baua:
Bundesanstalt für Arbeitsschutz
und Arbeitsmedizin
Federal Institute for Occupational
Safety and Health

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl acrylate

EC No. 241-527-8

and

3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl methacrylate

EC No. 218-407-9

Evaluating Member State: Germany

Dated: April 2023

Evaluating Member State Competent Authority

BAuA
Federal Institute for Occupational Safety and Health
Division 5 - Federal Office for Chemicals
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Year of evaluation in CoRAP: 2016

Before concluding the substance evaluation, a Decision for each of the two substances to request further information was issued on: 11 February 2019

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

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The Substances 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl acrylate (EC No. 241-527-8, CAS RN 17527-29-6, hereafter "6:2 FTA") and 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl methacrylate (EC No. 218-407-9, CAS RN 2144-53-8, hereafter "6:2 FTMA") were originally selected for substance evaluation in order to clarify concerns about:

- potential endocrine disrupting (ED) properties for the environment
- suspected PBT/vPvB properties
- high mobility in the environment
- exposure of the environment

During the evaluation no further concerns were identified.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

As precursors for undecafluorohexanoic acid (EC No. 206-196-6, CAS RN 307-24-4, "PFHxA"), 6:2 FTA and 6:2 FTMA were under the scope of a regulatory management option analysis (RMOA) on "undecafluorohexanoic acid (PFHxA) including its salts and precursors" prepared by the eMSCA in 2017[2]. The eMSCA has submitted a CLH dossier to classify PFHxA as Repr. 1B to ECHA for accordance check on 22 October 2022[3]. A restriction dossier on per-and polyfluorinated substances (PFAS) has been prepared by the eMSCA in cooperation with the Denmark CA, Netherlands CA, Norway CA, and Sweden CA[4]. The dossier has been submitted to ECHA on 13 January 2023 and is intended to also cover 6:2 FTA and 6:2 FTMA.

[2] ECHA website on PFHxA RMOA. https://echa.europa.eu/de/rmoa/-/dislist/details/0b0236e180b2ef59

[3] CLH process on PFHxA: https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e1877da3b1

[4] Restriction process on PFAS: https://echa.europa.eu/de/registry-of-restriction-intentions/-/dislist/details/0b0236e18663449b

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 Table 1 below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	x
Harmonised Classification and Labelling *(For 6:2 FTOH, the primary hydrolysis product of the two substance)*	x
Identification as SVHC (authorisation)	
Restrictions	x
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

The available data on 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol (EC No. 211-477-1, CAS RN 647-42-7, "6:2 FTOH"), the primary hydrolysis product of 6:2-FTA and 6:2 FTMA, is considered sufficient by the eMSCA to classify the substance as STOT RE 2, H373 and Aquatic Chronic 2, H411. The eMSCA has prepared and submitted a CLH dossier. The Committee for Risk Assessment (RAC) adopted the classification for STOT RE 2, H373 and Aquatic Chronic 1, H410 in its opinion on 26 November 2021[5].

The eMSCA recommends that the classification for 6:2 FTOH should also be applied by registrants and users of the parent compounds 6:2 FTA and 6:2 FTMA.

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

While the eMSCA has concerns regarding the ED, PBT/vPvB and PMT properties of 6:2 FTA and 6:2 FTMA, the eMSCA does not consider it proportionate to follow up on these with further information requirements or potential SVHC identification according to Art. 57 as it is expected that the two substances fall under the scope of the restriction of PFHxA and precursors (cf. section 4.1.3), thereby ultimately limiting their further impact on the environment.

[5] ECHA website on the CLH process for 6:2 FTOH: https://echa.europa.eu/de/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e1852e44c3

4.1.3. Restriction

In the environment and *in vivo*, 6:2 FTA and 6:2 FTMA are quickly hydrolysed to 6:2 FTOH. In the environment, this polyfluorinated alcohol is expected to ultimately undergo oxidation to PFHxA, which is highly persistent and mobile in the environment and exerts adverse effects on humans and environmental organisms.

Hence, in the view of the eMSCA, it is necessary to minimise the release of PFHxA and its precursors to the environment. The eMSCA has therefore prepared and submitted a restriction dossier for PFHxA in 2020[6]. The goal of this restriction is to limit the emissions of this man-made substance and its precursors from multiple sources. The opinions of the Risk Assessment Committee (RAC) and Socio-economic Analysis Committee (SEAC) were adopted in 2021[7].

In addition, a broad restriction proposal for PFASs has been submitted to ECHA by Denmark, Germany, the Netherlands, Norway, and Sweden in January 2023. The restriction aims at limiting the manufacture, placement on the market and use of PFASs and includes 6:2 FTA and 6:2 FTMA[8].

4.1.4. Other EU-wide regulatory risk management measures

N/A

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

N/A

[6] ANNEX XV RESTRICTION REPORT. PROPOSAL FOR A RESTRICTION of Undecafluorohexanoic acid (PFHxA), its salts and related substances. Available at: https://echa.europa.eu/documents/10162/c4e04484-c989-733d-33ed-0f023e2a200e

[7] ECHA website on the restriction of PFHxA, its salts and related substances, including adopted opinions and background documents: https://echa.europa.eu/de/registry-of-restriction-intentions/-/dislist/details/0b0236e18323a25d

[8] Restriction proposal for PFASs: https://echa.europa.eu/fi/registry-of-restriction-intentions/-/dislist/details/0b0236e18663449b

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

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
No additional follow-up actions proposed.	N/A	N/A

- As explained under section 4.1, follow-up regulatory actions have been already taken at the EU level: Harmonised classification of 6:2 FTOH for STOT RE 2, H373 and Aquatic Chronic 1, H410.
- restriction of PFHxA with the goal to limit the emissions of this man-made substance and its precursors from multiple sources.
- As PFAS, the eMSCA expects that 6:2 FTA and 6:2-FTMA will also be affected by the broad PFAS restriction for which an Annex XV dossier has been submitted in January 2023 by multiple member state competent authorities and which as of April 2023 is undergoing a public consultation.

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substances 6:2 FTA and 6:2 FTMA were originally selected for substance evaluation in order to clarify concerns about:

- potential endocrine disrupting (ED) properties for the environment
- suspected PBT/vPvB properties
- high mobility in the environment
- exposure of the environment

During the evaluation no further concerns were identified.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Endocrine disruption for the environment	**Concern confirmed.** The assessment focused on the two degradation products 6:2 FTOH and PFHxA. Available studies indicate that 6:2-FTOH interacts with the hypothalamus-pituitary-gonadal (HPG) axis and PFHxA interacts with the hypothalamus-pituitary-thyroid (HPT) and HPG axis. During substance evaluation, an Amphibian Metamorphosis Assay (AMA, OECD 231) on PFHxA and a Fish Sexual Development Test (FSDT, OECD 234) on 6:2 FTOH were requested. Based on the overall hazard profile of PFHxA, further risk management is required. The available data clarifies that the substance acts as an ED for the environment in accordance with the Endocrine Disruptor (ED) definition of the World Health Organisation (WHO). For 6:2 FTOH as well there is sufficient evidence that the substance acts as an ED for the environment in accordance with the WHO definition considering all data available.
Persistence	The eMSCA considers PFHxA the substance of relevance as it is the final degradation product of both substances. **Concern confirmed**, PFHxA is considered to meet the vP criterion.
Bioaccumulation	The eMSCA considers PFHxA the substance of relevance as it is the final degradation product of both substances. **Concern unresolved**, PFHxA does not meet the B or vB criteria for aquatic organisms but the bioaccumulation potential in air breathing organisms is unresolved.
Toxicity	The eMSCA considers PFHxA the substance of relevance as it is the final degradation product of both substances. **Concern confirmed**, based on the data, the eMSCA considers that the PFHxA warrants classification as Repro 1B, and thus would meet the T criteria for human health, if this classification would be formally adopted by the RAC.

High mobility in the environment	**Concern confirmed.** The high mobility of PFHxA, the final degradation product of both substances, is of high concern.
Exposure of the environment	**Concern confirmed.** Based on new information provided by the registrants, the eMSCA concludes that 6:2 FTMA and 6:2 FTA and their hydrolysis product 6:2 FTOH are released into the environment during the uses and life cycle of articles.

7.2. Procedure

Substance evaluation on 6:2 FTA and 6:2 FTMA started in March 2016 for both substances. The evaluation was conducted by assessing chemical safety reports (CSRs), original study reports obtained from the registrants and the eMSCA's own literature search.

In June 2016, the eMSCA invited the registrants for both substances for an expert meeting in order to discuss the current state of the evaluation and on-going tasks.

The substances were discussed via written procedure in 2016 and at the 8th Endocrine Disrupter Expert Group Meeting with other MSCAs. Furthermore, the substances, especially the potential PBT properties of their degradation product PFHxA, were discussed on the 12th Meeting of the PBT Expert Group and in written procedure in 2016.

At the end of the initial evaluation period, the eMSCA concluded that further information was necessary for both substances to clarify the concern for ED properties in the environment and regarding the exposure of the environment. Consequently, the eMSCA prepared a draft decision according to Art. 46(1) to request this information from the registrants of both substances. The decisions were adopted at the 62nd meeting of the Member State Committee and sent to the registrants[9], requesting a Fish Sexual Development Test (FSDT) according to OECD TG 234 on 6:2-FTOH, an Amphibian Metamorphosis Assay (AMA) according to OECD TG 231 on PFHxA and further information on uses and environmental releases of the substances.

The requested information was provided by the registrants throughout 2020 to 2021. The results of the studies and the ED properties of the substances were discussed at the 21st Endocrine Disrupter Expert Group Meeting with other MSCAs.

The eMSCA concluded the substance evaluation in 2022 based on all available information without the need for further information.

[9] Substance evaluation decision for 6:2-FTA dated 11 February 2019: https://echa.europa.eu/documents/10162/78bb05ac-42d0-76a7-02e7-c6c3eb80d645; substance evaluation decision for 6:2-FTMA dated 11 February 2019: https://echa.europa.eu/documents/10162/be125bab-7a63-18c9-4ca6-c7d79606a152

7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY OF 6:2 FTA	
Public name:	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl acrylate
EC number:	241-527-8
CAS number:	17527-29-6
Index number in Annex VI of the CLP Regulation:	-
Molecular formula:	$C_{11}H_7F_{13}O_2$
Molecular weight range:	418.1513 g/mol
Synonyms:	2-Propenoic acid, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl ester, 6:2 FTA

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

Structural formula:

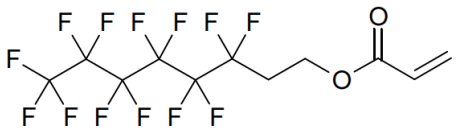
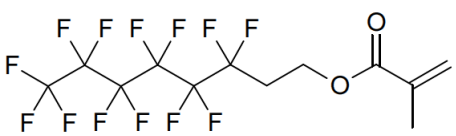


Table 5

SUBSTANCE IDENTITY OF 6:2 FTMA	
Public name:	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl methacrylate
EC number:	218-407-9
CAS number:	2144-53-8
Index number in Annex VI of the CLP Regulation:	-
Molecular formula:	$C_{12}H_9F_{13}O_2$
Molecular weight range:	432.1779 g/mol
Synonyms:	6:2 FTMA

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

Structural formula:



7.4. Physico-chemical properties

Table 6

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES OF 6:2 FTA	
Property	**Value**
Physical state at 20 °C and 101.3 kPa	colourless liquid, weak characteristic odour
Vapour pressure	at 20 °C 208 Pa, at 25 °C 259 Pa; The value was calculated from the regression of 13 individual measurements, which were performed in a temperature range of 17.8 - 62.6 °C.
Water solubility	0.185 mg/L at 25 °C and pH 5.9 - 6; mean value of three determinations.
Partition coefficient n-octanol/water (Log K_{ow})	log P_{ow} 5.067 at 25 °C, pH 6 - 7; mean value of three determinations.
Stability in organic solvents and identity of relevant degradation products	N/A
Dissociation constant	N/A

Table 7

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES OF 6:2 FTMA	
Property	**Value**
Physical state at 20 °C and 101.3 kPa	yellow liquid
Vapour pressure	at 20 °C 4.2 Pa, at 25 °C 8.6 Pa; The value was calculated from the regression of individual measurements, which were performed in a temperature range of 30 - 50 °C.
Water solubility	0.042 mg/L at 20 °C and pH 6.9 - 7; mean value of ten determinations.
Partition coefficient n-octanol/water (Log K_{ow})	log P_{ow} 5.3 at 25 °C, pH 7 HPLC method
Stability in organic solvents and identity of relevant degradation products	N/A
Dissociation constant	N/A

7.5. Manufacture and uses[10]

7.5.1. Quantities

Table 8

AGGREGATED TONNAGE (PER YEAR) OF 6:2 FTA				
☐ 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

Table 9

AGGREGATED TONNAGE (PER YEAR) OF 6:2 FTMA				
☐ 1 – 10 t	☒ 10 – 100 t	☐ 100 – 1,000 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 10

USES OF 6:2 FTA	Use(s)
Uses as intermediate	Use as monomer/intermediate during polymerisation reactions
Formulation	N/A
Uses at industrial sites	• Manufacture of the substance • Paper production • Polymerisation Use as an intermediate and in polymerisation process 6:2 FTA is used as an intermediate to manufacture another substance (ERC 6a) and as monomer for the manufacturing of thermoplastics (ERC 6c). The process categories cover use in closed processes with no likelihood of exposure (PROC 1), in closed, continuous process with occasional controlled exposure (PROC 2), in closed batch processes (PROC 3), and other processes where opportunity for exposure arises (PROC 4). Furthermore, 6:2 FTA or a preparation is transferred from/to vessels large container at non-dedicated facilities (PROC 8a) and at dedicated facilities (PROC 8b). 6:2 FTA or a preparation is transferred into small containers (PROC 9).

[10] ECHA dissemination database for 6:2 FTA (https://echa.europa.eu/de/substance-information/-/substanceinfo/100.037.737) and 6:2 FTMA (https://echa.europa.eu/de/substance-information/-/substanceinfo/100.016.735) accessed on 05 January 2023.

	Polymerisation 6:2 FTA is used as monomer for the manufacturing of thermoplastics (ERC 6c). In addition to above mentioned process categories (PROC 1-4, 8a, 8b and 9) 6:2 FTA is used as laboratory reagent (PROC 15). The mentioned product categories are coatings and paints, thinners, paint removers (PC 9a), polymer preparations and compounds (PC 32), textile dyes, finishing and impregnating products, including bleaches and other processing aids (PC 34). The sectors of end use are manufacture of rubber products (SU 11) and manufacture of plastics products (SU 12). (All information from ECHA dissemination website)
Uses by professional workers	N/A
Consumer Uses	N/A
Article service life	Use of paper packaging intended for food contact by consumers and by professionals.

Table 11

USES OF 6:2 FTMA	**Use(s)**
Uses as intermediate	Use as monomer during polymerisation reactions
Formulation	Formulation into mixtures
Uses at industrial sites	• Manufacture of the monomer • Use of the monomer in polymerization reactions • Textile finishing Use as an intermediate 6:2 FTMA is used as an intermediate to manufacture another substance (ERC 6a). The process categories cover use in closed processes with no likelihood of exposure (PROC 1), in closed, continuous process with occasional controlled exposure (PROC 2), in closed batch processes (PROC 3), and other processes where possibility for exposure arises (PROC 4), transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities (PROC 8a), and at dedicated facilities (PROC 8b), transfer of substance or preparation into small containers (dedicated filling line, including weighing) (PROC 9) and use as laboratory reagent (PROC 15). Polymerisation (inkl. surface active agents) 6:2 FTMA is used as monomer for the manufacture of thermoplastics (ERC 6c). Also, here 6:2 FTMA is used in the above given process categories (PROC 1-4, 8a, 8b, 9 and 15). The mentioned product categories are coating and paints, thinners, paint removers (PC 9a), polymer preparations and compounds (PC 32), textile dyes, finishing and

	impregnating products, including bleaches and other processing aids (PC 34). The sectors of end use are manufacture of rubber products (SU 11) and manufacture of plastics products (SU 12). Use of the monomer in polymerization reactions 6:2 FTMA is used as monomer for the manufacturing of thermoplastics (ERC 6c). Also, here 6:2 FTMA is used in the above given process categories (PROC 1-3, 8b, 9). The mentioned product category is intermediate (PC 19). The sectors of end use are manufacture of fine chemicals (SU 9). (All information from ECHA dissemination website)
Uses by professional workers	Use of textiles by professional workers
Consumer Uses	N/A
Article service life	Use of textiles by consumers Textile finishing (both professional and consumer)

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

There is no entry for 6:2 FTA and 6:2 FTMA in Annex VI of CLP.

7.6.2. Self-classification

For 6:2 FTA:

- In the registration(s):

STOT RE 2	H373 (Liver, teeth)

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

Skin Irrit. 2	H315
Eye Irrit. 2	H319
STOT SE 3	H335

For 6:2 FTMA:

- In the registration(s):

STOT RE 2	H373 (Liver and teeth)
Aquatic Chronic 1	H410 M(Chronic)=1

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

Skin Irrit. 2	H315
Eye Irrit. 2	H319
STOT SE 3	H335

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. Abiotic degradation

7.7.1.1.1. Hydrolysis

6:2 FTA is hydrolytically unstable at pH 4, 7 and 9 (Table 12).

Table 12

Summary of studies on hydrolysis of 6:2 FTA			
Method	**Results**	**Remarks**	**Reference**
OECD Guideline 111 (Hydrolysis as a Function of pH)	Half-life (DT50): (pH 4): 25.2 h at 50 °C; Rate constant: 0.0275 h-1 (pH 7): 32.9 h at 50 °C; Rate constant: 0.0211 h-1 (pH 9): 10.2 h at 50 °C; Rate constant: 0.0677 h-1 (pH 4): 37.3 h at 25 °C; Rate constant: 0.0186 h-1 (pH 7): 37.9 h at 25 °C; Rate constant: 0.0183 h-1 (pH 9): 44.8 h at 25 °C; Rate constant: 0.0154 h-1 (pH 4): 47.2 h at 10 °C; Rate constant: 0.0147 h-1 (pH 7): 50.7 h at 10 °C; Rate constant: 0.0137 h-1 (pH 9): 50.7 h at 10 °C; Rate constant: 0.0137 h-1	Rel. 1 (key study)	Registration dossier

The hydrolysis of 6:2 FTMA (Tier 2) was tested at only one temperature (20 °C) for a duration of 5 days (Table 13). According to OECD TG 111 the hydrolysis test should be conducted at three temperatures in the range of 10-70°C. Furthermore, the higher Tier tests should be conducted until 90% hydrolysis of the test substance is observed or for 30 days. The preliminary test at 50°C showed a loss of greater than 10% in 5 days for all three pH values.

Table 13

Summary of studies on hydrolysis of 6:2 FTMA			
Method	**Results**	**Remarks**	**Reference**
OECD Guideline 111 (Hydrolysis as a Function of pH)	Half-life (DT50): (pH 4): 17.3 d at 20 °C (Average of 2 replicates, confidence interval: 14%) (pH 7): 15.7 d at 20 °C (Average of 2 replicates, confidence interval: 31%) (pH 9): 18.7 d at 20 °C (Average of 2 replicates, confidence interval: 1%)	Rel. 1 (key study) eMSCA: Rel. 3	Registration dossier

7.7.1.1.2. Phototransformation /photolysis

No relevant information available.

7.7.1.2. Biodegradation

7.7.1.2.1. Biodegradation in aquatic compartment

Biodegradation of 6:2 FTA was tested in one screening test (Table 14). No further data is available.

Table 14

Summary of screening tests on ready biodegradability of 6:2 FTA

Method	Results	Remarks	Reference
OECD Guideline 301 C (Ready Biodegradability: Modified MITI Test (I))	under test conditions no biodegradation observed % Degradation of test substance: 6 after 28 days (BOD) (vessel No 2) 13 after 28 days (BOD) (Vessel No. 3) 12 after 28 days (BOD) (Vessel No.4) 10 after 28 days (BOD) (mean) 11 after 28 days (percentage biodegradation of test item (GC)) (mean)	Rel. 1 (key study)	Registration dossier

In conclusion, 6:2 FTA is not readily biodegradable.

Three studies according to OECD guideline 301 are available for 6:2 FTMA (Table 15). In contrast to the assessment of the registrant, the eMSCA considers one of the three studies as invalid. This is because the difference of extremes of replicate values was above the validity criterion of <20%. The two other studies according to OECD guideline 301B and OECD guideline 301D show that 6:2 FTMA is not readily biodegradable.

Furthermore, in an inherent biodegradation study according to OECD guideline 302C, 78.1% and 70.1% degradation were observed after 28 days based on biological oxygen demand (BOD) and residue analysis, respectively (Table 15). The result based on residue analysis does not reflect mineralization but rather dissipation. Only the test substance was analysed, the degradation products are unknown.

The formula of the theoretical oxygen demand, which is the basis for the calculation of percentage biodegradation from the oxygen consumption, considers halogens such as fluorine, but not the strong binding of the fluorine to carbon. This leads to a supposed higher degradation rate (applies to both ready and inherent biodegradation studies). Based on the general known stability of organic fluorine compounds, the perfluorinated part of 6:2 FTMA (carbon chain of six perfluorinated carbon atoms) is very resistant to degradation and will not be mineralized for 28 days. Hence, the degradation of 78.1% covers only the degradable residue ($C_6H_9O_2$). This residue is equivalent to approximately 26% of the overall molar mass.

Table 15

Summary of screening tests on ready biodegradability of 6:2 FTMA			
Method	**Results**	**Remarks**	**Reference**
OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test) activated sludge (adaptation not specified)	Not readily biodegradable % Degradation of test substance: 3 after 28 days (ThCO2)	Rel. 2 (key study)	Registration dossier
OECD Guideline 301 C (Ready Biodegradability: Modified MITI Test (I))	Not readily biodegradable % Degradation of test substance: 2 after 28 days (O2 consumption) (average determined by biological oxygen demand) 15 after 28 days (Test mat. analysis) (average determined by gas chromatography) => difference of extremes of replicate values 37% (≥20% = validity criteria not fulfilled)	Registrant: Rel. 1 (supporting study) eMSCA: Rel. 3	Registration dossier
OECD Guideline 301 D (Ready Biodegradability: Closed Bottle Test) activated sludge (adaptation not specified)	Not readily biodegradable % Degradation of test substance: 14 after 28 days (O2 consumption) (average determined by biological oxygen demand) 8 after 28 days (Test mat. analysis) (average determined by gas chromatography)	Rel. 2 (supporting study)	Registration dossier
OECD Guideline 302 C (Inherent Biodegradability: Modified MITI Test (II)) activated sludge, domestic, non-adapted	% Degradation of test substance: 48 after 14 days (BOD) 78.1 after 28 days (BOD) 70.1 after 28 days (based on residue analysis) Reference substance: Aniline (40% after 7 days and 65% after 14 days	Registrant: Rel. 1 (key study) eMSCA: 3	Registration dossier

7.7.1.2.2. Biodegradation in soil

No relevant information available.

7.7.1.2.3. Degradation studies of structurally similar substance

4:2 FTA (List No 808-163-7)

Butt et al. investigated the atmospheric chemistry of 4:2 fluorotelomer acrylate (4:2 FTA) (Butt et al., 2009). The atmospheric lifetime of 4:2 FTA is determined by its reaction with OH radicals and is approximately 1 day. The OH-radical-initiated oxidation in 700 Torr of air in the presence of NO (nitric oxide) gives HCHO with 4:2 fluorotelomer glyoxylate as the expected coproduct. The atmospheric fate of 4:2 fluorotelomer glyoxylate will be photolysis and reaction with OH radicals, which will lead to formation of 4:2 fluorotelomer aldehyde and ultimately perfluoroalkyl carboxylic acids. In conclusion, the atmospheric oxidation of FTA is expected to lead to the formation of perfluoroalkyl carboxylic acids (1-10% molar yield) in approximately 10 days. Therefore, the atmospheric oxidation of FTA is a potential source of perfluoroalkyl carboxylic acids in remote areas.

8:2 FTA (EC No 248-722-7)

Microbial transformation (microbially mediated hydrolysis) of 8:2 fluorotelomer acrylate (8:2 FTA) in aerobic soils was investigated by Royer et al. (Royer et al., 2015). 8:2 FTA was rapidly degraded with half-lives of 3-5 days. The substance was hydrolysed at the ester linkage as evidenced by the formation of 8:2 FTOH. 8:2 FTOH was further degraded via the known biotransformation pathway. 8 mol% perfluorooctanoic acid (PFOA) was formed after 105 days. Besides the stable metabolites like PFOA, 1.3 mol% PFHpA, < 0.4mol% perfluoroheaxanoic acid (PFHxA), and 2.3 mol% 7:3 acid, 38 mol% of intermediate metabolites (8:2 fluorotelomer carboxylic acid (FTCA), 8:2 fluorotelomer unsaturated carboxylic acid (FTUCA), 7:2 sFTOH) were observed at day 105. Total mass balance decreased with incubation time with 50% recovery at the end of 105 days incubation. Reasons for loss of mass balance could be reduced extractability, increased irreversibly bound metabolites over time, or additional metabolites which were not quantified or identified.

Metabolism

The biotransformation of 8:2 FTA was investigated in rainbow trout via dietary exposure (Butt et al., 2010). The parent, suspected intermediates and terminal metabolites were monitored in liver, blood, kidney, bile, and faeces during the 5-d uptake and 8-d elimination phases. Very low levels of the 8:2 FTA were detected in the internal tissues and faeces, suggesting that the 8:2 FTA was rapidly biotransformed in the gut or liver. Similarly, low concentrations of the 8:2 FTOH were accumulated in the fish tissues, although high concentrations were measured in faeces. In liver and kidney, a low but constant level of FTA and FTOH could be measured during the uptake phase. The 8:2 saturated fluorotelomer carboxylate (FTCA) was formed in the highest concentration. The 8:2 unsaturated fluorotelomer carboxylate (FTUCA) and 7:3 FTCA were also accumulated in high levels, at levels approximately 10-fold lower than the 8:2 FTCA. PFOA was also formed from the 8:2 FTA biotransformation, and tissue levels were above the method detection limit within 4 to 6 h after initial dosing. PFOA levels showed a rapid increase until approximately 24 h, followed by a much slower but constant increase throughout the uptake phase and a steady level during the initial 72 to 96 h of elimination, indicating continued formation from precursors still present in the body.

7.7.1.3. Degradation pathway and persistent metabolites

As mentioned above Royer et al. (2015) investigated the microbial transformation of 8:2 FTA, which is structurally similar to 6:2 FTA. Royer et al. observed the formation of PFOA via 8:2 FTOH. This pathway is also likely for 6:2 FTA (Figure 1). The degradation of 6:2 FTOH and PFHxA is demonstrated in the following chapter.

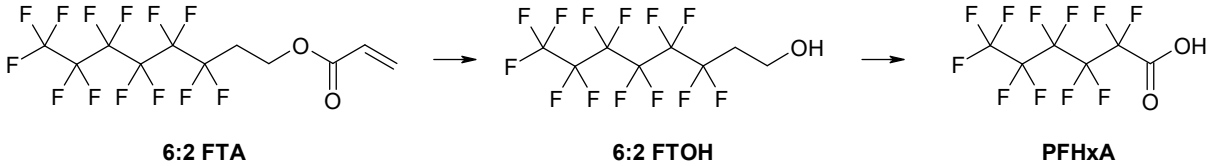


Figure 1: Degradation pathway for 6:2 FTA

6:2 FTOH

The aerobic biodegradation of 6:2 FTOH was performed in a flow through soil incubation system (Liu et al., 2010b). After 1.3 days, 50% of ^{14}C labelled 6:2 FTOH disappeared from soil, because of microbial degradation and volatilisation. The overall mass balance during the 84-day incubation averaged 77% and 87% for the live and sterile treatments, respectively. 16% [^{14}C] 5:2 sFTOH, 14% [^{14}C] 6:2 FTOH and 6% [^{14}C] CO_2 were measured in the airflow after 84 days. In soil the following stable transformation products were detected after 84 days: 5:3 acid (12%), PFHxA (4.5%), perfluoropentanoic acid (PFPeA; 4.2%), and perfluorobutanoic acid (PFBA; 0.8%). In soil-bound residues, the major transformation product was 5:3 acid. In a further study, the authors investigated the aerobic biodegradation of 6:2 FTOH (without ^{14}C-labelling) in soil (closed system) (Liu et al., 2010c). 6:2 FTOH primary gradation half-life was 1.6 days. The overall mass balance in aerobic soil was ~67% after 180 days (e.g., due to irreversible bond to soil). After 180 days the following substances were accounted: 30 % PFPeA, 8.1% PFHxA, 1.8% PFBA, 15% 5:3 acid, 1 % 4:3 acid, 3 % 6:2 FTOH, and 7.1% 5:2 sFTOH. 5:2 sFTOH, 5-3 acid and the intermediate 5:2 FT ketone were incubated with soil to elucidate the biodegradation pathway. 5:2 FT ketone yielded 5:2 sFTOH (78%), PFHxA (4%) and PFHeA (18%) after 90 days. Incubation with 5:2 sFTOH for 60 days yielded PFHxA (12%), PFPeA (85%) and small amounts of 5:2 FT ketone (<0.5%). Incubating with 5:3 acid 4:3 acid (2.3±0.4%) was the only metabolite after 60 days. The concentration of the initial 5:3 acid concentration decreased to 63%, this is likely due to the strong adsorption to soil (5:3 acid is becoming non-extractable).

Liu et al. also investigated the biodegradation of 6:2 FTOH in mixed bacterial culture (Liu et al., 2010c). Activated sludge was collected from an industrial wastewater treatment plant (WWTP) and was mixed with a nutrient medium. The sludge was pre-exposed to fluorinated chemicals. The bacterial culture itself was not pre-exposed to fluorinated chemicals. The primary degradation of 6:2 FTOH was rapid with an estimated half-life of 1.3 days. After 90 days, the overall mass balance was 60% (low mass balance can be attributed to unidentified or unquantified metabolites). PFHxA (5%), 6:2 FTCA (6%), 6:2 FTUCA (23%), 5:2 sFTOH (16%) and 5-3 acid (6%) were observed at the end of the study.

Zhao et al. investigated the aerobic biotransformation of 6:2 FTOH in activated sludge of two domestic WWTP (Zhao et al., 2013b). Primary biotransformation was rapid. More than 97 mol% converted within 3 days to at least nine transformation products. The most

abundant transformation product was the volatile 5:2s FTOH. After two months 40 mol% 5:2 sFTOH (30 mol% in the headspace) was detected. Further major biotransformation products were 5:3 acid (14 mol%), PFHxA (11 mol%), and PFPeA (4.4 mol%). PFBA and PFHpA were not observed within two months.

In an aerobic river sediment system, similar biotransformation products as in soil and activated sludge were detected (Zhao et al., 2013a). The recovery of 6:2 FTOH and quantifiable transformation products ranged between 71-88 mol% of initially applied 6:2 FTOH. The lower mass balance compared to sterile control (86-98 mol%) could be explained by formation of bound residues. After 100 days 22.4 mol% 5:3 acid, 10.4 mol% PFPeA, 8.4 mol% PFHxA, and 1.5 mol% PFBA were detected. PFHpA was not observed. Most of the 5:3 acid formed bound residues with sediment organic components, which can only be recovered by NaOH and ENVI-Carb™ carbon. In addition, 5:3 acid can be further degraded to 4:3 acid (2.7 mol%). Major intermediates during biotransformation of 6:2 FTOH were 6:2 FTCA, 6:2 FTUCA, 5:2 ketone, and 5:2 sFTOH (~28 mol% still remaining after 100 days). The 6:2 FTOH primary degradation half-life in sediment system was estimated to be 1.8 days. Figure 2 illustrates the proposed biodegradation pathway of 6:2 FTOH in aerobic sediment systems.

Anaerobic degradation of 6:2 FTOH under methanogenic conditions has been analysed by Zhang et al. (Zhang et al., 2013a). Anaerobic digester sludge was incubated dosed with 6:2 FTOH in two studies one for 90 and the other for 176 days. The half-life of 6:2 FTOH (primary degradation) was about 30 days. PFHxA formation was much lower compared with the results of the aerobic sludge and soil studies (0.2 mol% in the 90d-study, 0.4 mol% in the 176d-study). Approximately 30 mol% and 6 mol% of the added 100 mol% 6:2 FTOH still remained at day 90 and day 176, respectively. An average of 43 mol% of intermediate transformation products (sum of 6:2 FTCA and 6:2 FTUCA) were detected in both studies. 5:3 acid was detected as a stable degradation product (average 21 mol%). The results on anaerobic degradation obtained by Zhang et al. may be relevant for conditions such as landfill leachate and anaerobic WWTP sludge.

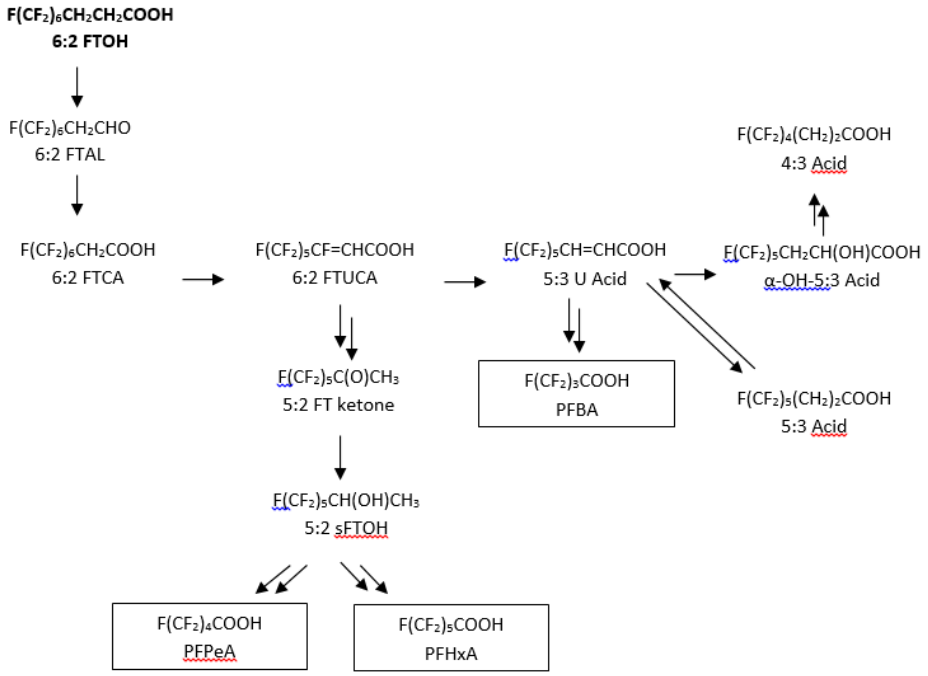


Figure 2: Proposed 6:2 FTOH aerobic biodegradation pathways. The single arrows indicate transformation steps based on observed transformation product and the double arrows indicate multiple transformation steps (based on (Zhao et al., 2013a)).

PFHxA

For PFHxA there is no degradation study under relevant environmental conditions available.

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

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

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

Based on their molecular properties it is clear that researchers could not measure degradation of the intensively studied PFOA or its salts. Considering the organic chemistry of this substance group the eMSCA considers it as very likely that PFHxA is as resistant to degradation as it has been shown for PFOA and PFHxA is therefore very persistent.

7.7.1.4. Summary and discussion on degradation

6:2 FTA

According to OECD guideline 111 the test item 6:2 FTA was determined to be hydrolytically unstable at pH 4, 7 and 9.

The substance has been shown to be not readily biodegradable according to the test method described in the OECD guideline 301 C.

No simulation tests on 6:2 FTA are available. Nevertheless, the microbial transformation of the structurally similar substance 8:2 FTA (two more CF_2-groups) was investigated in aerobic soil (Royer et al., 2015). 8:2 FTA was hydrolysed at the ester linkage with formation of 8:2 FTOH. 8:2 FTOH was further degraded to PFOA, which was the main stable transformation product at the end of the study (formation of PFOA: 8 mol% in 105 days). After 105 days approximately 50 mol% of intermediates and stable metabolites were observed.

There are no indications showing differences in transformation pathway of 8:2 FTA compared to 6:2 FTA. In analogy to the formation of 8:2 FTOH and PFOA from 8:2 FTA, also for 6:2 FTA, formation of PFHxA via 6:2 FTOH is expected.

6:2 FTMA

The substance 6:2 FTMA has been shown to be not readily biodegradable according to the test methods described in the OECD guideline 301 B and 301 C.

No simulation tests on 6:2 FTMA are available. Nevertheless, the microbial transformation of the structurally similar substance 8:2 FTMA (two more CF_2-groups) was investigated in aerobic soil (Royer et al., 2015). 8:2 FTMA was hydrolysed at the ester linkage with formation of 8:2 FTOH. 8:2 FTOH was further degraded to PFOA, which was the main stable transformation product at the end of the study (formation of PFOA: 10.3 mol% in 105 days). After 105 days approximately 69 mol% of intermediates und stable metabolites were observed. This shows that the result of an inherent biodegradation test (OECD guideline 302C) with 6:2 FTMA under optimized conditions (78.1% degradation after 28 days based on BOD), might overestimate the biodegradation of the registered substance and covers only the degradable residue of the registered substance (26% of the overall molar mass).

There are no indications showing differences in transformation pathway of 8:2 FTMA compared to 6:2 FTMA. In analogy to the formation of 8:2 FTOH and PFOA from 8:2 FTMA, also for 6:2 FTMA, formation of PFHxA via 6:2 FTOH is expected.

Overall conclusion

A number of studies on degradation of 6:2 FTOH show formation of PFHxA as stable transformation product (formation of PFHxA e.g., in soil: 4.5% after 84 days, in sediment 8.4 mol% after 100 days, in WWTP activated sludge 11 mol% after 60 days). PFHxA itself

is likely to be very persistent based on the general stability of organic fluorine compounds and read-across to the structurally similar substance PFOA, which is already identified as P and vP.

7.7.2. Environmental distribution

7.7.2.1. Adsorption

The adsorption of 6:2 FTA was determined according to OECD 121 guideline using the HPLC method. The log K_{oc} was estimated to be 4.18. The value indicates that the substance will be adsorbed by organic carbon in soil and sediment.

The adsorption of 6:2 FTMA was determined according to OECD 121 guideline using the HPLC method. The log K_{oc} was estimated to be 4.18. The value indicates that the substance will be adsorbed by organic carbon in soil and sediment.

For PFHxA no results from guideline testing are known. Adsorption was tested in different laboratory or semi-natural set-ups.

Vierke et al. investigated breakthrough of PFHxA and other PFASs in a column (surface areas 1 m^2, length 1 m) under environmental conditions (Vierke et al., 2013). The column is supposed to represent riverbank filtration and therefore was water saturated. The column was filled with coarse-grained medium sand. PFHxA and other PFASs were spiked as one initial pulse in the supernatant of the column. Concentration of PFHxA were determined in samples from 40 cm and 80 cm depth over a period of two weeks. Distribution coefficient calculated based on the breakthrough was log K_d = −0.18/0.46 (in 40 cm/80 cm) and log K_{OC} = 3.0/3.6 (in 40 cm/80 cm) for PFHxA. Furthermore, breakthrough of PFHxA through the column was faster than for long-chain PFCAs.

Gellricht et al. also performed a column study. The columns were 60 cm long and 5 cm in diameter filled with loamy sand. No distribution coefficient was calculated. But the results confirm the results of Vierke et al. when comparing the breakthrough of PFHxA to long-chain PFCAs: Breakthrough of PFHxA was always faster compared to longer-chain PFCAs indicating less sorption (Gellrich et al., 2012).

Due to this, indications on a high mobility of PFHxA in the aqueous environment, the environmental distribution of PFHxA especially with respect to a potential for long-range transport and for contamination of drinking water was further explored (ECHA, 2021a).

7.7.2.2. Long-range transport potential

PFHxA has no known natural source. Nevertheless, different studies report findings of PFHxA also in remote regions which indicates a potential for long-range transport. Two examples are given in the table below (ECHA, 2021a).

Table 16

Findings of PFHxA in remote areas				
Environmental Media	**Sampling location**	**PFHxA**	**Sampling year**	**Reference**
Surface Water	Atlantic and Canadian Arctic Oceans (cruises)	Atlantic: <0.0046 ng/L – 0.51 ng/L	2005-2009	(Benskin et al., 2012b)

Findings of PFHxA in remote areas				
Environmental Media	**Sampling location**	**PFHxA**	**Sampling year**	**Reference**
		Canadian Arctic: <0.0024 ng/L – 0.0048 ng/L		
Snow	European Alps (Colle Gnifetti; 10 m shallow firn core = 1996-2008)	0.06 ng/L – 0.34 ng/L 100% > LOQ	2008	(Kirchgeorg et al., 2013)

7.7.2.3. Occurrence in drinking water resources and drinking water

Drinking water is for example obtained from groundwater as well as from surface water.

Several studies report findings of PFHxA in these environmental media, see table 17, table 18 and table 19.

Table 17

PFHxA in surface water			
Sampling location	**PFHxA (in ng/L)**	**Sampling year**	**Reference**
Atlantic and Canadian Arctic Oceans (cruises)	Atlantic: <0.0046 – 0.51 Canadian Arctic: <0.0024 – 0.0048	2005-2009	(Benskin et al., 2012b)
Northern Europe, Atlantic Ocean, and Southern Ocean (cruise)	<0.003 – 0.117	2008	(Ahrens et al., 2010)
Greenland Sea, Atlantic and Southern Ocean (cruises)	Greenland Sea: <0.0059 – 0.038 Atlantic: <0.0059 – 0.120 Southern Ocean: <0.0059	2009-2011	(Zhao et al., 2012)
Seawater of the German Bight (North Sea)	0.47 – 9.56	2007	(Ahrens et al., 2009a)
Hessian (Germany) surface water (32 samples)	11 (median) 81% > LOQ	2009	(Gellrich et al., 2012)
10 Lakes around Shenyang, China	<0.63 – 25.0	2009	(Sun et al., 2011)
Guadalquivir and Ebro rivers (Spain)	9.6 – 31.4	2010	(Lorenzo et al., 2015)
40 river samples across the Hyogo prefecture, Japan	<0.5 – 6.9 65% > LOD	2010	(Takemine et al., 2014)
1 river sample downstream of a fluororesin manufacturer (effluent of WWTP)	2.300 – 16.000	2010-2012	
38 seawater samples across the Hyogo	1.5 – 510 100% > LOD	2010	

PFHxA in surface water			
Sampling location	**PFHxA (in ng/L)**	**Sampling year**	**Reference**
prefecture, Japan			

Table 18

PFHxA in groundwater			
Sampling location	**PFHxA (in ng/L)**	**Sampling year**	**Reference**
Hessian (Germany) groundwater (150 samples)	4 (median) 14% > LOQ	2008	(Gellrich et al., 2012)
Groundwater recharge area (several PFAA sources are present – a former landfill, a military base, and a small commercial/industrial area) (Netherlands) Observation wells (OW, n=1) Pumping wells (PW, n=5)) – travel distance > 25 years	OW: <0.45 – 670 PW: 0.22 – 0.41	2011	(Eschauzier et al., 2013)
164 individual groundwater samples from 23 European Countries	<0.5 0% > LOD	2008	(Loos et al., 2010)
Groundwater (n=26) from an unlined firefighter training area at Ellsworth U.S. Air Force Base (AFFF used between 1970 and 1990)	<100 – 320,000 96% > LOD	2011	(Houtz et al., 2013)

Table 19

PFHxA in drinking water			
Sampling location	**PFHxA (in ng/L)**	**Sampling year**	**Reference**
26 tap water samples from Germany	<1 – 6.4 23% > LOQ	Not mentioned	(Gellrich et al., 2013)
3 tap water samples from Norway (households receiving water from different water works)	<0.11 0.31 0.78	2008-2009	(Haug et al., 2010)
84 tap water samples from Spain and 5 from Germany	Spain: 3.0 (median) 11 (max) 18% > LOQ Germany 0.7 (median) 1.8 (max) 80% > LOQ	2010-2012	(Llorca et al., 2012a)
Raw water from public drinking water system (New	<5 – 17 23% > MRL	2009-2010	(Post et al., 2013)

PFHxA in drinking water Sampling location	PFHxA (in ng/L)	Sampling year	Reference
Jersey, USA) (12 surface water and 18 groundwater)			
Tap water samples in six European Countries (Sweden, Italy, Belgium, Netherlands, Norway, Germany) (n=7)	<0.38 – 5.15 86% > LOQ	2010	(Ullah et al., 2011)
26 waterworks along the Ruhr River (Germany)	<10 (median) 40 (max) 49% > LOD	2008-2009	(Wilhelm et al., 2010)
Drinking water production chain, Amsterdam (Netherlands)	Intake: 2.3 – 2.4 Finished drinking water: 3.8 – 5.3	2010	(Eschauzier et al., 2012)

7.7.2.4. Data from environmental monitoring

In addition to data given above on PFHxA in surface water, groundwater and drinking water, PFHxA is also found in effluent and sludge of WWTPs (Ahrens et al., 2009b), landfill leachates (Busch et al., 2010), soil and sediment (Klif, 2010), atmosphere (Jahnke et al., 2007), house dust and air (Shoeib et al., 2011).

6:2 FTOH was detected in the atmosphere, indoor air, dust, influent of wastewater treatment plants and in the air compartment above wastewater and landfill sites (Ahrens et al., 2011; Barber et al., 2007; Ericson Jogsten et al., 2012; Jahnke et al., 2007). An overview of these data is given in the tables below.

Table 20

PFHxA monitoring data in different environmental compartments Sampling location	PFHxA concentration	Sampling year	Reference
Wastewater treatment plant (WWTP) [ng/L]			
9 WWTP effluents along River Elbe, Germany	3.7 – 57.4	2007	(Ahrens et al., 2009b)
4 WWTP (1 industrial and 3 municipal) effluents, Finland	10.7 – 11.3	2009-2010	(Sun et al., 2011)
Sewage sludge of 10 WWTPs (4 domestic, 5 industrial, 1 hospital), Southwest Nigeria	<0.0105 – 0.2458 ng/g	2012	(Sindiku et al., 2013)
90 European WWTP effluents	5.7 (median) 23800 (max) 72% > LOQ	2010	(Loos et al., 2013)
Effluent from 3 WWTP, Hong Kong	<0.25	2012	(Loi et al., 2013)

PFHxA monitoring data in different environmental compartments			
Sampling location	**PFHxA concentration**	**Sampling year**	**Reference**
Sludge from 3 WWTP, Hong Kong	<0.066 ng/g d.w.		
Landfill leachates [ng/L]			
One Landfill leachate (Canada)	650 – 2500	2010	(Benskin et al., 2012a)
One Landfill leachate, Finland	49 -200	2009-2010	(Perkola and Sainio, 2013)
Landfill leachates (22 landfill sites in Germany)	<0.37 – 2509	-	(Busch et al., 2010)
Soil [µg/kg]			
Soil (0.6m below surface; n=16) and aquifer solids (5-6 m below surface; n= 10) from an unlined firefighter training area at Ellsworth U.S. Air Force Base (AFFF used between 1970 and 1990)	Soil: <0.8 –2,000 88% > LOQ Aquifer solids: 16 – 210 100% > LOQ	2011	(Houtz et al., 2013)
Soil (n=60) and aquifer solids (n=16) from a former firefighter training area at Ellsworth Air Force Base (USA)	Soil <0.05 – 2,761 93% > LOQ Aquifer Solids 0.445 – 297 100% > LOQ	2011	(McGuire et al., 2014)
Soil from a firefighting training ground at Flesland airport, Norway	0.18 – 18.5	2009	(Klif, 2010)
Sediment [pg/g]			
Sediment from 16 locations in Hong Kong	<17 – 95	2009	(Loi et al., 2013)
Surface sediment samples from 26 stations and sediment core samples (n=31) from 3 stations in Lake Ontario, Canada	Surface: <50 – 56 6% > LOQ Core: <50 – 409 23% > LOQ	2006 and 2008	(Yeung et al., 2013)
Sediment from Langavatnet near Flesland airport., Norway	<600 – 1,600	2009	(Klif, 2010)

Table 21

6:2 FTOH monitoring data in different environmental compartments				
Sampling location	**6:2 FTOH concentration**	**PFHxA concentration**	**Sampling year**	**Reference**
Atmosphere [pg/m³]				
Urban area	gas- phase:	-	2005	(Jahnke et al.,

6:2 FTOH monitoring data in different environmental compartments				
Sampling location	**6:2 FTOH concentration**	**PFHxA concentration**	**Sampling year**	**Reference**
(Hamburg, Germany) and rural area (Waldhof, Germany)	urban: 33 – 149 rural: 17 – 125 particle-phase: -			2007)
Air samples from Northwest Europe (UK - semirural and urban, Ireland - rural, Norway - rural)	gas-phase: 4.95 – 187 particle-phase: <0.1 – 1.8	particle-phase: 0.5 – 107	2005-2006	(Barber et al., 2007)
Air samples from two landfill sites and two reference sites (assumed to be not contaminated)	gas-phase: Landfill-sites: 8.2 – 102.8 Ref-sites: 7.4 – 36.8	particle-phase: Landfill-sites n.d./n. q – 16.9 Ref-sites: 0.1 – 0.8	2009	(Weinberg et al., 2011)
Landfill site (air concentrations), Ontario (Canada)	169 – 6462	0.51 – 62.0	2009	(Ahrens et al., 2011)
WWTP (air concentrations), Ontario (Canada)	89.9 – 12286	2.2 – 62.8		
Dust [ng/g] / indoor air [pg/m³]				
House dust (n=10) and indoor air (n=10) from selected homes in Catalonia, Spain	Dust: 0.008 – 0.060 Air: 3.0 - 47	Dust: 0.40 – 2.9 Air: not detected	2009	(Ericson Jogsten et al., 2012)
House dust (n=152) and indoor air (n=59 FTOH; n=39 PFCA) from selected homes in Vancouver, Canada	Dust: <0.05 – 4830 92% > MDL Air: <7.5 – 22890 97% > MDL	Dust: - Air: <0.02 – 205 94% > MDL	2007-2008	(Shoeib et al., 2011)
Residential dust (n=102) Vancouver, Canada			2007-2008	(De Silva et al., 2012)
Indoor dust from homes (n=30), offices (n=31) and vehicles (n=13) of 31 participants in the greater Boston, USA area	Offices: <50 – 2390 35% > LOQ Homes: <50 0% > LOQ Vehicles: <50 –2243 8% > LOQ	Offices: <5 – 102 68% > LOQ Homes: <5 – 1380 57% > LOQ Vehicles: <5 – 18.2 54% > LOQ	2009	(Fraser et al., 2013)

6:2 FTOH monitoring data in different environmental compartments				
Sampling location	**6:2 FTOH concentration**	**PFHxA concentration**	**Sampling year**	**Reference**
Samples from indoor locations in Tromsø, Norway (dust n=12, Indoor air n= 8)	Air: 16.0 – 9831	Dust: <0.3 – 363	2007-2008	(Huber et al., 2011)
Dust samples from homes (n=17), workplaces (n=5) and cars (n=9) from Cairo, Egypt	Homes: <0.05 – 6.79 78% > MDL Workplaces: 1.29 – 3.60 100% > MDL Cars: 0.91 – 4.20 100% > MDL	Homes: <0.20 – 0.74 21% > MDL Workplaces: - Cars: -	2013	(Shoeib et al., 2016)
Indoor air samples (n=11) – presence of materials with fluorine containing surface treatment (Carpets, surface-treated textiles, liquid care products for leather and textiles, and miscellaneous sources (e.g., food packaging, paints etc.).	150 – 46800	-	-	(Schlummer et al., 2013)

7.7.3. Bioaccumulation

There are two BCF studies provided in the registration dossiers for 6:2 FTA:

- OECD 305 conducted with 6:2 FTA resulted in BCF of < 80 and < 8
- OECD 305 conducted with 2-propenoic acid 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl ester resulted in BCF of ≤ 3.4 and ≤ 34

There are no BCF studies for 6:2 FTMA provided in the registration dossiers. Only one supporting study investigating metabolisms of 6:2 FTMA in trout hepatocytes predicts a BCF of 268.

BCF is known to not properly reflect the bioaccumulation potential of PFASs (European Chemicals Agency, 2013b). Therefore, no in-depth assessment of available BCF studies was conducted by the eMSCA.

Due to the environmental degradation pathways of 6:2 FTA and 6:2 FTMA described above with PFHxA being one main degradation product, the bioaccumulation assessment was focusing on PFHxA. Hereby, studies investigating PFHxA in biota, half-lives of PFHxA in humans and other organisms, protein binding of PFHxA and uptake of PFHxA in plants were taken into account.

7.7.3.1. PFHxA in biota

Occurrence of PFHxA in biota was investigated in several studies as can be seen in the table below. Overall, the results of the studies show that PFHxA can be found in biota.

Table 22

Concentration of PFHxA in different organisms			
Organisms	**PFHxA concentration**	**Sampling location**	**Reference / Sampling year**
Zooplankton Herring Sprat Guillemot egg	< 0.04 (MQL), < 0.2 (MQL) < 0.5 (MQL) 0.0026	Baltic Sea	(Gebbink et al., 2016) 2013-2014
Albatross liver Albatross muscle Albatross adipose	0.09 n. d. – 0.06 n. d. – 0.05	Midway Atoll (North Pacific Ocean	(Chu et al., 2015) 2011
Fish muscle Fish liver Prawn	68% recovery 44% recovery 38% recovery All samples < LOQ	Australia, contaminated estuaries	(Taylor and Johnson, 2016) 2015
Crucian carp blood Crucian carp liver Mandarin fish blood Mandarin fish liver	n. d. – 0.36 n. d. n. d. n. d.	Korea	(Lam et al., 2016) 2010 - 2012
Amphipod Damselfly Shrimp Sunfish Bullhead Turtle plasma	2.22 < MDL (0.25) < MDL (0.25) < MDL (0.25) < MDL (0.25) < MDL (0.1)	Hamilton, Canada. downstream of an airport	(De Solla et al., 2012) 2007 - 2010
Wild boar	0.49	Ingolstadt, Germany	(Klein et al., 2016) 2011 - 2012
Fish skin Fish liver Fish muscle Roe fish Algae Guano Algae Penguin dung Penguin tissue	< MLOD – 12.3 207 – 232 < MLOD - 72 1.44 – 2.31 3.4 – 240 < MLOD – 1190 < MLOQ 17.3 – 237 0.26 – 0.61	Tierra del Fuego Antarctica	(Llorca et al., 2012b)

Concentration of PFHxA in different organisms			
Organisms	**PFHxA concentration**	**Sampling location**	**Reference / Sampling year**
Beaver, liver Cod, blood Velvet scoter Eider duck Long-tailed duck Long-tailed duck Red-throated diver Razorbill	0.08 0.17 (pg/mL) < 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	Poland	(Falandysz et al., 2007) 2003
Roe deer	Recovery 5.5% < 0.02 (LOQ) – 0.7	Germany	(Falk et al., 2012) (2010)
Zooplankton Arctic cod Ringed seal Bearded seal	n.d. (LOD 0.3) n.d. (LOD 0.3) n.d. (LOD 0.3) n.d. (LOD 0.3)	Canadian Western Arctic	(Powley et al., 2008) 2004
PFHxA detected in 3 of 16 monitored organisms: White shrimp Whitebait Gobies	 2.11 0.815 3.29	Taihu Lake China	(Fang et al., 2014) 2012
Fish homogenates	4.67	Ohio, Missouri, and Upper Mississippi Rivers	(Ye et al., 2008)
Fish plasma (European chub)	0.2 (Not found in liver, gills, gonads, muscle)	Orge River (nearby Paris)	(Labadie and Chevreuil, 2011) 2010
Soft tissue of seafood samples, PFHxA detected in 1 of 16 monitored organisms: Swimming crab	 0.29	Fish market China	(Gulkowska et al., 2006) 2004
Ice amphipod Polar cod Black guillemot Glaucous gull	n.d. (LOD 0.41) 6:2 FTS: n.d. – 1.68 (LOD) 0.64 – 5.38 n.d. – 0.39 (LOD 0.22) n.d. – 1.55 (LOD 0.22)	Barants Sea	(Haukas et al., 2007) 2004

7.7.3.2. Half-lives of PFHxA in humans and animals

Studies in the following tables list derived half-lives of PFHxA in humans and other organisms. No threshold for defining a substance of being bioaccumulative above a certain half-live is available. For being able to compare half-lives of PFHxA with half-lives of a substance which is already known to be bioaccumulative, (benchmarking) data for PFOA were also included in the tables.

When comparing half-lives of PFHxA with those of PFOA it can be seen that PFHxA is eliminated faster from human bodies as well as from animals. However, regarding PFHxA half-lives in human only one study is available. As the half-lives of PFOA do not automatically set a threshold for bioaccumulation, the eMSCA considers that it cannot be concluded whether PFHxA is bioaccumulative in air-breathing organisms or not.

Table 23

Half-Lives of PFOA and PFHxA in rats, mice, pigs, and monkeys				
Organism	**Half-life PFOA (M = male, F = female)**	**Reference** **Remark**	**Half-life PFHxA (M = male, F = female)**	**Reference** **Remark**
Rat	M: 5.6d, F: 0.08d	(Ohmori et al., 2003) Mean β-phase of two compartment model with first-order elimination, single IV dose of 48.64 mmol/kg bw	M: 1.0h, F: 0.42h	(Chengelis et al., 2009a) Mean β-phase of two compartment model with first-order elimination, single IV dose of 10 mg/kg
	M: 13d	(Benskin et al., 2009) β-phase elimination rate, single oral dose, 0.5 mg/kg	M: 2.2h, F: 2.7h [9]	(Chengelis et al., 2009a) Mean β-phase of two compartment model with first-order elimination, repeated oral dose of 50 mg/kg, day 25
	M: 9.1d	(De Silva et al., 2009) β-phase elimination rate, repeated dose, 12-week exposure to 0.40 mg/kg in diet	M: 2.7h, F: 2.4h	(Chengelis et al., 2009a) Mean β-phase of two compartment model with first-order elimination, repeated oral dose of 150 mg/kg, day 25
			M: 2.8h, F: 2.3h	(Chengelis et al., 2009a) Mean β-phase of two compartment model with first-order elimination, repeated oral dose of 300 mg/kg, day 25
			M: 1.7h, F: 0.5h	(Gannon et al., 2011) Mean β-phase of one compartment model with first-order elimination, single oral dose of 2 mg/kg
			M: 1.5h, F: 0.7h [13]	(Gannon et al., 2011) Mean β-phase of one compartment model

Half-Lives of PFOA and PFHxA in rats, mice, pigs, and monkeys				
Organism	**Half-life PFOA (M = male, F = female)**	**Reference** **Remark**	**Half-life PFHxA (M = male, F = female)**	**Reference** **Remark**
				with first-order elimination, single oral dose of 100 mg/kg
Mice	M: 22d, F: 16d	(Lou et al., 2009) Mean β-phase of one compartment model with first-order elimination, single oral dose of 1 or 10 mg/kg	1-2h	(Russell et al., 2013)
Pig	M, F: 236d	Data from Numata et al., 2014. Mean β-phase of two compartment model with first-order elimination, 21d exposure to 22.4 µg/kg dw in diet	M, F: 4.1d	(Numata et al., 2014) Mean β-phase of two compartment model with first-order elimination, 21d exposure to 48 µg/kg dw in diet
Monkey	M: 21d, F: 33d	(Butenhoff et al., 2004) Mean β-phase of non-compartment model with first-order elimination, single IV dose of 10 mg/kg	M: 5.3h, F: 2.4h	(Chengelis et al., 2009a) Mean β-phase of two compartment model with first-order elimination, single IV dose of 10 mg/kg
	M: 20d [22]	(Butenhoff et al., 2004) Mean β-phase of non-compartment model with first-order elimination, repeated dose, six-month oral dosing of 10 mg/kg	1-2d	(Russell et al., 2013)
	M: 21d [23]	(Butenhoff et al., 2004) Mean β-phase of non-compartment model with first-order elimination, repeated dose, six-month oral dosing of 20 mg/kg		

Table 24

Half-lives of PFOA and PFHxA in humans				
Human	**Half-life PFOA (M = male, F = female)**	**Reference** **Remark**	**Half-life PFHxA (M = male, F = female)**	**Reference** **Remark**
Retired and non-retired workers	M, F: 3.6y (AM), 3.5y (GM)	(Olsen et al., 2007) Mean β-phase of non-compartment model with first-order elimination, periodic blood samples collected over 5 years	32d (GM)	(Russell et al., 2013)
Young females	2.3y (AM), 1.7y (GM), 2.0y (Med)	(Zhang et al., 2013b) β-phase estimate based on one compartment modelling of urine and blood samples. Should according to the authors be considered as upper limit estimates.	-	
Males and older females	2.8y (AM), 1.2y (GM), 1.8y (Med)	(Zhang et al., 2013b) β-phase estimate based on one compartment modelling of urine and blood samples. Should according to the authors be considered as upper limit estimates.	-	

7.7.3.3. Protein binding of PFHxA

Bischel et al. investigated the binding of PFCAs to bovine serum albumin (BSA) using equilibrium dialysis (Bischel et al., 2011). >99% of PFHxA bound to BSA and the log K_{PW} was 4.05. The benchmarking with PFOA (known to be bioaccumulative) shows that both were bound >99 % and that the K_{PW} of PFHxA is higher than for PFOA (although not significantly). Even though there seems to be some agreement that protein binding is the relevant pathway for bioaccumulation of PFASs, it is unclear how this can be used in the assessment of the bioaccumulation potential.

7.7.3.4. Uptake of PFHxA in plants

Several studies investigated the uptake of PFHxA and other PFASs from the surrounding environment into plants (see Table 25).

So far it is unclear how such data can be used in the assessment of substances under REACH. As PFOA (C8-PFCA) and other long-chain PFCAs are known to be bioaccumulative, the uptake in plants of PFHxA was compared to the uptake of these longer-chain PFCAs.

Based on study results it cannot be excluded that PFHxA is more effectively taken up by plants and transferred to edible parts within plants compared to longer-chain PFCAs.

Table 25

Uptake of PFHxA and other PFCAs in plants				
Method	**Plant**	**Regarded PFCAs**	**Results**	**Reference**
Field study (biosolid amended soils)	Wheat (*Triticum aestivum L.*) (roots, straws, husks, grains)	C4-12 PFCAs	roots: no correlation between the root concentration factor (RCF) and carbon chain length **PFHxA = 3.28 (ng/g_{root})/(ng/g_{soil})** straws: - transfer factor from roots to straws (TF_{r-s}) for short-chain higher than for long-chain PFCAs Straw concentration factor (SCF) **PFHxA = 1.24 (ng/g_{straw})/(ng/g_{soil})** TF_{r-s} **PFHxA = 0.392 (ng/g_{straw})/(ng/g_{root})** grains: transfer factor from straws to grains (TF_{s-g}) for short-chain higher than for long-chain PFCAs Grain concentration factor **(GCF)** **PFHxA = 0.311 (ng/g_{grain})/(ng/g_{soil})** TF_{s-g} **PFHxA = 0.253 (ng/g_{grain})/(ng/g_{straw})**	(Wen et al., 2014)
Climate chamber (100 µg/L of each individual PFCA per litre of nutrient solution)	Maize (*Zea mays*)	C4-10 PFCAs	- Uptake rate by roots: C4 PFCA = 2.46 µg/g root DW/d=> decrease until C7 PFCA (0.12 µg/g root DW*/d) => increase until C10 PFCA (1.95 µg/g root DW/d) **PFHxA = 0.35 µg/g root DW/d** - shoot:root ratios: C4-7 PFCAs = 2-4 (transferred to shoot); C8-10 PFCAs <1 (accumulated in roots) **PFHxA = 2.25**	(Krippner et al., 2014)
Pot experiment (Soil was spiked with an aqueous solution of 0.25 mg individual PFCA/kg soil and 1.00 mg individual PFCA/kg soil)	Maize (*Zea mays*)	C4-10 PFCAs	Straw: - Concentrations of PFCAs in straw decreased significantly with increasing chain length - Transfer factor (TF_{straw}): Decrease from C4 PFCA 63.64 (0.25 mg/kg treatment) and 35.23 (1.00 mg/kg treatment) to C10 PFCA (TF_{straw} = 0.03 and 0.04). **PFHxA = 3.19 (0.25 mg/kg treatment) and 2.82 (1.00 mg/kg treatment)**	(Krippner et al., 2015)

Uptake of PFHxA and other PFCAs in plants				
Method	**Plant**	**Regarded PFCAs**	**Results**	**Reference**
			Kernel: - Transfer factor ($TF_{kernels}$) Decrease from C5 PFCA = 0.366 (0.25 mg/kg treatment) and 0.380 (1.00 mg/kg treatment) to C10 PFCA ($TF_{kernels}$< LOQ) **PFHxA = 0.123** (0.25 mg/kg treatment) **and 0.216** (1.00 mg/kg treatment)	
Field study (biosolid amended fields)	Grass (from biosolid-amended fields)	C6-14 PFCAs	- grass/soil accumulation factors (GSAF): **PFHxA (mean) = 3.8** => decrease until C9 (mean decrease of 32-fold) => further decrease until C14 PFCA (mean decrease 2-fold)	(Yoo et al., 2011)
Greenhouse – hydroponic system (PFCA-spiked nutrient solution with nominal concentration of 10 ng/L to 10 µg/L of each spiked PFCA)	Lettuce (*Lactuca sativa*)	C4-14 PFCAs	- Roots concentration factor (RCF): **PFHxA ≈ 1.2** (lowest RCF of all PFCAs) - Foliage to root concentration factor (FRCF): C4 and C5 PFCAs > 1 (decrease with increasing chain length) **PFHxA ≈ 0.98** - Transpiration stream concentration factor (TSCF): only PFCA C4 > 1 (all other PFCAs inhibited) **PFHxA ≈ 0.07**	(Felizeter et al., 2012)
Greenhouse – hydroponic system (PFCA-spiked nutrient solution with nominal concentration of 10 ng/L to 10 µg/L of each spiked PFCA)	Tomato (*Solanum lycopersicum* var. Moneymaker), cabbage (*Brassica Oleracea* convar. *capitata var. alba*) and zucchini (*Cucurbita pepo* var. Black Beauty)	C4-14 PFCAs	- Root concentration factor (RCF): RCF increases with increasing chain length *cabbage:* **PFHxA ≈ 15.8** (highest C11 PFCA ≈ 370) *zucchini:* **PFHxA ≈7.5** (highest C14 PFCA ≈ 740) *tomato:* **PFHxA ≈ 3.6** (highest C11 PFCA ≈ 340) - Stem concentration factor (SCF): *cabbage:* SCF C4 to C7 PFCA decrease, C7 to C11 PFCA increase, C11 to C14 PFCA decrease; highest C11PFCA with SCF of ≈ 10 **PFHxA ≈ 2.4** *zucchini:* SCF C4 to C9 PFCA increase, C9 to C14 PFCA decrease; highest C9 PFCA with SCF of ≈ 11.4 **PFHxA ≈ 5.5** *tomato:* SCF C4 to C10 PFCA increase, C10 to C14 PFCA decrease;	(Felizeter et al., 2014)

Uptake of PFHxA and other PFCAs in plants				
Method	**Plant**	**Regarded PFCAs**	**Results**	**Reference**
			highest C10 PFCA with SCF of ≈ 13.8 **PFHxA ≈ 2.3** - Leaf concentration factor (LCF) *cabbage*: LCF C4 to C5 PFCA decrease, C5 to C8 PFCA increase, C8 to C14 PFCA decrease; highest C8 PFCA with LCF of ≈ 11 **PFHxA ≈ 6.9** *zucchini*: LCF C12 to C14 < 1 (C4 to C6 PFCA decrease, C6 to C8 PFCA increase, C8 to C14 PFCA decrease; highest C4 PFCA with LCF of ≈ 19) **PFHxA ≈ 10** *tomato*: LCF C12 to C14 < 1 (C4 to C5 PFCA decrease, C5 to C8 PFCA increase, C8 to C14 PFCA decrease; highest C8 PFCA with LCF of ≈ 58) **PFHxA ≈ 19** => leaves show the highest concentration factors compared to other above-ground parts of the plants; the leaves also store the largest mass of all of the PFCAs - Twig concentration factor (TCF) *cabbage*: (no twig) *zucchini*: TCF C4 to C7 PFCA constant, C7 to C10 PFCA increase, C10 to C14 PFCA decrease; highest C10 PFCA with TCF of ≈ 5.3 **PFHxA ≈ 1.7** *tomato*: TCF C4 to C5 PFCA decrease, C5 to C10 PFCA increase, C10 to C14 PFCA decrease, highest C10 PFCA with TCF of ≈ 14 **PFHxA ≈ 5.3** - Edible part concentration factor (ECF) (cabbage head and tomato and zucchini fruit) No concentration above LOQ for C12-C14 *cabbage*: ECF C4 to C5 PFCA increase, C5 to C11 PFCA decrease; highest C5 PFCA with ECF of ≈ 8 **PFHxA ≈ 3.6** *zucchini*: ECF C4 to C5 PFCA increase, C5 to C11 PFCA decrease; highest C5 PFCA with ECF of ≈ 1.8) **PFHxA ≈ 0.9**	

Uptake of PFHxA and other PFCAs in plants				
Method	**Plant**	**Regarded PFCAs**	**Results**	**Reference**
			tomato: ECF C4 to C5 PFCA increase, C5 to C11 PFCA decrease; highest C5 PFCA with ECF of ≈ 7.6) **PFHxA ≈ 4.4** - Transpiration stream concentration factor (TSCF) TSCF < 1 for all compounds in all plant species => transfer from the nutrient solution to the vegetative parts of the plants was inhibited *cabbage*: TCSF C4 to C6 PFCS decrease, C6 to C8 PFCA increase, C8 to C14 PFCA decrease; highest C8 PFCA with TCSF of ≈ 0.21 **PFHxA ≈ 0.12** *zucchini*: TCSF C4 to C5 PFCS decrease, C5 to C7 PFCA constant, C7 to C9 PFCA increase, C9 to C14 PFCA decrease; highest C9 PFCA with TCSF of ≈ 0.08 **PFHxA ≈ 0.06** *tomato*: TCSF C4 to C6 PFCS decrease, C6 to C8 PFCA increase, C8 to C14 PFCA decrease; highest C8 PFCA with TCSF of ≈ 0.23 **PFHxA ≈ 0.12** - Edible part/leaf transfer factor: All factors were < 1, which indicates that leafy crops with open leaves (spinach or some lettuce) accumulate higher amounts in the edible part than fruit-bearing crops. Leafy crops pose a higher risk for human exposure	

7.7.3.5. Summary and discussion on bioaccumulation

PFHxA, as one degradation product of 6:2 FTA as well as 6:2 FTMA in the environment, was in the focus of assessing the potential for bioaccumulation. As BCFs are known to not properly reflect the bioaccumulation potential of PFCAs other endpoints were considered:

- Data from environmental monitoring show that PFHxA is present in biota.
- PFHxA half-lives in organisms and humans range from a few hours to a few days.
- PFHxA is taken up by plants and is transferred to edible parts of plants.

For none of these endpoints standardized concepts within the assessment of bioaccumulation are available. One possible approach is to compare results for PFHxA with results of a substance, which is already known to be bioaccumulative (benchmarking-approach), here PFOA:

- Monitoring data, e.g., concentrations in biota, cannot be compared between PFOA and PFHxA, because biota exposure concentrations might be different.
- PFOA has longer half-lives (a few days up to a few years) in animals and humans compared to PFHxA.
- Binding of PFOA and PFHxA to proteins is comparable.
- PFHxA and PFOA are both taken up by plants and are transferred to edible parts of plants, whereby the extent of both processes seems to depend on the study design.

In conclusion it cannot be excluded that PFHxA does bioaccumulate in air-breathing organisms.

7.8. Environmental hazard assessment

The following chapter contains ecotoxicological information of the evaluated substances (6:2 FTA and 6:2 FTMA) as well as the stable transformation product (PFHxA). The results of the available studies give no indication that the T criterion might be fulfilled. Therefore, no in-depth evaluation of each study was performed by the eMSCA.

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

Table 26

Summary of effects on fish			
Substance	**Method/ Organism**	**Results**	**Reference**
6:2 FTA	OECD Guideline 203 (Fish, Acute Toxicity Test) Semi-static Limit test *Oryzias latipes*	LC_{50} (48h) > 0.306 mg/L (meas. (geom. mean)) based on mortality LC_{50} (96h) > 0.306 mg/L (meas. (geom. mean)) based on mortality LC_{50} (96h) > 100 mg/L (nominal) based on mortality	Registration dossier
6:2 FTMA	OECD Guideline 203 (Fish, Acute Toxicity Test) Semi-static	LC_{50} (96h) > 0.077 mg/L dissolved (meas. TWA) based on mortality	Registration dossier

Summary of effects on fish			
Substance	**Method/ Organism**	**Results**	**Reference**
	Oncorhynchus mykiss		
6:2 FTMA	OECD Guideline 203 (Fish, Acute Toxicity Test) Static-renewal *Pimephales promelas*	LC_{50} (96h) > 14.5 mg/L (meas.) based on mortality LC_{50} (96h) > 30 mg/L (nominal) based on mortality	Registration dossier
6:2 FTMA	OECD Guideline 203 (Fish, Acute Toxicity Test) Semi-static *Oryzias latipes*	LC_{50} (96h) > 0.133 mg/L (meas. (geomean)) based on mortality	Registration dossier
6:2 FTMA	OECD Guideline 203 (Fish, Acute Toxicity Test) Static-renewal *Gobiocypris rarus*	LC_{50} (96h) > 29.3 mg/L (meas. (arithm. Mean) LC_{50} (96h) > 30 mg/L (nominal)	Registration dossier
PFHxA	*Oncorhynchus mykiss*	LC_{50} (96h) > 99.2 mg/L	(Danish Environmental Protection Agency, 2015)
PFHxA	*Oncorhynchus mykiss*	NOEC (56d) = 10.1 mg/L (reprod.)	(Danish Environmental Protection Agency, 2015)
6:2 FTOH	OECD Guideline 203 (Fish, Acute Toxicity Test) static *Pimephales promelas*	LC_{50} (96h) = 4.84 mg/L (mean meas.)	Registration dossier Anonymous 1, 2007
6:2 FTOH	OECD Guideline 203 (Fish, Acute Toxicity Test) static *Oncorhynchus mykiss*	LC_{50} (96h) = 9 mg/L (nominal)	Registration dossier Anonymous 2, 2005 Reliability: 4 (registrant reliability: 1)
6:2 FTOH	OECD Guideline 203 (Fish, Acute Toxicity Test) static *Oryzias latipes*	LC_{50} (96h) = 5.78 mg/L (mean meas.)	Registration dossier Anonymous 3, 2007 Reliability: 4 (registrant reliability: 1)

Summary of effects on fish			
Substance	**Method/ Organism**	**Results**	**Reference**
6:2 FTOH	OECD Guideline 234 (Fish Sexual Development Test) Flow-through Medaka *Oryzias latipes* 0.00933, 0.0137, 0.0231, 0.0537 and 0.0953 mg/L (mean measured)	NOEC (122d) = 0.0231 mg/L (mm) (hatching success) NOEC (122d) = 0.0537 mg/L (mm) (survival and growth – male + female body length and weight) NOEC (122d) ≥ 0.0953 mg/L (mm) (genetic + phenotypic sex ratio) NOEC (122d) = 0.0137 mg/L (mm) (VTG male) NOEC (122d) = 0.0537 mg/L (mm) (sec. sex char. – male anal fin papillae count)	Registration dossier

No long-term toxicity test for 6:2 FTA or 6:2 FTMA on fish is available.

7.8.1.2. Aquatic invertebrates

Table 27

Summary of effects on aquatic invertebrates			
Substance	**Method/ Organism**	**Results**	**Reference**
6:2 FTA	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) Semi-static Limit test	EC_{50} (24h) > 0.141 mg/L (meas. (geom. mean)) based on mobility EC_{50} (48h) > 0.141 mg/L (meas. (geom. mean)) based on mobility LC_{50} (48h) > 100 mg/L (nominal) based on mortality	Registration dossier
6:2 FTMA	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) Semi-static *Daphnia magna*	EC_{50} (48h) > 0.017 mg/L dissolved (meas. TWA) based on mobility	Registration dossier
6:2 FTMA	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) Static *Daphnia magna*	EC_{50} (48h) > 120 mg/L (nominal) based on mobility	Registration dossier

6:2 FTMA	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) Semi-static *Daphnia magna*	EC_{50} (48h) > 0.038 mg/L (meas. (geom. mean) based on mobility	Registration dossier
PFHxA	*Daphnia magna*	LC_{50} (48h) > 96.5 mg/L	(Danish Environmental Protection Agency, 2015)
PFHxA	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test) *Daphnia magna*	EC_{50} (48h) = 1048 mg/L based on mobility	(Barmentlo et al., 2015)
PFHxA	OECD Guideline 211 (Daphnia magna Reproduction test) *Daphnia magna*	EC_{50} (21d) = 776 mg/L (per capita no. offspring) EC_5 (21d) = 724 mg/L (per capita no. offspring) EC_{50} (21d) = 853 mg/L (per capita population growth rate) EC_5 (21d) = 779 mg/L (per capita population growth rate)	(Barmentlo et al., 2015)
6:2 FTOH	OECD Guideline202 (Daphnia spa. Acute Immobilisation Test) Static *Daphnia magna*	EC_{50} (48h) = 7.84 mg/L (Mean measured) Based on mobility	Registration dossier
6:2 FTOH	OECD Guideline 211 (Daphnia magna Reproduction Test) EPA 797.1330 Semi-static *Daphnia magna*	NOEC (21d) = 2.16 mg/L (Mean measured) Based on adult survival, total live young per female, total immobile young per surviving female and length and weight of surviving females	Registration dossier

No long-term toxicity test for 6:2 FTA or 6:2 FTMA on aquatic invertebrates is available.

7.8.1.3. Algae and aquatic plants

Table 28

Summary of effects on algae and aquatic plants			
Substance	**Method/ Organism**	**Results**	**Reference**
6:2 FTA	OECD Guideline 201 (Algae Growth Inhibition Test) *Selenastrum capricornutum* (new name: *Pseudokirchnerella subcapitata*) Limit test	EC_{50} (72h) > 0.0215 mg/L (meas. (geom. mean)) based on growth rate NOEC (72h) ≥ 0.0215 mg/L (meas. (geom. mean)) based on growth rate EC_{50} (72h) > 100 mg/L (nominal) based on growth rate	Registration dossier
6:2 FTMA	OECD Guideline 201 (Algae Growth Inhibition Test) static *Scenedesmus subspicatus* (new name: *Desmodesmus* Registration dossier *subspicatus*	EC_{50} (72h) > 0.008 mg/L dissolved (meas. (geom. mean) based on growth rate EC_{50} (72h) > 0.008 mg/L dissolved (meas. (geom. mean) based on biomass NOEC (72h) ≥ 0.008 mg/L dissolved (meas. (geom. mean) based on growth rate	Registration dossier
6:2 FTMA	OECD Guideline 201 (Algae Growth Inhibition Test) static *Pseudokirchnerella subcapitata*	EC_{50} (72h) > 24.6 mg/L (nominal) based on growth rate, biomass (healthy cell count), and cell number (healthy cell count yield) NOEC (72h) ≥ 24.6 mg/L (meas. (arithm. mean)) based on present inhibition of cell count, cell count yield and growth rate	Registration dossier
6:2 FTMA	OECD Guideline 201 (Algae Growth Inhibition Test) static *Pseudokirchnerella subcapitata*	EC_{50} (72h) > 0.013 mg/L (meas. (geom. mean) Based on growth rate NOEC (72h) ≥ 0.013 mg/L (meas. (geom. mean) Based on growth rate	Registration dossier
PFHxA	*Pseudokirchnerella subcapitata*	EC_{50} (72h) > 100 mg/l based on biomass EC_{50} (72h) > 100 mg/l based on growth rate	(Danish Environmental Protection Agency, 2015)
PFHxA	*Geitlerinema amphibium*	IC_{50} (72h) = 998.7 mg/L based on optical density	(Danish Environmental

			Protection Agency, 2015)
PFHxA	*Scenedesmus subspicatus*	EC_{50} (72h) = 86 mg/l based on growth rate NOEC (72h) = 50 mg/L based on growth rate	(Danish Environmental Protection Agency, 2015)
6:2 FTOH	OECD Guideline 201 (Algae Growth Inhibition Test) Static *Pseudokirchneriella subcapitata*	EC_{50} (72h) = 14.8 mg/L (measured) Based on growth rate NOEC (72h) = 2.22 mg/L (measured) Based on growth rate	Registration dossier
6:2 FTOH	OECD Guideline 201 (Algae Growth Inhibition Test) Static *Desmodesmus subspicatus*	EC_{50} (72h) = 7.8 mg/L (measured) Based on growth rate NOEC (72h) = 1.3 mg/L (measured) Based on growth rate	Registration dossier Reliability: 4 (Registrant reliability: 1)
6:2 FTOH	OECD Guideline 201 (Algae Growth Inhibition Test) Static *Pseudokirchneriella subcapitata*	EC_{50} (72h) > 5.19 mg/L (measured) Based on growth rate NOEC (72h) = 1.47 mg/L (measured) Based on growth rate	Registration dossier Reliability: 4 (Registrant reliability: 1)

7.8.1.4. Sediment organisms

No relevant information available.

7.8.1.5. Other aquatic organisms

Table 29

Summary of effects on other aquatic organisms			
Substance	**Method/ Organism**	**Results**	**Reference**
PFHxA	OECD Guideline 231 (Amphibian Metamorphosis Assay) *Xenopus laevis* 1.07, 10.7 and 107 mg/L (nominal)	NOEC (21d) = 10.7 mg/L (developmental stage, HLL increased) NOEC (21d) ≥ 107 mg/L (thyroid gland histopathology) NOEC (7d) = 1.07 mg/L (wet weight, snout-vent length increased)	Registration dossier

7.8.2. Terrestrial compartment

No relevant information available.

7.8.3. Microbiological activity in sewage treatment systems

Table 30

Summary of effects on microorganisms				
Substance	**Method**	**Results**	**Remarks**	**Reference**
6:2 FTA	OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test) Sewage, domestic	NOEC (3h) ≥ 1000 mg/L (nominal) EC_{10} (3h) > 1000 mg/L (nominal) EC_{50} (3h) > 1000 mg/L (nominal)		Registration dossier
6:2 FTMA	OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test) Activated sludge of a predominantly domestic sewage	EC_{50} (3h) > 100 mg/L (nominal) based on respiration rate	Evaluation of the registrant: Rel. 1 (key study)	Registration dossier

7.8.4. PNEC derivation and other hazard conclusions

Not part of the substance evaluation.

7.8.5. Conclusions for classification and labelling

Based on the available information, the eMSCA considers that classification of 6:2 FTA and 6:2 FTMA as Aquatic Chronic 1 is warranted. This classification is currently being implemented in the registration dossiers of both substances.

7.9. Human Health hazard assessment

The eMSCA considered not only data on 6:2 FTA (EC-No 241-527-8) and 6:2 FTMA (EC-No 218-407-9) but also data on 6:2 FTOH (EC-No 211-477-1) as the hydrolysis product and PFHxA (EC-No 244-479-6) as a potential metabolite of 6:2 FTOH. Data on the additional compounds were only presented when considered necessary for the evaluation as supporting information in a weight of evidence approach.

7.9.1. Toxicokinetics

<u>6:2 FTA</u>

Experimental data on the toxicokinetics of 6:2 FTA are not available.

The registrants concluded that the substance would be well absorbed by the gastrointestinal (GI) tract based on a molecular weight of 418.15 g/mol and underlined the assumption by systemic findings which were observed in an oral 28 d repeat dose toxicity

study (7.9.4.1). Concerning the dermal route, a default absorption rate of 10 % was assumed based on a molecular weight of 418.15 g/mol and a log P_{ow} value of 5.067. The inhalation route was considered irrelevant due to the high boiling point.

The registration of 6:2 FTA proposed that the substance has potential for bioaccumulation and that acrylic acid is formed during degradation of 6:2 FTA. Metabolites of acrylic acid are expected to distribute in liver, blood, or forestomach, and those of 6:2 FTOH will distribute in liver, blood, kidney, and other tissues (spleen, gonads, and brain) as well as fat.

With respect to metabolism, it was proposed by the registrant that perfluorooctyl acrylate would be slowly hydrolysed by esterase to corresponding acrylic acid and 6:2 FTOH. A scheme for further metabolism of 6:2 FTOH was proposed based on knowledge on 6:2 FTOH suggesting 6:2 fluorotelomer carboxylate (FTCA), 6:2 fluorotelomer unsaturated carboxylate (FTUCA), 5:3 FTUCA and 5:3 FTCA as intermediate metabolites and - following elimination of hydrogen fluoride (HF) - perfluorohexanoic acid (PFHxA) and perfluoroheptanoic acid (PFHpA) (anticipated to be minor amounts) as terminal metabolites. Sulfation and glucuronidation of 6:2 FTOH were also suggested. With respect to acrylic acid as a further metabolite resulting from initial ester cleavage it was proposed that it is degraded in the organism via hydroxypropionic acid and malonyl semialdehyde to acetyl-CoA, which is then oxidized to CO_2 in the citric acid cycle based on an oral study performed with acrylic acid in rats.

Parent compound and metabolites are suggested to be excreted via respiration, urine, and faeces.

The eMSCA concurs with the assumption of 10% dermal absorption. As the data available do not allow estimating the extent of oral absorption and as oral absorption has been evidenced by an oral 28 d repeat dose toxicity study, an oral absorption percentage of 100% is proposed.

The proposed metabolic fate appears plausible, however no quantitative figures on amounts of metabolites formed, distributed, and excreted can be made.

The eMSCA remarks that the statements made by the registrants are not supported by data.

<u>6:2 FTMA</u>

<u>*In vitro* studies</u>

In a study by DuPont (2011b), Hepatocytes from male and female Crl:CD(SD) rats and male and female Crl:CD1(ICR) mice were used to determine (1) the metabolic clearance of the test compound (6:2 FTMA, purity: 97.59%) by using a concentration of 5 µM and incubation times of 5, 15, 30, 45, 60, 90, and 120 minutes; heat inactivated cells were used as negative controls as well as (2) metabolites by using a concentration of 200 µM and incubation times of 10 and 30 min, respectively. Metabolites were identified by LC/MS and GC/MS.

(1) Metabolic clearance

Less than 5% of parent compound was detectable in hepatocytes from male and female rats at the first sampling point (5 min) indicating rapid metabolism. Also, data from male

mouse hepatocytes indicated rapid metabolism (no quantitative figures given). Data on female mouse hepatocytes were not given.

(2) Metabolism

Metabolites in male and female rat hepatocytes after 10 and 30 minutes were not quantified. A variety of metabolites was detected but not all of them were identified. Results indicated some sex-specific differences. From metabolite intensities, some major metabolites (quantities not given) were identified as 6:2 uFTOH-GH (major metabolite), 6:2 GTOH-Gluc, 6:2 diOH-diGluc, 6:2 FTOH (by GC/MS), 6:2 FMA-GS (male hepatocytes only), 6:2 FTOH-Sulf (female hepatocytes only), and 6:2 UAL-GS (isomer II DNPH deriv.) (female hepatocytes only). The abbreviations are not further explained in the dossier.

Concerning mouse liver hepatocytes only results from males were given. The major metabolite appeared to be 6:2 uFTOH-GS. Metabolites identified differed from those identified using rat hepatocytes.

For both studies, there were some differences compared to metabolites obtained from incubations using 6:2 FTOH. Further, data from rat hepatocytes indicated sex differences in metabolism. As only data on male mouse hepatocytes have been reported it is unclear whether sex specific metabolic differences are also present in the mouse.

Further, metabolism data indicated strain-specific metabolic differences.

In hepatocytes from male Crl:CD1(ICR) rats' rapid metabolism was observed using a concentration of 5 µM. In incubations using 200 µM the following metabolites (abbreviations not explained) were identified (quantities not given): 6:2 uFTOH-GH (major metabolite), 6:2 UA-GS, 6:2 uFTOH-N-acetyl-GS, 5:2 OH-Gluc, 6:2 FTOH-Gluc, and 5-2 Ketone (DNPH deriv.). The abbreviations are not explained in the dossier.

In vivo studies

Male Crl:CD(SD) rats (in groups of 3) received a single oral (not further specified) dose of 300 mg/kg 6:2 FTMA (purity: 97.59 %; dose vehicle: 0.5% methylcellulose, 0.1% TWEEN-80; dose volume: 4 ml/kg) (DuPont, 2011a). Blood was sampled before (0 hr) and 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hrs post dose. Livers and fat were sampled 6, 12, 24, 48, 72, 96, 120, 144, and 168 hrs post-dose.

At 1, 2 and 4 hr post dose, less than 40 ng/ml of parent substance was detected in plasma. At 6, 12, and 24 hr post-dose concentration of parent compound in liver was < 20 ng/ml. At 6, 12, 24, 48, 72 and 96 hr post-dose, concentration in fat ranged from < 20.0 to 99.9 ng/g.

The study points to rapid metabolism of the parent compound. No conclusion on the extent of absorption can be derived from that study.

A further *in vivo* study performed with 6:2 FTOH according to EPA OPPTS 870.3100 was used as a supporting study in order to inform on metabolism. Male and female Crl:CD(SD) rats (no information on number of animals used) received daily oral gavage doses of 0, 5, 25, 125 and 250 mg/kg bw/d 6:2 FTOH for 90 days. Plasma level of parent substance was below the limit of quantitation. 5-3A ($F(CF_2)_5CH_2CH_2COOH$) was the major metabolite observed in the plasma, liver, and fat samples at all dose levels and in both sexes (unclear at which time points measured). ($F(CF_2)_4CH_2CH_2COOH$) and PFPeA ($F(CF_2)_4COOH$) were also observed but in much lower concentrations than 5-3A.

For both studies, there were some differences compared to metabolites obtained from incubations using 6:2 FTOH. Further, data from rat hepatocytes indicated sex differences in metabolism. As only data on male mouse hepatocytes have been reported it is unclear whether sex specific metabolic differences are also present in the mouse.

Further, metabolism data indicated strain specific metabolic differences.

Thus, as metabolism of 6:2 FTMA appears to be complex, is not identical to that of 6:2 FTOH, as there are sex- and strain-specific differences and as metabolites have not been quantified the study cannot be used to justify read across between 6:2 FTOH and 6:2 FTMA. However, as 6:2 FTOH can be considered as one metabolite formed under the conditions of the experiments described above, information from toxicological studies performed with 6:2 FTOH can be used as supporting information in the context of weight–of-evidence considerations.

7.9.2. Acute toxicity and Corrosion/Irritation

These endpoints were not in the scope of the substance evaluation.

7.9.3. Sensitisation

This endpoint was not in the scope of the substance evaluation.

7.9.4. Repeated dose toxicity

7.9.4.1. 6:2 FTA

The registrant presented two subacute oral studies with the test compound in rats.

A 28-day study was performed in rats with three doses (5, 25 and 125 mg 6:2 FTA/kg bw/d) (Hita, 2007a). The test compound was dissolved in olive oil and applied via gavage. Effects were observed on the incisors, livers, and kidneys.

Decreased iron pigments and irregular alignment of the ameloblasts at the maturation stage and cyst formation in the papillary layer were observed in both sexes of the 125 mg/kg bw group. At necropsy, mottled teeth of the incisor were observed in both sexes, even after the recovery period.

In the liver, the following effects were observed in the 125 mg/kg bw/d group: periportal hypertrophy, periportal prominent nucleoli and single cell necrosis of the hepatocytes in males and diffuse hypertrophy of the hepatocytes in females. At necropsy, enlargement of the liver was observed in both sexes of the 125 mg/kg/d bw group. As for the organ weights, relative liver weight in males and absolute and relative liver weights in females were increased in the 125 mg/kg bw/d group. In the blood chemical examinations, increased levels of aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) and decreased levels of total cholesterol were found as the related changes in males of the 125 mg/kg group. In females of the same group, increased levels of gamma-glutamyl transpeptidase (gamme-GTP), triglyceride, albumin, albumin to globulin (A/G) ratio and total bilirubin and decreased level of cholinesterase were found.

In the kidney the following effects were observed in the 125 mg/kg bw/d group: Dilatation of the tubules in males and ballooning of the tubular epithelium in females. Furthermore, relative kidney weight in males given 25 mg/kg bw/d and more and absolute and relative kidney weights in females given 125 mg/kg bw/d were increased.

At the end of the recovery period of 14 days, absolute and relative testis weight was significantly increased (without histological effects) in the 125 mg/kg bw/d group as compared to the control recovery.

Another 28-day study was performed in rats with two doses (30 and 120 mg/kg bw/d) and control group for (Hita, 2014). The test compound was dissolved with olive oil including 0.5% w/v Tween 80 and applied via gavage.

Total bilirubin was increased in males of the 120 mg/kg bw/d group. Absolute weight of the liver was increased in males (+30%) and females (+35%) of the 120 mg/kg bw/d group. Relative weight of the liver was increased in males of the 30 and 120 mg/kg bw/d groups (+14 and +25%) and females of the 120 mg/kg bw/d group (+36%). Hypertrophy of the hepatocytes was observed in males and females of the 120 mg/kg bw/d group.

Relative kidney weights were increased (+11%) of the 120 mg/kg bw/d group without histopathological lesions.

Mottled teeth were observed in males and females of the 120 mg/kg bw/d group.

No data were available for the dermal or the inhalative route.

7.9.4.2. 6:2 FTMA

The registrant presented two subacute oral studies with the test compound in rats.

A 14-day study was performed with three dose levels (100, 500 and 1000 mg 6:2 FTMA/kg bw/d) over a period of 14 days (males) and 15 days (females) (DuPont, 2009). The compound was dissolved in deionized water and applied via gavage.

A 28-day study was performed with three dose levels (1, 5 and 25 mg/kg bw/d) (Hita, 2007b). The compound was dissolved in olive oil and applied via gavage.

No effects of the test substance were observed on clinical signs, body weight or food intake. The following effects were observed at the end of the dosing period: increased relative kidney weights in males and increased absolute and relative liver weights in females of the 25 mg/kg bw/d. In the histopathological examinations, decreased iron pigment of the ameloblasts at maturations stage in the incisors was observed in both sexes of the 25 mg/kg/d bw group. No abnormalities were observed in the hematology, blood chemical examinations or urinalysis.

In the recovery group after recovery, treatment-related changes (concerning incisor, kidneys, and livers) were not observable anymore apart from mottled teeth at necropsy, which was, however, not accompanied by histopathological changes in the ameloblasts. Therefore, the effects observed were considered reversible.

Considering all data derived from the test substance (subacute testing) and using data obtained from 6:2 FTOH as supporting information (sub chronic testing and one generation

reproductive toxicity study), the registrants classify the substance as STOT RE2 with effects on liver and teeth. Based on the available information the eMSCA supports this conclusion.

7.9.4.3. 6:2 FTOH

Details are provided in the CLH report or 6:2 FTOH prepared by the eMSCA available on ECHA dissemination site (ECHA, 2021b).

Data obtained from 6:2 FTOH was used by the registrant as supporting information based on a weight of evidence approach for some repeated dose toxicity studies of 6:2 FTA and 6:2 FTMA.

7.9.4.4. PFHxA

Details are provided in the Annex XV Restriction Proposal for PFHxA available on ECHA dissemination site (ECHA, 2021a). PFHxA is of relevance as it is considered as the final environmental degradation product of 6:2 FTA.

In a sub chronic toxicity study, main effects on SD rats included reduced body weight of males at the highest tested doses of 50 and 200 mg/kg bw/d. Further, increased liver enzymes and relative liver weight of males at 200 mg/kg bw/d (incl. histopathological changes), and increased relative kidney weights in males and females at 50 and 200 mg/kg bw/d were reported (Chengelis et al., 2009b).

In a combined sub chronic/reproductive toxicity study in SD rats, the main effects included decreased mean body weight in male rats in the highest tested dose of 500 mg/kg bw/d group (Loveless et al., 2009). Liver weights were increased, and other liver parameters changed in male rats dosed with 100 and 500 mg/kg bw/d. Minimal hypertrophy of thyroid follicular epithelium was present in male and female rats in the 500 mg/kg bw/d group. The effects were reversible after 90 days of recovery but not following 30 days of recovery.

In a combined chronic toxicity/carcinogenicity study in SD rats, a significant dose-related decrease in survival rates was observed in females (Klaunig et al., 2015). Histopathological changes were observed in the liver, kidney, and lung at the highest tested dose of 200 mg/kg bw/d.

In a subacute study in SD rats, the main effects included effects on the liver (organ weight, hypertrophy, liver enzymes) at 500 mg/kg bw/d, and increased kidney weight in males and females at the highest tested doses of 500 and 1000 mg/kg bw/d with chronic progressive nephropathy in females at the highest dose (NTP, 2019). Thyroid hormones in plasma were reduced (-18 % of T3, -25 % for fT4, -20 % for T4) in males; in females no significant changes of thyroid hormones were reported. TSH was not altered in either sex. The sperm counts in the cauda epididymis were reduced by -25 % and epididymal weight was reduced by -5 % at the highest tested dose of 1000 mg/kg bw/d.

7.9.4.5. Conclusion on repeated dose toxicity testing

Considering all data from the test substances (subacute testing) and using data obtained from 6:2 FTOH as supporting information, the registrants classify the substance as STOT RE2 with effects on liver and teeth. Based on the available information the eMSCA can support this conclusion.

7.9.5. Mutagenicity

This endpoint was not in the scope of the substance evaluation.

7.9.6. Carcinogenicity

This endpoint was not in the scope of the substance evaluation.

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

7.9.7.1. Fertility

7.9.7.1.1. 6:2 FTA

No studies addressing fertility effects of 6:2 FTA are available.

7.9.7.1.2. 6:2 FTMA

No studies addressing fertility effects of 6:2 FTMA are available.

7.9.7.1.3. 6:2 FTOH

Details are provided and discussed in the CLH report on 6:2 FTOH prepared by the eMSCA which is available on ECHA dissemination site (ECHA, 2021b).

7.9.7.1.4. PFHxA

Details are provided in the Annex XV Restriction Proposal for PFHxA available on ECHA dissemination site (ECHA, 2021a).

7.9.7.2. Developmental toxicity

7.9.7.2.1. 6:2 FTA

No studies addressing developmental toxicity of 6:2 FTA are available.

7.9.7.2.2. 6:2 FTMA

A Prenatal Developmental Toxicity Study according to OECD guideline 414 was performed for FTMA in rats (Toxi-Coop Zrt, 2017). Wistar rats (22-24 sperm-positive females) were exposed to three dose levels (100, 320, and 1000 mg 6:2 FTMA/kg bw/d) for 15 days (gestational day 5-19). The test substance was administered in olive oil as a vehicle by oral gavage.

No clinical signs or mortality were observed. Body weight reduced in 1000 mg/kg bw/d group on gestational day (GD) 8 and 20. Body weight gain was lower in the periods GD 5-8, GD 17-20, GD 5-20, and GD 0-20. Corrected body weight on GD 20 and corrected bw gain from GD 0-20 was also reduced, which was interpreted by the registrant as indicative for maternal toxicity. Reduced body weight and body weight gain was associated with reduced food consumption throughout exposure period in 1000 mg/kg bw/d group. Food consumption was also lowered in the 320 mg/kg bw/d group (GD 3-5, GD 5-8, GD 17-20) and in the 100 mg/g bw/d group (GD 17-20). For one dam in the 1000 mg/kg bw/d group, a distended stomach, weak and foamy appearance of the small intestine, pale and enlarged

kidneys were observed. Another female in the 1000 mg/kg bw/d group had pin-head-sized hemorrhages in the stomach.

Mean placental weight (mean of litter mean values) was reduced in the 1000 mg/kg bw/d group. Reduced pre-implantation loss and total intrauterine mortality per litter in 100 and 1000 mg/kg bw/d groups were observed compared to control. Total early embryonic death was statistically significantly increased in the 320 mg/kg bw/d group but reduced in the 1000 mg/kg bw/d group. Fetal body weights (mean of litter mean values) were lower in the 1000 mg/kg bw/d group compared to control group. No effects were observed for the number of live offspring of sex ratio. Significant increases of incomplete ossification of one or more skull bones, delayed ossification of sacral arches and centra, and incidence of wavy ribs were observed in the 1000 mg/kg bw/day group.

7.9.7.2.3. 6:2 FTOH

Details are provided in the CLH report for 6:2 FTOH available on ECHA dissemination site (ECHA, 2021b).

7.9.7.2.4. PFHxA

Details are provided in the Annex XV Restriction Proposal for PFHxA available on ECHA dissemination site (ECHA, 2021a). In a recent proposal, a harmonised classification for PFHxA and its salts as Repr. 1B (H360D; May damage the unborn child) has been submitted (ECHA, 2021b).

7.9.7.3. Conclusion on reproductive toxicity

No study for either fertility or developmental toxicity has been provided performed with 6:2 FTA. In a prenatal developmental toxicity study with 6:2 FTMA, reduced maternal and pup body weights and/or body weight gains were observed at 1000 mg/kg bw/d. In conclusion, 6:2 FTA and 6:2 FTMA themselves cannot directly be considered reproductive toxicants.

Further reproductive toxicity studies are available for the degradation products 6:2 FTOH and PFHxA. For 6:2 FTOH, the available data do not suggest direct reproductive toxicity. However, PFHxA (as the final degradation product of 6:2 FTA, 6:2 FTMA and 6:2 FTOH) increased pup mortality in studies with mice (Iwai and Hoberman, 2014). The proposal for a harmonised classification of PFHxA and its salts as Repr. 1B (H360D; May damage the unborn child) has been submitted recently (ECHA, 2021b).

7.9.8. Hazard assessment of physico-chemical properties

Not considered relevant for the substances of this substance evaluation.

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

The eMSCA decided to omit an exposure assessment for the consumer. Therefore, it was also decided to omit the development of DNELs. The human health hazard assessment was restricted to the identification of human health hazards.

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

Considering all data from the test substances (subacute testing) and using data obtained from 6:2 FTOH as supporting information, the registrants classify the substances as STOT RE 2 with effects on liver and teeth. Based on the available information the eMSCA can support this conclusion. However, the majority of notifiers do not apply this self-classification for 6:2 FTA and 6:2 FTMA.

No study for either fertility or developmental toxicity performed with 6:2 FTA has been provided. In the prenatal developmental toxicity study with 6:2 FTMA, reduced maternal and pup body weights and/or body weight gains were observed at 1000 mg/kg bw/d. Further reproductive toxicity studies are available for the degradation products 6:2 FTOH and PFHxA.

Details are provided in the Annex XV Restriction Proposal for PFHxA available on ECHA dissemination site (ECHA, 2021a) as well as in the CLH report or 6:2 FTOH available on ECHA dissemination site (ECHA, 2021b).

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

Studies assessing endocrine disrupting properties of 6:2 FTA and 6:2 FTMA, 6:2 FTOH and PFHxA are discussed in this chapter. According to chapter 7.7, 6:2 FTOH and PFHxA are potential degradation products of 6:2 FTA as well as 6:2 FTMA. Data on endocrine disrupting properties are not available for 6:2 FTA as well as 6:2 FTMA and for degradation products other than 6:2 FTOH and PFHxA (such as FTCA, FTUCA). Nevertheless, for other PFASs – especially long-chain PFASs - studies are available showing endocrine properties. As effects of long-chain PFASs have been investigated more thoroughly compared to short-chain PFASs the information on long-chain PFASs is given as supplementary information to show that concerns on ED effects of this group of substances cannot be excluded and therefore attention should be given to them. A tabular view of the results organized according to individual substances and substance classes is presented in Annex III (Table 12-A / 12-B / 12-C).

7.10.1.1. Endocrine disrupting properties of 6:2 FTA

No data on endocrine disrupting properties are available for 6:2 FTA. Benninghoff et al. (2011) assessed the *in vitro* binding properties of 8:2 FTA to trout hepatic estrogen receptor (ER) up to 1mM finding no binding in a competitive assay with estradiol (E_2).

7.10.1.2. Endocrine disrupting properties of 6:2 FTMA

No data on endocrine disrupting properties are available.

7.10.1.3. Endocrine disrupting properties of 6:2 FTOH

Interaction with the hypothalamus-pituitary-thyroid axis

Regarding interaction with the hypothalamus-pituitary-thyroid (HPT) axis only two studies using *in vitro* assays are available:

Ren et al. (2015)
Method: using GH3 rat pituitary cancer cells a T-screen assay was performed. The competitive binding of 6:2 FTOH to the human thyroid receptor (TR) ligand binding domain was tested in presence of fluorescein tagged T_3.
Results: no competitive binding was found in the concentration range tested (1-100000 nM) for 6:2 FTOH. 6:2 FTOH showed also no agonistic or antagonistic activity in the T-screen assay in concentrations of 5-500 µM and 125 µM, respectively.
→ Klimisch reliability 2- well documented, non-guideline study without analytical determination of the test concentrations.

Weiss et al. (2009)
Method: using radiolabeled ^{125}I-labeled T_4 (thyroxine) the competitive binding of 6:2 FTOH with T_4 to the human TTR (transthyretin) was assessed for a concentration range of 10-10000 nM.
Results: no competitive binding was found in the concentration range tested for 6:2 FTOH.
→ Klimisch reliability 2- well documented, non-guideline study without analytical determination of the test concentrations.

Interaction with the hypothalamus-pituitary-gonadal (HPG) axis

To assess the interaction with the estrogen signalling both *in vitro* and *in vivo* data are available:

Benninghoff et al. (2011)
Method in vitro: an ER competitive binding assay was conducted by incubating liver cytosols from E_2 exposed trout in the presence of [3H]-estradiol and increasing concentrations of 6:2 FTOH (10^{-7}-10^{-3} M) for 24h.
Results in vitro: 6:2 FTOH exhibited no response in the tested concentration range.
Method in vivo: The same study investigated the *in vivo* VTG (vitellogenin) induction in blood plasma of juvenile trout in a subchronic dietary exposure (14d) to 250 ppm 6:2 FTOH. VTG was determined using ELISA (Enzyme Linked Immunosorbent Assay).
Results in vivo: 6:2 FTOH exhibited an 8-fold increase VTG induction compared to the control.
→ Klimisch reliability 2- well documented, non-guideline study without analytical determination of the test concentrations.

Ishibashi et al. (2007)
Method: estrogenic effects of 6:2 FTOH in a concentration range of 0.01-1000 µM were assessed during 4h exposure using a yeast two-hybrid assay with human ERα or ERβ and coactivator TIF2 using β-galactosidase as reporter.
Results: Interaction was visible starting at 0.1 µM; EC_{10} for ERα and ERβ were 2.3 and 4.1 µM, respectively. These results show a possible stimulation of human ER mediated target gene transcription, thus giving an indication for estrogenic mode of action.
→ Klimisch reliability 2 – well documented, non-guideline study without analytical determination of the test concentrations.

Ishibashi et al. (2008)
Method in vitro: estrogenic effects of 6:2 FTOH in a concentration range of 0.01-1000 µM were assessed during 4h exposure using a yeast two-hybrid assay with medaka ERα and coactivator TIF2 using β-galactosidase as reporter.
Results in vitro: the authors reported a concentration-dependent interaction between medaka ERα and coactivator TIF2 for 6:2 FTOH showing a possible stimulation of medaka ER mediated target gene transcription, thus giving an indication for estrogenic mode of action. Effects of 6:2 FTOH (EC_{10} = 0.26 µM) started at 0.1 µM and FTOH had a 0.16% affinity, compared to the positive control E_2 (EC_{10} = 410 pM).
Method in vivo: In the same study, *in vivo* estrogenic effects were also assessed by exposing adult medakas to 6:2 FTOH treated water (nominal concentration of 0.01, 0.1, 1, 10 and 100 µM) over three days for hepatic VTG analysis and 8h for gene expression analysis of ERα, ERβ, VTGI and VTGII. VTG analysis was performed using ELISA, gene expression analysis was conducted with qPCR (quantitative polymerase chain reaction).
Results in vivo: 6:2 FTOH caused a concentration-dependent induction of VTG and hepatosomatic index (HSI) in male medakas. Significant changes were seen starting at 1 µM 6:2 FTOH regarding VTG levels, and 10 µM regarding HSI, respectively. In addition, a significant increase in gene expression induction exhibited by 6:2 FTOH for ERα, and for two VTG genes, but not for ERβ was observed. Concentration-dependent gene expression changes were seen for ERα only, starting at 10 µM.
→ *Klimisch reliability 2 – well documented, non-guideline study without analytical determination of the test concentrations.*

Liu et al. (2007)
Method: VTG induction exhibited by 6:2 FTOH was assayed in primary cultured hepatocytes of freshwater male tilapia and compared with that of known estrogenic compounds. Time-course (0-96h with 14µM 6:2 FTOH) and dose dependent (48h with 1.4-54 µM 6:2 FTOH) VTG induction were assessed using single-compound-exposure. Binary exposures (0.22-22 µM 6:2 FTOH) to E_2 or tamoxifen (anti-estrogen, selective estrogen-receptor modulator) were investigated to elucidate ER-mediated effects. Hepatocyte viability was determined by comparing mitochondrial MTT activity. A non-competitive ELISA was employed to determine the VTG production.
Results: Hepatocyte cell viability was unchanged in all treatment groups compared to controls. Significant VTG induction took place after 12h (1.4×10^{-5} M 6:2 FTOH), and the VTG production increased further after 96h of exposure. A concentration-dependent induction of VTG was observed in E_2, 4-nonylphenol (4-NP) and 6:2 FTOH-treated cells. The estimated 48h EC_{50} values for E_2, 4-NP and 6:2 FTOH were 0.47 µM, 7.1 µM and 28 µM, respectively. Reduction of VTG induction could be observed when 30 µM 6:2 FTOH was applied in combination with 10 µM tamoxifen meaning that 6:2 FTOH presumably exhibits its effect via the ER directly or an involved co-factor. Interestingly, 6:2 FTOH showed also anti-estrogenic effects (IC_{50}=1.1 µM) when applied in combination with E_2. The different mode of action of 6:2 FTOH can be accounted for conformational changes of the ER provoked by the substance.
→ *Klimisch reliability 2 – well documented, non-guideline study without analytical determination of the test concentrations.*

Liu et al. (2009)
Method: An *in vivo* study was conducted by Liu et al. (2009) using adult zebrafish exposed to 0.03, 0.3 and 3.0 mg/l (0.08, 0.8 and 8.2 µM) 6:2 FTOH for 7 days. Effects on plasma sex hormone levels and gene expression of selected genes of the HPG axis were measured

in liver, gonad, and brain. Sex hormones were measured using ELISA, gene expression was analysed with qPCR.
Results: Exposure to 6:2 FTOH significantly increased plasma E_2 and testosterone (T) levels in both males and females (LOEC 0.08 and 0.8 µM, respectively). Furthermore, the ratio of T/E_2 was reduced in females while increased in males (LOEC 0.08 and 0.8 µM, respectively). In females, the increase of E_2 was accompanied by upregulated hepatic VTG (VTG1 and VTG3, LOEC 0.08 µM), downregulation of gonad ERα and ERβ (LOEC 0.8 and 0.08 µM, respectively) and upregulation of the brain activin and activin receptor (putative mediator of gonadotropin-induced oocyte maturation, LOEC 8.2 µM) gene expression. In males, the elevation of the T level is consistent with the altered regulation of some enzymes playing a role in the steroid biosynthesis (CYP17 and CYP19A). In males, gonadal CYP19B, ERα, ERβ, GnRH2 and FSH were upregulated (LOEC 0.08, 0.08, 0.8, 8.2 and 0.08 µM, respectively), while hepatic VTG showed a concentration-dependent decreasing upregulation with increasing concentration (LOEC 0.08 µM).
→ *Klimisch reliability 2 – well documented, non-guideline study without analytical determination of the test concentrations.*

Maras et al. (2006)
Method: Maras et al. (2006) investigated estrogen-like properties of 6:2 FTOH using a combination of three *in vitro* assays: E-screen assay using MCF-7 breast cancer cells (incubation for 6d to 0.1-30 µM 6:2 FTOH, analysis of proliferation with CyQuant assay), cell cycle analysis (incubation for 24 h to 0.3-30 µM 6:2 FTOH, cell cycle distribution and apoptosis were analysed with flow cytometer), and gene expression analysis using qPCR of estrogen-responsive biomarker genes exposed for 48 h to 30 µM 6:2 FTOH (trefoil factor 1, progesterone receptor, ER, ERBB2 and PDZK1).
Results: By means of an E-screen assay, the proliferation-promoting capacity of 10 µM 6:2 FTOH was detected. Exposure to 6:2 FTOH stimulated resting MCF-7 cells to reenter the synthesis phase of the cell cycle. Furthermore, similar to E_2 and 4-NP 6:2 FTOH induced the expression of some estrogen responsive genes, although showing lower fold induction changes. Based on this latter finding, the authors hypothesized different estrogenic mode of action of 6:2 FTOH compared with that of E_2.
→ *Klimisch reliability 2 – well documented, non-guideline study without analytical determination of the test concentrations.*

Fish Sexual Development Test from registration dossier provided to fulfil the information requirements from the SEV decision (2021)
Method: The study was conducted according to OECD Guideline 234 under semi static conditions with daily renewal of media using medaka (*Oryzias latipes*). The test duration was 122 days (112 days post-hatch). The endpoints measured were growth, secondary sex characteristics, genetic sex, vitellogenin (VTG in liver homogenate measured via ELISA). In addition, gonad histology was performed to determine the phenotypic sex of the fish. The fish was exposed to the nominal concentrations: 0.030, 0.096, 0.30, 0.96 and 3.0 mg/L (mean measured concentrations: 0.00933, 0.0137, 0.0231, 0.0537 and 0.0953 mg/L). A high variability in the measured concentrations was reported owing to the volatility of the test substance.
Results: Statistically significant effects on hatching success at nominal 0.96 mg/L (0.0537 mg/L measured) and higher as well as on survival and growth (male and female body length and weight) at the highest nominal test concentration of 3.0 mg/L (0.0953 mg/L measured) were reported. Sex ratio was not significantly affected in terms of either genetic or phenotypic sex ratio. There were no significant effects on female liver VTG. A significant

increase in male liver VTG at 0.3 mg/L (0.0231 mg/L measured) as well as a significant decrease in the number of anal fin papillae was observed at the 3.0 mg/L (0.0953 mg/L measured) in males.
→ *Klimisch reliability 1 – guideline study with analytical determination of the test concentrations.*

More information is available to ED properties of other FTOH homologues (see Annex III). E.g., 8:2 FTOH exhibits disruption of sex hormone biosynthesis and interacts with reproduction of zebrafish.

Table 31

(Anti)estrogenic properties of 6:2 FTOH					
Test system	**Endpoint**	**Effect concentration of positive control (µM)**	**Effect concentration of 6:2 FTOH (µM)**	**Reference**	**Reliability**
Cytosol isolated from rainbow trout liver	Competitive binding to ER (exposure 24h)	E_2: IC_{50} =0.0139 EE_2: IC_{50} =0.0589 4-NP: IC_{50} =842 Based on nominal concentrations	LOEC>1000 Based on nominal concentrations	Benninghoff et al., 2011	2 – well documented, non-guideline study without analytical determination of the test concentrations
Trout *in vivo* subchronic dietary exposure	VTG induction (Exposure 14d)	E_2: LOEC=5 ppm in diet caused 10^4-fold change induction Based on nominal concentrations	LOEC=250 ppm in diet caused 8-fold change induction Based on nominal concentrations	Benninghoff et al., 2011	2 – well documented, non-guideline study without analytical determination of the test concentration
Yeast two-hybrid assay with human ERα or ERβ and coactivator TIF2	Induction of human ER mediated transcriptional activity (exposure of 4h)	E_2: LOEC≈3×10^{-5}* E_2: EC_{10}=9×10^{-5} Based on nominal concentrations	LOEC=0.1* $EC_{10ERα}$=2.3 $EC_{10ERβ}$=4.1 Concentration-dependent effects	Ishibashi et al., 2007	2 – well documented, non-guideline study without analytical determination of the test concentration
Yeast two-hybrid assay with medaka ERα and coactivator TIF2	Induction of medaka ER mediated transcriptional activity (exposure of 4h)	E_2: LOEC≈0.000156* E_2: EC_{10}=0.00041 Based on nominal concentrations	LOEC≈0.1* EC_{10}=0.26 Based on nominal concentrations Concentration-dependent effects	Ishibashi et al. 2008	2 – well documented, non-guideline study without analytical determination of the test concentrations
Medaka *in vivo* acute waterborne exposure	VTG, HSI induction (exposure 3d) Gene expression induction of ERα, ERβ, VTG I, VTGII (exposure 8h)	Not measured	$LOEC_{VTG}$=1 $LOEC_{HSI}$=10 $LOEC_{Gene\ exp\_ERα}$=100 $LOEC_{Gene\ exp\_ERβ}$>100 $LOEC_{Gene\ exp\_VTGI}$=100 $LOEC_{Gene\ exp\_VTGII}$=100 Based on nominal concentrations Concentration-dependent effects were seen for VTG, HIS and Gene expression ERα	Ishibashi et al. 2008	2 – well documented, non-guideline study without analytical determination of the test concentrations

(Anti)estrogenic properties of 6:2 FTOH					
Test system	**Endpoint**	**Effect concentration of positive control (µM)**	**Effect concentration of 6:2 FTOH (µM)**	**Reference**	**Reliability**
Medaka *in vivo* fish sexual development test (OECD TG 234)	growth, secondary sex characteristics, genetic sex, VTG (Exposure 122d)		LOEC = 0.0537 mg/L (mm) (hatching success) LOEC = 0.0953 mg/L (mm) (survival and growth – male + female body length and weight) LOEC ≥ 0.0953 mg/L (mm) (genetic + phenotypic sex ratio) LOEC = 0.0231 mg/L (mm) (VTG male) LOEC = 0.0953 mg/L (mm) (sec. sex char. – male anal fin papillae count)	Registration dossier	1 – guideline study with analytical determination of the test concentrations
Primary cultured hepatocytes of freshwater male tilapia	VTG induction Inhibition of VTG induction when co-treated with E_2 (Exposure of 48h)	E_2: $LOEC_{VTG\ induction}$=0.18 E_2: $EC_{50\_VTG\ induction}$=0.47 4-NP: $LOEC_{VTG\ induction}$=2.3 4-NP: $EC_{50\_VTG\ induction}$=7.1 Based on nominal concentrations	$LOEC_{VTG\ induction}$=2.7 $EC_{50\_VTG\ induction}$=28 $IC_{50\_VTG\ inhibition}$=1.1 Based on nominal concentrations Both VTG induction and inhibition concentration-dependent	Liu et al., 2007	2 – well documented, non-guideline study without analytical determination of the test concentrations
Zebrafish *in vivo* subchronic waterborne exposure	Gene expression (ER_α, ER_β, VTG1, VTG3, CYP17, CYP19A, CYP19B, Activin) Changes of sex hormone levels BSI, HSI, GSI (Exposure 7d)	Not measured	$LOEC_{HSI}$=8.2 $LOEC_{GSI}$=0.8 $LOEC_{BSI}$>8.2 $LOEC_{T\_males}$=0.08 $LOEC_{T\_females}$=0.8** $LOEC_{E2\_males}$=8.2 $LOEC_{E2\_females}$=0.8** $LOEC_{gene\ exp\_males}$=0.08 $LOEC_{gene\ exp\_females}$=0.08 Based on nominal concentrations	Liu et al., 2009	2 – well documented, non-guideline study without analytical determination of the test concentration

(Anti)estrogenic properties of 6:2 FTOH					
Test system	**Endpoint**	**Effect concentration of positive control (µM)**	**Effect concentration of 6:2 FTOH (µM)**	**Reference**	**Reliability**
E-screen assay and gene expression assay using real-time PCR	Proliferation of MCF-7 cells, increased percentage of cells in S-phase Upregulation of trefoil factor 1, progesterone receptor, ERα, and PDZK1 Down-regulation of ERBB2	E_2: $LOEC_{prolif}$≈0.00001* E_2: $LOEC_{gene\ exp}$=0.001* (for all measured genes, except for ERα) 4-NP: $LOEC_{prolif}$≈3* 4-NP: $LOEC_{gene\ exp}$=0.001* (for all measured genes, except for ERα and progesterone receptor) Based on nominal concentrations	$LOEC_{prolif}$=10* $LOEC_{gene\ exp}$=30*(for all measured genes) Based on nominal concentrations	Maras et al., 2006	2 – well documented, non-guideline study without analytical determination of the test concentration

*No statistical analysis was provided. Value was derived by visual observation of the results for comparison.
**Not concentration-dependent

7.10.1.4. Endocrine disrupting properties of PFHxA

Interaction with the hypothalamus-pituitary-thyroid axis

Regarding interaction with the HPT axis four *in vitro* assays are available:

Ren et al. (2015)
Method: using GH3 rat pituitary cancer cells a T-screen assay was performed to test for thyroid hormone dependent cell proliferation in order to identify thyroid hormone receptor active compounds. The competitive binding of 200-500000 nM PFHxA to the human TR ligand binding domain was tested in presence of fluorescein tagged T_3.
Results: PFHxA exhibited very low binding (0.06%) in comparison with T_3. PFHxA showed no agonistic or antagonistic activity in the T-screen assay in the concentrations tested (5-500 µM and 15 µM).

→ *Klimisch reliability 2 - well documented, non-guideline study without analytical determination of the test concentrations.*

Naile et al. (2012)
Method: Rat H4IIE hepatoma cells were exposed for 72h to 0.1-100 µM PFHxA and changes in mRNA abundance of thyroid-, cholesterol- and lipoprotein related genes were quantified by qPCR. Regarding thyroid-related genes PAX and HEX expression playing a role in thyroid development were tested. Cell viability was assessed visually.
Results: The cell viability was not affected by the treatment. An upregulation of the gene expression could be observed for PFHxA starting at 0.1µM, although not in a concentration-dependend way.

→ *Klimisch reliability 2 - well documented, non-guideline study without analytical determination of the test concentrations.*

Vongphachan et al. (2011)
Method: A gene expression assay was performed on avian embryonic neuronal cells of domestic chicken and herring gull investigating key genes in the thyroid hormone pathway (iodothyronine 5'-deiodinase 2 and 3 –D2/D3, transthyretin –TTR, neurogranin –RC3, octamer motif-binding factor –Oct-1, and myelin basic protein –MBP). Primary cultures of chicken embryonic neuronal cells and herring gull embryonic neuronal cells were prepared from the cerebral cortices of day 11 or 14 embryos, respectively. PFHxA was administered at concentrations of 0.01, 0.1, 1, 3, 10 and 50µM (the latter was applied only for chicken neuronal cells) over 24h. Cell viability was estimated using the Calcein-AM assay. After RNA isolation and cDNA synthesis, qPCR was performed to investigate gene expression changes. In chicken cells, expression of genes of D2, D3, TTR, RC3, Oct-1, in herring gull cells, genes of D2, RC3, Oct-1 were investigated. As positive control for the gene expression analyses, T_3 was used.
Results: PFHxA decreased the cell viability in concentrations > 10µM, therefore this was the highest applied concentration in the definitive test. PFHxA altered significantly the expression of MBP (LOEC 10µM), D2 (LOEC 10 µM) and D3 (LOEC 3µM) genes in chicken embryonic neuronal cells, although for D3 not in a concentration-dependent way (for MBP and D2 only the highest tested concentration exhibited significant expression changes). For the rest of the genes, no changes in gene expression were observed. The positive control, T_3 exhibited gene expression induction for D2, RC3 (LOEC 3 nM) but not for TTR, D3, Oct-1 and MBP. In herring gull embryonic neuronal cells changes were seen only for

Oct-1 expression following PFHxA exposure (LOEC 3 µM). The positive control T_3 was effective for RC3 (LOEC 300 nM) but not for D2 and Oct-1.

→ Klimisch reliability 2 – well documented, non-guideline study without analytical determination of the test concentrations.

Weiss et al. (2009)
Method: using radiolabeled ^{125}I-labeled T_4 the competitive binding of PFHxA with T_4 to the human TTR was assessed for a concentration range of 10-10000 nM.
Results: PFHxA exhibited 0.7% of activity of T_4 with IC_{50} of 8220 nM (IC_{50} of T_4 = 61 nM) showing a concentration-dependent inhibition of T_4 bound to TTR.

→ Klimisch reliability 2 – well documented, non-guideline study without analytical determination of the test concentrations.

Amphibian Metamorphosis Assay from registration dossier provided to fulfil the information requirements from the SEV decision (2021)
Method: OECD TG 231 exposing *Xenopus laevis* (NF stage 51) in a flow-through test over 21 days to 1.07, 10.7 and 107 mg/L (nominal; mean recovery values of 95, 98% and 105%). Endpoints measured were hind limb length (HLL), snout to vent length (SVL), developmental stage, wet weight, and thyroid histology. Survival and behaviour were monitored daily. The study encompassed 4 treatment groups (3 concentrations of the test item and a negative control) with 4 replicates each containing 20 tadpoles.
Results: The AMA provides evidence for thyroidal effects as the developmental stage as well as the hind limb length were significantly increased at 107 mg/L on day 21. Wet weight and snout-vent length were significantly increased at 10.7 mg/L on day 7.

More studies are available investigating thyroid disruption of other PFCAs. Both PFOA and PFNA altered the expression of genes playing a role in thyroid morphogenesis, TH synthesis, transport or metabolism and caused histopathological alterations in the thyroid follicles in fish (see Annex III).

<u>Interaction of PFHxA with the hypothalamus-pituitary-gonadal axis</u>

To assess the interaction with the estrogen signaling the following studies are available:

Benninghoff et al. (2011)
Method in vitro: an ER competitive binding assay was conducted by incubating liver cytosols from E_2 exposed trout in the presence of [3H]-estradiol and increasing concentrations of PFHxA (10^{-6}-10^{-2} M) for 24h.
Results in vitro: PFHxA exhibited an IC_{50} of 1.22 mM and a 0.0011% binding affinity relative to E_2 (LOEC and binding affinity for ethinylestradiol EE_2 were 0.0589µM and 23.7% and for 4-NP 842µM and 0.0017%, respectively). Higher homologues of PFHxA were also investigated. These showed similar IC_{50} values ranging between 0.234-2.89 mM. There was no correlation between fluorinated alkyl chain length and relative binding affinity.

Method in vivo: The same study investigated the *in vivo* VTG induction in blood plasma of juvenile trout in a subchronic dietary exposure (14 d) to 250 ppm PFHxA. VTG was determined using ELISA.
Results in vivo: PFHxA did not exhibit significantly the VTG induction compared to the control. However, a slight increase in VTG could be observed. Higher homologues of PFHxA were also investigated. Until 11 fluorinated alkyl chain, these homologues induced

significantly the VTG expression, the magnitude of induction was dependent on the fluorinated alkyl chain length. Some of these homologues were assessed in more concentrations (2, 50, 250 ppm) showing concentration dependent VTG expression. Presumably, the low effect of PFHxA *in vivo* is related to the toxicokinetic characteristics of PFHxA (i.e., low internal concentration), as just slight and not fluorinate alkyl-chain dependent differences in affinity could be seen *in vitro* between C6-C11 homologues.

→ *Klimisch reliability 2- well documented, non-guideline study without analytical determination of the test concentrations.*

Gorrochategui et al. (2014)
Method: aromatase inhibition of 500 µM PFHxA using human placental choriocarcinoma cell line (JEG-3) was assessed.
Results: PFHxA exhibited no effects in contrast to other PFASs tested (PFOS, PFOA and PFBS- see Annex III).
→ *Klimisch reliability 2- well documented, non-guideline study without analytical determination of the test concentrations.*

Wielogorska et al. (2015)
Method: A reporter gene assay using MMV-Luc cell line was used to assess the effects of PFHxA on estrogen nuclear receptor transactivation. Both agonistic and antagonistic (in presence of 10^{-10}M E2) activities were tested with a single concentration of 10µM PFHxA (24h exposure). Cell viability was determined by comparing mitochondrial MTT activity.
Results: PFHxA exhibited neither agonistic nor antagonistic effects.
→ *Klimisch reliability 2- well documented, non-guideline study without analytical determination of the test concentrations.*

Higher PFCA homologues were shown to affect expression of genes, proteins, and hormones in HPG axis in zebrafish. Histopatholgical alterations were observed followed by exposure to PFOA in gonads of rare minnows. Developmental effects in offspring of PFOA-exposed medaka were also noticed (see Annex III).

Table 32

Thyroid disrupting properties of PFHxA					
Test system	**Endpoint**	**Effect concentration of positive control (µM)**	**Effect concentration of PFHxA (µM)**	**Reference**	**Reliability**
Human TR binding assay	Competitive (with T_3) binding to TR	T_3: IC_{50}=0.3	IC_{50}~500*	Ren et al., 2015	2 – well documented, non-guideline study without analytical determination of the test concentrations
Rat hepatoma cells	Gene expression of PAX8 and HEX	Not measured	LOEC=0.1**	Naile et al., 2012	2 – well documented, non-guideline study without analytical determination of the test concentrations
Domestic chicken/ herring gull embryonic neuronal cells	Gene expression of D2, D3, MBP, TTR, RC3, Oct1	T_3 in Domestic chicken: $LOEC_{D2}$=0.003 $LOEC_{RC3}$=0.003 $LOEC_{TTR, D3, Oct-1, MBP}$ >0.03 T_3 in Herring gull: $LOEC_{RC3}$=0.3 $LOEC_{D2, Oct-1}$>0.3	Domestic chicken: $LOEC_{D2}$=10 $LOEC_{D3}$=3** $LOEC_{MBP}$=10 $LOEC_{TTR, RC3, Oct1}$>10 Herring gull: $LOEC_{Oct1}$=3 $LOEC_{D2, RC3,}$>10	Vongphachan et al., 2011	2 – well documented, non-guideline study without analytical determination of the test concentrations
Human TTR binding assay	Competitive (with T_4) binding to TTR	T_4: IC_{50}=0.061	IC_{50}=8.2	Weiss et al., 2009	2 – well documented, non-guideline study without analytical determination of the test concentrations
Amphibian Metamorphisis Assay according to OECD TG 231	Developmental stage, wet-weight (d7, d21), snout-vent length (d7, d21), hind limb length (normalised by SVL; d7, d21), survival, behaviour, thyroid gland histopathology		LOEC= 107 mg/L (d21; developmental stage, HLL increased) LOEC ≥ 107 mg/L (thyroid gland histopathology) LOEC = 10.7 mg/L (d7; wet weight, snout-vent length increased)	Registration dossier	1 – guideline study with analytical determination of the test concentrations

*Estimated by linear interpolation.
**Not concentration-dependent

7.10.2. Endocrine disruption - Human health

Studies assessing endocrine disrupting properties of 6:2 FTA, 6:2 FTMA, 6:2 FTOH and PFHxA are discussed in this chapter. According to chapter 7.9.1, 6:2 FTOH and PFHxA are potential degradation products of 6:2 FTA and 6:2 FTMA. Data on endocrine disrupting properties are not available for 6:2 FTA, 6:2 FTMA and other degradation products (such as FTCA, FTUCA).

7.10.2.1. Endocrine disrupting properties of 6:2 FTA and 6:2 FTMA

No data on endocrine disrupting properties are available either for 6:2 FTA or for 6:2 FTMA. Benninghoff et al. (2011) assessed the *in vitro* binding properties of 8:2 FTA, a structurally similar compound, to trout hepatic estrogen receptor (ER) up to 1mM finding no binding in a competitive assay with estradiol (E_2).

Zhang et al. (2020) modelled half-maximal effective concentrations (EC_{50}) of the activation of human pregnane X receptor (hPXR) for 6:2 FTA (-logEC_{50}=3.03) and compared it to observed and predicted EC_{50} values of other perfluorinated compounds, a.o., PFHxA (observed/predicted -logEC_{50} = 4.70/4.65) and 6:2 FTOH (observed/predicted –logEC_{50} = 5.01/5.15, Zhang et al., 2017, 2020). Hence, it can be assumed that all three compounds bind to hPXR, which is postulated by the authors to be associated with endocrine disruption.

For 6:2 FTA, two subacute 28-day oral repeated-dose toxicity studies with rats are available in the registration dossier (equivalent to OECD TG 407 (Hita, 2007a; Hita, 2014), more details in 7.9.4). In one of the two studies, histopathology was performed in the following organs relevant for endocrine effects: testes, epididymis, prostate, seminal vesicle, ovaries, uterus, vagina, pituitary gland, thyroid (with parathyroids) and adrenals. Organ weights showed statistically significant increases in absolute and relative testis weights of the recovery group of the 125 mg/kg bw/d group at the end of the recovery period; no such effect was observed at the termination of the dosing period (Hita, 2007a).

For 6:2 FTMA, one subacute 28-day oral repeated-dose toxicity study with rats is available in the registration dossier (equivalent to OECD TG 407; (Hita, 2007b), more details in 7.9.4) and one 14-day oral repeated dose toxicity study (DuPont, 2009). Histopathology was performed in the following organs relevant for endocrine effects: testes, epididymis, prostate, seminal vesicle, ovaries, uterus, vagina, pituitary gland, thyroid (with parathyroids) and adrenals. In the 14-day study, thyroid weights were significantly elevated at the two highest doses (500 and 1000 mg/kg bw/d) without changes in thyroid histopathology (DuPont, 2009). No other endocrine related effects were observed.

The effects of 6:2 FTA in a subacute toxicity study on the relative testis weight at the end of the recovery period in the highest dose group were considered as irrelevant since no such effects were observed in the highest dose group at termination of the dosing and no associated morphological lesions were found. Likewise, the elevated thyroid weights after 14-day exposure to 6:2 FTMA was not associated to histopathological changes and is therefore not considered relevant evidence for endocrine disruption. The limited data gave no evidence for endocrine disruption caused by 6:2 FTA or 6:2 FTMA.

7.10.2.2. Endocrine disrupting properties of 6:2 FTOH

Mammalian in vivo studies

The available data contained in the registration dossier on 6:2-FTOH in rats and mice has been compiled in a CLH report by the DE CA (ECHA, 2021b). Effects relevant for endocrine disrupting properties listed in the CLH report are briefly summarised in the following (more details in sections 7.9.4 and 7.9.7):

- OECD TG 407 (rat, 28-days, oral; (Hita, 2007c)): ↑ rel. testis weight and ↑ rel. ovary weight (in high dose only at 125 mg/kg bw/d at the end of recovery period);
- OECD TG 408 (rat, 90-days, oral; (Serex et al., 2014)): ↑ rel. testis weight at all doses tested (5, 25, 125 and 250 mg/kg bw/d); ↑ abs. and rel. thyroid weights in female rats in the 250 mg/kg bw/day group at the 1-month recovery time point and the abs. thyroid weight in the 25, 125, and 250 mg/kg/day groups at the 3-month recovery time point;
- OECD TG 415 (mouse, one-generation reproduction toxicity study, oral (DuPont, 2013)): ↑ rel. testis weight and ↑ rel. uterus weight (in high dose only at 100 mg/kg bw/d);

In summary, no clear effects with a potential link to endocrine disrupting properties were recorded in repeated-dose toxicity studies with 6:2 FTOH in mammals. Increased relative testis weights in three oral studies (rats and mice), increased relative ovary weights in one oral rat study, increased relative uterus weights in one oral mouse study, and increased thyroid weights in one oral rat study were observed. However, no histological correlations were reported. Therefore, the available in-vivo data do not indicate a potential specific ED-mediated concern.

In vitro and non-mammalian studies

Interaction with the hypothalamus-pituitary-thyroid axis

Regarding interaction with the hypothalamus-pituitary-thyroid (HPT) axis only two studies using *in vitro* assays are available:

Ren et al. (2015)

Method: using GH3 rat pituitary cancer cells a T-screen assay was performed. The competitive binding of 6:2 FTOH to the human thyroid receptor (TR) ligand binding domain was tested in presence of fluorescein tagged T_3.
Results: no competitive binding was found in the concentration range tested (1-100000 nM) for 6:2 FTOH. 6:2 FTOH showed also no agonistic or antagonistic activity in the T-screen assay in concentrations of 5-500 µM and 125 µM, respectively.
→ Klimisch reliability 2 - well documented, non-guideline study without analytical determination of the test concentrations.

Weiss et al. (2009)

Method: using radiolabeled ^{125}I-labeled T_4 (thyroxine) the competitive binding of 6:2 FTOH with T_4 to the human TTR (transthyretin) was assessed for a concentration range of 10-10000 nM.
Results: no competitive binding was found in the concentration range tested for 6:2 FTOH.

→ Klimisch reliability 2 - well documented, non-guideline study without analytical determination of the test concentrations.

Interaction with the hypothalamus-pituitary-gonadal axis

To assess the interaction with the estrogen signaling both *in vitro* and *in vivo* data are available: The studies of Benninghoff et al. (2011), Ishibashi et al. (2007), Ishibashi et al. (2008), Liu et al. (2007), Liu et al. (2009), Maras et al. (2006) have been discussed in section 7.10.1.3.

7.10.2.3. Endocrine disrupting properties of PFHxA

Mammalian in vivo studies

Various mammalian repeated-dose toxicity studies with PFHxA in rats and mice are available. Effects potentially relevant for endocrine disrupting properties are briefly summarized in the following (more details in sections 7.9.4 and 7.9.7):

- OECD TG 407 (similar to guideline, rat, 28 days, oral; (NTP, 2019)): ↓ total thyroxine, ↓ free thyroxine, ↓ total triiodothyronine, and ↓ cholesterol, in males but not in females; no changes in TSH, thyroid weight, or thyroid histopathology; ↑ gene expression of PPARα constitutive androstane receptor and (CAR)-related genes (Acox1, Cyp4a1, Cyp2b1, Cyp2b2);
- OECD TG 422 (rat, 28 day, oral; (WIL, 2005)): No effects on thyroid weights or thyroid histopathology; thyroid hormones not examined;
- OECD TG 414 (rat, oral; (Loveless et al., 2009)): No effects observed with regard to number of abortions, pre- and post-implantation loss, total litter losses by resorption, early or late resorptions, dead foetuses, pregnancy duration, no changes in sex ratio;
- RDT 90-day study (rat, oral, no guideline; (Chengelis et al., 2009b)): no macroscopic or microscopic findings on thyroid tissue; thyroid not weighed; thyroid hormones not examined.
- OECD TG 408 (rat, 90 days, oral (Loveless et al., 2009)): ↑ testes weight in high dose (500 mg/kg bw/d); thyroid minimal follicular cell hypertrophy in high dose (500 mg/kg bw/d) in males and females, ↑ thyroid weight in females at the 500 mg/kg bw/d only at the end of recovery, no examination of thyroid hormones in serum;
- OECD TG 415 (rat, one-generation reproduction toxicity study, oral; (Loveless et al., 2009)): in F1: ↓ rel. testis weight, ↓ rel. epididymides weight; no effects on estrous cycle, sperm measures, mating, fertility, gestation length or number of implantation sites; ↓ bodyweight in P0 and F1
    - Testes weight and relative testis weight (relative to bw) were decreased by 7 and11% (statistically significant) in the 20 and 500 mg/kg bw/day males, respectively, compared with the control. However, this effect was not dose related, as there were no statistically significant changes in testes weight in the 100 mg/kg bw/day group.
    - Statistically significant decreases in epididymides weights relative to body weight (in 20 mg/kg bw/day males) and relative to brain weight (in 100 mg/kg bw/day males) were not dose related and were thus not considered treatment related.
- OECD TG 453 (rat, 104-week study, oral (Klaunig et al., 2015)): No treatment-related effects in hormone parameters (hormone analysis only on estradiol, luteinizing hormone, testosterone and cholecystokinin; no measurements of thyroid hormones),

weight of thyroid and reproductive organs not reported in this study (unclear whether it was measured); liver effects (↑ Hepatocellular necrosis); kidney effects (↑ papillary necrosis)

In summary, effects with potential link to endocrine disrupting properties were recorded in repeated-dose toxicity studies with PFHxA in mammals. Such effects include increased testis weights (without histological findings) in one oral study (rats, highest dose only), increased minimal follicular cell hypertrophy in thyroid in one oral rat study, decreased total thyroxine, free thyroxine, and total triiodothyronine in one rat study, and increased gene expression of PPARα constitutive androstane receptor and (CAR)-related genes (Acox1, CYP4A1, CYP2B1, CYP2B2). Together, the *in vivo* data raise some concern for ED-mediated toxicity in particular with regard to the HPT axis.

In vitro and non-mammalian studies

Interaction with the hypothalamus-pituitary-thyroid axis

Regarding interaction with the HPT axis four *in vitro* assays are available. The studies (Ren et al. (2015), Naile et al. (2012), Vongphachan et al. (2011), Weiss et al. (2009)) have been discussed in section 7.10.1.4

More studies are available investigating thyroid disruption of other PFCAs. Both PFOA and PFNA altered the expression of genes playing a role in thyroid morphogenesis, TH synthesis, transport or metabolism and caused histopathological alterations in the thyroid follicles in fish (see Annex III).

During the Substance Evaluation of FTA and FTMA an Amphibian Metamorphosis Assay (AMA, OECD TG 231) was performed using PFHxA. This study shows an accelerated metamorphosis (increase in snout-vent length and hind limb length as well as an increase in the developmental stage) after treatment with PFHxA. Thus, the AMA study provides evidence for a thyroid agonistic activity and related adverse effects.

Interaction of PFHxA with the hypothalamus-pituitary-gonadal axis

To assess the interaction with the estrogen signaling several studies are available: Benninghoff et al. (2011), Gorrochategui et al (2014) and Wielogorska et al. (2015) have been discussed in section 7.10.1.4.

Higher PFCA homologues were shown to affect expression of genes, proteins, and hormones in HPG axis in zebrafish. Histopathological alterations were observed followed by exposure to PFOA in gonads of rare minnows. Developmental effects in offspring of PFOA-exposed medaka were also noticed (see Annex III).

In summary, the available *in vitro* assays conducted with PFHxA give rise to the concern that PFHxA might interact with the thyroid hormone signaling and/or transport (**Error! Reference source not found.**). Based on the available *in vitro* and *in vivo* test results, PFHxA seems to be not interacting with the sex steroid signaling pathways. However, its structural homologues show some interactions, therefore, concerns on ED effects of this group of substances cannot be excluded and therefore attention should be given to them.

7.10.3. Conclusion on endocrine disrupting properties

Environment

Information on endocrine disrupting properties was evaluated according to the definition of the WHO/IPCS 2002 [*"An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations."* (WHO/IPCS, 2002)], which means that the identification of an ED substance should fulfil the following conditions:

- Having an endocrine mode of action
- Causing adverse effects
- Providing a plausible link between adverse effects and endocrine mode of action

Assessment of data on endocrine properties was mainly based on the OECD guidance document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption (OECD, 2012) and the ECHA/EFSA Guidance to assess endocrine disruptors (EFSA, 2018). The available tests were put in context of the OECD Conceptual Framework (CF) for the Screening and Testing of Endocrine Disrupting Chemicals which defines five levels of a tiered assessment of endocrine disrupting properties.

Indicators of an endocrine mode of action may be provided by biomarkers that are known to be specific for a certain endocrine mode of action (such as expression changes of genes encoding for thyroid hormone synthetizing enzymes or vitellogenin (VTG) induction). Furthermore, histological changes of the thyroid follicles or gonads are likely to be a direct response to an endocrine mode of action. This information can be obtained from tests giving information equivalent to OECD CF 1-3 (such as read across to other substances, *in vitro* tests, and *in vivo* screening tests e.g., H295R steroidogenesis assay according to OECD TG 456 or amphibian metamorphosis assay according to OECD TG 231).

Alteration of the endocrine system may cause adverse effects that are endocrine specific but may also influence endpoints that are not endocrine specific (Kendall et al., 1998; Knacker et al., 2010; OECD, 2004). Changes of onset of metamorphosis, secondary sex characteristics and sex ratio are apical endpoints that are considered to be endocrine specific. Other endpoints such as development, growth, sexual maturity, reproduction, and behaviour are known to be sensitive to endocrine disruptors (IPCS, 2002; OECD, 2004; OECD, 2011), although they could be also influenced by systemic toxicity. Information on endocrine mediated adverse effects can be obtained from tests giving information equivalent to OECD CF 4-5 (such as larval amphibian growth and development assay according to OECD TG 241 or medaka extended one generation reproduction test according to TG 240).

Data on endocrine disrupting properties on 6:2 FTA and its degradation products, 6:2 FTOH and PFHxA were collected. Respective data were, however, mainly for 6:2 FTOH and PFHxA available. Furthermore, data on homologues of 6:2 FTOH and PFHxA were also assessed and used as supporting information compiled in Annex III to this report. Endpoints regarding effects on both the HPG and HPT axes were investigated.

<u>Endocrine disrupting properties of 6:2 FTA</u>

The effects of 6:2 FTA in a subacute toxicity study on the relative testis weight at the end of the recovery period in the highest dose group were considered as irrelevant since no

such effects were observed in the highest dose group at termination of the dosing and no associated morphological lesions were found. The limited data gave no evidence for endocrine disruption caused by 6:2 FTA.

Endocrine disrupting properties of 6:2 FTOH

Regarding interactions with the HPT axis, two studies assessed the interaction of 6:2 FTOH with the thyroid pathway finding no binding to the human thyroid hormone transport protein or to the human thyroid hormone receptor. However, no interaction with thyroid hormone biosynthesis, bioactivation or metabolism was assessed and therefore cannot be excluded. Given the limited data, it is not possible to derive a concern for thyroid disruption for FTOHs.

More data are available for disruption of the HPG axis (see an overview in **Error! Reference source not found.**12-A, Annex III): four (out of five) *in vitro* studies report on 6:2 FTOH (anti)estrogenic activity in µM concentrations. This is supported by three *in vivo* studies finding concentration-dependent induction of VTG and HSI or increased sex hormone concentrations and altered estrogen signalling pathway related gene expression changes observed from µM ranges of 6:2 FTOH. However, effect concentrations seem to be species and exposure route dependent.

A Fish Sexual Development Test (FSDT, OECD TG 234), which is an OECD CF level 4 test, was requested and submitted under the substance evaluation process to clarify whether or not the estrogen mediated mode of action of 6:2 FTOH provoke adverse and population relevant effects in fish. Although no statistically significant effect on sex ratio was observed, there was a clear and significant effect on the secondary sex characteristics (decrease in the number of anal fin papillae) and indication of feminisation at the highest test concentration tested. This concentration was considered very low due to difficulties to reach levels even close to the nominal concentrations. **In summary, it is concluded that both *in vitro* and *in vivo* data in different fish species provide sufficient evidence to prove an estrogenic activity of the substance** that can be linked to adverse and population relevant effects like femininization of male fish and the loss of male specific secondary sex characteristics, possibly leading to an impairment of mating success.

Endocrine disrupting properties of PFHxA

Regarding data on interaction with the HPT axis, the available *in vitro* assays conducted with PFHxA give rise to the concern that PFHxA might interact with the thyroid hormone signalling (see an overview in **Error! Reference source not found.**32). Given that no data are available with PFHxA for aquatic species, this concern was further investigated in a substance evaluation. Furthermore, information on interaction with the HPT axis of higher homologues of PFHxA (e.g., PFOA) is available. These *in vivo* fish tests report on expression changes of genes related to the HPT axis, histopathological alteration of the thyroid follicles and altered thyroid hormone levels in the animals. An Amphibian metamorphosis assay according to OECD TG 231 was requested and submitted under the substance evaluation process. This AMA provides clear evidence for agonistic thyroidal effects and for T-mediated adversity since e.g., an advanced development was observed in the study. This evidence fits to the available *in vitro* data for PFHxA e.g., agonistic binding to TR as well as to the observed histopathological changes of the thyroid in fish observed for PFOA. The adverse effects on development are concluded to be of population relevance. This is also supported by the ECHA/EFSA Guidance which states in respect to the interpretation of the AMA:

Accelerated and asynchronous development (characterised by disruption of the relative timing of the morphogenesis or development of different tissues and the inability to clearly establish the developmental stage of an animal by morphological landmarks) are thyroid-mediated effects. Changes in development in amphibians even if observed in the absence of investigation of thyroid histopathology are considered population relevant effects. However, the degree of delay or acceleration in the development that can be considered adverse at population level is uncertain (Marty et al., 2017). Therefore, such effects should be considered relevant at the population level unless available information demonstrates the contrary.

In summary, it is concluded that *in vitro* and amphibian *in vivo* data provide sufficient evidence to prove a thyroid agonistic activity of PFHxA that can be linked to adverse and population relevant effects (accelerated development in amphibians).

Regarding data on interaction with the HPG axis, *in vitro* test results are contradictory: some show estrogenic activity of PFCA including PFHxA, some not. The only *in vivo* fish study available for PFHxA show slight but not significant VTG induction in contrast to higher PFCA homologues. Whereas, in the same study no differences could be observed regarding *in vitro* estrogenic activity of short and middle chain PFCAs. Presumably, the low effect of PFHxA *in vivo* is related to the toxicokinetic characteristics of PFHxA (i.e., lower internal concentrations compared to that of higher homologues), as just slight differences – independently from the length of the fluorinated alkyl chain- in affinity could be seen *in vitro* between C6-C11 homologues. Furthermore, higher PFCA homologues were shown to affect expression of genes, proteins, and hormones in HPG axis in zebrafish. Histopathological alterations were observed followed by exposure to PFOA in gonads of rare minnows and common carps.

Several high tier studies in mammals gave no evidence for any ED-related effect. One sub chronic toxicity study in rats showed in the highest dose of 500 mg/kg bw/d some effects on the thyroid. Thyroid weight was statistically increased in female rats of the 500 mg/kg bw/d 30-day recovery group. Similar changes were not seen in female rats at the end of the dosing period, after 90 days recovery, or in male rats at any time point. Minimal hypertrophy of thyroid follicular epithelium was present in male and female rats in the 500 mg/kg bw/d group. The effects were reversible after 90 days of recovery but not following 30 days of recovery. Due the very limited effects the results were not considered to cause a sufficient concern. However, these data can be considered to be supportive for the concern substantiated for environmental endocrine disrupting properties driven by PFHxA interaction with the HPT axis. Additionally, it should be considered that the PFHxA formation from 6:2 FTA in mammals is low at best. There are no information requirements for the section human health.

Overall conclusion on endocrine disruption for the environment

The assays requested for 6:2FT-OH and PFHxA during the substance evaluation of FTA and FTMA, together with available *in vitro* data, leads the eMSCA to the conclusion that the WHO/IPCS criteria for an endocrine disruptor in the environment are fulfilled for both substances under evaluation. Thus, there is a need for further regulatory action on both substances to minimise environmental exposure as much as possible.

As stated under Part A of this Conclusion document SVHC identification based on Art. 57 (f) of REACH is not seen as the most effective next step in risk management as the ongoing

restriction activities with respect to PFHxA and its precursor substances are expected to sufficiently address the environmental concern clarified within this substance evaluation.

Human Health

For 6:2 FTA or 6:2 FTMA, the limited data gave no evidence for endocrine disruption relevant for human health.

For 6:2 FTOH no clear effects with a potential link to endocrine disrupting properties were recorded in repeated-dose toxicity studies in mammals. Increased relative testis weights in three oral studies (rats and mice), increased relative ovary weights in one oral rat study, increased relative uterus weights in one oral mouse study, and increased thyroid weights in one oral rat study were observed. However, no histological correlations were reported. Therefore, the available *in vivo* data do not indicate a potential specific ED-mediated concern.

For PFHxA, effects with potential link to endocrine disrupting properties were recorded in repeated-dose toxicity studies with PFHxA in mammals. Such effects include increased testis weights (without histological findings) in one oral study (rats, highest dose only), increased minimal follicular cell hypertrophy in thyroid in one oral rat study, decreased total thyroxine, free thyroxine, and total triiodothyronine in one rat study, and increased gene expression of PPARα constitutive androstane receptor and (CAR)-related genes (Acox1, CYP4A1, CYP2B1, CYP2B2). Together, the *in vivo* data raise some concern for ED-mediated toxicity in particular with regard to the HPT axis.

7.11. PBT and vPvB assessment

6:2 FTA as well as 6:2 FTMA are degraded in the environment and one of the final degradation products is PFHxA. Because of the biodegradation in PFHxA, 6:2 FTA as well as 6:2 FTMA are not persistent substances. Details are provided in the section 7.7.1.2. The PBT assessment is focusing on the degradation product PFHxA.

Persistence

6:2 FTA

6:2 FTA is not readily biodegradable. No simulation tests are available. Nevertheless, the microbial transformation of the structurally similar substance 8:2 FTA (two more CF_2-groups) was investigated in aerobic soil. 8:2 FTA was hydrolysed at the ester linkage with formation of 8:2 FTOH. 8:2 FTOH was further degraded to PFOA, which was the main stable transformation product at the end of the study (formation of PFOA: 8 mol% in 105 days). This transformation pathway is also likely for 6:2 FTA, i.e., transformation of PFHxA via 6:2 FTOH. A number of studies on degradation of 6:2 FTOH show formation of PFHxA as stable transformation product (formation of PFHxA e.g., in soil: 4.5% after 84 days, in sediment 8.4 mol% after 100 days, in WWTP activated sludge 11 mol% after 60 days).

6:2 FTMA

No simulation tests on 6:2 FTMA are available. Nevertheless, the microbial transformation of the structurally similar substance 8:2 FTMA (two more CF_2-groups) was investigated in aerobic soil (Royer et al., 2015). 8:2 FTMA was hydrolysed at the ester linkage with formation of 8:2 FTOH. 8:2 FTOH was further degraded to PFOA, which was the main stable

transformation product at the end of the study (formation of PFOA: 10.3 mol% in 105 days). This transformation pathway is also likely for 6:2 FTMA, i.e., transformation of PFHxA via 6:2 FTOH. A number of studies on degradation of 6:2 FTOH show formation of PFHxA as stable transformation product (formation of PFHxA e.g., in soil: 4.5% after 84 days, in sediment 8.4 mol% after 100 days, in WWTP activated sludge 11 mol% after 60 days).

PFHxA

PFHxA itself is considered to be very persistent based on the general stability of organic fluorine compounds (Siegemund et al., 2000) and read-across to the structurally similar substance PFOA, which is already identified as P and vP.

In the RAC opinion on the PFHxA (C6-PFCA) restriction proposal it was concluded that PFHxA exceeds by far the trigger of being very persistent and clearly exceeds the threshold values for being "very persistent" (vP) as defined in REACH Annex XIII.

Bioaccumulation

As BCFs are known to not properly reflect the bioaccumulation potential of PFCAs other endpoints were considered:

- Data from environmental monitoring show that PFHxA is present in biota.
- PFHxA half-lives in organisms and humans range from a few hours to a few days.
- PFHxA is taken up by plants and is transferred to edible parts of plants.

For none of these endpoints standardized concepts within the assessment of bioaccumulation are available. One possible approach is to compare results for PFHxA with results of a substance, which is already known to be bioaccumulative (benchmarking-approach), here PFOA:

- Monitoring data, e.g., concentrations in biota, cannot be compared between PFOA and PFHxA, because biota exposure concentrations might be different.
- PFOA has longer half-lives (a few days up to a few years) in animals and humans compared to PFHxA.
- Binding of PFOA and PFHxA to proteins is comparable.
- PFHxA and PFOA are both taken up by plants and are transferred to edible parts of plants, whereby the extent of both processes seems to depend on the study design.

In conclusion it cannot be excluded that PFHxA bioaccumulates in air-breathing organisms.

Toxicity

No short-term toxic effects were observed up to the water solubility of 6:2 FTA as well as 6:2 FTMA. No long-term data for fish and aquatic invertebrates is available. As the PBT assessment is focusing on the persistent degradation product PFHxA, the T criterion for 6:2 FTA and 6:2 FTMA were not evaluated in-depth.

From a human health point of view the T-criterion for 6:2 FTA would be fulfilled due to the STOT RE2 classification. This is also true for 6:2 FTMA.

Based on the long-term toxicity data on aquatic organisms (lowest NOEC = 10.1 mg/L), PFHxA does not fulfil the criteria of Annex XIII.

A CLH dossier for classification of PFHxA as Repr. 1B has been prepared and submitted to ECHA for accordance check by the eMSCA. If adopted by the RAC, classification as Repr. 1B would fulfil the T criterion according to Annex XIII with regard to human health.

Overall conclusion

PFHxA is considered to be very persistent. It cannot be excluded that PFHxA bioaccumulates in air-breathing organisms. The toxicity criterion is not fulfilled neither from an environmental point of view nor from a human health point of view. Nevertheless, because of other concerns like its high mobility in the environment, it cannot be excluded that PFHxA is a substance of very high concern similar to a PBT-substance

7.12. Exposure assessment

7.12.1. Human health

Not performed in the context of this substance evaluation.

7.12.2. Environment

As stated in the registrations 6:2 FTA as well as 6:2 FTMA are used as intermediate and for polymerisation. In some of the registration dossiers, emission scenarios are given for manufacturing of the substance as well as use as intermediate and in polymerization. For analysing risk management options to address above-described concerns on PFHxA and 6:2 FTOH it is necessary to have knowledge about the uses and releases of 6:2 FTA and 6:2 FTMA into the environment during the whole life cycle. This knowledge is of relevance because emissions of 6:2 FTA and 6:2 FTMA are at the same time a source for PFHxA and 6:2 FTOH (see chapter: Degradation of 6:2 FTA in the environment).

For fluorinated polymers – the polymer formed out of 6:2 FTA as well as 6:2 FTMA is also a fluorinated polymer – information is available on uses and emissions from the public literature. For example textiles is one of the use areas of fluorinated polymers (European Chemicals Agency, 2015). Textiles are also mentioned by a registrant as one of the product categories for use of 6:2 FTA and 6:2 FTMA in polymerization. Fluorinated polymers are used for making textiles water-, dirt- and stain repellent (Lacasse and Baumann, 2004). When extracting such textiles, e.g. outdoor textiles, non-polymeric fluorinated substances, beyond others PFHxA and 6:2 FTOH, can be found (Greenpeace International, 2016; Gremmel et al., 2016; Kotthoff et al., 2015). Textiles release these substances into air during use as well as into water during washing (Knepper et al., 2014). It is not clear whether these findings and releases of non-polymeric PFASs, like PFHxA and 6:2 FTOH, are coming from degradation of the polymers, degradation of residues or if these non-polymeric PFASs are residues and impurities in the polymer (Dinglasan-Panlilio and Mabury, 2006).

Several studies investigated the degradation of fluorinated polymers in soil, especially acrylate-based polymers (C8-based instead of C6-based as given by 6:2 FTA). Half-lives ranged from 8 years to > 2000 years (Rankin et al., 2014; Russell et al., 2008; Washington et al., 2009; Washington and Jenkins, 2015). All studies show the formation of non-polymeric PFASs whereby it can mostly not be excluded those parts of that is coming from residues and degradation of residues. Modelled data indicate much shorter half-lives with 170 – 270 years in marine systems and < 1 year in landfills (calculated with SPARC) (Rayne and Forest, 2010).

Overall studies show that fluorinated polymers are a source of non-polymeric PFASs into the environment. Only a few registrants address these issues described above from the general literature in their registration. This is a citation of one study on polymer degradation without taking other studies into account and reference to analysis of residues in the polymer.

7.13. Risk characterisation

Not performed in the context of this substance evaluation.

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9. Abbreviations

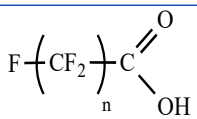
4-NP	4-nonylphenol
AM	Arithmetic mean
AMA	Amphibian metamorphosis assay
AR	Androgen receptor
BSI	Body somatic index
CoRAP	Community rolling action plan
D	Deiodinase
E_2	Estradiol
EE_2	Ethinylestradiol
EC_{50}	Half maximal effect concentration
ED	Endocrine disruptor
ELISA	Enzyme linked immunosorbent assay
ER	Estrogen receptor
CLH	Harmonised classification and labelling
CLP	Classification, Labelling and Packaging
FT	Fluorotelomer compounds
	4:2 FTOH 4:2-Fluorotelomer alcohol
	6:2 FTOH 6:2-Fluorotelomer alcohol
	8:2 FTOH 8:2-Fluorotelomer alcohol
	6:2 FTA 6:2 Fluorotelomer acrylate
	8:2 FTA 8:2 Fluorotelomer acrylate
	6:2 FTMA 6:2 Fluorotelomer methacrylate
	FTUCA Fluorotelomer unsaturated acid
	FTCA Fluorotelomer carboxylate
	PAPs Polyfluoroalkyl phosphoric acid esters
FSDT	Fish sexual development test
GM	Geometric mean
GSI	Gonadosomatic index
HPG	Hypothalamus-pituitary-gonadal
HPT	Hypothalamus-pituitary-thyroid
HSI	Hepatosomatic index
IC_{50}	Half maximal inhibitory concentration
LAGDA	Larval amphibian growth and development assay
LOD	Limit of detection
LOEC	Lowest observed effect concentration
LOQ	Limit of quantification
MBP	Myelin basic protein
MDL	Method detection limit
Med	Median
MQL	Method quantification limit
n.d.	not detected
NIS	Sodium iodide symporter
Oct-1	Octamer motif-binding factor
OECD	Organisation for Economic Co-operation and Development
PBT	Persistent, bioaccumulative and toxic
PFASs	Per- and polyfluoroalkyl substances
PFCAs	Perfluoroalkyl carboxylic acids
	PFBA Perfluorobutanoic acid
	PFPeA Perfluoropentanoic acid
	PFHxA Perfluorohexanoic acid
	PFHpA Perfluoroheptanoic acid
	PFOA Perfluorooctanoic acid
	AFPO Ammonium perfluorooctanoate
	PFNA Perfluorononanoic acid

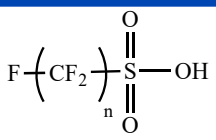
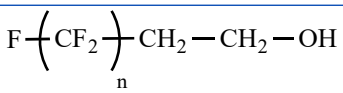
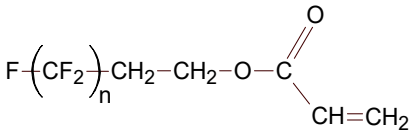
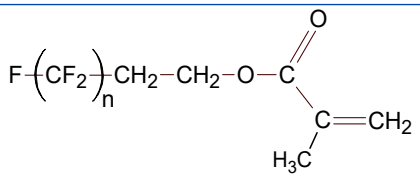
	PFDA	Perfluorodecanoic acid
	PFUnDA	Perfluoroundecanoic acid
	PFDoDA	Perfluorododecanoic acid
	PFTrDA	Perfluorotridecanoic acid
	PFTeDA	Perfluorotetradecanoic acid
PFSAs	Perfluoroalkane sulfonic acids	
	PFBS	Perfluorobutane sulfonic acid
	K-PFBS	Potassium perfluorobutane sulfonate
	PFHxS	Perfluorohexane sulfonic acid
	PFOS	Perfluorooctane sulfonic acid
	PFDS	Perfluorodecane sulfonate anion
qPCR	Quantitative polymerase chain reaction	
RAC	Risk Assessment Committee	
RC3	Neurogranin	
RMOA	Risk management option analysis	
SVHC	Substances of Very High Concern	
T	Testosterone	
T_3	Triiodothyronine	
T_4	Thyroxin	
TG	Test guideline	
TR	Thyroid receptor	
TTR	Transtyretin	
VTG	Vitellogenin	
vPvB	Very persistent, very bioaccumulative	

10. Annex I: Introduction to PFASs, their concerns and regulatory status

PFASs are characterized by an aliphatic structure containing a per- or polyfluorinated alkyl chain connected to a functional group (Table 8-A). For perfluorinated substances, all hydrogen atoms in the aliphatic chain of the molecule are exchanged by fluorine, while polyfluorinated substances contain at least one C-H bond. The most thoroughly investigated classes of PFASs are perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) as they are the final degradation products; therefore, they have the highest environmental relevance that is shown by various monitoring data measured in different environmental compartments. None of the final degradation products are registered under REACH, therefore a significant part of the emissions to the environment in Europe are related to the production and use of the precursor substances, such as fluorotelomer compounds (FTOHs, FTAs, FTMAs, etc.).

Table 10-A: Structure and acronym of some selected subgroups of PFASs

Structure and acronym of some selected subgroups of PFASs		
Structure	**Acronym substance class**	**Acronym single compounds**
Final persistent degradation products		
	PFCA (Perfluoroalkyl carboxylic acid)	PF*X*A n≥6 long-chain; n<6 short-chain

Structure and acronym of some selected subgroups of PFASs		
Structure	**Acronym substance class**	**Acronym single compounds**
	PFSA (Perfluoroalkane sulfonic acid)	PF*X*S n≥6 long-chain; n<6 short-chain
Precursor substances		
	FTOH (Fluorotelomer alcohol)	n:2-FTOH
	FTA (Fluorotelomer acrylate)	n:2-FTAC
	FTMA (Fluorotelomer methacrylate)	n:2-FTMAC

In general, the persistence of PFCAs and PFSAs can be explained by the shielding effect of the fluorine atoms, blocking e.g., nucleophilic attacks to the carbon chain. High electronegativity, low polarizability and high bond energies make highly fluorinated alkanes the most stable organic compounds. Because of the very strong C-F bond, PFASs exhibit extreme chemical and heat stability. Furthermore, due to the hydrophobic properties of the perfluoroalkyl tail, and hydrophilic functional group they are able to decrease the surface tension, therefore some of them are applied as surfactants. Owing to these physicochemical properties, they are used in a wide variety of industrial and commercial applications: metal plating, coating formulation (such as non-stick cookware), fire-fighting foams, lubricants, water/oil/dirt repellents for leather, paper, textiles, etc.

Their stability is also reflected by their environmental persistence; therefore, they are ubiquitously found in the environment, also in remote places, far from emissions. Since long-chain PFASs tend to bind to blood proteins, they can accumulate in blood rich tissues. Therefore, they can be found in top predators, also in the human blood. Some of the long-chain PFCAs were shown to be toxic to reproduction. Due to these properties of concern, for some of the PFCAs regulatory activities has been taken place under REACH as shown in Table 10B.

Table 10-B:

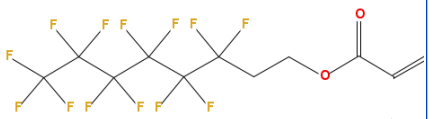
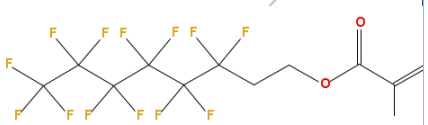
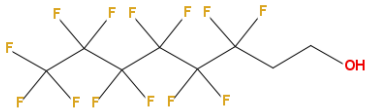
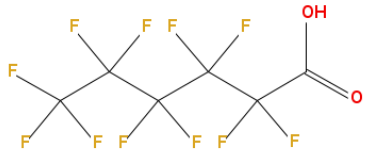
Regulatory status of selected PFASs under REACH			
Substance	**Regulatory activity under REACH**	**Status of the regulatory activity**	**Year and Member State performing regulatory activity**
C_{11}-C_{14} PFCA	SVHC	Listed as vPvB	2012, Germany
C_8 PFCA (PFOA) and		Listed as PBT and	2013, Germany and

its NH_4^+ salt		CMR	Norway
C_9 PFCA (PFNA) and its Na/NH_4^+ salts		Listed as PBT and CMR	2015, Sweden and Germany
C_{10} PFCA (PFDA) and its Na/NH_4^+ salts		Dossier submitted (PBT and CMR)	2017, Sweden and Germany
C_8 PFCA and related substances	Restriction	Proposal under consideration by EU Commission	Ongoing, Germany & Norway
C_9-C_{14} PFCA and related substances		Update of RMOA in preparation	Ongoing, Germany & Sweden
EC 700-403-8, precursor of C_7 PFCA	Substance evaluation	Further information requested	2013, Belgium
EC 700-161-3, precursor of C_7 PFCA		Further information requested	2013, the Netherlands
6:2 FTA & 6:2 FTMA		In preparation	2016, Germany

11. Annex II: Information on degradation products

11.1. General information

Table 11-A:

General information to the substances discussed in this document				
	6:2 FTA	**6:2 FTMA**	**6:2 FTOH**	**PFHxA**
EC number:	241-527-8	218-407-9	211-477-1	206-196-6
CAS number:	17527-29-6	2144-53-8	647-42-7	307-24-4
EC name:	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl acrylate	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl methacrylate	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol	Perfluorohexanoic acid
IUPAC name:	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl acrylate	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl methacrylate	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol	Undecafluorohexanoic acid
Mol. formula:	$C_{11}H_7F_{13}O_2$	$C_{12}H_9F_{13}O_2$	$C_8H_5F_{13}O$	$C_6HF_{11}O_2$
Mol. weight:	418.1513 g/mol	432.1779 g/mol	364.1 g/mol	314.05 g/mol
Molecular structure:				
Classification and labelling:	not listed in Annex VI of the CLP regulation Notified self-classifications: STOT SE 3 H335 Skin Irrit. 2 H315 Eye Irrit. 2 H319	not listed in Annex VI of the CLP regulation Notified self-classifications: STOT SE 3 H335 Skin Irrit. 2 H315 Eye Irrit. 2 H319	not listed in Annex VI of the CLP regulation (but RAC opinion available) Acute Tox 4. H302 Eye Irrit. 2 H319 Skin Irrit.2 H315 STOT SE 3 H335 Aquatic Chronic 2 H411 STOT RE 1 H372	not listed in Annex VI of the CLP regulation Skin Corr. 1B H314 Met. Corr. 1 H290 Eye Dam. 1 H318 Acute Tox. 3 H301, H311 Acute Tox. 2 H330

11.2. Physicochemical properties

Table 11-B

Physicochemical properties as published in the ECHA dissemination site.				
Property	**6:2 FTA**	**6:2 FTMA**	**6:2 FTOH**	**PFHxA***
Physical state at 20°C and 101.3 kPa	liquid	liquid	liquid	liquid
Melting/ freezing point	<-20 °C	-38 °C	-33.09 °C	14 °C
Boiling point	210.2 °C	214 °C at 1021 hPa	n.d.	157 °C
Vapour pressure	208 Pa at 20 °C, 259 Pa at 25 °C	4.2 Pa at 20 °C, 8.6 Pa at 25 °C	713 Pa at 25°C	n.d.
Density	1.558 g/ml at 20 °C	1.52 g/mL at 25 °C	n.d.	1.759 g/mL at 20 °C
Water solubility	0.1858 mg/L at 25°C	0.042 mg/L at 20 °C	18.8 mg/L at 22.5 °C	n.d.
Partition coefficient n-octanol/ water (log value)	5.067	5.3	4.54	n.d.

*No data on ECHA dissemination site. Source www.chemicalbook.com

12. Annex III: Studies on endocrine disrupting properties of other PFASs

The tables 12-A / 12-B / 12-C provide an overview on the endocrine effects discussed by studies available in the scientific literature. A discussion organized according to the different endocrine axes can be found below where we summarize first *in silico*, then *in vitro* and *finally in vivo* studies.

Table 12-A

Effects of different PFASs on the hypothalamus-pituitary-gonadal axis.				
Substance	**Test system**	**Endpoints**	**ED-Effect**	**Reference**
PFCA				
PFPeA	Human, *in vitro* (reporter gene assay using MMV-Luc cell line)	Binding to ER	-	Wielogorska, E., et al. (2015)
PFHxA	Rainbow trout, *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding + VTG -	Benninghoff, A. D., et al. (2011)
	Human, *in vitro* (reporter gene assay using MMV-Luc cell line)	Binding to ER	-	Wielogorska, E., et al. (2015)
	Human, *in vitro* (human placental choriocarcinoma cell line JEG-3)	Aromatase inhibition	-	Gorrochategui, E., et al. (2014)
PFHpA	Rainbow trout, *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding + VTG +	Benninghoff, A. D., et al. (2011)
	Human, *in vitro* (reporter gene assay using MMV-Luc cell line)	Binding to ER	-	Wielogorska, E., et al. (2015)
PFOA	Rainbow trout, *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding + VTG +	Benninghoff, A. D., et al. (2011)
	Medaka, *in vitro/in vivo*	VTG induction, binding to ER	VTG-, ER binding-	Ishibashi, H., et al. (2008)
	Medaka, *in vivo*	Fecundity in adults (number of eggs), developmental effects in offspring (hatching, swim-up, survival)	+	Ji, K., et al. (2008)
	Zebrafish, *in vivo*	Expression of steroidogenic enzyme genes (CYP17, CYP19a, CYP19b) and ER1, ER2b	ER1+, rest -	Du, G., et al. (2013b)
	Rare minnow, *in vivo*	Gene expression of ERβ, VTG, protein expression of VTG, gonadal histophathology	All+	Wei, Y., et al. (2007)
	Common carp, *in vivo*	GSI, HSI, gonadal histopathology	GSI, HSI – Gonadal histopathology+	Giari, L. et al. (2016)
	Freshwater tilapia, *in vitro*	VTG induction	+	Liu, C., et al. (2007)

Effects of different PFASs on the hypothalamus-pituitary-gonadal axis.				
Substance	**Test system**	**Endpoints**	**ED-Effect**	**Reference**
	Human, *in vitro*	Binding to ER/AR (reporter gene assay using CV-1 cell line) E2, SF-1 and T levels, gene expression of steroidogenesis genes in H295R cell	ER+, AR- T+, E_2+, gene expression +, SF-1+	Du, G., et al. (2013b)
	Human, *in vitro* (yeast two-hybrid assay)	Binding to human ERα and ERβ	-	Ishibashi, H., et al. (2007)
	Human, *in vitro* (E-Screen assay using MCF-7 cells)	Promotion of proliferation	-	Maras, M., et al. (2006)
	Human/rat, *in vitro* (microsomal preparations of human and rat testes)	Enzyme inhibition assays (3β-HSD and 17β-HSD3)	3βHSD+, 17βHSD3+	Zhao, B., et al. (2010)
	Human, *in vitro* (reporter gene assay using MMV-Luc cell line)	Binding to ER	-	Wielogorska, E., et al. (2015)
	Human, *in vitro* (human placental choriocarcinoma cell line JEG-3)	Aromatase inhibition	+	Gorrochategui, E., et al. (2014)
	Human, *in vitro* (E-Screen assay, binding assays with MVLN cells)	Promotion of proliferation, binding to ER	-	Wang, C., et al. (2012)
PFNA	Rainbow trout, *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding + VTG +	Benninghoff, A. D., et al. (2011)
	Medaka, *in vitro/in vivo*	VTG induction, binding to ER	VTG-, ER binding-	Ishibashi, H., et al. (2008)
	Human *in vitro* (E-Screen assay using MCF-7 cells)	Promotion of proliferation	-	Maras, M., et al. (2006)
	Human, *in vitro* (reporter gene assay using MMV-Luc cell line)	Binding to ER	-	Wielogorska, E., et al. (2015)
	Human, *in vitro* (human placental choriocarcinoma cell line JEG-3)	Aromatase inhibition	-	Gorrochategui, E., et al. (2014)
PFDA	Rainbow trout, *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding + VTG +	Benninghoff, A. D., et al. (2011)
	Zebrafish, *in vivo*	E2, T, 11-ketotestosterone levels and ratios, gene expression of VTG 1	VTG1+ in males, VTG1 – in females Hormone levels -, E_2/T+, E_2/11-KT+	Jo, A. et al. (2014)
	Human, *in vitro* (H295R cells)	E2 and T and transcription of steroidogenic genes (CYP19A, CYP17A, CYP11A1, BYP11B2, 3BHSD2, 17BHSD4)	-	Jo, A. et al. (2014)
	Human, *in vitro* (reporter gene assay using MMV-Luc cell line)	Binding to ER	-	Wielogorska, E., et al. (2015)
PFUnDA	Rainbow trout, *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding + VTG +	Benninghoff, A. D., et al. (2011)

Effects of different PFASs on the hypothalamus-pituitary-gonadal axis.				
Substance	**Test system**	**Endpoints**	**ED-Effect**	**Reference**
	Human, *in vitro* (reporter gene assay using MMV-Luc cell line)	Binding to ER	-	Wielogorska, E., et al. (2015)
PFDoDA	Rainbow trout, *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding - VTG -	Benninghoff, A. D., et al. (2011)
	Human, *in vitro* (reporter gene assay using MMV-Luc cell line)	Binding to ER	-	Wielogorska, E., et al. (2015)
	Human, *in vitro* (human placental choriocarcinoma cell line JEG-3)	Aromatase inhibition	-	Gorrochategui, E., et al. (2014)
PFTrDA	Rainbow trout, *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding - VTG -	Benninghoff, A. D., et al. (2011)
	Zebrafish, *in vivo*	E2, T, 11-ketotestosterone levels and ratios, gene expression of VTG 1	VTG1- in males, VTG1 + in females E_2+, T-, 11-KT-, E_2/T+, E_2/11-KT+	Jo, A. et al. (2014)
	Human cells, *in vitro* (H295R cells)	E2 and T and transcription of steroidogenic genes (CYP19A, CYP17A, CYP11A1, BYP11B2, 3BHSD2, 17BHSD4)	T, CYP17A, CYP11A1 + Rest -	Jo, A. et al. (2014)
PFTeDA	Rainbow trout, *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding - VTG -	Benninghoff, A. D., et al. (2011)
PFSA				
PFBS	Xenopus laevis, *in vivo*	Gonadal histopathology, reproductive parameters, expression of ER, AR, brain aromatase	AR/ER expression +, rest -;	Lou, Q. Q., et al. (2013)
	Mallard and Northern bobwhite, *in vivo*	Gonadal histopathology, reproductive parameters	-	Newsted, J. L., et al. (2008)
	Human, *in vitro* (human placental choriocarcinoma cell line JEG-3)	Aromatase inhibition	+	Gorrochategui, E., et al. (2014)
	Human/rat, *in vitro* (microsomal preparations of human and rat testes)	Enzyme inhibition assays (3β-HSD and 17β-HSD3) testing K-PFBS	3βHSD-, 17βHSD3-	Zhao, B., et al. (2010)
PFHxS	Human/rat, *in vitro* (microsomal preparations of human and rat testes)	Enzyme inhibition assays (3β-HSD and 17β-HSD3)	3βHSD-, 17βHSD3-	Zhao, B., et al. (2010)
	Human, *in vitro* (reporter gene assay using MMV-Luc cell line)	Binding to ER	-	Wielogorska, E., et al. (2015)

Effects of different PFASs on the hypothalamus-pituitary-gonadal axis.				
Substance	**Test system**	**Endpoints**	**ED-Effect**	**Reference**
PFOS	Xenopus laevis, *in vivo*	Gonadal histopathology, reproductive parameters, expression of ER, AR, brain aromatase	AR/ER expression +, brain aromatase -, spermatogonia +	Lou, Q. Q., et al. (2013)
	Rainbow trout, *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding + VTG -	Benninghoff, A. D., et al. (2011)
	Medaka, *in vitro/in vivo*	VTG induction, binding to ER	VTG-, ER binding-	Ishibashi, H., et al. (2008)
	Freshwater tilapia, *in vitro*	VTG induction	+	Liu, C., et al. (2007)
	Fathead minnow, *in vivo*	Fecundity, gonadal histopatholgy, developmental effects in offspring, levels of T/11-ketotestosterone/E_2/VTG/ brain aromatase activity	Fecundity +, gonadal histopatholgy +, developmental effects in offspring-, Males: levels of T+/11-ketotestosterone +/E_2-/VTG-/ brain aromatase activity- Females: all -	Ankley, G. T., et al. (2005)
	Medaka, *in vivo*	Fecundity in adults (number of eggs), developmental effects in offspring (hatching, swim-up, survival)	+	Ji, K., et al. (2008)
	Zebrafish, *in vivo*	Expression of steroidogenic enzyme genes (CYP17, CYP19a, CYP19b) and ER1, ER2b	All +	Du, G., et al. (2013a)
	Zebrafish, *in vivo*	CYP19a, CYP19b gene expression	+	Shi, X., et al. (2008)
	Marine medaka, *in vivo*	Expression of VTG1, VTG2, ERα, ERβ, ERγ, ChgL, ChgH	All +	Fang, C., et al. (2012)
	Zebrafish, *in vivo*	VTG expression, gonadal histopathology, fecundity, fertilization rate	VTG+, rest -	Keiter, S., et al. (2012)
	Human, *in vitro*	Binding to ER/AR (reporter gene assay using CV-1 cell line) E2 and T levels, gene expression of steroidogenesis genes in H295R cell	ER+, AR- T+, E_2+, gene expression +	Du, G., et al. (2013a)
	Human, *in vitro* (yeast two-hybrid assay)	Binding to human ERα and ERβ	-	Ishibashi, H., et al. (2007)
	Human, *in vitro* (E-Screen assay using MCF-7 cells)	Promotion of proliferation	-	Maras, M., et al. (2006)
	Human/rat, *in vitro* (microsomal preparations of human and rat testes)	Enzyme inhibition assays (3β-HSD and 17β-HSD3)	3βHSD+, 17βHSD3+	Zhao, B., et al. (2010)

Effects of different PFASs on the hypothalamus-pituitary-gonadal axis.				
Substance	**Test system**	**Endpoints**	**ED-Effect**	**Reference**
	Human, *in vitro* (reporter gene assay using MMV-Luc cell line)	Binding to ER	-	Wielogorska, E., et al. (2015)
	Human, *in vitro* (human placental choriocarcinoma cell line JEG-3)	Aromatase inhibition	+	Gorrochategui, E., et al. (2014)
PFDS	Rainbow trout, *in vitro/in vivo*	Binding to ER, VTG induction	*In vitro* binding +, VTG -	Benninghoff, A. D., et al. (2011)
Fluorotelomer alcohols and acrylates				
4:2 FTOH	Freshwater tilapia, *in vitro*	VTG induction	-	Liu, C., et al. (2007)
6:2 FTOH	Zebrafish, *in vivo*	E_2 and T levels, gene expression of ERα, VTG, CYP17, CYP19A	Females: T/E_2 ↓, ERα and VTG↑ Males: T/E_2↑, CYP17↑, CYP19A↓	Liu, C., et al. (2009)
	Rainbow trout, *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding - 8-fold VTG induction	Benninghoff, A. D., et al. (2011)
	Medaka, *in vitro/in vivo*	VTG induction, binding to ER	VTG+, ER binding+	Ishibashi, H., et al. (2008)
	Freshwater tilapia, *in vitro*	VTG induction	+	Liu, C., et al. (2007)
	Human, *in vitro* (yeast two-hybrid assay)	Binding to human ERα and ERβ	+	Ishibashi, H., et al. (2007)
	Human *in vitro* (E-Screen assay using MCF-7 cells)	Promotion of proliferation	+	Maras, M., et al. (2006)
8:2 FTOH	Zebrafish, *in vivo*	Disruption of sex hormone biosynthesis and impaired reproduction	+	Liu, C., et al. (2010)
	Freshwater tilapia, *in vitro*	VTG induction	-	Liu, C., et al. (2007)
	Medaka, *in vitro/in vivo*	VTG induction, binding to ER	VTG+, ER binding+	Ishibashi, H., et al. (2008)
	Rainbow trout *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding - VTG -	Benninghoff, A. D., et al. (2011)
	Human, *in vitro* (yeast two-hybrid assay)	Binding to human ERα and ERβ	+	Ishibashi, H., et al. (2007)
	Human *in vitro* (E-Screen assay using MCF-7 cells)	Promotion of proliferation	+	Maras, M., et al. (2006)
8:2 FTA	Rainbow trout, *in vitro/in vivo*	Binding to ER VTG induction	*In vitro* binding - VTG -	Benninghoff, A. D., et al. (2011)

+ effective, - non-effective, n.a. not analysed

Table 12-B

Effects of different PFASs on the hypothalamus-pituitary-thyroid axis.				
Substance	**Test system**	**Endpoints**	**ED-Effect**	**Reference**
PFCAs				
PFBA	Domestic chicken/ herring gull, *in vitro* (embryonic neuronal cells)	Gene expression Domestic chicken: D2, D3, TTR, RC3, Oct1, MBP Herring gull: D2, RC3, Oct1	Domestic chicken: D2-, D3-, TTR-, RC3-, Oct1-, MBP- Herring gull: D2-, RC3-, Oct1-	Vongphachan, V., et al. (2011)
	Human, *in vitro*	Binding to TTR	-	Weiss, J. M., et al. (2009)
	Human, *in vitro*	Intrinsic/competitive binding to TR	-/-	Ren, X. M., et al. (2015)
	Rat, *in vitro*	HEX and PAX8 gene expression	+	Naile, J. E., et al. (2012)
PFPeA	Rat, *in vitro*	HEX and PAX8 gene expression	+	Naile, J. E., et al. (2012)
PFHxA	Domestic chicken/ herring gull, *in vitro* (embryonic neuronal cells)	Gene expression Domestic chicken: D2, D3, TTR, RC3, Oct1, MBP Herring gull: D2, RC3, Oct1	Domestic chicken: D2+, D3+, TTR-, RC3-, Oct1-, MBP+ Herring gull: D2-, RC3-, Oct1+	Vongphachan, V., et al. (2011)
	Human, *in vitro*	Binding to TTR	+	Weiss, J. M., et al. (2009)
	Human, *in vitro*	Intrinsic/competitive binding to TR	-/+	Ren, X. M., et al. (2015)
	Rat, *in vitro*	HEX and PAX8 gene expression	+	Naile, J. E., et al. (2012)
PFHpA	Domestic chicken/ herring gull, *in vitro* (embryonic neuronal cells)	Gene expression Domestic chicken: D2, D3, TTR, RC3, Oct1, MBP Herring gull: D2, RC3, Oct1	Domestic chicken: D2+, D3-, TTR-, RC3-, Oct1-, MBP- Herring gull: D2-, RC3+, Oct1-	Vongphachan, V., et al. (2011)
	Human, *in vitro*	Binding to TTR	+	Weiss, J. M., et al. (2009)
	Human, *in vitro*	Intrinsic/competitive binding to TR	-/+	Ren, X. M., et al. (2015)
PFOA	Zebrafish, *in vivo*	HEX and PAX8 gene expression	+	Du, G., et al. (2013b)
	Medaka, *in vivo*	Developmental (hatching, swim-up, survival) and histopathological effects of the thyroid in offspring	+	Ji, K., et al. (2008)
	Domestic chicken/ herring gull, *in vitro* (embryonic neuronal cells)	Gene expression Domestic chicken: D2, D3, TTR, RC3, Oct1, MBP Herring gull: D2, RC3, Oct1	Domestic chicken: D2-, D3-, TTR-, RC3-, Oct1-, MBP-	Vongphachan, V., et al. (2011)
	Human, *in vitro*	Intrinsic/competitive binding to TR	+/+	Ren, X. M., et al. (2015)
	Human, *in vitro*	Binding to TTR	+	Weiss, J. M., et al. (2009)
	Human, *in vitro*	Binding to TR (reporter gene assay using CV-1 cell line)	TR+ (antagonist)	Du, G., et al. (2013b)

Effects of different PFASs on the hypothalamus-pituitary-thyroid axis.				
Substance	**Test system**	**Endpoints**	**ED-Effect**	**Reference**
PFNA	Domestic chicken/ herring gull, *in vitro* (embryonic neuronal cells)	Gene expression Domestic chicken: D2, D3, TTR, RC3, Oct1, MBP Herring gull: D2, RC3, Oct1	Domestic chicken: D2+, D3-, TTR-, RC3-, Oct1-, MBP-	Vongphachan, V., et al. (2011)
	Zebrafish, *in vivo*	Thyroid histopathology, plasma T_3 and T_4, gene expression of TTR, DIO2, TRα, TRβ, DIO1, UGT1A5, UGT2A1, TSHβ, SLC5A5, TPO, TG, CTSb	Thyroid histopathology (hypertrophy, hyperplasia) + T_3 +, T_4- TTR (induction), Ugt1A5 and Ugt2A1 (inhibition) +, Rest -	Liu, Y. et al. (2011)
	Rat, *in vitro*	T-screen (promotion of T_3 dependent proliferation of GH3 cells)	+	Long, M., et al. (2013)
	Human, *in vitro*	Intrinsic/competitive binding to TR	+/+	Ren, X. M., et al. (2015)
	Human, *in vitro*	Binding to TTR	+	Weiss, J. M., et al. (2009)
PFDA	Domestic chicken/ herring gull, *in vitro* (embryonic neuronal cells)	Gene expression Domestic chicken: D2, D3, TTR, RC3, Oct1, MBP Herring gull: D2, RC3, Oct1	Domestic chicken: D2-, D3-, TTR-, RC3-, Oct1-, MBP-	Vongphachan, V., et al. (2011)
	Human, *in vitro*	Binding to TTR	+	Weiss, J. M., et al. (2009)
	Human, *in vitro*	Intrinsic/competitive binding to TR	+/+	Ren, X. M., et al. (2015)
	Rat, *in vitro*	T-screen (promotion of T_3 dependent proliferation of GH3 cells)	-	Long, M., et al. (2013)
PFUnDA	Domestic chicken/ herring gull, *in vitro* (embryonic neuronal cells)	Gene expression Domestic chicken: D2, D3, TTR, RC3, Oct1, MBP Herring gull: D2, RC3, Oct1	Domestic chicken: D2-, D3-, TTR-, RC3-, Oct1-, MBP-	Vongphachan, V., et al. (2011)
	Human, *in vitro*	Binding to TTR	+	Weiss, J. M., et al. (2009)
	Human, *in vitro*	Intrinsic/competitive binding to TR	+/+	Ren, X. M., et al. (2015)
	Rat, *in vitro*	T-screen (promotion of T_3 dependent proliferation of GH3 cells)	+	Long, M., et al. (2013)
PFDoDA	Domestic chicken/ herring gull, *in vitro* (embryonic neuronal cells)	Gene expression Domestic chicken: D2, D3, TTR, RC3, Oct1, MBP Herring gull: D2, RC3, Oct1	Domestic chicken: D2-, D3-, TTR-, RC3-, Oct1-, MBP-	Vongphachan, V., et al. (2011)
	Human, *in vitro*	Binding to TTR	+	Weiss, J. M., et al. (2009)
	Human, *in vitro*	Intrinsic/competitive binding to TR	+/+	Ren, X. M., et al. (2015)

Effects of different PFASs on the hypothalamus-pituitary-thyroid axis.				
Substance	**Test system**	**Endpoints**	**ED-Effect**	**Reference**
	Rat, *in vitro*	T-screen (promotion of T_3 dependent proliferation of GH3 cells)	-	Long, M., et al. (2013)
PFTrDA	Human, *in vitro*	Binding to TTR	+	Weiss, J. M., et al. (2009)
	Human, *in vitro*	Intrinsic/competitive binding to TR	+/+	Ren, X. M., et al. (2015)
PFSAs				
PFBS	Domestic chicken/ herring gull, *in vitro* (embryonic neuronal cells)	Gene expression Domestic chicken: D2, D3, TTR, RC3, Oct1, MBP Herring gull: D2, RC3, Oct1	Domestic chicken: D2, D3, TTR, RC3, Oct1, MBP Herring gull: D2-, RC3-, Oct1+	Vongphachan, V., et al. (2011)
	Human, *in vitro*	Binding to TTR	+	Weiss, J. M., et al. (2009)
	Human, *in vitro*	Intrinsic/competitive binding to TR	-/+	Ren, X. M., et al. (2015)
	Rat, *in vitro*	HEX and PAX8 gene expression	+	Naile, J. E., et al. (2012)
PFHxS	Domestic chicken/ herring gull, *in vitro* (embryonic neuronal cells)	Gene expression Domestic chicken: D2, D3, TTR, RC3, Oct1, MBP Herring gull: D2, RC3, Oct1	Domestic chicken: D2, D3, TTR, RC3, Oct1, MBP Herring gull: D2-, RC3-, Oct1+	Vongphachan, V., et al. (2011)
	Human, *in vitro*	Binding to TTR	+	Weiss, J. M., et al. (2009)
	Human, *in vitro*	Intrinsic/competitive binding to TR	-/+	Ren, X. M., et al. (2015)
	Rat, *in vitro*	HEX and PAX8 gene expression	+	Naile, J. E., et al. (2012)
	Rat, *in vitro*	T-screen (promotion of T_3 dependent proliferation of GH3 cells)	+	Long, M., et al. (2013)
PFOS	Xenopus laevis, *in vivo*	Gene expression (BTEB, TRβ-A, ST3, IFABP, CPO, SLC6A19)	All + but not concentration-dependent	Ren, X. M., et al. (2015)
	Zebrafish, *in vivo*	Gene expression (TTR, TR, NIS, TSHβ, D1) Body length Growth Whole body T_3/T_4	T_4 – Rest +	Shi, X. et al. (2009)
	Medaka, *in vivo*	Developmental (hatching, swim-up, survival) and histopathological effects of the thyroid in offspring	+	Ji, K., et al. (2008)
	Zebrafish, *in vivo*	HEX and PAX8 gene expression	+	Shi, X., et al. (2008)
	Zebrafish, *in vivo*	HEX and PAX8 gene expression	+	Du, G., et al. (2013a)
	Human, *in vitro*	Binding to TTR	+	Weiss, J. M., et al. (2009)

Effects of different PFASs on the hypothalamus-pituitary-thyroid axis.				
Substance	**Test system**	**Endpoints**	**ED-Effect**	**Reference**
	Rat, *in vitro*	T-screen (promotion of T_3 dependent proliferation of GH3 cells)	+	Long, M., et al. (2013)
	Human, *in vitro*	Intrinsic/competitive binding to TR	-/+	Ren, X. M., et al. (2015)
	Human, *in vitro*	Binding to TR (reporter gene assay using CV-1 cell line)	TR+ (antagonist)	Du, G., et al. (2013a)
Fluorotelomer alcohols and acrylates				
6:2 FTOH	Human, *in vitro*	Binding to TTR	-	Weiss, J. M., et al. (2009)
	Human, *in vitro*	Intrinsic/competitive binding to TR	-/-	Ren, X. M., et al. (2015)
8:2 FTOH	Human, *in vitro*	Binding to TTR	-	Weiss, J. M., et al. (2009)
	Human, *in vitro*	Intrinsic/competitive binding to TR	-/-	Ren, X. M., et al. (2015)

+ effective, - non-effective

Table 12-C

Effects of different PFASs on the hypothalamus-pituitary-adrenal/interrenal axis				
Substance	**Test system**	**Endpoints**	**ED-Effect**	**Reference**
PFSAs				
PFBS	Human/Rat, *in vitro*	11βHSD1 enzyme inhibition	-	Ye, L., et al. (2012)
	Human/Rat, *in vitro*	11βHSD2 enzyme inhibition	+	Zhao, B., et al. (2011)
PFHxS	Human/Rat, *in vitro*	11βHSD1 enzyme inhibition	-	Ye, L., et al. (2012)
	Human/Rat, *in vitro*	11βHSD2 enzyme inhibition	+	Zhao, B., et al. (2011)
PFOS	Human/Rat, *in vitro*	11βHSD1 enzyme inhibition	+	Ye, L., et al. (2012)
	Human/Rat, *in vitro*	11βHSD2 enzyme inhibition	+	Zhao, B., et al. (2011)

+ effective, - non-effective

12.1. Interaction with the hypothalamus-pituitary-gonadal axis

12.1.1. *In silico* studies

According to the *in silico* binding study of Kovarich et al. (2012) from the tested short-chain PFASs only 6:2 FTOH and 1,1,2,2-tetrahydroperfluorohexyl iodide can potentially bind to the human ER. A binding potency to the human ER has also been confirmed for long-chain PFASs (such as PFOS, 8:2 FTOH), where also experimental data support this. In addition, 1,1,2,2-tetrahydroperfluorohexyl iodide and polyfluorinated compounds containing an aromatic ring were identified as binder to the human AR (androgen receptor), as well.

In silico predictions of the study of Benninghoff et al. (2011) classified all tested PFASs (C_5-C_{14} PFCA, PFOS, PFDS, 6:2 FTOH, 8:2 FTOH and 8:2 FTA) as weak or very weak agonist for mouse, trout and human ER.

12.1.2. *In vitro* studies

In vitro binding studies with rainbow trout liver cells (Benninghoff et al., 2011) showed a concentration-dependent response for C_5-C_{14} PFCA, PFOS and PFDS. IC_{50} values were ranged between 15.2–289 mM. 6:2 FTOH, 8:2 FTOH and 8:2 FTA showed no binding.

In vitro assessment on FTOHs by Liu et al. (2007) using VTG induction in primary cultured hepatocytes of freshwater male tilapia showed for 6:2 FTOH, PFOA and PFOS a concentration-dependent VTG induction, while 4:2 FTOH and 8:2 FTOH provoked no response.

VTG induction was assessed using an *in vitro* yeast two-hybrid assay involving medaka ERα and ERβ (Ishibashi et al., 2008). VTG induction was exhibited by 6:2 FTOH, 8:2 FTOH and 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-1-decanol but not by C_7-C_{11} PFCA and PFOS.

According to *in vitro* studies on estrogenic activities, fluorinated iodine alkanes and fluorinated diiodine alkanes showed activity using the E-screen and MVLN assays. While none of the fluorotelomer iodides and PFOA showed estrogenic effects in the tests. This study states that the estrogenic potencies of fluorinated iodine and diiodine alkanes correlate well with the carbon chain length of the chemicals indicating an optimum for six carbons (Wang et al., 2012).

The reporter gene assay using MMV-Luc cell line was used also by Wielogorska et al. (2015) for assessing estrogenic effects of short-chain perfluoroalkyl acids (PFAAs). However, the studied PFHxS, C_5-C_{12} PFCA exhibited no estrogenic responses.

Further *in vitro* assays using MCF-7 cells also confirm these finding: using the E-screen assay and gene expression analysis for 6:2 FTOH and 8:2 FTOH (but not for PFOS and PFOA) a concentration-dependent interaction could be observed between the human ER (Maras et al., 2006).

Using the yeast two hybrid assay, both of 6:2 FTOH and 8:2 FTOH (but not PFOS and PFOA) showed a concentration-dependent interaction between the human ERα and ERβ (Ishibashi et al., 2007).

A potential interaction with steroidogenesis has been hypothesized by the study of Gorrochategui et al. (2014) using an *in vitro* test system (human placental choriocarcinoma cell line) indicating aromatase inhibition activity of PFBS, PFOS and PFOA.

Another study by Zhao et al. (2010) investigated the inhibition of enzymes taking part in the production of testosterone using human and rat testis microsomes. PFHxS-K and PFBS-K had no effect at concentrations up to 250 µM while PFOS and PFOS-K were potent inhibitor of the human or rat 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase 3.

The effect of PFOS on steroidogenesis was assessed both at hormone levels in the supernatant and at expression levels of hormone-induced genes in the H295R cell. Reporter gene assays wit CV-1 cells were used to detect receptor-mediated (anti-) estrogenic and (anti-) androgenic activities. The results indicate that PFOS can act as an ER agonist. Exposure to PFOS decreased supernatant T, increased E2 concentrations in H295R cell medium and altered the expression of several genes involved in steroidogenesis (Du et al., 2013a).

The hormone levels of E_2 and T, the expression of major steroidogenic genes and the key steroidogenic gene regulator steroidogenic factors 1 (SF-1) were measured after PFOA exposure in H295R steroidogenesis assay. PFOA is able to interfere with hormone receptor ER using CV-1 cells. In H295R cells, PFOA could increase the E2 production and decrease the T production, altered the expression of major steroidogenic genes and regulator SF-1 (Du et al., 2013b).

Production of sex hormones (E_2 and T) and transcription of steroidogenic genes (CYP19A, CYP17A, CYP11A1, BYP11B2, 3BHSD2, 17BHSD4) were measured after *in vitro* exposure of H295R cells to PFNA and PFTrDA for 48 h. Exposure to PFTrDA resulted in reduced production of T along with lesser expression of CYP17A and CYP11A1 mRNA in H295R cells. PFDA did not induce any changes (Jo et al., 2014).

12.1.3. *In vivo* studies

FTOHs

Ishibashi et al. (2008) assessed *in vivo* estrogenic effects exposing adult medakas over three days to 6:2 FTOH, 8:2 FTOH and 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-1-decanol treated water. All the three compounds caused a concentration-dependent induction of VTG and increase in the hepatosomatic index (HSI) in male medakas. There was also a significant increase in gene expression induction for ERα, and for two VTG genes, but not for ERβ.

An *in vivo* study was conducted by Liu et al. (2009) using zebrafish exposed to 6:2 FTOH for 7 days. Effects on plasma sex hormone levels were measured followed by use of real-time PCR to examine selected gene expression in HPG axis and liver. Exposure to 6:2 FTOH significantly increased plasma E_2 and testosterone (T) levels in both males and females. Furthermore, the ratio of T/E_2 was reduced in females while increased in males. In females, the increase of E_2 was accompanied by upregulated hepatic VTG (VTG1 and VTG3), downregulation of gonad ERα and ERβ and upregulation of the brain activin and activin receptor (putative mediator of gonadotropin-induced oocyte maturation) gene expression. In males, the elevation of the T level is consistent with the altered regulation of some enzymes playing a role in the steroid biosynthesis (CYP17 and CYP19A). In males gonadal

CYP19B, ERα, ERβ, GnRH2 and FSH were upregulated, while hepatic VTG showed a concentration-dependent decreasing upregulation with increasing concentration.

Impairment of reproduction has been shown for 8:2 FTOH: disruption of sex hormone biosynthesis (increased T and E2 levels in females, decreased T and increased E2 levels) and impaired reproduction in adult zebrafish (poor sperm and egg quantity and quality), ultimately resulting in decreased hatching rates in the offspring (Liu et al., 2010a).

PFCAs

The study of Benninghoff et al. (2011) assessed estrogenic effect *in vivo* via dietary exposure of trout assessing plasma VTG. Only C_7-C_{11} PFCA exhibited significant different VTG concentration from control in contrast to C_5-C_6 and C_{12}-C_{14} PFCA, PFBS and PFOS. For C_8-C_{11} PFCA a concentration-dependent increase in VTG concentration could be observed.

The lower dose effect of PFOA on development and endocrine related gene expression (ESR1, ESR2B, CYP17, CYP19A, and CYP19B) were assessed in a short-term zebrafish assay *in vivo*. Exposure of zebrafish embryo to PFOA resulted in higher expression of ESR1. There was a concentration-dependent but not significant induction in CYP19B expression. No lethal or sublethal effects were seen in zebrafish embryos (Du et al., 2013b).

Wei et al. investigated the effects of waterborne exposure to PFOA over 28 days in adult rare minnows on the expression of hepatic estrogen-responsive genes VTG, ERβ, VTG protein levels and histopathological alteration of gonads (Wei et al., 2007). Gene expression changes of ERβ showed concentration-dependent induction both for females and males after 14 days of exposure, which was dissipated by the end of the 28-day exposure. Gene expression of VTG showed significant induction in all treatment groups after 14- and 28-day exposure, as well. However, concentration-dependent changes were seen only at the end of the treatment. Hepatic VTG protein was shown to be significantly induced both in females and males, concentration-dependent changes were seen after 14 days but not after 28 days of exposure. Higher concentration of PFOA induced the development of testes-ova in male rare minnows. The ovaries of PFOA exposed females underwent degeneration.

Giari et al. exposed adult common carps to PFOA over 56 days (Giari et al., 2016). There were no significant differences in condition factor, HSI and GSI of the treated and untreated fish. Occurrence of atretic oocytes and a paucity of spermatozoa were documented in the highest treatment group.

Two-generation fish toxicity tests using medakas showed that parental exposure to PFOA affected the performance of offspring. Unexposed progeny-generation (F1) fish exhibited elevated mortality and poor hatching and swim-up success that were correlated with exposure in the parental generation (F0) (Ji et al., 2008).

Effects of PFDA and PFTrDA on sex steroid hormones and expression of mRNA of selected genes in HPG axis were evaluated after 120-day exposure of zebrafish. In zebrafish, significant upregulation of VTG1 was observed in males exposed to PFDA, whereas down-regulation was observed in females exposed to PFTrDA. In male zebrafish, concentrations of E_2 were significantly increased at 0.01 mg/L PFTrDA. Significant increases in ratios of E_2/T and E_2/11-ketotestosterone were observed in male zebrafish after exposure to PFDA or PFTrDA, indicating estrogenic potentials of these compounds (Jo et al., 2014).

PFSAs

Higher tier effects on sexual development were tested using xenopus tadpoles in a chronic test by Lou et al. (2013). PFBS and PFOS caused hepatohistological impairment at higher concentrations (100 and 1000 µg/L). Unlike PFBS, PFOS at all concentrations did not alter the sex ratio and induce intersex but caused degeneration of spermatogonia in testes except for the lowest concentration. PFOS and PFBS promoted expression of ER and AR, but not affected aromatase expression in the brain. The increase in expression of ER and AR suggests an increase in the responsiveness to the corresponding sex hormone and potential effects on sexual development.

Sexually mature fathead minnow was exposed via the water for 21 d to PFOS, and effects on reproductive capacity and endocrinology were assessed. Effects on fecundity and histopathology of ovaries were observed. Furthermore, males exhibited decreased aromatase activity and elevated concentrations of plasma 11-ketotestosterone and T, while females did not show any alteration in levels of E_2, VTG, T and brain aromatase activity. No developmental effects were noticed in offspring. It is unclear whether the observed effects were related to systemic toxicity (fish were died in the highest treatment group within the first week of exposure) or interference with the HPG axis (Ankley et al., 2005).

PFOS decreased steroidogenic enzyme gene (CYP17, CYP19A, CYP19B) expression, and changed the expression pattern of estrogen receptor production genes (upregulation for ER1, downregulation for ER2B) in zebrafish embryos (Du et al., 2013a).

The expression of CYP19A and of CYP19B was significantly downregulated at all PFOS exposure concentrations in zebrafish embryos (Shi et al., 2008). Systemic toxicity of PFOS, which was visible by sublethal effects, might confound the gene expression data.

Fang et al. (2012) examined the transcriptional responses and PFOS accumulation in the marine medaka embryos at the early and late developmental stages of 4 and 10 dpf upon PFOS exposure. The mRNA expression levels of ERα and ERγ were not significantly altered, but the estrogenic marker genes (VTG1, VTG2, ChgH and ChgL) were downregulated upon PFOS exposure at 4 days post fertilization. Conversely, ERs and related marker genes all were significantly upregulated at 10 days post fertilization. The expressions of CYP19A and CYP19B were regulated by PFOS in a stage-specific manner. Since lethal and sublethal effects were also documented in the tested concentrations, these effects might mask the findings on gene expression alteration provoked by PFOS.

Long-term endocrine effects of PFOS were tested using three generations of zebrafish by Keiter et al. (2012). VTG levels were generally found to decrease with increasing PFOS-exposure in both F1 and F2 generations. Histological analyses of F1 and F2 fish revealed hepatocellular vacuolization, predominantly in males, following PFOS-exposure. Hepatotoxicity by PFOS might explain the suppressed VTG response seen in PFOS-exposed F1 and F2 males. Fecundity and fertilization rate were not influenced by PFOS.

Two-generation fish toxicity tests using medakas showed that parental exposure to PFOS affected the performance of offspring. Unexposed progeny-generation (F1) fish exhibited elevated mortality and poor hatching and swim-up success that were correlated with exposure in the parental generation (F0) (Ji et al., 2008).

PFBS exhibited no effects on the reproduction of northern bobwhite quail in a chronic dietary study (Newsted et al., 2008).

12.2. Interaction with the hypothalamus-pituitary-thyroid axis

12.2.1. *In vitro* studies

A gene expression assay was performed on avian (domestic chicken and herring gull) neuronal cells investigating key genes in the thyroid hormone pathway (iodothyronine 5'-deiodinase 2 and 3, transthyretin, neurogranin, octamer motif-binding factor, and myelin basic protein). Short-chained PFCs (less than eight carbons) altered the expression of TH-responsive genes (D2, D3, TTR, and RC3) in chicken embryonic neuronal cells to a greater extent than long-chained PFCs (more than or equal to eight carbons). Variable transcriptional changes were observed in herring gull embryonic neuronal cells exposed to short-chained PFCs; mRNA levels of Oct-1 and RC3 were upregulated (Vongphachan et al., 2011).

By assessing the T_3-dependent proliferation of rat pituitary cells, seven PFAAs (PFOS, PFHxS, C_8-C_{12} PFCA) inhibited the GH3 cell growth, and four PFAAs (PFOS, PFHxS, PFNA, and PFUnA) antagonized the T_3-induced GH3 cell proliferation. At the highest test concentration, PFHxS showed a further increase of the T_3-induced GH3 growth (Long et al., 2013).

Using rat hepatoma cells, expression of two genes (PAX8 and HEX) playing a role in thyroid development was assessed (Naile et al., 2012). These genes have been shown previously to be responsive for PFOS. An upregulation could be observed for PFBS, PFHxS, PFBA, PFPeA, PFHxA. When comparing the gene expression induction of PFHxS and PFBS to PFOS, PFBS was similar to PFOS while PFHxS provoked stronger effects. When comparing PFBA, PFPeA, PFHxA to PFOA, HEX levels were almost the same, while PAX8 was higher expressed in all the shorter chain PFAAs.

The binding to the human thyroid receptor (TR) was assessed by Ren et al. (2015) where the intrinsic binding studies showed no activity for the assessed PFBA, PFHxA, PFHpA, PFBS, PFHxS, 8:2 FTOH, 6:2 FTOH in contrast to C_8-C_{18} PFCA and PFOS. However, PFHxA, PFHxS and PFHpA showed very weak but concentration-dependent binding affinity to TR in the competitive binding assay with T_3 similarly to C_8-C_{18} PFCA, PFBS and PFOS. PFBA, 6:2 FTOH and 8:2 FTOH exhibited no effect, and the highest binding affinity was shown for PFDA.

Weiss et al. (2009) assessed the competitive binding of various PFASs (C_4-C_{14}) to the human thyroid hormone transport protein, transthyretin (TTR). TTR binding potency varied with carbon chain length, with a maximum potency for PFOA among PFCA (IC_{50} = 949 nM) and for PFHxS (IC_{50} = 717 nM) among PFSAs. The binding potency is clearly associated with the degree of fluorination of the alkyl chain. TTR binding potencies were significantly higher for compounds containing a sulfonate functional group than for those containing a carboxylic acid functional group.

Reporter gene assay using CV-1 cells was used to detect receptor-mediated (anti-)thyroid hormone activity of PFOS and PFOA. The results indicate that both PFOS and PFOA can act as a TH receptor antagonist (Du et al., 2013a; Du et al., 2013b).

12.2.2. *In vivo* studies

PFCAs

The lower dose effect of PFOA on development and endocrine related gene expression (HHEX, PAX8) were assessed in a short-term zebrafish assay *in vivo*. Exposure of zebrafish embryo to PFOA resulted in higher expression of HHEX and PAX. No lethal or sublethal effects were seen in zebrafish embryos (Du et al., 2013b).

Two-generation fish toxicity tests using medakas showed that parental exposure to PFOA affected the performance of offspring. Unexposed progeny-generation (F1) fish exhibited elevated mortality and histopathological changes of the thyroid that were correlated with exposure in the parental generation (F0) (Ji et al., 2008).

Zebrafish were exposed to different concentrations of PFNA (0, 0.05, 0.1, 0.5, and 1 mg/l) from their early life stages (F (0), 23-day post-fertilization dpf), and the exposure period lasted for 180 days. At the end of the exposure period, thyroid follicle histology and plasma thyroid hormone levels in male zebrafish were evaluated as direct endpoints for the specific thyroid toxicities, while gene expression relative to the HPT axis was also investigated to study the underlying mechanisms. In addition, offspring embryos (F (1)) from the PFNA exposure parental zebrafish was reared in water either without PFNA or under continual exposure to PFNA for an additional 180 days to investigate effects of multi-generational exposures on the circulating T_3 levels and thyroid-associated gene expression. The results demonstrate significantly elevated plasma T_3 levels were observed in both F (0) and F (1) adults, as well as PFNA-induced histological changes in the thyroid follicles of F (0) male zebrafish. In the liver, the abundance of gene transcript encoding the protein TTR was significantly induced, while the expression of UDP-glucuronosyltransferases in F (0) adult males was inhibited. The induced thyroid-disrupting effects also demonstrated a trans-generational effect that was reflected by altered gene expression related to TH synthesis and metabolism in F(1) larvae (Liu et al., 2011).

PFSAs

Ren et al. (2015) assessed PFOS effects on gene expression of Xenopus laevis tadpoles. After two days of exposure intestinal tissues of tadpoles were collected for gene expression analysis of three TH upregulated genes (TRβ-A, ST3, BTBE) and three TH downregulated genes (IFABP, CPO, SLC6A19). PFOS caused similar effects in gene expression like T_3, although to lesser extent.

In zebrafish embryos the expression of HHEX was significantly up-regulated at all concentrations tested, whereas PAX8 expression was significantly up-regulated only upon exposure to lower concentrations of PFOS (0.1, 0.5, 1 mg/L) (Shi et al., 2008). Systemic toxicity of PFOS, which was visible by sublethal effects, might confound the gene expression data.

PFOS increased early thyroid development gene expression of HHEX from 500 µg/L and PAX8 from 100 µg/L in a concentration-dependent manner in zebrafish embryos (Du et al., 2013a).

In zebrafish larvae PFOS exposure could alter gene expression in the HPT axis and that mechanisms of disruption of thyroid status by PFOS could occur at several steps in the synthesis, regulation, and action of thyroid hormones (NIS, TTR, TG, D1). The whole body T_4 content remained unchanged, whereas T_3 levels were significantly increased. In the

highest PFOA concentration (400µg/L), wet weight and length were also significantly affected (Shi et al., 2009).

Two-generation fish toxicity tests using medakas showed that parental exposure to PFOS affected the performance of offspring. Unexposed progeny-generation (F1) fish exhibited elevated mortality and histopathological changes of the thyroid that were correlated with exposure in the parental generation (F0) (Ji et al., 2008).

12.3. Interaction with the hypothalamus-pituitary-adrenal/ interrenal axis

12.3.1. *In vitro* studies

Interaction with glucocorticoid metabolism was investigated *in vitro* by assessing the inhibition potency of PFASs on two key enzymes playing a role in glucocorticoid metabolism. Despite PFOA and PFOS, PFHxS and PFBS provoked no effect on the human and the rat 11β-hydroxysteroid dehydrogenase 1 (Ye et al., 2012).

A competitive inhibition by PFOA, PFOS, PFHxS and PFBS was shown for the human and rat 11β-hydroxysteroid dehydrogenase 2 (Zhao et al., 2011).

12.4. Conclusion on endocrine disrupting properties

In summary, findings on interaction with the HPG axis are contradictory for short-chain PFSAs and PFCAs but rather convincing for FTOHs and long-chain PFCAs. Since PFSAs exhibited sublethal effects at concentrations where endocrine effects were reported, it is unclear whether they were related to systemic toxicity or interference with the HPG axis. Several studies indicate interactions of PFSAs and PFCA with the thyroid hormone signalling. Given the wide variety of mode of actions for thyroid disruption, it is difficult to assess the whole spectrum of molecular initiating actions of thyroid disruption using *in vitro* assays. Unfortunately, aquatic *in vivo* assays are lacking for the short-chain PFASs but the ones available for the long-chain members indicating interaction with the thyroid hormone signalling. Only limited number of studies gives first indication on interaction with the glucocorticoid pathway. Further evidence would be needed to evaluate concerns on this pathway.