EUROPEAN CHEMICALS AGENCY

Helsinki, 31 August 2015

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**DECISION ON SUBSTANCE EVALUATION PURSUANT TO ARTICLE 46(1) OF REGULATION (EC) NO 1907/2006**

**For reaction mass of mixed (3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl) phosphates, ammonium salt, CAS No not available (EC No 700-161-3)**

**Addressees: Registrant(s)[1] of reaction mass of mixed (3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl) phosphates, ammonium salt**

This decision is addressed to all Registrants of the above substance with active registrations on the date on which the draft for the decision was first sent, with the exception of the cases listed in the following paragraph. A list of all the relevant registration numbers subject to this decision is provided as an annex to this decision.

Registrants meeting the following criteria are *not* addressees of this decision: i) Registrants who exclusively use the above substance as an on-site isolated intermediate and under strictly controlled conditions and ii) Registrants who have ceased manufacture/import of the above substance in accordance with Article 50(3) of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation) before the decision is adopted by ECHA.

Based on an evaluation by Bureau REACH on behalf of the Ministry of Infrastructure and the Environment as the Competent Authority of the Netherlands (evaluating MSCA), the European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 52 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

This decision is based on the registration dossier(s) on 23 January 2015 , i.e. the day until which the evaluating MSCA granted an extension for submitting dossier updates which it would take into consideration.

This decision does not imply that the information provided by the Registrant(s) in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on the dossier(s) of the Registrant(s) at a later stage, nor does it prevent a new substance evaluation process once the present substance evaluation has been completed.

## I. Procedure

Pursuant to Article 45(4) of the REACH Regulation the Competent Authority of the Netherlands has initiated substance evaluation reaction mass of mixed (3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl) phosphates, ammonium salt, CAS No not available (EC No 700-161-3) based on registration(s) submitted by the Registrant(s) and

[1] The term Registrant(s) is used throughout the decision, irrespective of the number of registrants addressed by the decision.

other relevant and available information and prepared the present decision in accordance with Article 46(1) of the REACH Regulation.

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to its persistence, bioaccumulation and toxicity (PBT) properties reaction mass of mixed (3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl) phosphates, ammonium salt was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2013. The updated CoRAP was published on the ECHA website on 20 March 2013. The Competent Authority of the Netherlands was appointed to carry out the evaluation.

The evaluating MSCA considered that further information was required to clarify the abovementioned concerns. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 20 March 2014.

On 29 April 2014 ECHA sent the draft decision to the Registrant(s) and invited them pursuant to Article 50(1) of the REACH Regulation to provide comments within 30 days of the receipt of the draft decision.

**Registrant(s) commenting phase**

By 5 June 2014 ECHA received comments from the Registrant(s) of which it informed the evaluating MSCA without delay.

The evaluating MSCA considered the comments received from the Registrant(s). The information contained therein is reflected in the Statement of Reasons (Section III) whereas no amendments to the Information Required (Section II) were made.

**Commenting by other MSCAs and ECHA**

In accordance with Article 52(1) of the REACH Regulation, on 5 March 2015 the evaluating MSCA notified the Competent Authorities of the other Member States and ECHA of its draft decision and invited them pursuant to Articles 52(2) and 51(2) of the REACH Regulation to submit proposals to amend the draft decision within 30 days of the receipt of the notification.

Subsequently, Competent Authorities of the Member States and ECHA submitted proposals for amendment to the draft decision.

On 10 April 2015 ECHA notified the Registrant(s) of the proposals for amendment to the draft decision and invited them pursuant to Articles 52(2) and 51(5) of the REACH Regulation to provide comments on those proposals for amendment within 30 days of the receipt of the notification.

The evaluating MSCA reviewed the proposals for amendment received and amended the draft decision.

**Referral to Member State Committee**

On 20 April 2015 ECHA referred the draft decision to the Member State Committee.

By 11 May 2015, in accordance to Article 52(2) and Article 51(5), the Registrant(s) provided comments on the proposal(s) for amendment. The Member State Committee took the

comments of the Registrant(s) on the proposal(s) for amendment into account.

A unanimous agreement of the Member State Committee on the draft decision was reached on 28 May 2015 in a written procedure launched on 15 May 2015. ECHA took the decision pursuant to Article 52(2) and Article 51(6) of the REACH Regulation.

## II. Information required

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit the following information using the indicated test methods (in accordance with Article 13 (3) and (4) of the REACH Regulation) and the sodium or potassium salt of the degradation product 2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoroheptanoic acid (PFHpA), CAS No 375-85-9 (EC No 206-798-9) of the registered substance subject to the present decision:

**Reproduction/Developmental Toxicity Screening Test in mice, oral route (OECD 422) extended to 90 days for the pre-mating and mating period and extended to 21 days post weaning.**

The study shall follow the test method described in the updated draft OECD test guideline 422 (OECD 2015), with some modifications: The premating exposure period for the P generation shall be extended to 90 days to ensure that steady-state exposure conditions in P males and females are achieved before mating. The post-weaning period of the F1-generation shall be adjusted to ensure that the animals are exposed for at least 21 days after weaning.

The test shall include 5 extra females in the control group and 5 extra females in the highest dose group. Blood samples shall be taken from 5 males and 5 females randomly selected from each group at the same time (prior to mating) in order to detect any difference of sensitivity during the repeat dose toxicity test. The additional animals shall be necropsied after the 90 day period and assessed in the same way as the remaining animals. In this way a gender comparison can be set up without gestational influence.

The Registrant(s) shall follow the physical development of the offspring by recording the body weight gain, and sexual maturation (age and body weight at vaginal opening or balano-prepution separation). The ano-genital distance shall also be recorded at weaning. Moreover, the Registrant(s) are requested to examine the mammary gland development of the offspring using the whole mounts method. The toxicity of the liver shall also be evaluated by doing a histological examination of liver at necropsy.

The required combined protocol shall generate information on repeated dose toxicity equivalent to a repeated dose toxicity study (EU B.26 /OECD TG408).

Pursuant to Article 46(2) of the REACH Regulation, the Registrant(s) shall submit to ECHA by **07 December 2017** an update of the registration(s) containing the information required by this decision[2], including robust study summaries and updated Chemical Safety Reports addressing the new information.

## III. Statement of reasons

*(To enhance the readability of this section, acronyms according to Buck et al. (2011) are used for the constituents of the registered substance and their degradation products. The*

[2] The deadline set by the decision already takes into account the time that registrants may require to agree on who is to perform any required tests and the time that ECHA would require to designate a registrant to carry out the test(s) in the absence of the aforementioned agreement by the registrants (Article 53(1) of the REACH Regulation).

*corresponding molecular structures, IUPAC names and synonyms are given in Annex I.)*

Based on the evaluation of all relevant information submitted on reaction mass of mixed (3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl) phosphates, ammonium salt and other relevant and available information, ECHA concludes that further information is required in order to enable the evaluating MSCA to complete the evaluation of whether the substance constitutes a risk to human health or the environment.

Reaction mass of mixed (3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl) phosphates, ammonium salt is a multi-constituent substance. [redacted] All constituents, [redacted], are highly fluorinated and thus potentially (very) persistent.

Initial grounds for concern related to the PBT properties of the registered substance. PBT assessment can be based on the registered substance, a specific constituent or a formed degradation product. The latter option was advocated by the Registrant(s) in the last update of the registration dossier (May 21st, 2013) as the following bioaccumulation waiver was included: "Since the notified substance (TNS) rapidly degrades to form 6:2 FTOH, and 6:2 FTOH and its terminal metabolite PFHxA have been demonstrated to have a low bioconcentration and bioaccumulation potential, it is appropriate to conclude that TNS will pose no concern for bioaccumulation". Besides this waiver, there is also increasing evidence in the public literature that fluorotelomer-based compounds are ultimately biotransformed to PFCAs (reviewed by Butt et al. (2013)). Therefore, it is considered appropriate to base the PBT/vPvB assessment of the registered substance on a degradation product. While the Registrant(s) proposed PFHxA, extensive evaluation of the available data showed that primary biodegradation, atmospheric degradation and/ or metabolism of the constituents yields a number of terminal degradation products, including PFHpA (CAS No 375-85-9, EC No 206-798-9). PFHpA is one fluorinated carbon longer than PFHxA and thus in regard to PBT properties more relevant. Therefore, this substance evaluation focusses on the degradation product PFHpA.

PFHpA has six perfluorinated carbons. Carbon-fluoride bonds are very strong, and highly fluorinated alkanes are generally considered to be the most stable organic compounds (Siegemund et al., 2000). Several structurally similar longer chained PFCAs have already been identified as PBT/vPvB substances, and it is very likely that PFHpA will also meet the criteria for P and/or vP as specified in REACH, Annex XIII.

Considering the B-criterion, there is evidence that PFHpA bioaccumulates in earthworms (Zhao et al., 2013c) and humans (Freberg et al., 2010, Nilsson et al., 2010) with a geometric mean elimination half-life in ski-wax technicians of 98 day (range 23 to 599 days). In addition, it has also been shown that PFHpA is maternally transferred in polar bears (Bytingsvik et al., 2012) and humans (Zhang et al., 2013b). In contrast, trophic magnification of PFHpA was investigated but not observed in an artic (Kelly et al., 2009), a subtropical pelagic (Loi et al., 2011), nor a remote terrestrial (Müller et al., 2011) food web. Also, there are human maternal transfer studies that did not detect PFHpA in maternal and/or cord blood (Monroy et al., 2008, Kim et al., 2011, Gutzkow et al., 2012). Thus, data from public literature on the bioaccumulation potential of PFHpA are equivocal, and at this moment it is too premature to conclude upon the B-criterion.

In general, information regarding the bioaccumulation potential of PFHpA would be requested first, instead of toxicity data. However, standardized bioconcentration and

bioaccumulation studies with fish have already been performed (Martin et al., 2003a, Martin et al., 2003b), but are not suitable to assess the bioaccumulation potential of PFCAs as was previously demonstrated for PFOA. PFOA has recently been identified as a substance of very high concern based on its PBT properties (Article 57 e), as well as its toxicity to reproduction (Article 57 c), but had bioconcentration factor (BCF) values ranging from 4 to 27 in a rainbow trout bioconcentration study (Martin et al., 2003a). It was concluded that since PFOA is notably water soluble and mainly occurs in protein rich tissues, e.g. blood and liver, bioconcentration in gill breathing organisms and the accumulation in lipids may not be the most relevant endpoint to consider. Consequently, to demonstrate bioaccumulation of the structurally similar compound PFHpA additional monitoring studies, preferably in humans, may have to be requested. Requesting such complex and time-consuming studies is considered unjustified if PFHpA does not meet the T-criterion in addition to the P-/vP-criterion. Therefore, the information requested in Section II constitutes the first tier in a testing strategy to clarify the concerns for potential PBT properties of the registered substance or its degradation products.

Based on the result from the requested OECD TG422 study, a second tier of the PBT testing strategy may be triggered. If it is concluded that PFHpA, i.e. the degradation product of the registered substance, meets the criteria for P and/or vP, and T as specified in REACH, Annex XIII, then PFHpA will need to be evaluated with regard to the criteria for bioaccumulation (REACH, Annex XIII). A human monitoring study may be requested in a second draft decision in which the study design will need to be specified. Hence, the evaluating MSCA will review the information submitted by the Registrant(s) as an outcome of this decision and evaluate if further information should be requested in order to clarify the PBT properties.

In the following sections, the above stated information from the registration dossier and public literature will be discussed in more detail.

***Rationale to conduct PBT assessment with PFHpA***

*Persistence of the registered substance*
Chemically, [redacted] are capable of undergoing hydrolysis reactions under acid, base and neutral conditions to form their corresponding alcohol and acid [redacted]. Hydrolysis of the [redacted] constituents of the registered substance would yield 6:2 FTOH and [redacted]. Nonetheless, it was demonstrated that the registered substance is hydrolytically stable in the presence of strong acids and bases, with half-lives of more than 1 year at 25 °C and pH 4, 7 and 9 ([redacted]).

Biodegradation of the registered substance was investigated in two GLP compliant screening studies. In the closed bottle test (OECD 301D) 1.87 mg a.i/L was used, and it was shown that the registered substance is not ready biodegradable as only 11.5% was degraded after 28 days based on oxygen consumption ([redacted]). In the modified MITI test (II) (OECD 302C) that used 30.5 mg a.i./L, biodegradation was assessed by measurement of the oxygen consumption and the residual amounts of the [redacted] ([redacted]). Both analytical approaches showed that the registered substance is not inherently biodegradable as degradation after 28 days amounted to 16.3% and 15.7%, respectively. Thus, as the registered substance is neither ready nor inherently biodegradable, it meets the P screening criteria as stated in REACH, Annex XIII.

Nevertheless, it seems unlikely that the registered substance will meet the definitive P criteria, as there is increasing evidence that fluorotelomer-based compounds are ultimately biotransformed to PFCAs (Butt et al., 2013). The ready biodegradation study did not contradict this, as oxygen consumption monitoring merely showed that the registered substance was not ultimately biodegraded, and as such it did not exclude primary

biodegradation of the constituents to one or more persistent degradation products. In contrast, the monitoring of the residual amounts of the [redacted] constituent in the inherent biodegradation study showed that the [redacted] constituent is not inherently biodegradable under the prevailing test conditions. Since persistence of the [redacted] constituent could not be excluded, this constituent may be assessed at a later stage of the substance evaluation process if needed.

*Biodegradation of the major constituents* [redacted] investigated the aerobic biodegradation of [redacted] (1 µg test substance/mL) in a mixture of raw wastewater, sewage sludge and [redacted] mineral medium for 92 days. It was shown that [redacted] are microbially hydrolysed to 6:2 FTOH, which is then further oxidized via transient transformation products, i.e. 6:2 FTCA and 6:2 FTUCA, to primarily PFHpA (8.4 and 7.3 mol% expressed as percent [redacted] present in the aqueous phase at the start of the experiment) and in lesser amounts to PFHxA (2.1 and 6.2 mol%), PFPeA (0.7 and 1.5 mol%) and 5:3 Acid (0.1 and 1.5 mol%). The PFCAs add up to 11 and 16 mol% of the [redacted] present in the aqueous phase at the start of the experiment, respectively. These yields might have been higher if the test vessels were not continuously purged. This resulted in the stripping of the volatile degradation product 6:2 FTOH from the system (6:2 FTOH collected in the XAD-cartridges amounted to approximately 14 and 54 mol%, respectively) and thus reduced its degradation to PFCAs. It should also be noted that the [redacted] concentrations in the aqueous phase were very low at the start of the experiment, corresponding to 10, respectively, 33% of the added test substance. These losses could have been caused by several processes, e.g. binding to the polypropylene bottle walls, septa and/or caps, evaporation due to continuous purging, and binding to biosolids. The latter process seems especially relevant for [redacted] as it rapidly disappeared from the aqueous phase of the sterile controls. Therefore, only qualitative interpretation of the study is appropriate, and it can be concluded that two major constituents of the registered substance (amounting to [redacted]), are PFHpA sources upon biodegradation.

*Biodegradation of intermediate transformation product* [redacted] *6:2 FTOH*
Considering that [redacted] are biodegraded to 6:2 FTOH [redacted], and [redacted], the fate of this compound was further investigated. Several studies from public literature were available on 6:2 FTOH biodegradation. All aerobic studies reported rapid 6:2 FTOH biotransformation with most of the 6:2 FTOH disappeared from the test systems within a few days, regardless of the environmental matrix. None of the studies detected degradation in the sterile controls, indicating microbial degradation.

Zhao et al. (2013b) investigated aerobic biodegradation of $^{14}C$-labeled 6:2 FTOH in activated (diluted and native) sludge for 60 days. Detected transient intermediate transformation products were 6:2 FTCA, 6:2 FTUCA, 5:3 Uacid, 5:2 sFTOH and 5:2 ketone, followed by the terminal transformation products 5:3 Acid (14.1 mol%), PFHxA (11 mol%), PFPeA (4.4 mol%) and 4:3 Acid (1.3 mol%). These were average yields. In the native sludge setup (undiluted) PFBA was also detected (1 mol%).

Liu et al. (2010b) investigated aerobic biodegradation of 6:2 FTOH in a mixed bacterial culture for 90 days. Detected transient intermediate transformation products were 6:2 FTUCA, 5:2 sFTOH and 6:2 FTCA, followed by the terminal transformation products 5:3 Acid (6 mol%), PFHxA (5 mol%) and trace quantities of PFBA and PFPeA (<0.5 mol%).

Liu et al. (2010b) also investigated aerobic biodegradation of 6:2 FTOH in a soil for 180 days. Detected transient intermediate transformation products were 6:2 FTCA, 6:2 FTUCA, 5:2 ketone and 5:2 sFTOH, followed by the terminal transformation products PFPeA (30

mol%), 5:3 Acid (15 mol%), PFHxA (8 mol%), PFBA (2 mol%) and 4:3 Acid (1%).

Liu et al. (2010a) investigated aerobic biodegradation of $^{14}$C-labeled 6:2 FTOH in a flow-through soil incubation system for 84 days. Detected transient intermediate transformation products were 6:2 FTCA, 6:2 FTUCA, 5:3 Uacid, 5:2 sFTOH and 5:2 ketone, followed by the terminal transformation product 5:3 Acid (12 mol%), PFHxA (4.5 mol%), PFPeA (4.2 mol%), 4:3 Acid (1 mol%) and PFBA (0.8 mol%).

Zhao et al. (2013a) investigated aerobic biodegradation of 6:2 FTOH in river sediments for 100 days. Detected transient intermediate transformation products were 6:2 FTCA, 6:2 FTUCA, 5:2 ketone and 5:2 sFTOH, followed by the terminal transformation product 4:3 Acid (23.7 mol%), 5:3 Acid (22.4 mol%), PFPeA (10.4 mol%), PFHxA (8.4 mol%) and PFBA (1.5 mol%).

Zhang et al. (2013a) investigated anaerobic biodegradation of $^{14}$C-labeled 6:2 FTOH in digester sludge for 90 and 176 days. Biotransformation was considerably slower than in aerobe systems, with an estimated disappearance half-life of 30 days. At the end of the experiments, intermediate and terminal transformation products were detectable, i.e. 6:2 FTCA, (32-43 mol%), 6:2 FTUCA (1.8-8.0 mol%), 5:2 sFTOH (2.5-0.6 mol%), 5:3 Acid (23-18 mol%) and PFHxA (0.2-0.4 mol%).

Thus, it was shown that 6:2 FTOH is rapidly aerobically biodegraded in different environmental matrices and that anaerobic biodegradation also occurs albeit considerably slower. The reported metabolite profiles differed even when aerobic biodegradation studies were performed in the same environmental matrix, e.g. Liu et al. (2010a) reported equal yields of PFPeA (4.2 mol%) and PFHxA (4.5 mol%) in soil, while Liu et al. (2010b) detected considerably more PFPeA (30 mol%) than PFHxA (8 mol%). These differences can be due to covalent bonding of transformation products, e.g. 5:3 Acid, to organic matter thus limiting further transformation. The more fundamental differences between studies, i.e. if certain PFCAs are formed, reflects most probably the type of microbial community present. Besides the differences reported in the above discussed biodegradation studies, there are literature reports that show that individual bacterial strains can biotransform 6:2 FTOH via different or preferred transformation pathways (Kim et al., 2012) and that addition of an enzyme inducer or an external reducing energy source can modify the metabolite profile of a bacterial strain (Kim et al., 2013). The formation of PFHxA in all 6:2 FTOH biodegradation studies demonstrates that β-oxidation is an important microbial degradation mechanism. While none of the studies discussed in this section detected PFHpA, it should be noted that the fact that [redacted] observed PFHpA formation upon biodegradation of 6:2 FTOH (that was formed by microbial hydrolysis of [redacted]), suggests that there are bacterial strains that can degrade 6:2 FTOH via α-oxidation.

*Abiotic degradation of intermediate transformation product and minor constituent 6:2 FTOH*

6:2 FTOH is a very volatile compound with a Henry's law constant (log $K_{p,\,AW}$) at 25 °C of 1.92 ± 0.24 Pa.m$^3$.mol$^{-1}$ (Lei et al., 2004), and is thus expected to partition into the atmosphere upon release to the environment. It is therefore relevant to know the atmospheric persistence of 6:2 FTOH and the formed degradation products.

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

Ellis et al. (2004) investigated the degradation products that are formed upon atmospheric degradation of 4:2, 6:2 and 8:2 FTOHs. The FTOHs were incubated with Cl-atoms in trapped smog chamber. Oxidation of 6:2 FTOH produced PFHpA, PFHxA, PFPeA, PFBA, PFPrA, TFA, 6:2 FTCA, 6:2 FTAL, PFHxAL and 6:1 FTOH. Yields were only reported for the 8:2 FTOH oxidation products and amounted for the PFCAs: PFNA (1.6%), PFOA (1.5%), PFHpA (0.32%), PFHxA (0.24%), PFPeA (0.10%), PFBA (<0.1%), PFPrA (<0.1%), TFA (<0.1%). Based on these yields, it is very likely that PFHpA and PFHxA are the most abundantly formed PFCAs upon oxidation of 6:2 FTOH.

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

Thus, it was shown that 6:2 FTOH is very volatile (Lei et al., 2004) and has a long atmospheric half-life of 20 days allowing long-range atmospheric transport (Ellis et al., 2003). In the atmosphere 6:2 FTOH oxidizes to a range of PFCAs with PFHpA and PFHxA being the most abundant ones (Ellis et al., 2004). Removal from the atmosphere also occurs by binding to natural and artificial surfaces where especially at metal-rich surfaces, 6:2 FTOH is photooxidized to PFHxA and in lower amounts to PFPeA and PFHpA (Styler et al., 2013). Thus, abiotic degradation of 6:2 FTOH is a source of PFHpA, especially upon oxidation in the atmosphere.

*Biodegradation of [redacted] 6:2 FTI*
Ruan et al. (2013) investigated the aerobic biotransformation of 6:2 FTI in soil for 91 days. Primary biotransformation was rapid with an estimated disappearance half-life of about 4.5 days. Detected transient intermediate transformation products were 6:2 FTCA, 6:2 FTUCA, 5:2 ketone, 5:2 sFTOH, and 5:2 Uacid. These were followed by the terminal transformation products PFPeA (20 mol%), PFHpA (16 mol%), 5:3 Acid (16 mol%) and PFHxA (3.8 mol%). It was postulated that 6:2 FTI biotransformation in soil occurs via two major routes: one via 6:2 FTUI to PFHpA and the other via 6:2 FTOH to PFPeA, PFHxA, 4:3 acid and 5:3 acid. The authors could not verify this hypothesis as a 6:2 FTUI standard was lacking. It can therefore not be excluded that PFHpA is also formed via 6:2 FTOH.

Thus, it was shown that 6:2 FTI, [redacted], is also a source of PFHpA.

*Metabolization of a compositionally similar analogue of the registered substance*
An *in vivo* rat study with a compositionally similar analogue of the registered substance was included in the registration dossier as supporting study after read-across ([redacted]). The read-across seems plausible. Both multi-constituent substances are formulations with ~[redacted] active ingredient as solids in water. Their constituents as well as the typical concentrations of their constituents are almost identical, i.e. [redacted] amounted to [redacted] and [redacted]% in the registered substance and the analogue, [redacted] to [redacted] and [redacted]%; [redacted] to [redacted] and [redacted] to [redacted] and [redacted] to [redacted] and [redacted]%; [redacted] to [redacted] and [redacted]%. The only substantial difference between the two substances is the counterion, which is ammonium in the registered substance and [redacted] in the analogue.

In the study, the analogue was administered by oral gavage to 11-weeks old male and

female rats in a single dose of either 10 mg a.i./kg or 30 mg a.i./kg. Plasma, fat and liver tissue were regularly sampled. It was observed that the levels of [redacted] constituents rapidly decreased, indicating metabolism in rats. In all tissues, [redacted] was the most abundantly present parent constituent. It was also the only parent constituent that was consistently detected in both fat and liver tissue. A half-life between 37 and 53 hours was estimated for [redacted]. The transient metabolites 6:2 FTCA and 6:2 FTUCA were detected in plasma, as well as the terminal metabolites PFHpA, PFHxA, PFBA and 5:3 Acid. Plasma concentrations of 6:2 FTOH were not quantified (<LOQ). It was suggested that the metabolic rate of 6:2 FTOH is faster than the rate of formation, resulting in non-detectable concentrations in vivo. Yields were not reported.

The Registrant(s) submitted in the commenting period, a modified one-generation reproduction study in mouse according to EPA OPPTS 870.3550 for the compositionally similar analogue of the registered substance that is being evaluated by the Belgian Competent Authority (EC number 700-403-8) ([redacted]). In this study, the analogue was administered by oral gavage to $P_1$ adult CD-1 mice (20/sex/group) daily at 0, 30, 300, 750, or 1000 mg/kg body weight/day (mg/kg/day) for 10 weeks (males) and 2 weeks (females) during the premating period, and then up until the day before scheduled sacrifice. The formation of metabolites was not monitored.

Thus, based on the study with the structural analogue in rat, it can be concluded that the registered substance is metabolized in rats and that several PFCAs are formed, amongst which PFHpA.

Metabolization of the minor constituent and intermediate metabolite 6:2 FTOH
In an in vivo rat study, where 6:2 FTOH was administered by oral gavage to three male rats in a single dose of 300 mg/kg ([redacted]), metabolism of 6:2 FTOH was investigated. Regular sampling of blood, fat and liver tissue (up to 168 h) detected the following metabolites: 6:2 Acid, 6:2 FTUA, 4:3 Acid, 5:3 Acid, PFBA, PFPeA, PFHxA and PFHpA. Yields were not reported.

In an in vivo rat study, where sexual mature male and female rats (5 rat/dose/sex) were nose-only exposed to 6:2 FTOH (estimated inhaled doses of 1.8 and 16.6 mg/kg bw) for a single 6-hour period, followed by a recovery period of 18 h ([redacted]), metabolism of 6:2 FTOH was investigated. Regular sampling of blood identified the intermediate metabolites 6:2 FTCA and 6:2 FTUCA. 18h post exposure 5:3 Acid was the predominant terminal metabolite (males: 0.29-0.45%; females: 0.42-0.51%), followed by PFBA (males: 0.21-0.25%; females: ≤0.009%) and PFHpA (males: 0.084-0.28%; females: ≤0.009%). The sum of all analytes after 24 hours ranged from 0.82-0.84% in male rats and 0.42-0.56% in female rats.

In an oral gavage repeated dose 90-day toxicity study of 6:2 FTOH in rats according to EPA OPPTS 870.3100 [redacted] metabolism of 6:2 FTOH was investigated. The applied doses in this GLP compliant study were 0, 5, 25, 125, and 250 mg/kg/day for 90 days. The study design consisted of 10 rats/sex/dose (main study), 10 rats/sex/dose (1-month recovery), 5 rats/sex/dose (3-month recovery). Following the last dosing, rats were sacrificed and blood plasma, liver and fat were analysed. Metabolite yields were not reported, except that 5:3 Acid was the major metabolite observed in plasma, liver and fat samples at all dose levels and in both sexes, followed by 4:3 Acid and PFPeA that were detected in much lower concentrations.

The Registrant(s) submitted in the commenting period, a modified one-generation reproduction study of 6:2 FTOH in mouse according to EPA OPPTS 870.3550 [redacted]. In this study, 6:2 FTOH was administered by oral gavage to $P_1$ adult CD-1 mice

(15/sex/group) daily at 0, 1, 5, 25, or 100 mg/kg body weight/day (mg/kg/day) for 10 weeks (males) and 2 weeks (females) during the premating period, and then up until the day before scheduled sacrifice. The formation of metabolites was not monitored.

Thus, these studies show that 6:2 FTOH is metabolized in rats. The reported metabolite profiles differed slightly between the three in vivo rat studies. Most metabolites were detected in the 168-hour oral gavage study that reported the formation, but not the yields of 6:2 Acid, 6:2 FTUA, 4:3 Acid, 5:3 Acid, PFBA, PFPeA, PFHxA and PFHpA ([redacted]). The 24-hour nose-only inhalation study detected the intermediate metabolites 6:2 FTCA and 6:2 FTUCA, and four terminal metabolites. At the end of the experiment, 5:3 Acid was detected at the highest concentration followed by PFBA and PFHpA. Yields for PFHxA were not reported ([redacted]). The 90-day repeated dose study reported only the formation of three metabolites, with 5:3 Acid being the major terminal metabolite followed by much lower quantities of 4:3 Acid and PFPeA ([redacted]). Considering these studies, it is clear that 6:2 FTOH is metabolized in rats to a number of PFCAs, including PFHpA.

*Conclusion on the degradation of the registered substance and relevance of PFHpA*
It was shown that the main constituents of the registered substance, i.e. [redacted], are aerobically biodegraded to 6:2 FTOH, which is further biodegraded to at least four PFCAs, including PFHpA. Considering that 6:2 FTOH is very volatile, atmospheric degradation was also investigated. It was also shown that 6:2 FTOH is oxidized in the atmosphere to PFHpA, as well as all shorter chained PFCAs. Upon binding of 6:2 FTOH to metal-rich surfaces photooxidation was shown to produce PFHpA, even though PFHxA was the primary product. Metabolization of [redacted] in rats yields three PFCAs, including PFHpA. The same was observed for 6:2 FTOH that is metabolized in rats to up to four PFCAs including PFHpA. In addition, it was shown that aerobic biodegradation of the minor constituent 6:2 FTI yields PFPeA and PFHpA. Taken all these data together, it can be concluded that PFHpA will be formed upon degradation of the registered substance.

A recent monitoring study determined the presence of $C_{6-14}$-PFCAs and a range of [redacted] in human plasma (n=420) (Yeung et al., 2013). The samples were collected in two German cities in the period 1982-2009. All samples had detectable concentrations $C_{7-12}$-PFCAs. The concentration range was for PFHpA 0.0191-2.24 ng/mL, for PFOA 0.092-39.4 ng/mL and for PFNA 0.200-2.70 ng/mL. PFHxA was detected in less than 10% of the samples and was present in much lower concentrations (<0.0017-0.056 ng/mL). [redacted] This study showed that PFHpA and its precursor [redacted] are present in relevant quantities in the environment.

Overall, it can be concluded that degradation of the registered substance will result in the formation of PFHpA at levels higher than 0.1%, and that PFHpA is already detected in the environment. Due to the voluntary phase-out of PFOA and its precursors (e.g. [redacted]), it is expected that the amount of PFHpA and its precursor [redacted] will probably increase over time. Therefore, it is justified to conduct the PBT assessment for the registered substance with the degradation product PFHpA

***Evaluation of PFHpA persistence***

PFHpA ($C_7$-PFCA) has no degradation studies available, except for one abiotic degradation study that investigated photodegradation of $C_{4-8}$-PFCAs. Chen et al. (2007) reported that following 6h irradiation by 185 nm light, degradation of PFBA ($C_4$) was >40%, while for the other four PFCAs it was ≥90%. These testing conditions are, however, not representative for abiotic degradation of PFHpA under relevant environmental conditions.

For other PFCAs, especially PFOA ($C_8$-PFCA) and PFNA ($C_9$-PFCA) more information is available. PFOA, and its ammonium salt APFO, have been identified as substances of very high concern based on their persistent, bioaccumulating and toxic properties (Article 57 e), as well as their toxicity to reproduction (Article 57 c). The $C_{11-14}$-PFCAs have also been identified as substances of very high concern based on their very persistent and very bioaccumulative properties (Article 57 e). During the persistence assessment of the $C_{11-14}$-PFCAs, a read-across approach has been applied within $C_{8-14}$-PFCAs. It was shown that the $C_{8-14}$-PFCAs contain a highly similar chemical structure, a perfluorinated carbon chain and a carboxylic acid group. The compounds differ only in the number of $CF_2$-groups. Comparison of experimental and estimated physicochemical data of $C_8$-PFCA (the analogue substance) with experimental and estimated data on $C_{11-14}$-PFCAs showed that with increasing chain length water solubility decreased and the sorption potential increased. It was concluded, that with a sufficient reliability it could be stated that the behaviour of these chemicals follow a regular pattern.

The structure of PFHpA is highly similar to the $C_{8-14}$-PFCAs, being only one $CF_2$-group shorter than PFOA. PFHpA, is as expected, more water soluble than PFOA (Bhhatarai and Gramatica, 2011). Consequently, because of the structural similarity and the regular pattern of physico-chemical properties, $C_{7-14}$-PFCAs may be considered as a group or a category of substances for the purpose of the PBT/vPvB assessment and the read-across approach can be applied within this group.

In general, the persistence of PFCAs can be explained by the shielding effect of the fluorine atoms, blocking e.g. nucleophilic attacks to the carbon chain. High electronegativity, low polarizability and high bond energies make highly fluorinated alkanes to the most stable organic compounds (Siegemund et al., 2000). It is not expected that the carboxylic group in PFCAs alters the persistence of these chemicals. This fact is confirmed by a hydrolysis study which obtained a DT50 of >92 years for PFOA in water. Screening studies of $C_{8,9,12,14}$-PFCAs showed no biodegradation within 28 days. Non-standard abiotic degradation tests with PFOA could not detect any degradation products under environmentally relevant conditions. Furthermore, screening biodegradation studies on $C_{8,9,12,14}$-PFCAs and one non-standard anaerobic biodegradation simulation test with PFOA provide evidence of high persistence. Additionally, elements of non-standard higher tier aerobic biodegradation studies on PFOA provide further support that no biodegradation in water, soil and sediment occurs.

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

***Evaluation of PFHpA bioaccumulation***

Assessing the bioaccumulation potential of PFHpA ($C_7$-PFCA) is not straightforward, as standardized bioconcentration studies do not seem to be suitable.

Martin et al. (2003a) investigated tissue distribution and bioconcentration of $C_{5-8,10-12,14}$-PFCAs in rainbow trout exposed in a flow-through system for 12 days followed by a depuration time of 33 days in fresh water. It was shown that BCFs increase with increasing length of the perfluoroalkyl chain, i.e. $BCF_{carcass}$ ranged from 4 to 23000 for $C_8$- and $C_{14}$-PFCA, respectively, the $BCF_{blood}$ from 27 to 30000, and the $BCF_{liver}$ from 8 to 30000. The shorter chained $C_{5-7}$-PFCAs were not consistently detected in the sampled tissues, and no BCF values were reported. It was only stated that their bioaccumulation potential in rainbow trout is considered insignificant.
Martin et al. (2003b) also investigated dietary bioaccumulation of $C_{5-12,14}$-PFCAs in rainbow

trout exposed in a flow-through system for 34 days followed by a depuration time of 41 days in fresh water. It was reported that only PFCAs with more than six perfluoroalkyl carbons were detectable in fish tissues at all given sampling times. Consequently, no bioaccumulation factor (BAF) was reported for PFHpA. It was shown that BAFs increase with increasing length of the perfluoroalkyl chain, i.e. $BCF_{carcass}$ ranged from 0.038 to 1.0 for $C_8$- and $C_{14}$-PFCA, respectively.

Based on these studies, PFHpA would not be considered bioaccumulative. This also accounts for PFOA ($C_8$-PFHpA) whose low BCF and BAF values imply low bioaccumulation potential. However, PFOA has only recently been identified as a substance of very high concern based on its PBT properties (Article 57 d). In the annex XV dossier it was concluded that since PFOA is notably water soluble and mainly occurs in protein rich tissues, e.g. blood and liver, bioconcentration in gill breathing organisms and the accumulation in lipids may not be the most relevant endpoint to consider. The bioaccumulation potential of PFOA was demonstrated in the Annex XV dossier using monitoring data.

For PFHpA, less monitoring data are available in the public literature. In addition, the available studies are not unequivocal. Some studies suggest that PFHpA does not bioaccumulate, i.e. trophic magnification of PFHpA was investigated but not observed in an arctic (Kelly et al., 2009), a subtropical pelagic (Loi et al., 2011), nor a remote terrestrial (Müller et al., 2011) food web, and several human maternal transfer studies did not detect PFHpA in maternal and/or cord blood (Monroy et al., 2008, Kim et al., 2011, Gutzkow et al., 2012). In contrast to these studies, there is also increasing evidence that PFHpA does bioaccumulate.

Zhao et al. (2013c) investigated bioaccumulation of $C_{6-12}$-PFCAs in earthworms (*Eisenia fetida*) exposed in spiked soil for 30 days followed by a depuration time of 28 days in clean soil. All the studied PFCAs were shown to bioaccumulate in the earthworms and the biota-to-soil accumulation factors (BSAFs) increased with perfluorinated carbon chain length, i.e. 0.087 ± 0.008 for PFHxA, 0.122 ± 0.006 for PFHpA, 0.131 ± 0.012 for PFOA, 0.213 ± 0.004 for PFNA, 0.542 ± 0.010 for PFDA, 1.366 ± 0.049 for PFUnDA, and 3.408 ± 0.739 for PFDoA. It was reported that bioaccumulation of short-chained PFCAs ($C_{6-7}$) could be due to the active ingestion of soil through the gut and the high protein content of the earthworms.

Bytingsvik et al. (2012) examined plasma levels of perfluoroalkyl substances, including $C_{4-14}$-PCFAs, in polar bear mothers in relation to their suckling cubs-of-the-year collected in 1998 (n=12, respectively, n=16) and 2008 (n=9, respectively, n=12) in Norway. PFHpA was detected in the plasma of 83 and 44% of the polar bear mothers in 1998 and 2008, respectively, and averaged (± SEM) 1.1 ± 0.2 and 0.4 ± 0.2 ng/g w.w., respectively. PFHpA was also detected in 100 and 78% of the polar bear cups in 1998 and 2009, respectively, and averaged (± SEM) 1.2 ± 0.3 and 1.1± 0.2 ng/g w.w., respectively. The mean cub to mother ratios ranged from 0.15 for PFNA to 1.69 for PFHpA. This study showed $C_{7-13}$-PFCAs are maternally transferred, with PFHpA being most efficiently transferred followed by PFOA and the longest chained PFCAs (i.e. PFUnDA, PFDoDA, and PFTrDA), whereas transfer of the intermediate chained PFCAs (i.e. PFNA and PFDA) was least efficient.

Zhao et al. (2013c) investigated maternal transfer of perfluoroalkyl substances, including $C_{5-12}$-PCFAs, in humans by analysing matched maternal blood (n=31), placenta (n=29), cord blood (n=29) and amniotic fluid (n=29) samples collected in Tianjin, China. PFHpA concentrations were above LOQ in 94% of the maternal blood, 83% of placenta, 97% of the cord blood and 17% of the amniotic fluid samples. Maternal transfer efficiencies were calculated from maternal blood to cord blood ($T_{MB-CB}$). A U-shaped trend in $T_{MB-CB}$ of $C_{7-12}$-PFCAs with increasing carbon chain length was found, i.e. the $T_{MB-CB}$ amounted to 1.2 for

PFHpA, 0.57 for PFOA, 0.35 for PFNA, 0.25 for PFDA, 0.28 for PFUnDA, and 0.81 for PFDoDA. This study showed that maternal-foetal transmission of PFHpA takes place.

Freberg et al. (2010) investigated the temporal trend of the levels of perfluoroalkyl substances, including $C_{4-14}$-PCFAs, in the serum of 13 ski wax technicians before, during and after the 2008-2009 ski season. A positive correlation was observed between years exposed as a ski wax technician and concentration of PFHpA in serum. A positive correlation was also observed for the $C_{8-13}$-PFCA, but not for the $C_{4-6}$-PFCAs.

Nilsson et al. (2010) investigated the temporal trend of the levels of perfluoroalkyl substances, including $C_{4-11}$-PCFAs, in the blood of 11 ski wax technicians before, during and after the 2007-2008 skiing season. It was shown that PFHpA levels increase in humans after using fluorinated ski wax. This study also reported a positive correlation between the number of years working as ski wax technician and the levels of PFHpA in the blood. This positive correlation was also observed for $C_{8-11}$-PCFAs, but not for $C_{4-6}$-PFCAs. By applying first order kinetics, the evaluating MSCA was able to calculate for six technicians PFHpA elimination half-lives that ranged between 23 and 599 days, with the geometric mean human elimination half-life from blood being 98 days. In comparison, from the same data Russell et al. (2013) calculated for PFHxA ($C_6$-PFCA) elimination half-lives that ranged between 14 and 49 days, with the geometric mean human elimination half-life being 32 days. While for PFOA ($C_8$-PFCA) human elimination half-lives between 2 and 4 years were reported in the annex XV dossier.

Zhang et al. (2013c) investigated the elimination rates of linear $C_{7-11}$-PFCAs, PFHxS and PFOS, as well as branched PFOA and PFOS, in humans by analysing paired blood and urine samples (n = 86) collected in Tianjin, China. PFHpA, PFNA, PFDA and PFUnDA were reported to be below LOD in some samples, but the exact percentages were not specified. The number of blood-urine pairs amounted to 31 for PFHpA, 66 for PFOA, 50 for PFNA, 60 for PFDA and 63 for PFUnA. For all PFCAs except PFUnA, levels in urine correlated positively with levels in blood. PFCAs were excreted more efficiently than PFSAs, and shorter PFCAs were excreted more efficiently than longer ones. Urinary excretion was the major elimination route for short PFCAs ($C \leq 8$), but for longer PFCAs, PFOS and PFHxS, other routes of excretion likely contributed to overall elimination. Biological elimination half-lives of PFCAs were estimated using a one-compartment model in young woman, and males & older females together. The estimated geometric mean elimination half-lives are 1.0 and 0.82 years for PFHpA, 1.7 and 1.2 years for PFOA, 1.7 and 3.2 years for PFNA, 4.0 and 7.1 years for PFDA, and 4.0 and 7.4 for PFUnDA, respectively. This study shows that while PFHpA elimination is faster than that of PFOA and PFNA it still takes substantial time, indicating bioaccumulation potential of PFHpA in humans.

Thus, even though several human monitoring studies indicate bioaccumulation of PFHpA in humans, data from public literature on the bioaccumulation potential of PFHpA are equivocal, and it cannot be stated with certainty that this degradation product will meet the B-criterion.

*Conclusion on PFHpA bioaccumulation*

Standardized bioconcentration studies with fish are not suitable to assess the bioaccumulation potential of PFHpA, and presently available monitoring data is insufficient to conclude that PFHpA will meet the B-criterion. Consequently, to demonstrate bioaccumulation of the degradation product PFHpA additional monitoring studies, preferably in humans, may have to be requested. Such complex and time-consuming studies are not considered proportional at this stage of the substance evaluation process, and maybe requested if the degradation product PFHpA meets the T criterion in addition to the P/vP-criteria.

***Evaluation of PFHpA toxicity***

For the degradation product PFHpA to meet the T-criterion it must exhibit chronic toxicity to aquatic organisms (NOEC/EC10 < 0.01 mg/L) and/or meet the criteria for classification according to the CLP Regulation as either carcinogenic (Carc. Cat. 1A or 1B), germ cell mutagenic (Muta. Cat. 1 or 1B), toxic for reproduction (Repr. Cat. 1A, 1B or 2), or specific target organ toxicity after repeated exposure (STOT RE Cat. 1 or 2). The limitedly available toxicity data are discussed in the following sections.

*Environmental hazard assessment of PFHpA*
Regarding the $T_{environment}$-criterion, there is no information on long-term effects of PFHpA on marine or freshwater organisms, and consequently it cannot be determined if the $T_{environment}$-criterion is met.

It should be noted that the registered substance meets the $T_{environment}$-criterion, as the NOEC derived for aquatic invertebrates is 0.0093 mg/L (after correction for [redacted] a.i.) [redacted]. Considering that the current PBT assessment is based on the degradation product PFHpA, the observed toxicity should also be linked to PFHpA. As the invertebrates were exposed to the registered multi-constituent substance, the toxicity can not be attributed to PFHpA and this NOEC is considered insufficient to conclude on the T-criterion at this stage.

Limited information is available on acute toxicity to algae and aquatic invertebrates. Latała et al. (2009) tested the 72-h toxicity of $C_{6\text{-}9}$-PFCAs to three representative marine algae in the Baltic Sea. For PFHpA, EC50 values of 1636 ± 95, 754 ± 44 and 446 ± 4 mg/L were obtained for the green alga *Chlorella vulgaris*, the diatom *Skeletonema marinoi* and the blue-green alga *Geitlerinema amphibium*, respectively. Comparison of the toxicity of the four PFCA showed that for every extra $CH_2$-group in the alkyl chain the toxicity increased twofold. Hoke et al. (2012) compared publicly available toxicity data of $C_{4\text{-}10}$-PFCAs and reported a 48-h LC50 value for PFHpA in daphnids of >100 mg/L that could not be verified as it was obtained from a MSc thesis (Boudreau, 2002). Their conclusion was in line with Latała et al. (2009) as they also observed that aquatic toxicity of PFCAs increases with increasing number of fluorinated carbons.

Thus, the limitedly available environmental toxicity data on PFHpA suggest low acute toxicity to algae and daphnids. For the structurally similar compound PFOA, acute and chronic toxicity data for environmental species are available. In the annex XV dossier of PFOA, it was stated that these studies show low toxicity of PFOA to environmental species, and the $T_{environment}$-criterion was not further investigated. Since PFOA is one fluorinated carbon longer, and thus expected to be more toxic than PFHpA, it was decided not to proceed with elucidating long-term toxicity of PFHpA to aquatic organisms at this stage.

*Human health hazard assessment of PFHpA*
There is no harmonized classification for PFHpA in the C&L Inventory, nor is there information available on reproductive toxicity or repeated dose toxicity of this degradation product in the registration dossier or public literature, and consequently it cannot be determined if the $T_{mammalian}$-criterion is met. The available toxicity data on PFHpA is discussed below.

*Acute toxicity of PFHpA to bacteria and mammalian cell lines*
Nobels et al. (2010) compared the mode of action of $C_{4\text{-}12}$-PFCAs, $C_{4,6,8}$-PFSAs, and 6:2 and 8:2 FTOH using a bacterial gene profiling assay. The assay consisted of 14 different *Escherichia coli* strains that due to their specific *lacZ* constructs allowed the measurement of the induction of 14 different stress responsive genes. The results were categorized in five

modes of action mechanisms, i.e. oxidative stress, general cell lesions, osmotic stress, DNA damage, membrane damage and specific stress caused by heavy metals. All investigated substances induced stress responsive genes. However, few inductions were observed after exposure to PFBA, PFPeA, PFHxA and PFHpA at equimolar concentrations (0.0156–1 mM). In ranking the potency of the investigated substances the following overall ranking was presented: PFOA > PFNA > PFUnDA > PFOS > PFDoDA > PFBS > PFDA > PFHxS > PFHxA > 6:2 FTOH > PFBA > 8:2 FTOH > PFHpA > PFPeA. The ranking for PFCAs, i.e. $C_8$ > $C_9$ > $C_{11}$ > $C_{12}$ > $C_6$ > $C_4$ > $C_7$ > $C_5$, did not show a pattern of increasing toxicity with increasing chain-length. This ranking that is based on gene expression in *E. coli* suggests that only PFPeA is less toxic than PFHpA. This is in contrast with the *in vitro* studies that are discussed below (Mulkiewicz et al., 2007, Buhrke et al., 2013, Rand et al., 2014) and that clearly show that PFHpA is more toxic than PFHxA, and especially PFBA. Furthermore, these studies also observed that toxicity of PFCAs increases with chain length.

Mulkiewicz et al. (2007) investigated the acute toxicity of $C_{6\text{-}10}$-PFCAs using different in vitro test systems: cytotoxicity towards two mammalian cell lines (promyelocytic leukemia rat cell line (IPC-81) and the rat glioma cell line (C6)), bioluminescence inhibition in the marine bacterium *Vibrio fischeri*, and enzyme (acetylcholinesterase and glutathione reductase) inhibition. For PFHpA, EC50 values of 558 ± 15, 1250 ± 100 and 948 ± 25 mg/L were obtained for IPC-81, C6 and *V. fischeri*, respectively. The acute toxicity of the other PFCAs was also in the milligrams range, with the lowest EC50 values found for PFNA and PFDA. In all test systems, a relationship was observed between the toxicity of the PFCAs and the perfluorocarbon chain length. It was concluded, that the longer the perfluorocarbon chain the more toxic the acid.

Buhrke et al. (2013) compared the toxicity of $C_{4,6\text{-}10,12}$-PFCAs. By using the human hepatocarcinoma cell line HepG2 as an *in vitro* model for human hepatocytes, cytotoxic effects of these compounds as well as their impact on apoptosis (caspase 3/7 activity) and cellular proliferation were examined. Cytotoxicity IC50 were >1000, 344, 128, 47, 23, 15 and 7 µM for PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA and PFDoDA, respectively, and cell proliferation responses IC50 were >100, 74.3, 23.6, 3.0, 2.3, 3.1 and 0.3 µM, respectively. A positive correlation between PFCA carbon chain length and cytotoxicity, respectively, cell proliferation was observed. There was, however, no indication of an apoptotic mechanism for cytotoxicity, as both an Ames test and a micronucleus assay were negative for all PFCAs. Using a gene reporter assay, it was demonstrated that all investigated PFCAs activate human peroxisome proliferator-activated receptor alpha (PPARα) with the c20% values being 27.4, 12.2, 5.3, 0.9, 21.3, 28.4 and >20 µM for PFBA, PFHxA, PFHpA, PFOA, PFNA, PFDA and PFDDA, respectively. The PFCAs showed weak potential to activate PPARγ (>1000, 43.2, 43.2, 19.7, >100, 57.3, and 18.0, respectively), and hardly activated PPARδ (>1000, >200, 51.0, 40.6, >100, >100, and >20, respectively). Ranking based on gene expression shows that PFOA is the most toxic PFCA followed by PFHpA, but it does not show a pattern of increasing toxicity with increasing carbon-chain length, which is in contrast to the cytotoxicity and cell proliferation data that do show such a pattern. Similarly, Nobels et al. (2010) also did not observe a pattern in their gene expression study. Collectively, the *in-vitro* data shows that PFHpA is less toxic than PFOA, but more toxic than PFHxA. From this study it can be concluded that that the toxicity of PFCAs increases with their carbon-chain length, and that the toxicity of PFHpA is intermediate between PFOA and PFHxA.
Rand et al. (2014) compared the toxicity of $C_{4\text{-}10}$-PFCAs and their metabolic precursors (e.g. 4:2, 6:2, 8:2 and/or 10:2 FT(U)CA, FT(U)AL) using an in vitro test system. Analytes were separately incubated with human liver epithelial (THLE-2) cells to assess how varying the functional group and the fluorinated chain length affects cell viability. Potential for 8:2 FTUALs to form DNA adducts was investigated, but not detected. The EC50 values for FTUCAs and FTCAs were similar, with values ranging from 22 ± 9 and 24 ± 9 µM for the 10:2 congeners to 1004 ± 20 and 1004 ± 24 µM for the 4:2 congeners, respectively. EC50

values for PFCAs varied from 65 ± 41 (PFDA) to 1361 ± 146 (PFBA) µM, and increased with chain length. Individual PFCAs EC50 values were not exactly reported, but were shown in a figure. The range of toxicity between PFCAs and their acid precursors were similar. The metabolic precursors of PFCAs were shown to be more toxic, with the order being: FTUALs ≥ FTALs > FTUCAs ≥ FTCAs > PFCAs. For all PFCAs and metabolic precursors, toxicity depended on the length of the fluorinated chain, with the longer chain lengths being more toxic. This is in agreement with the study by Mulkiewicz et al. (2007) and Buhrke et al. (2013).

From the above *in vitro* data (Mulkiewicz et al., 2007, Buhrke et al., 2013, Rand et al., 2014) it is clear that toxicity of PFCAs increases with carbon-chain length. The only discrepancy comes from the gene expression studies where no clear pattern was observed by neither Nobels et al. (2010) nor Buhrke et al. (2013). It should be noted that both identified PFOA as most toxic based on gene expression data, but while in the first study PFHpA was ranked as one of the least potent PFCAs, in the second study it was the second most potent PFCA. Toxicity based on cytotoxicity and cell proliferation is considered more reliable and shows that PFHpA is less toxic than PFOA, but considerably more toxic than PFHxA and other shorter chained PFCAs.

*Reproductive and repeated dose toxicity of PFHpA to mammals*

Kudo et al. (2000) investigated the relationship between the induction of peroxisomal beta-oxidation and hepatic accumulation of $C_{7-10}$-PFCAs in the liver of male and female rats. 6-week old rats were intraperitoneally administered with $C_{7-10}$-PFCAs at doses ranging from 2.5 to 20 mg/kg body weight once a day for 5 days. Livers were excised and homogenized for the enzymatic assays. In male rats, PFHpA had minimal effect, while PFOA, PFNA and PFDA had marked increases in beta-oxidation at much lower doses (up to a factor 30). Neither PFHpA nor PFOA induced beta-oxidation in female rates, while PFNA and PFDA did. The activity of peroxisomal b-oxidation was induced in a dose-dependent manner by PFOA, PFNA and PFDA in male rats, and by PFNA and PFDA in female rats. PFHpA was not detected in the livers of male or female rats. A significant correlation was reported between the induction and hepatic concentrations of $C_{8-10}$-PFCAs in the liver, regardless of their carbon chain length. Hepatic concentrations of PFOA and PFNA were higher in males than females. It was concluded that the difference in accumulation between $C_{8-10}$-PFCAs in the liver was responsible for the different potency of the induction of peroxisomal beta-oxidation between $C_{8-10}$-PFCAs with different carbon chain lengths and between sexes.

Kudo et al. (2006) investigated the relationship between the induction of hepatomegaly, peroxisomal beta-oxidation and microsomal 1-acylglycerophoshocholine acyltransferase of $C_{6-9}$-PFCAs in the liver of male and female mice. 8-week old mice were intraperitoneally administered with PFCAs at the doses ranging from 50 to 150 mg/kg of body weight for PFHxA, 25 to 100 mg/kg of body weight for PFHpA, and 2.5 to 20 mg/kg of body weight for PFOA and PFNA. Treatment did not significantly affect the growth or survival. Livers were excised and homogenized for the enzymatic assays. All PFCAs examined induced hepatomegaly and peroxisomal beta-oxidation and the potency was in the order of PFNA, PFOA, PFHpA and PFHxA when compared with the relative doses to induce the two parameters. Microsomal 1-acyl-GPC-acyltransferase was induced by PFHpA, PFOA and PFNA. No significant sex-related difference was observed in the induction of peroxisomal beta-oxidation by any PFCAs examined. PFNA and PFOA accumulated in the liver of both male and female mice in a dose-dependent manner. PFHpA also accumulated in the liver, but to a lesser extent, while PFHxA hardly accumulated in the liver. PFHpA, PFOA and PFNA caused enlargement of the liver in a dose-dependent manner, while PFHxA only slightly increased liver weight in female mice. The magnitude of liver weight increases was larger with PFOA and PFNA than PFHpA. The doses required to increase relative liver weight 1.5 times over

controls for PFHxA, PFHpA, PFOA and PFNA were approximately 150, 40, 2.5 and 2.5 mg/kg body weight for male mice, and 150, 100, 7.5 and 2.5 mg/kg of body weight for female mice, respectively. Hepatic concentrations of PFNA, PFOA and PFHpA were higher in male mice than those in female mice. One linear regression line was confirmed between the activities of peroxisomal beta-oxidation and hepatic concentrations of PFHxA, PFHpA, PFOA and PFNA in male mice regardless of their carbon chain lengths, and the activities were saturable at the concentrations over approximately 500 nmol/g liver. A similar linear regression line was obtained between the two parameters in female mice. It was suggested that (I) the longer the perfluoroalkyl chain becomes, the more PFCA accumulates in the liver of both male and female mice, (II) the accumulated PFCAs induce hepatomegaly, peroxisomal b-oxidation and microsomal 1-acyl-GPC acyltransferase, and (III) the difference observed in the accumulation of PFHpA, PFOA and PFNA in the liver between male and female mice is not enough to produce obvious sex-related difference in the induction of peroxisomal b-oxidation.

*Reproductive and repeated dose toxicity of PFHxA, PFOA and PFNA to mammals*
As shown above, there is a relationship between the carbon-chain length of the PFCAs and the exhibited toxicity. Therefore, the toxicity of PFHxA (one fluorinated carbon shorter), PFOA (one fluorinated carbon longer) and PFNA (two fluorinated carbons longer) is discussed below.

Chengelis et al. (2009) investigated toxicity of PFHxA to rats by administrating by oral gavage doses up to 200 mg/kg/day for 90 days. Lower bodyweight gains were noted in the 10, 50 and 200 mg/kg/day group males (not dose-responsive) throughout dosing. Other changes included lower red blood cell parameters, higher reticulocyte counts and lower globulin in the 200 mg/kg/day group males and females, higher liver enzymes in males at 50 and 200 mg/kg/day, lower total protein and higher albumin/globulin ratio, and lower cholesterol, calcium in males at 200 mg/kg/day. Minimal centrilobular hepatocellular hypertrophy was present in 200 mg/kg/day group males and correlated with higher liver weights and slightly higher peroxisome beta-oxidation activity at the end of the dosing period. Based on liver histopathology and liver weight changes, the no-observed-adverse-effect level (NOAEL) for oral administration was 50 mg/kg/day for males and 200 mg/kg/day for females. It was concluded that the effects of PFHxA observed in the current study were of a limited nature. PFHxA was considered much less toxic than PFOA.

Experimental toxicity data on PFOA is numerous, and has led to the classification of PFOA and its ammonium salt, APFO as Repr. 1B and STOT RE 1 (liver). Increased mortality and liver toxicity in mice, rats and monkeys following oral exposure to PFOA was reported in the CLH report for APFO. The effects, i.e. hepatocellular hypertrophy, degeneration and/or focal to multifocal necrosis, increased in severity between doses of 1.5 to 15 mg/kg bw/day in rats and mice. Developmental toxicity of PFOA in CD-1 mouse, leading to early pregnancy loss, compromised postnatal survival, delays in general growth and development, and sex-specific alterations in pubertal maturation was demonstrated by Lau et al. (2006). Other studies showed an effect of PFOA on the development of the mammary gland (White et al., 2007, White et al., 2009) in CD-1 mice.

Recently (December 2013), a proposal for Harmonized Classification and Labelling of PFNA has been submitted by the Swedish Competent Authority. This proposal aims to classify PFNA as Repr. 1B and STOT RE 1 (liver), and is based on toxicity data with PFNA as well as read-across to PFOA and its ammonium salt, APFO, especially for reproductive toxicity.

*Species sensitivity to PFCAs*
Tatum-Gibbs et al. (2011) compared the pharmacokinetic properties of PFNA ($C_9$-PFCA) in

Sprague-Dawley rats and CD-1 mice. PFNA was administered by oral gavage in a single dose of 1, 3 or 10 mg/kg. Blood, liver and kidneys were sampled. In the rat, serum elimination of PFNA was mostly linear with exposure doses. Major sex differences in the rate of elimination were observed, with an estimated half-life of 30.6 days for males and 1.4 days for female rats. PFNA was stored preferentially in the liver but not in the kidneys. In the mouse, the rates of PFNA serum elimination were non-linear with exposure dose and were slightly faster in females than males, with terminal estimated serum half-lives of 25.8–68.4 days and 34.3–68.9 days, respectively. PFNA was also stored preferentially in the mouse liver but not in the kidneys. Hepatic uptake appeared to be more efficient and storage capacity greater in male mice than in females. These data suggest that (I) PFNA is more persistent in the mouse than in the rat; (II) there is a major sex difference in the serum elimination of PFNA in the rat, but much less so in the mouse; and (III) there is a significantly higher hepatic accumulation of PFNA in male mice than in females.

Ohmori et al. (2003) also reported large sex differences in the rate of elimination in male and female rats. This study compared the toxicokinetics between PFHpA, PFOA, PFNA and PFDA in male and female rats. Half lives in male and female rats were calculated to be 0.10 and 0.05 days, respectively, for PFHpA, 5.63 and 0.08 days for PFOA, 29.5 and 2.44 days for PFNA and 39.9 and 58.6 days for PFDA.

Lou et al. (2009) investigated the half-life of PFOA in mouse and reported a greatly diminished sex difference in PFOA toxicokinetics in the mouse. Half-lives could not be estimated for the 60 mg/kg single dose experiment, nor the 20 mg/kg repeated dose experiment. The 1 and 10 mg/kg single doses experiments yielded serum half-lifes of 21.7 days for males and 15.6 days for females.

Since there are differences between species, it is necessary to use the species that shows the highest resemblance to humans. The half-live in humans for PFOA has been estimated to be between 2.3 (Bartell et al., 2010) and 3.5 years (Olsen et al., 2007), showing that PFOA is persistent and bioaccumulative in humans. The above study by Lou et al. (2009) and Tatum-Gibbs et al. (2011) show that PFOA (15.6 to 21.7 days) and PFNA (25.8 to 68.9 days) have considerably longer half-lives in mouse than in rat (0.08 to 5.6 days for PFOA and 1.4 to 30.6 days for PFNA), indicating that mice are more representative for humans than rats.

In the PFOA CLH report, the following was stated regarding renal clearance of PFOA:
*'As regards the renal clearances of PFOA in humans a study by Harada et al. (2005) showed that the renal clearances of PFOA were almost negligible in both sexes in humans, in clear contrast to the large active excretion in the female rat. Due to the similar lack of sex-difference in PFOA elimination among humans and mice, more weight should be put on the findings reported in the mice studies in the decision on classification of PFOA/APFO for developmental effects in offspring.'*

In the recent proposal for Harmonized Classification and Labelling of PFNA the following was stated in respect to the preferred use of mouse to test PFCAs:
*'PFNA as well as APFO/PFOA are very slowly eliminated from blood and have long durations in mice. It is very likely that due to the similarities between PFOA and PFNA both with regards to physicochemical properties and long elimination half-lives in exposed animals, that the elimination half-life for PFNA in humans is extremely long and within the same range as the ones recorded for PFOA. If anything, according to the elimination half-life in serum for CD-1 mice, PFNA is more slowly eliminated as compared to PFOA. The data suggests that there is a major sex difference in the serum elimination of PFNA in the rat; this is also true for PFOA (Ohmori et al., 2003). In mice the difference between the*

*elimination of PFNA and PFOA in serum between the sexes is less pronounced. Median human PFNA and PFOA serum concentrations in children are very similar for girls and boys (Schecter et al., 2012). Altogether, this suggests that the mouse is the preferred animal model. Both PFNA and PFOA can cross the placenta and the animal data suggests that the elimination via lactation could be substantial. PFNA (as well as PFOA) has been found in cord blood as well as in human breast milk which indicates that exposure during gestation and lactation also will occur in humans.'*

Thus, the way PFOA and PFNA affect humans bears more resemblance to the way mice are affected than rats. Therefore, mouse is the preferred animal model to test PFCAs.

**Reproduction/Developmental Toxicity Screening Test in CD-1 mice, oral route (OECD 422) extended to 90 days for the pre-mating and mating period and extended to 21 days post weaning**

The previous section showed there is limited toxicity data available on the degradation product PFHpA.

There are no chronic toxicity studies for PFHpA with aquatic organisms. Based on the low acute toxicity of PFHpA to algae and invertebrates (Boudreau, 2002, Latała et al., 2009, Hoke et al., 2012), and the fact that PFOA shows low acute and chronic toxicity to environmental species, it seems unlikely that PFHpA will meet the $T_{environment}$-criterion.

There are also no studies available on the reproductive toxicity or repeated dose toxicity of PFHpA. Thus, it can not be determined if PFHpA fulfils the criteria for classification as Repr. (1A, 1B or 2) and/or STOT RE (1 or 2), which are part of the $T_{mammalian}$-assessment under PBT-evaluation. There are, however, data available on the chronic toxicity of PFHxA and PFOA, PFCAs that are one fluorinated carbon shorter, respectively, longer. PFHxA was shown to be not very toxic to rats in a 90-day repeated dose toxicity study. PFOA on the other hand, has been classified based on its reproductive toxicity and repeated dose toxicity as Repr. 1B and STOT RE 1 (liver). In addition, for PFNA, which is two fluorinated carbons longer, only recently such a proposal for Harmonized Classification and Labelling has been submitted by the Swedish Competent Authority. Considering that the toxicity of PFCAs increases with the length of their fluorinated carbon chain (Kudo et al., 2006, Mulkiewicz et al., 2007, Latała et al., 2009, Hoke et al., 2012) it is not unlikely that PFHpA might also fulfil the criteria for classification as Repr. 1B and/or STOT RE 1.

In accordance with REACH, the usage of laboratory animals should be avoided to the extent that this can be done without compromising the basis for chemicals safety assessment. Such avoidance is currently not possible. However, in order to generate information on repeated dose and reproductive toxicity but reduce the number of animals tested as much as possible, a combined repeated dose toxicity/reproductive toxicity study is requested. It should be noted that the degradation product PFHpA is potentially bioaccumulating and that a standard 28-day repeated dose toxicity study might therefore be of too short a duration for PFHpA to reach a steady state in the test organism. The required combined protocol should generate information on repeated dose toxicity equivalent to a repeated dose toxicity study (EU B.26 /OECD TG408).

Mouse, in contrast to rat, is probably affected in a similar way by PFNA and PFOA as humans. Therefore, the proposed PFHpA toxicity test should be performed with this animal model, using the CD-1 strain that has been demonstrated to be sensitive for developmental effects.

It has been shown that gestational exposure of mice to the related compounds PFOA and PFNA reduced the number of live pups at birth as well as the survival of the offspring during the post-natal period (Wolf et al., 2007, Wolf et al., 2010).

PFOA caused whole litter loss (Lau et al., 2006) and post-natal developmental disorders after 10 and 20 days including effects on the mammary gland (White et al., 2007, White et al., 2009). Therefore, there is concern over the ability of a standard OECD TG422 to detect such effects (Reuter et al., 2003). For this reason, the standard OECD 422 study design is modified by following the F1 offspring till day 21 post weaning, with continued exposure and assessment of reproductive structures and functions.

It is important to follow the physical development of the offspring by recording the body weight gain and the sexual maturation (age and body weight at vaginal opening or balano-prepution separation). The ano-genital distance should also be recorded at weaning. Moreover, the Registrant(s) are requested to examine the mammary gland development of the offspring using the whole mounts method as PFOA showed an effect on the development of this gland. The toxicity of the liver should also be evaluated by doing a histological examination of liver at necropsy.

Thus, information on reproductive toxicity and repeated dose toxicity of the degradation product PFHpA are needed to determine if the criteria for classification as Repr. Cat. (1A 1B or 2) or STOT RE (1 or 2) are fulfilled. These hazard classes are part of the $T_{mammalian}$-assessment under PBT-evaluation.

*Comments and proposals for amendments received*
During the consultation process several comments were received from the Registrant(s) of the substance under evaluation and several proposals for amendments were received from ECHA and other Member States. Various remarks were formulated on the exact protocol for performing the combined toxicity study. The overall testing strategy reflected in a combined toxicity study has been retained, but certain elements of the originally proposed protocol have been adapted. A summary of the Registrant(s) comments (original and related to the PfAs) and the proposals for amendment from the Member States with the responses is provided below:

a) Proportionality of study requirements to product volume
The Registrant(s) argued that as a result of emissions of the substance into the environment, the calculated potential environmental concentrations of PFHpA are very low in all environmental compartments, both on the local scale and on the regional scale. Consequently, the Registrant(s) considered the potential PFHpA concentations in the environment to be 'negligible' and therefore further testing was not justified in their view.

ECHA notes that PFHpA, which is recognized as a potential degradation product that can be formed at levels higher than 0.1%, is a potential PBT substance. The PBT character is an intrinsic property of the substance and the identification of such PBT or vPvB substances is independent of measured or estimated concentrations in environmental compartments. For PBT and vPvB substances a 'safe' concentration in the environment cannot be established using the methods currently available with sufficient reliability for an acceptable risk to be determined in a quantitative way. In general, performing reliable estimations of the concentration of PBT substances that are released over longer periods of time is not feasible. Moreover, perfluorocarboxylic acids are extremely persistent and are known to belong to the most stable organic compounds.
Taking into account the suspected high persistency of PFHpA, it is reasonable to assume

that concentrations after years of environmental releases will be much higher than what is calculated by the Registrant(s) using a local emission scenario. Therefore, ECHA considers further testing of PFHpA appropriate.

In addition, in his comments and responses to the PfAs the Registrant(s) argued that any toxicity deriving from a potential minor metabolite, including PFHpA, would have been encompassed in available studies with a similar design. ECHA considers that the levels of PFHpA are so low in the available toxicity studies with the registered substance that effects do not become apparent. Therefore, ECHA considers further testing of PFHpA appropriate.

b) Elimination kinetics and toxicity of PFHpA in mammals
The Registrant(s) of the substance under evaluation argued that PFHpA has elimination kinetics in mammals and a lack of bioaccumulation and toxicity that is comparable with short-chain perfluorocarboxylic acids.

ECHA considers this an arbitrary categorization. "Long-chain perfluorinated chemicals" refer to PFCAs with carbon chain lengths $C_8$ and higher, perfluoroalkyl sulfonates with carbon chain lengths $C_6$ and higher, as well as precursors of these substances (http://www.oecd.org/ehs/pfc/). This categorization into "short-chain PFCAs" ($\leq C_7$-PFCA) and "long-chain PFCAs" ($\geq C_8$-PFCA) is based on the trend that both toxicity and bioaccumulation potential of PFCAs increases with increasing carbon chain length. However, this increase is gradual, and as such there is no clear cut off between the two categories. PFOA ($C_8$-PFCA) has been identified as a PBT substance, while the longer chained $C_{11\text{-}14}$-PCFAs have been identified as vPvB substances. PFHpA is a $C_7$-PFCA, and as such a borderline case.

Furthermore, the Registrant(s) proposed read-across to the analogous substances ω-H-PFCA. ECHA is of the opinion that this read-across is not appropriate, as suffient studies with PFCAs are available, as it is unclear how the substitution affects the PBT properties of the ω-H-PFCAs in comparison to PFCAs, and moreoverly as these studies have been published in a German journal 30 years ago and were not available to the evaluating MSCA.

c) Leave the option to replace the range-finding study by allowing additional groups of mice to be added in the main test
The Registrant(s) pointed out that performing a preliminary range-finding study would be a time-consuming process that is not necessarily an efficient way to proceed in terms of animal welfare. The Registrant(s) proposed to utilize an additional dose level in the main test as this would provide equivalent information.

ECHA is still of the opinion that for this case performing a range-finding study is necessary as it expects difficulties to correctly determine the dose levels in a combined test, especially when the systemic toxicity of the substance can interfere with the results of the developmental study. Moreover, the available studies on PFHpA were conducted using intraperitoneal administration which excludes first-pass metabolism/elimination or liver storage. This range-finding study is therefore considered as particularly important. The Registrant(s) requested an extension of the deadline if the range-finding study would be required. Taking into account the non-standard extension of the premating and mating period and extension of the post partum period, this request is considered appropriate and the deadline for submitting the requested information has been changed from 21 months to 27 months.

d) Conduct the requested study using the sodium or potassium salt of perfluoroheptanoic acid
The Registrant(s) noted that since the administration route is by oral gavage, continued administration of an acidic substance will induce unnecessary animal suffering and portal of entry irritation or degeneration. On the contrary the administration of a neutralized solution will greatly reduce irritation.

ECHA agrees with this adaptation of the protocol as administering a neutralized solution will avoid unnecessary animal suffering without affecting the dose reaching the blood circulation of the test animals.

e) Provide clarity on the necessity for a 90-day exposure duration

The Registrant(s) noted that the premating period in the proposed test protocol is 90 days which is much longer than what is normally required in the OECD 422 test guideline. They claim that the available TK data on PFHpA suggest that the elimination half-life from blood is 0.1 day, so at least in rats, steady state conditions should be attained within a single day. Therefore the Registrant(s) state that a 28-day study should be of sufficient duration and that there is no need to extend dosing to a longer period.

ECHA recognizes that a premating period of 90 days is effectively in contrast to the typical premating period required in the OECD 422 guideline. However, ECHA still considers it to be appropriate for two main reasons:
Concerning the time to reach the steady-state, there are no data on toxicokinetic behaviour of PFHpA in mice and the toxicokinetcs of PFOA is particularly complex. As highlighted by the studies conducted by Lou et al. (2009), the toxicokinetics with single low doses of PFOA is different from the toxicokinetcs with single high doses or repeated doses of PFOA. The values reported by the Registrant(s) for the PFOA half-lives in mice are moreover not correct. The half-life estimated by Lou et al. was 21.7 days for males and 15.6 days for females. These authors also highlighted the difficulty to extrapolate from one species to another. ECHA considers that it is rather speculative to predict the time to reach steady-state after repeated oral exposure in mice on the value of the half-life from blood in rats after intravenous injection as suggested by the Registrant(s). Moreover, recent data (Russell et al., 2015) suggest that the half-life of PFHpA in rats would be higher than one day at low plasma concentrations.

A second reason to ask this extension is to detect any adverse effects on the liver. In this perspective a 90-day study can generally be considered to be a more definitive study than a 28-day study.

f) Elimination of several developmental landmarks in the initially proposed test protocol
Both the Registrant(s) and a Member State made the remark that several developmental landmarks in the initial test protocol are not typical guideline endpoints. One foresees that the recordings of these landmarks will be complicated and that the interpretation of the results could be compromised particularly due to a lack of historical control data.

ECHA notes that while in general body weight will affect the physical development, other mechanisms can also affect these parametersindependently of body weight, as was shown amongst others for preputial separation by Lau et al. (2006). That said, ECHA agrees that recording some of the landmarks can be difficult to perform and, as it remains uncertain if that will provide valuable additional information, it is deemed

appropriate not to require anymore the recording of the ear and eye opening, tooth eruption and hair growth. Moreover, ECHA agrees to record the anogenital distance at weaning. The parameters indicative for the physical development of the offspring (body weight gain and sexual maturation) shall be followed as initially indicated.

g) Retain the F1-generation longer than initially indicated in section II of the Draft Decision
In the initial version of the Draft Decision it was indicated that the reproduction / developmental toxicity screening test had to be extended to"21 days post-partum" while in the next paragraph an extension of "at least 21 days after weaning" was mentioned. Both the Registrant(s) and a Member State noticed that the indication "21 days post partum" is wrong as in general mice only reach sexual maturation after 30-40 days.

ECHA recognizes that the initial Draft Decision contained contradictory instructions and agrees with the argumentation brought forward in the comments. It is crucial that the F1 offspring has reached at least sexual maturation in the test. Therefore, in section II of this decision the requirement was modified to "21 days post weaning".

h) Describe more precisely how the mammary glands shall be examined
A Member State pointed out that recently several studies were published in relation to perinatal and peripubertal exposure of mice to PFOA. Whole mount analysis of the mammary glands at different time points showed reduced development of perinatally exposed mice. Other studies investigating peripubertal exposure to PFOA showed similar delayed growth of female mammary glands in mice. Although there is also another study that reports contradictory results with regard to the development of mammary glands, the weight of evidence points to a delayed development of mammary glands in females exposed to PFOA early in life. In these studies whole mounts of the glands were analysed and therefore it is deemed appropriate to examine the influence of exposure to PFHpA in the same way being aware that this may increase the number of animals to be used as indicated by the Registrant(s) in their comments.

ECHA recognizes the relevance of this remark and has included this instruction within the present decision.

i) The usefulness of having additional animals to evaluate gender differences is questioned by the Registrant(s)
The Registrant(s) proposed in their comments and in their response to the PfAs to eliminate extra groups for evaluation of gender differences since the proposal to include these extra animals was not clear.

ECHA clarifis that the systemic toxicity in non-pregnant females and males is to be assessed and therefore the additional satellite animals shall be sacrificed after the same exposure time as the males. The blood samples that are to be taken from these additional satellite animals shall, as stated in this decision, be taken at the same time as from the other animals (prior to mating) and shall be tested for standard clinical biochemistry as is specified in paragraph 55 of the updated draft OECD test guideline 422 (OECD 2015).

*Conclusion*
Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following study using the sodium or potassium salt of the degradation product 2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoroheptanoic acid (PFHpA) of the registered substance subject to this decision: Reproduction/Developmental Toxicity Screening Test in

mice, oral route (OECD 422) extended to 90 days for the pre-mating and mating period and 21 days post weaning.

The premating exposure period for the P generation shall be extended to 90 days to ensure that steady-state exposure conditions in P males and females are achieved before mating. The post-weaning period of the F1-generation shall be adjusted to ensure that the animals are exposed for at least 21 days after weaning.

The test shall include 5 extra females in the control group and 5 extra females in the highest dose group. Blood samples shall be taken from 5 males and 5 females randomly selected from each group at the same time (prior to mating) in order to detect any difference of sensitivity during the repeat dose toxicity test. The additional animals shall be necropsied after the 90 day period and assessed in the same way as the remaining animals. In this way a gender comparison can be set up without gestational influence.

The Registrant(s) shall follow the physical development of the offspring by recording the body weight gain, and sexual maturation (age and body weight at vaginal opening or balano-prepution separation). The ano-genital distance shall also be recorded at weaning. Moreover, the Registrant(s) are requested to examine the mammary gland development of the offspring using the whole mounts method. The toxicity of the liver shall also be evaluated by doing a histological examination of liver at necropsy.

The required combined protocol shall generate information on repeated dose toxicity equivalent to a repeated dose toxicity study (EU B.26 /OECD TG408).

It should be noted that an identical study has been requested by ECHA in a decision pursuant to Art 46(1) for the substance with EC number 700-403-8 evaluated by the Belgian Competent Authority. This UVCB contains [redacted] constituents of which [redacted] are also major constituents in reaction mass of mixed (3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl) phosphates, ammonium salt. To reduce testing, the Competent Authorities of Belgium and the Netherlands agreed to assess the toxicity of the common degradation product PFHpA by requesting from the Registrant(s) one study for both substances.

IV. Adequate identification of the composition of the tested material

In relation to the required reproduction/developmental toxicity screening study with the degradation product 2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoroheptanoic acid (EC No 206-798-9), the used sample shall have a composition that matches with an identification as monoconstituent substance with EC No 206-798-9. It is the responsibility of all the Registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation. Finally, the test(s) must be shared by the Registrant(s).

V. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Articles 52(2) and 51(8) of the REACH Regulation. Such an appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on the ECHA's internet page at

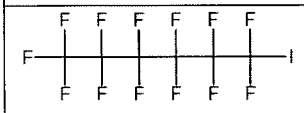
http://echa.europa.eu/web/guest/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

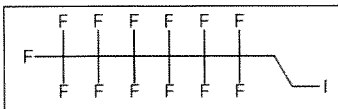
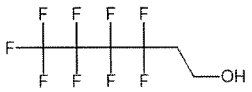
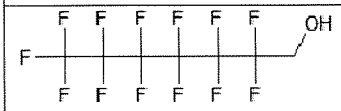
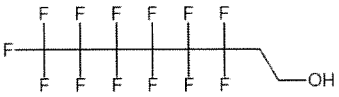
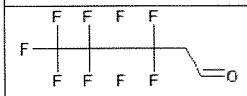
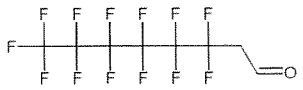
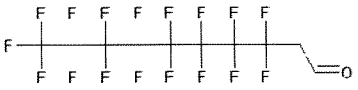
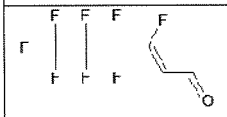
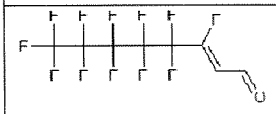
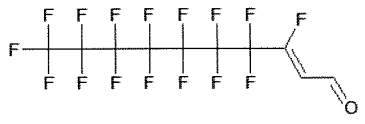
Authorised[3] by Leena Ylä-Mononen, Director of Evaluation

Annex: List of registration numbers for the addressees of this decision. This annex is confidential and not included in the public version of this decision

[3] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Annex I - Glossary of the perfluorinated compounds discussed in the decision

| Structure | IUPAC name | Synonym | Acronym |
|---|---|---|---|
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
|  | 1,1,1,2,2,3,3,4,4,5,5,6, 6-tridecafluoro- 6-iodohexane | perfluorohexyl iodide | PFHxI |

| | | | |
|---|---|---|---|
|  | 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoroiodooct-8-ene | 6:2 fluorotelomer iodide | 6:2 FTI |
|  | 3,3,4,4,5,5,6,6,6-nonafluoro-1-hexanol | 4:2 fluorotelomer alcohol | 4:2 FTOH |
|  | 2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoro-1-heptanol | 6:1 fluorotelomer alcohol | 6:1 FTOH |
|  | 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-1-octanol | 6:2 fluorotelomer alcohol | 6:2 FTOH |
|  | 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-1-decanol | 8:2 fluorotelomer alcohol | 8:2 FTOH |
|  | 3,3,4,4,5,5,6,6,6-nonafluorohexanal | 4:2 fluorotelomer saturated aldehyde | 4:2 FTAL |
|  | 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanal | 6:2 fluorotelomer saturated aldehyde | 6:2 FTAL |
|  | 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecanal | 8:2 fluorotelomer saturated aldehyde | 8:2 FTAL |
|  | 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-henicosafluorododecanal | 10:2 fluorotelomer saturated aldehyde | 10:2 FTAL |
|  | 3,4,4,5,5,6,6,6-octafluoro-2-hexenal | 4:2 fluorotelomer unsaturated aldehyde | 4:2 FTUAL |
|  | 3,4,4,5,5,6,6,7,7,8,8,8-dodecafluoro-2-octenal | 6:2 fluorotelomer unsaturated aldehyde | 6:2 FTUAL |
|  | 3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-hexadecafluoro-2-decenal | 8:2 fluorotelomer unsaturated aldehyde | 8:2 FTUAL |

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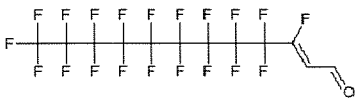
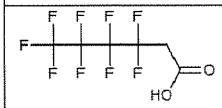
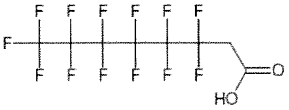
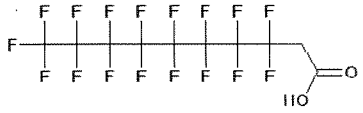
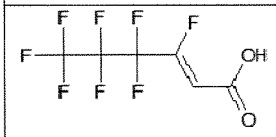
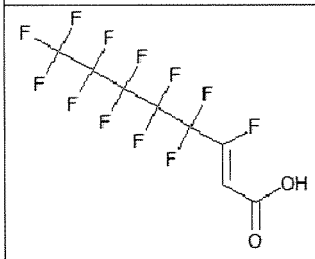
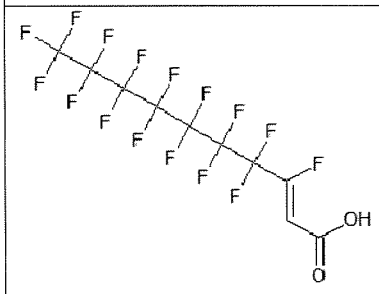
|  | 3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-henicosafluoro-2-dodecenal | 10:2 fluorotelomer unsaturated aldehyde | 10:2 FTUAL |
|  | 3,3,4,4,5,5,6,6,6-nonafluorohexanoic acid | 4:2 fluorotelomer saturated carboxylic acid | 4:2 FTCA |
|  | 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanoic acid | 6:2 fluorotelomer saturated carboxylic acid | 6:2 FTCA |
|  | 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecanoic acid | 8:2 fluorotelomer saturated carboxylic acid | 8:2 FTCA |
|  | 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-henicosafluorododecanoic acid | 10:2 fluorotelomer saturated carboxylic acid | 10:2 FTCA |
|  | 3,4,4,5,5,6,6,6-octafluorohex-2-enoic acid | 4:2 fluorotelomer unsaturated carboxylic acid | 4:2 FTUCA |
|  | 3,4,4,5,5,6,6,7,7,8,8,8 - dodecafluorooct-2-enoic acid | 6:2 fluorotelomer unsaturated carboxylic acid | 6:2 FTUCA |
|  | 3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-hexadecafluorodec-2 enoic acid | 8:2 fluorotelomer unsaturated carboxylic acid | 8:2 FTUCA |

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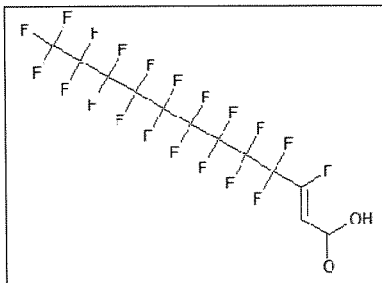
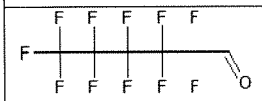
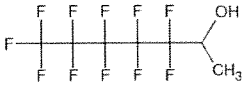
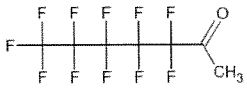
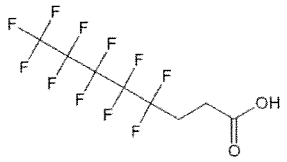
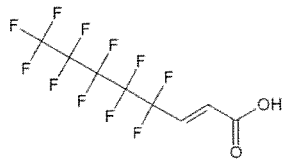
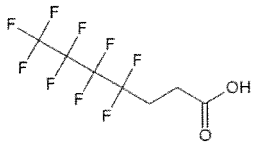
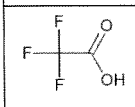
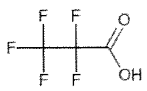
|  | 3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-henicosafluorododec-2-enoic acid | 10:2 fluorotelomer unsaturated carboxylic acid | 10:2 FTUCA |
|  | 2,2,3,3,4,4,5,5,6,6,6-undecafluorohexanal | perfluorinated hexanal | PFHxAL |
|  | 3,3,4,4,5,5,6,6,7,7,7-undecafluoro-2-heptanol | 5:2 Secondary polyfluorinated alcohol | 5:2 sFTOH |
|  | 3,3,4,4,5,5,6,6,7,7,7-undecafluoro-2-heptanone | 5:2 fluorotelomer ketone | 5:2 FT ketone |
|  | 4,4,5,5,6,6,7,7,8,8,8-undecafluorooctanoic acid | 5:3 polyfluorinated acid | 5:3 Acid |
|  | 4,4,5,5,6,6,7,7,8,8,8-undecafluorooctenoic unsaturated acid | 5:3 polyfluorinated unsaturated acid | 5:3 Uacid |
|  | 4,4,5,5,6,6,7,7,7-nonafluoroheptanoic acid | 4:3 polyfluorinated acid | 4:3 Acid |
| - | - | perfluorocarboxylates ; perfluorinated carboxylic acids | PFCAs |
|  | 2,2,2-trifluoroacetic acid | trifluoroacetic acid | TFA |
|  | 2,2,3,3,3-pentafluoropropionic acid | perfluoropropionic acid | PFPrA |

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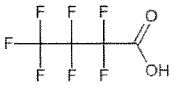
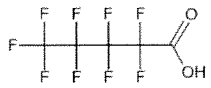
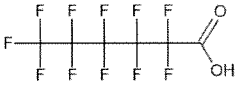
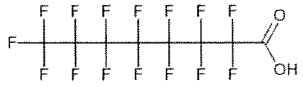
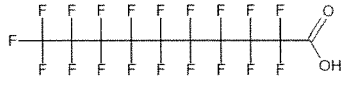
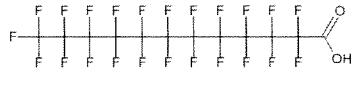
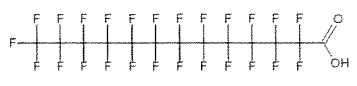
|  | 2,2,3,3,4,4,4-heptafluorobutanoic acid | perfluorobutanoic acid | PFBA |
|  | 2,2,3,3,4,4,5,5,5-nonafluoropentanoic acid | perfluoropentanoic acid | PFPeA |
|  | 2,2,3,3,4,4,5,5,6,6,6-undecafluorohexanoic acid | perfluorohexanoic acid | PFHxA |
|  | 2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoroheptanoic acid | perfluoroheptanoic acid | PFHpA |
|  | 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid | perfluorooctanoic acid | PFOA |
|  | 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluorononanoic acid | perfluorononanoic acid | PFNA |
|  | 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluorononanoic acid | perfluorodecanoic acid | PFDA |
|  | 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-henicosafluoroundecanoic acid | perfluoroundecanoic acid | PFUnDA |
|  | 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-tricosafluorododecanoic acid | perfluorododecanoic acid | PFDoDA |
|  | 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,13-pentacosafluorotridecanoic acid | perfluorotridecanoic acid | PFTrDA |
| - | - | perfluorinated sulfonic acids | PFSAs |

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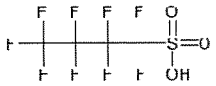
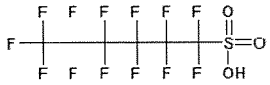
|  | 1,1,2,2,3,3,4,4,4-nonafluoro-1-butanesulfonic acid | perfluorobutane sulfonic acid | PFBS |
|  | 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-1-hexane sulfonic acid | perfluorohexane sulfonic acid | PFHxS |
|  | 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid; | perfluorooctane sulfonic acid | PFOS |

Annex II - References

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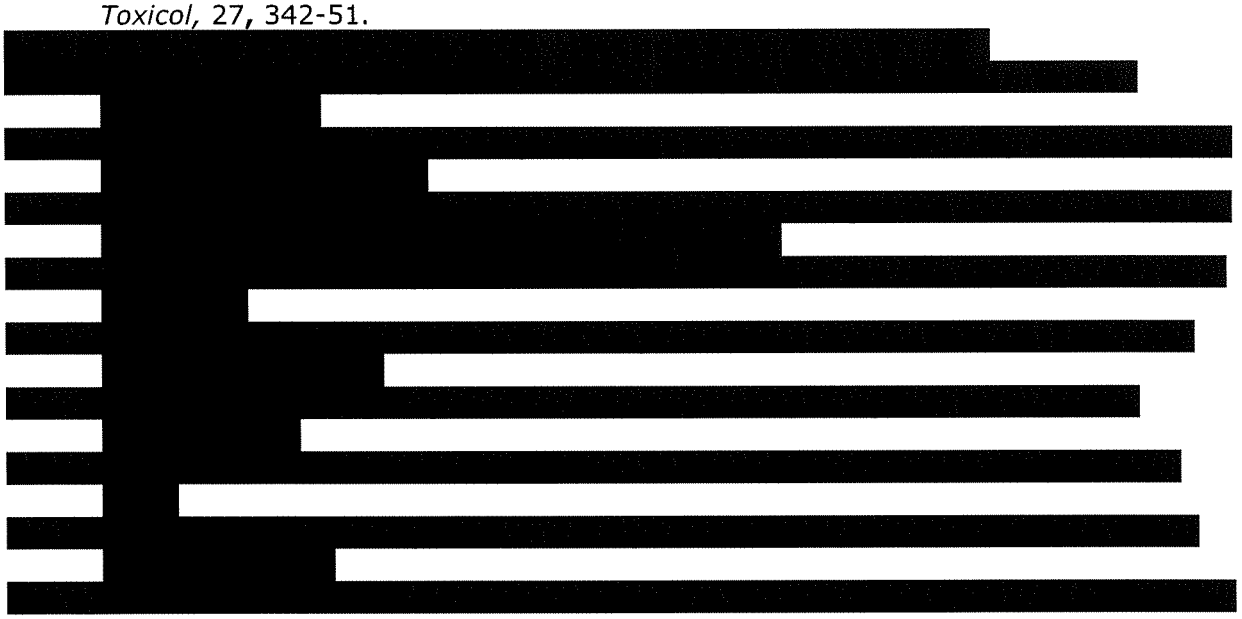

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