interaction observed for T4. However, only PFCs with a carbon chain length longer than 12 could fill the TBG ligand-binding pocket.

The authors conclude their study with an estimation showing that competitive displacement of T4 from TTR, by PFOS and PFOA, could be significant in the case of occupationally exposed workers.

PFHpA also demonstrated weak competitive binding to the human TRα-ligand-binding domain (LBD).

Ren *et al*. (2015)

Ren *et al*. (2015) studied binding affinity of 16 PFCs with human TRα-LBD. In a intrinsic tryptophan (Trp) fluorescence measurement, relative fluorescence intensity was compared after adding PFC-stock solutions to TRα-LBD. PFOA and PFOS, amongst others, managed to decrease Trp fluorescence intensity ($p<0.05$), suggesting binding to TRα-LBD.

A fluorescence competitive binding assay was also performed. For this purpose a fluorescence probe fluorescein-triiodothyronine (F-T3) was used. An IC_{50} value was derived, together with a relative potency (RP)-value (calculated by dividing the IC_{50} of T3 by that of the chemical). For PFHpA, a weak competitive binding to human TRα-LBD was shown with a calculated IC_{50} of 226 µM and a RP of 0.0013. PFOA exhibited a relatively high competitive binding with a IC_{50} of 42 µM and a RP of 0.0071. The relative potency of PFOS was even higher with a value of 0.01875 and a IC_{50} of 16 ± 4 µM.

Agonistic and antagonistic activities of PFCs on TR pathway were studied by performing a T-screen cell proliferation assay. For agonistic study purposes, GH3 rat pituitary cancer cells were treated with different concentrations of PFCs solely submitted. For antagonistic study purposes, GH3 cells were treated with different concentrations of PFCs together with 0.2 nM T3. PFOS was observed to have TR agonistic potency, since it increased cell growth when treated solely. When submitted together with 0.2 nM T3, PFOS also showed a synergistic effect on GH3 cell growth.

A molecular docking study was performed to evaluate the binding mode of PFCs to TRα-LBD. All PFC's discussed above, namely PFHpA, PFOA and PFOS, formed hydrogen bonds with ARG 228.

PFHpA was seen to induce peroxisome proliferator-activated receptor-alpha (PPARα), a transcription factor related to lipid metabolism in the liver.

Wolf *et al*. (2012)

PPARα has been shown to be activated by PFHpA in transiently transfected COS-1 cells with mouse or human PPARα-luciferase reporter plasmid (Wolf *et al*., 2012). An NOEC of 3 µM and an LOEC of 5 µM (1.82 µg/mL) was reported for mouse ($p<0.0001$) regarding PPARα transactivation. An NOEC of <0.5 µM and an LOEC of 0.5 µM (0.18 µg/mL) was reported for human ($p<0.01$).

OECD Level 3 : *in vivo* assay providing data about selected endocrine mechanism(s) and pathway(s)

Limited *in vivo* data are available for PFHpA, which is why a read-across with the C8-perfluorocarboxylic acid (PFOA) and the C8-perfluorosulfonic acid (PFOS) was performed.

PFOA and PFOS were observed to induce genes related to the thyroid system, *in vivo*.

Du *et al*. (2013)

Du *et al*. (2013) not only performed *in vitro* assays, but also an *in vivo* zebrafish embryo assay. After induction of spawning zebrafish adults, fertilized eggs were harvested and microscopically examined to determine effects of PFO on target genes. Normally developing embryos were selected for the assay. Zebrafish embryos were exposed to 100 µg/L, 200 µg/L and 500 µg/L in Petri dishes from 4 hours post-fertilization (hpf) to 120 hpf. A DMSO control was applied. Total RNA was extracted and first-strand complementary DNA (cDNA) synthesis was performed. mRNA expression levels of target genes were measured by applying Q-RT-PCR. For the treatment group, changes in mRNA expression were normalized to the fold above the vehicle control.

PFOA was seen to induce two genes related to the thyroid system, *hhex* and *pax8*, at concentrations of 200 and 500 µg/L ($p<0.05$). Since these genes are related to thyroid gland development, PFOA may be involved in the disruption of thyroid activity. PFOA was also seen to have an effect on estrogen receptor genes, since exposure to 500 µg/L of PFOA resulted in an upregulation of *esr1* ($p<0.05$).

Ren *et al*. (2015)

Ren *et al*. (2015) also performed an *in vivo* TH-response gene expression assay in *Xenopus laevis* to determine the effects of PFOS on TR pathway. Stage 48 tadpoles were subjected to different concentrations of PFOS (0.01 µM, 0.1 µM, 1 µM, 10 µM) and 1 nM T3 as a positive control. Intestinal tissues of the tadpoles were collected after 2 days and submitted to gene expression analysis. PFOS-concentrations of 0.01 µM to 1 µM promoted the expression of TRβ-A, BTEB and ST3, however to a lesser extent than T3. PFOS-concentrations of 0.1 µM to 10 µM downregulated IFABP, SLC6A19 and CPO in a way comparable to that of T3. PFOS thus seems to perform a similar mode of action regarding TR pathway as T3.

Single-oral dose exposure to PFOA and PFOS has been associated with increased protein levels related to synapse formation, synaptic plasticity and sprouting in male mouse pups.

Johansson *et al*. (2009)

This study also investigated possible developmental neurotoxic effects in mice, induced by PFOS and PFOA, with a focus on the proteins associated with normal brain development. Again, pregnant NMRI mice were housed individually in plastic cages. After birth, each litter was adjusted to a size of 10-12 mice. At first litters consisted of pups of both sexes, but only male mice were used for protein measurements.

Treatment groups were exposed to a single-oral dose of either 21 µmol/kg body weight of PFOS or PFOA via a metal gastric-tube at the age of 10 days. Control mice were treated with 10 ml/kg body weight of a 20% fat emulsion vehicle. Four litters were exposed to PFOS, four litters were exposed to PFOA and three litters were used as control. Animals were sacrificed in order to obtain cortex and hippocampus, which were frozen until assayed. Changes in protein levels were studied by performing a slot blotting procedure.

Treatment with 21 µmol/kg b.w. PFOS and PFOA resulted in significantly higher CaMKII protein levels in the hippocampus, with a 57% increase ($p<0.001$) in PFOS-treated mice and a 58% increase ($p<0.001$) in PFOA-treated mice, compared to the control group. GAP-43 protein levels were also significantly increased in the hippocampus after PFOS-treatment (22% increase, $p<0.01$) and after PFOA-treatment (17% increase, $p<0.05$).

An increase in synaptophysin protein levels, compared to the control group, could be observed in as well the hippocampus as the cerebral cortex. For the hippocampus, an increase of 48% ($p<0.001$) was observed after PFOS-treatment and an increase of 52% ($p<0.001$) after PFOA-treatment. For the cerebral cortex synaptophysin protein levels were even higher, with an increase of 59% ($p<0.01$) in PFOS-treated mice and an increase of 82% ($p<0.001$) in PFOA-treated mice.

Finally, significant increases in tau protein levels could be observed for PFOS- and PFOA-treatment groups, compared to the control. Namely an increase of 92% ($p<0.05$) after PFOA-treatment in the hippocampus, an increase of 80% ($p<0.05$) after PFOS-treatment and an increase of 142% ($p<0.01$) after PFOA-treatment in the cerebral cortex.

Both CaMKII and synaptophysin are involved in synapse formation and synaptic plasticity. Overexpression of GAP-43 has been associated with excessive sprouting. Since the proteins mentioned above are involved in normal brain development, PFOS and PFOA can influence brain development by altering these protein levels.

Reductions in total and free serum thyroxine (T4) were demonstrated in pregnant and non-pregnant female rats after exposure to PFOS.

Thibodeaux *et al*. (2003)

Thibodeaux *et al*. (2003) studied maternal and developmental toxicity of PFOS in rats and mice. For this purpose timed-pregnant Sprague-Dawley rats were bred within a 4h period and CD-1 mice overnight. In a separate study, mature female rats weighing 200 g were used. Each animal was housed individually in a polypropylene cage.

Rats

In rats, PFOS was administered daily at concentrations of 1, 2, 3, 5 and 10 mg/kg by gavage from gestational day 2 until gestational day 20. Controls were given vehicle alone. Information about maternal body weights, food and water consumption was recorded. Blood samples were taken on gestational day 7 and 14, and on gestational day 21 after decapitation. Serum samples were analysed for PFOS concentration, thyroid hormones, corticosterone, prolactin, cholesterol and lipid content. Maternal and foetal livers, uteruses and live foetuses were collected from some animals and analysed for PFOS concentration and/or submitted to teratological evaluation.

In a separate study on rats, adult (non-pregnant) females were treated daily with either 3 or 5 mg/kg PFOS for 20 days. Controls were given vehicle alone. Blood samples were taken at day 3, 7 and 14 after first PFOS-exposure. After 20 days of PFOS-treatment, trunk blood was collected from decapitation. Serum samples were submitted to triiodothyronine (T3), T4 and thyroid-stimulating hormone (TSH) analysis.

Maternal weight gain was reduced dose-dependently by PFOS-treatment, significantly in the 2 mg/kg and higher dosage groups. At the same time, a decrease in food and water consumption throughout gestation was observed. PFOS concentrations in maternal serum were consistently higher in each dosage group, compared to the control. Also PFOS concentrations in maternal and foetal livers at term increased with the submitted dosage.

Total and free serum T4 was decreased in all treatment groups in the study with pregnant females, compared to the control, starting from gestational day 7. For serum T3 a reduction in the dosage groups was visible too, but to a lesser extent. TSH serum concentrations showed no obvious differences between control and treatment groups. The separate study with adult non-pregnant females showed similar results. Reductions were observed in total and free serum T4, as well as serum T3. Serum TSH showed an initial increase in the 3 mg/kg PFOS-group and a decrease in the 5 mg/kg group, compared to the control. After 20 days of treatment however, these alterations were attenuated.

Teratological evaluation of foetuses exposed to the highest PFOS-dosage group (10 mg/kg) showed an increase in the incidence of cleft palate, defective sternebrae and anasarca.

Mice

In mice, PFOS was administered daily at concentrations of 1, 5, 10, 15 and 20 mg/kg by gavage from gestational day 1 until gestational day 17. Information about maternal body weights, food and water consumption was recorded. Blood samples were taken at gestational day 6, 12 and 18 from sacrificed mice. Serum samples were analysed for PFOS concentration, lipid content and T4-levels. Maternal livers, uteruses and live foetuses were collected from some animals and analysed for PFOS concentration and/or submitted to teratological evaluation.

A reduction of maternal weight gain was not as pronounced in mice as in rats, with only a significant difference compared to the control in the 20 mg/kg treatment group at high gestational age. Also reductions in food and water consumption were less apparent. Maternal serum and liver PFOS concentrations at term showed a similar increase with dose as was observed in rats.

Again, a dose-dependent reduction in total serum T4 was observed compared to the control at gestational day 6, but there is no longer a significant difference with the control during the last week of pregnancy. Teratological evaluation of foetuses exposed to the highest PFOS-dosage group (20 mg/kg) showed an increase in the incidence of cleft palate, defective sternebrae, enlargement of the right atrium and ventricular septal defects.

PFOS preferentially accumulated in the liver, with a ratio of serum to liver concentration of about 1:4 in both rats and mice. PFOS caused reductions in T4, which can have grave consequences since an unavailability of maternal T4 to the developing brain can increase the risk of poor neuropsychological development. Furthermore, increased incidences of malformations were seen in animals exposed to the highest PFOS-dosage group.

In utero exposure to PFOS resulted in postnatal reductions of free serum T4 in rat pups.

Lau *et al*. (2003)

Following the previous study, Lau *et al*. (2003) studied postnatal effects after *in utero* PFOS-exposure. For this purpose pregnant Sprague-Dawley rats were bred within a 4h period in the afternoon and CD-1 mice overnight. Each animal was housed individually in a polypropylene cage.

Rats

In rats, PFOS was administered daily at concentrations of 1, 2, 3, 5 or 10 mg/kg by gavage from gestational day 2 until gestational day 21. Controls were given vehicle alone. Information on time of parturition for each animal, number of live offspring and conditions of the newborns was recorded. The following day was described as postnatal day 1. Ages at which rat offspring opened their eyes and reached puberty were recorded. Estrous cycles were monitored in females.

10 Control and 10 PFOS-exposed litters were subdivided evenly into four groups. Two groups consisting of control pups remaining with their dams, and PFOS-exposed pups remaining with their dams. The other two groups were cross-fostered and consisted of PFOS-exposed pups transferred to control dams and control pups transferred to PFOS-exposed dams.

In a separate study, pregnant rats were administered PFOS-concentrations of 0, 1, 2, 3 or 5 mg/kg as previously described. Four pups from each litter were sacrificed by decapitation within 2-4 h after birth. Trunk blood and liver were obtained and subjected to PFOS and thyroid hormone analyses. Randomly chosen pups were sacrificed by decapitation on postnatal days 2, 5, 9, 15, 21, 28 and 35. Also here, trunk blood was collected for PFOS and thyroid hormone analyses. Liver weights were recorded, hippocampus and prefrontal cortex were obtained by dissection.

Postnatal survival for rat offspring was dose-dependently reduced after *in utero* PFOS-exposure, compared to the control. This result was not improved by cross-fostering the PFOS-exposed pups to a control dam. Yet, all control pups cross-fostered by PFOS-exposed dams survived for the duration of the study. Rat serum PFOS levels increased for each dose at birth. Rat liver PFOS concentrations at birth also increased dose-dependently, however liver PFOS-levels were similar to those in serum.

Pups treated with 2 mg/kg and higher doses of PFOS significantly lagged in body weight compared to control pups. Also slight, but significant delays in eye opening were observed for these treatment groups. Relative liver weight was significantly increased in all PFOS-treatment groups, compared to the control.

Total and free T4 serum levels were reduced in all PFOS dosage groups. Total T4 concentrations were seen to recover by weanling age. However, effects on free T4 persisted through the duration of the study. No significant differences between control and treatment groups were observed for serum T3 or TSH levels. As far as the prefrontal cortex was concerned, a slight but significant alteration in ChAT activity was observed for the 3 mg/kg PFOS-treatment group.

Mice

In mice, PFOS was administered daily at concentrations of 1, 5, 10, 15 and 20 mg/kg by gavage from gestational day 1 until gestational day 17. Controls were given vehicle alone. The day of birth was described as postnatal day 0. Condition of pups, litter size, weight gain and age of eye opening was monitored.

In a separate study, pregnant mice were treated with PFOS as mentioned above. Randomly selected pups were sacrificed within 2-4 h after birth and on postnatal days 3, 7, 14, 21, 28 and 35. Trunk blood was collected for thyroid hormone analysis and liver weight was recorded.

A similar dose-dependent decrease in postnatal survival after *in utero* exposure to PFOS was observed for mice pups as for rats. The increase in liver weight was even more pronounced for the mouse as for the rat. Nonetheless, rats were seen to be more sensitive in terms of morbidity and mortality than mice. Also, a significant delay in eye-opening could be observed for PFOS-treated mice pups. Changes in total T4 serum levels were however less apparent for mice compared to the pattern which was observed for PFOS-exposed rat pups.

It was suggested by the authors that PFOS could interfere with cellular or functional maturation of target organs via effects on thyroid hormones. Reductions of T4 and T3 in pregnant rodents exposed to PFOS, could lead to a deprivation of key endogenous signals for the developing foetus.

OECD Level 4 : *in vivo* assays providing data on adverse effects on endocrine relevant endpoints

Single-oral dose exposure to PFOA and PFOS has been associated with a lag in exhibited spontaneous behaviour, which is a measure of cognitive function, in male mouse pups.

Johansson *et al*. (2008)

Johansson *et al*. (2008) studied whether PFOS and PFOA caused developmental neurotoxic effects in mice. For this purpose, pregnant NMRI mice were housed individually in plastic cages. After birth, each litter was adjusted to contain 10-12 pups. Treatment groups were exposed to a single-oral dose of either 1.4 or 21 µmol/kg body weight of PFOS, PFOA or PFDA (perfluorodecanoic acid) via a metal gastric-tube at the age of 10 days. Control mice were treated with 10 ml/kg body weight of a 20% fat emulsion vehicle. At 4-5 weeks, females were killed and only males were retained for behavior testing, which were raised in groups of 4-7. Mice from 3 to 4 different litters were included in each treatment group.

Males were submitted to a spontaneous behavior test at the age of two and four months. This test measured motor activity during a 60 min-period, divided in three 20-min spells, in a automated device. Measured parameters were locomotion, rearing and total activity.

Following the spontaneous behavior test at four months of age, mice were given a single s.c. injection of 80 µg nicotine base/kg body weight. Directly after this injection a nicotine-induced behavior test was performed following the same protocol mentioned above.

Results from the first spontaneous behavior test showed that mice from the 21 µmol/kg bw PFOS- and PFOA-treatment group presented significantly lower activity in the beginning (0-20 min), while a significantly increased activity was observed at the end of the 60 min-period (40-60 min), compared to the control group. This pattern was observed in mice aged two and four months.

The second nicotine-induced behavior test showed high activity values for control mice in the beginning of the test (60-80 min), while low activity values were observed at the end (100-120 min). For the 21 µmol/kg b.w. PFOS- and PFOA-treatment group however the pattern was reversed, with significantly reduced activity in the first 20-min spell (60-80 min) and significantly increased activity during the last 20-min spell (100-120 min), compared to the control.

Spontaneous behavior is a sign of the ability of an animal to process new information and to habituate to a new environment. Since this can be considered a measure of cognitive function, the results above show that PFOS and PFOA can disrupt cognitive function and motoric behavior.

7.10.3. Conclusion on endocrine disrupting properties (combined / separate)

Based on information in the registration dossier and information from the published literature (as detailed above), there is a concern that PFHpA may be an endocrine disruptor for human health according to the World Health Organisation/International Programme on Chemical Safety working definition (WHP/IPCS, 2002). The currently available information is not sufficient to conclude on potential ED properties of PFHpA. Further information would be needed, but due to the ongoing restriction proposal for PFHxA and related substances and the identification of PFHpA as SVHC, further testing has not been requested.

7.11. PBT and vPvB assessment

Based on the argumentation elaborated in § 7.7.1. the eMSCA has concluded that the parent constituents are most probably not persistent. Further, examination of the degradation pattern of the polyfluorinated constituents (= 6:2 mono- and diPAPs) has shown that depending on environmental conditions several degradation steps will take place whose rates in field conditions cannot be reliably assessed. It is also concluded that in practice degradation will stop when the stage of the perfluorinated carboxylic acids is reached. The main recalcitrant endproduct is PFHx(A), while PFHp(A) will be formed in lesser amount. Because PFHp(A) is a relevant and hazardous stable degradation product of the Substance, focus of this PBT assessment was on this specific compound.

Persistence of PFHpA/PFHp.

In general, the persistence of PFHpA can be explained by the shielding effect of the fluorine atoms, blocking e.g., nucleophilic attacks to the carbon chain. High electronegativity, low polarisability and high bond energies make highly fluorinated alkanes one of the most stable organic compounds. It is not expected that the carboxylate moiety in perfluorinated carboxylic acids or their salts alters the stability of the perfluorinated carbon chain.

A simulation test is not available for PFHpA, but considering the extreme stability of carbon-fluorine bonds and based on a read-across approach with PFOA, PFNA, PFDA and $C_{11\text{-}14}$ PFCAs, the eMSCA has concluded that PFHpA and its salts will undergo, no or extremely limited degradation in the environment and that the very persistent criterion is met. In the Member State Committee Support Document (ECHA Member State Committee, 2022) the very persistent character of PFHpA is recognised. Also, in the RAC and SEAC final opinion on the PFHxA restriction proposal, the persistence of PFHxA was emphasised (RAC and SEAC, 2021): '*RAC notes that it is well established that PFCAs, including PFHxA, are very stable organic substances whose persistence in the environment greatly exceeds the very persistent (vP) criterion in Annex XIII of REACH*.'

Monitoring data support the above conclusion. The detection and/or quantification of PFHpA in remote areas such as the Arctic (in air, snow, fresh- and marine water (including sediments and soil) (Routti *et al*., 2017*)* and the Antarctic (snow) (Schiavone *et al.*, 2009), in locations far away from point sources, points towards high persistence of PFHpA.

Finally, 'Perfluoroheptanoic acid and its salts' (EC No: -; CAS No: -) have been identified as a substance of very high concern (SVHC), meeting the criteria set out in Article 57 c, d, e and f[15]. On 17 January 2023, "Perfluoroheptanoic acid and its salts" was included as an SVHC in the Candidate List for eventual inclusion in Annex XIV (ECHA Decision, 2022).

Bioaccumulation of PFHpA/PFHp.

The bioaccumulation potential of PFHpA/PFHp was examined both for water- and air-breathing organisms. PFHpA/PFHp is not bioaccumulative for aquatic organisms as the BCF is far below the threshold value of 2000. In order to assess the bioaccumulation potential of PFHpA/PFHp in air-breathing organisms, its half-life has been determined in studies with various organisms. These studies were performed with usual laboratory animals like mice and rats, with pigs and with human populations that were unintentionally exposed to PFASs. The studies show that there is considerable variation of the half-lives of PFHpA in blood and serum, not only between different populations but also between individuals. This is also true for studies that used the same methodology and so one may

[15] Registry of SVHC intentions until outcome. Perfluoroheptanoic acid and its salts (EC No: -; CAS no: -). https://echa.europa.eu/nl/registry-of-SVHC-intentions/-/dislist/details/0b0236e187714636

conclude that this variability is not triggered by the experimental setup or the calculation method.

The half-life for both female and male rats proved to be less than 1 day and this does not indicate a potential for bioaccumulation. In contrast, for pigs a large variability was observed with a half-life range of 10 to 500 days and a geometric half-life of 74 days. Based on this result a biomagnification factor in pigs of 2.7 is calculated. In the studies on human populations the following geometric half-lives were found: 220 days (Freberg et al., 2010), 86 days (Nilsson et al., 2010), 365 days (young females) & 299 days (rest of population) (Zhang et al., 2013), 74 days (Xu et al., 2020). All these values are quite high and they indicate a substantial bioaccumulation potential. As explained in the Support Document for SVHC identification PFHpA is considered to be very bioaccumulative because the observed half-lives in humans are greater than 30 days and such values lead to a BMF in humans considerably greater than 1.

Finally, 'Perfluoroheptanoic acid and its salts' (EC No: -; CAS No: -) have been identified as a substance of very high concern (SVHC), meeting the criteria set out in Article 57 c, d, e and f[16]. On 17 January 2023, "Perfluoroheptanoic acid and its salts" was included as an SVHC in the Candidate List for eventual inclusion in Annex XIV (ECHA Decision, 2022).

Toxicity of PFHpA/PFHp.

The ecotoxicological profile of PFHpA/PFHp is not examined.

PFHpA is covered by index number 607-761-00-3 of Regulation (EC) No 1272/2008 in Annex VI, part 3, Table 3 (the list of harmonised classification and labelling of hazardous substances) and it is classified in the hazard class toxic for reproduction category 1B (H360D: 'May damage the unborn child') and STOT RE 1 (H373) (liver). Therefore, the toxicity criterion of REACH Annex XIII is fulfilled.

Overall Conclusion on the PBT assessment.

The eMSCA has concluded that PFHpA is a hazardous stable degradation product of the Substance that in the environment is formed in relevant amounts. PFHpA fulfils the P, B and T criteria and its identification as a PBT substance is confirmed by the addition to the Candidate List of substances of very high concern for Authorisation on 17 January 2023 based on Articles 57(c)(d)(e)(f). Therefore, the eMSCA concludes that the Substance is also a PBT substance.

7.12. Exposure assessment

Not applicable.

7.13. Risk characterisation

Not applicable.

[16] Registry of SVHC intentions until outcome. Perfluoroheptanoic acid and its salts (EC No: -; CAS no: -). https://echa.europa.eu/nl/registry-of-SVHC-intentions/-/dislist/details/0b0236e187714636

7.14. References

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7.15. Abbreviations

5:2 sFTOH	5:2 secondary fluorotelomer alcohol
5:3 FTCA	5:3 fluorotelomer carboxylate
6:2 diPAP	ammonium bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl) phosphate
6:2 FTAL	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanal
6:2 FTCA	6:2 fluorotelomer carboxylate
6:2 FTOH	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol; 6:2 fluorotelomer alcohol
6:2 FTUCA	6:2 unsaturated fluorotelomer carboxylate
6:2 monoPAP	diammonium 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl phosphate
8:2 diPAP	ammonium bis(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-septadecafluorodecyl) phosphate
*	$p<0.05$
**	$p<0.01$
abs	absolute
ALC	approximate lethal concentration
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
B	bioaccumulative
BAF	bioaccumulation factor
BCA	bicinchoninic acid
BCF	bioconcentration factor
BMF	biomagnification factor
bw	body weight
CA	Competent Authority
CF	conceptual framework
conc.	Concentration
CoRAP	Community Rolling Action Plan
DMSO	dimethyl sulfoxide
E2	estradiol
EC3	Effective Concentration inducing a stimulation index of 3 in the LLNA test
ED	endocrine disruptor
eMSCA	evaluating Member State Competent Authority
ER	estrogen receptor
FBW	final body weight
FOB	functional observational battery
GD	gestational day
GLP	good laboratory practice
HCD	historical control data
HGEN	herring gull embryonic neuronal
Hpf	hours post-fertilization
HPLC	high-performance liquid chromatography
IQ	intelligence quotient
IV	intravenous
LC_{50}	concentration that is lethal for 50% of the organisms
LD	lactation day
LD_{50}	dose that is lethal for 50 % of the organisms
LLNA	local lymph node assay
Meas.	measured
MS	mass spectrometry
NA	not applicable
no	number
NOAEL	no observed adverse effect level

NOEC	no observed effect concentration
NZW	New Zealand White
OECD	Organisation for Economic Co-operation and Development
P	persistent
PAP(s)	polyfluoroalkyl phosphate(s)
PBT	persistent, bioaccumulative and toxic
PFAS	perfluoroalkyl substance
PFBA	perfluorobutanoic acid
PFBS	perfluorobutanesulfonic acid
PFC	perfluorinated compound
PFCA	perfluorocarboxylic acid
PFDA	perfluorodecanoic acid
PFHp	perfluoroheptanoate
PFHpA	perfluoroheptanoic acid
PFHpS	perfluorheptanesulfonic acid
PFHx	perfluorohexanoate
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexanesulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
PFPe	perfluoropentanoate
PFPeA	perfluoropentanoic acid
PFPeS	perfluoropentanesulfonic acid
PFPrA	perfluoropropanoic acid
PND	postnatal day
PPARα	peroxisome proliferator-activated receptor-alpha
RA	read-across
RAC	Risk Assessment Committee
Rel.	reliability
Rela	relative
Repr.	reproductive toxicity
RP	relative potency
SD	Sprague-Dawley
SEAC	Socio-Economic Assessment Committee
SI	stimulation index
STOT RE	specific target organ toxicity after repeated exposure
STOT SE	specific target organ toxicity after single exposure
SVHC	substance of very high concern
T	toxic
T3	triiodithyronine
T4	thyroxine
TBG	thyroxine-binding globulin
TFA	trifluoroacetic acid
TG	test guideline
TH	thyroid hormone
TMF	trophic magnification factor
Tox.	toxicity
TR	thyroid hormone receptor
Trig	triglyceride
TSH	thyroid-simulating hormone
TTR	transthyretin
USA	United States of America
vB	very bioaccumulative
vP	very persistent
vPvB	very persistent and very bioaccumulative
WWTP	wastewater treatment plant