Annex XV report

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

Substance Name(s): 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid, its salts and its acyl halides (covering any of their individual isomers and combinations thereof)

EC Number(s): -

CAS number(s): -

Submitted by: The Netherlands

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CONTENTS

TABLES

Abbreviations

A/G ratio	Albumin/globulin ratio
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APFO	Ammonium pentadecafluorooctanoate
AST	Aspartate aminotransferase
BAC	Biological Activated Carbon
BAF	Bio Accumulation Factor
BCF	Bio Concentration Factor
BMD	Benchmark dose
BUN	Blood urea nitrogen
CAR	Constitutive androgen receptor
CCK	Cholecystokinin
CTD	Characteristic Travel Distance
dwt	Dry weight
EDL	Effect dose lower limit
EDU	Effect dose upper limit
FEP	Fluorinated ethylene propylene
FRD-902	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate
FRD-903	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid
fwt	Fresh weight
GAC	Granulated Activated Carbon
GD	Gestation day
GGT	γ-glutamyl transferase
H-28308	A specific purity grade of the ammonium salt 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate
H-28307	A specific purity grade of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid
H-28397	A specific purity grade of the ammonium salt 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate
HFPO-DA	Dimer acid of HFPO; 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid/ deprotonated form, used to indicate the group of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, its salts and its acyl halides (covering any of their individual isomers and combinations thereof)

HFPO-TA	Trimer acid of HFPO
hl-FABP	Human liver fatty acid binding protein
HNF4α	Hepatocyte nuclear factor 4α
H-PFESA	Hydrogen substituted polyfluoroalkyl ether sulfonic acid
LOD	Limit Of Detection
LOQ	Limit Of Quantification
LRTP	Long Range Transport Potential
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MRP	Multidrug resistance protein
NC	North Carolina
NOAEL	No observed adverse effect level
OAT	Organic anion transporter protein
PAC	Powdered Activated Carbon
PCB	Poly Chlorinated Biphenyl
PFAS	Per- and polyfluoroalkyl substance
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutanesulfonic acid
PFCA	Perfluoroalkyl carboxylic acid/ perfluoroalkyl carboxylate
PFECA	Perfluoroalkyl ether carboxylic acid
PFHxA	Perfluorohexanoic acid
PFSA	Perfluoroalkyl sulphonic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane-1-sulphonic acid
POP	Persistent Organic Pollutant
PPAR	Peroxisome proliferator-activated receptor
PTFE	Polytetrafluoroethylene
QSAR	Quantitative Structure-Activity Relationship
RBC	Red blood cell
RPF	Relative Potency Factor
SDH	Sorbitol dehydrogenase
TDAR	T cell –dependent antibody response
tTDI	tentative Tolerable Daily Intake
WTP	Water Treatment Plant
WWTP	Waste Water Treatment Plant

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

Substance Names: 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid, its salts and its acyl halides (covering any of their individual isomers and combinations thereof)

EC Number(s): -

CAS number(s): -

It is proposed to identify the substances as substances of equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of Regulation (EC) No 1907/2006 (REACH) according to Article 57(f) of REACH Regulation.

Summary of how the substance meets the criteria set out in Article 57 of the REACH Regulation

2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid, its salts and its acyl halides (covering any of their individual isomers and combinations thereof), further denoted as HFPO-DA, are identified as substances of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) because in water under environmental conditions these substances exist in the form of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate, for which there is scientific evidence of probable serious effects to the environment and human health which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of REACH.

As HFPO-DA is very persistent and very mobile in the aquatic environment, continuous emission into the environment will lead to increasing concentrations of HFPO-DA in the environment and to a world-wide distribution, also to pristine areas and groundwater, which will become irreversibly affected. Available information already provides evidence that HFPO-DA emissions have resulted in a ubiquitous presence in the environment in the low ng/l range. Environmental monitoring data show the presence of local emission sources that can be attributed to (industrial) human activities involving HFPO-DA and diffuse and uncertain emissions without any identifiable source.

HFPO-DA adversely impacts human health at a daily intake that could be as low as 21 ng/kg bw/day (tTDI). Key target organs are the liver, the kidney, the blood and the immune system. HFPO-DA may also be a human carcinogen, but data are currently insufficient to fully determine its carcinogenic potential. The carcinogenicity of FRD-902 is currently under investigation in an ongoing Substance Evaluation. Based on the information available, it is concluded that the effects of HFPO-DA are severe. The effect on human health may come with a possible delay between the moment of exposure and the onset of any observable adverse effect (i.e. cancer), but may also already be relevant upon short-term exposure (i.e. immune effects, haematological effects, liver effects, kidney effects). Further concern arises as a consequence of the very high persistence and chronic background concentrations in the environment resulting in continuous exposure that may lead to the irreversibility of adverse effects that are normally considered reversible upon the removal of exposure in standard toxicity studies.

Based on the hazard and fate properties there is a serious concern that effects in the general human population are unavoidable in the future and, when occurring, are irreversible. Cessation of emissions will not necessarily result in a reduction in substance concentration and continuous exposure over very long times can lead to inter-generational effects that will impact the quality

of life and are of high societal concern. Furthermore, due to the potential for wide spread distribution and long range transport of HFPO-DA, effects will not only occur at the point of release but also far away from its point of release and will affect a very large number of people. The substance will be distributed world-wide and pristine areas and groundwater will become irreversibly affected. The demonstrated presence of HFPO-DA in drinking water in several areas across the globe, in fish and in vegetables indicate the daily exposure of humans, which is of high societal concern. The co-exposure of HFPO-DA and other very persistent fluorochemicals such as PFOA, PFHxA and PFBS present in the environment lead to combination effects on human health, which add further onto the societal concern for this substance.

The above given arguments demonstrate that there is scientific evidence of probable serious effects to the environment and humans, which gives rise to an equivalent level of concern according to article 57(f) of REACH.

Registration dossiers submitted for the substance? Yes, for ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902; CAS 62037-80-3).

PART I

Justification

1. Identity of the substance and physical and chemical properties

1.1 Name and other identifiers of the substances

This Annex XV dossier applies to 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid, its salts and its acyl halides (covering any of their individual isomers and combinations thereof). The characteristics of those substances belonging to this group for which there is a registration or preregistration under REACH are summarised below.

First is the registered ammonium salt ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902; CAS 62037-80-3, see Table 1). FRD-902 belongs to the group of perfluorinated substances (PFAS), and is a member of perfluorinated alkyl acids and therein its subgroup of PFECAs (per- and polyfluoroether carboxylic acids). In several of its properties, FRD-902 resembles the properties of other perfluorinated alkyl acids, more in detail, short chain perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs).

FRD-902 is manufactured by mixing the second substance, FRD-903 (EC 236-236-8; CAS 13252-13-6, see Table 2 and Figure 2), with an ammonium hydroxide solution (Beekman et al., 2016). FRD-903 is not registered under REACH but is manufactured in the US.

Under environmental conditions in a water environment, FRD-902 and FRD-903 dissociate to form HFPO-DA (see Table 5 and Figure 5, and Section 1.4 for further details). HFPO-DA consists of two C3 moieties, with the carboxylic acid on the first carbon atom, and a propoxy (propyl ether) group attached to the second carbon atom of the first C3 moiety. The chemical is perfluorinated, i.e. no hydrogen but only fluoro atoms are attached to the two carbon chains.

In addition to FRD-902 and FRD-903, the ECHA dissemination site makes reference to the pre-registered highly similar potassium salt of HFPO-DA (potassium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate; EC 266-578-3; CAS 67118-55-2, see Table 3 and Figure 3). It is expected that under environmental conditions, this salt will behave in a similar way as FRD-902 and FRD-903.

Currently available information suggests that only one other substance besides the acid (FRD-903), the ammonium (FRD-902) and the potassium salts could lead to HFPO-DA in the environment. This substance is hexafluoropropylene oxide (HFPO). It is used in manufacturing processes of fluoropolymers. HFPO is a well-known versatile synthetic building block in the manufacturing of fluoropolymers (such as polyfluoroalkoxy plastics)[1] as well as a number of poly- and per-fluorinated intermediates. HFPO can react to form the HFPO-dimer acid fluoride, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoyl fluoride acid (EC 218-173-8, see Table 4 and Figure 4) during oligomerisation, or other manufacturing processes (Siegemund, et al. 2012). Acyl halides in general are very reactive towards water (acid catalysed hydrolysis) forming the carboxylic acid (in this case HFPO-DA and a hydrogen halide (hydrogen fluoride) (Hopkins et al 2018). This hydrolysis is the most heavily exploited reaction for acyl halides as it occurs in the industrial synthesis of acetic acid. The strong reactivity with water makes acyl halides in general

[1] HFPO-DA datasheet Dupont
http://www2.dupont.com/FluoroIntermediates/en_US/assets/downloads/k05132.pdf

irritants to the eyes, skin and mucous membranes. Therefore, after release in the environment of the HFPO-dimer via e.g. air, contact with water would readily lead to the formation of the HFPO-dimer acid via hydrolysis. A likely airborne precursor to HFPO-DA is the C3 dimer acid fluoride, which readily hydrolyzes to form deprotonated HFPO–DA when it comes into contact with water (Oppenheimer et al. 2007). The presence of this HFPO dimer acid (HFPO-DA) in the environment could thus also be due to residual leaching from commercial products or direct release during the manufacturing processes that involve HFPO as the building block for fluoropolymer synthesis.

This annex XV report covers 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid, its salts and its acyl halides (covering any of their individual isomers and combinations thereof). For simplicity, the substances are denoted all together by the abbreviation HFPO-DA in this document, unless specified otherwise.

Table 1: Substance identity FRD-902, registered

EC number:	n/a
EC name:	n/a
CAS number (in the EC inventory):	-
CAS number: **Deleted CAS numbers:**	62037-80-3
CAS name:	Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-, ammonium salt (1:1)
IUPAC name:	Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate
Index number in Annex VI of the CLP Regulation	-
Molecular formula:	C6H4F11NO3
Molecular weight range:	347.1
Synonyms:	• FRD-902 (ammonium salt) • GenX • C3 Dimer salt Other synonyms noted in literature are: • Cheminox P0-2-AM60 • Ammonium perfluoro(2-methyl-3-oxahexanoate)

Note: the individual isomers of FRD-902 are:

- ammonium (2*R*)-2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate; and
- ammonium (2*S*)-2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate.

Structural formula:

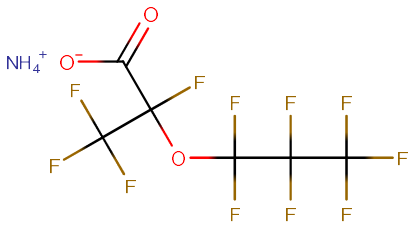

Figure 1: Chemical structure of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoate (FRD-902)

Table 2: Substance identity FRD-903, preregistered

EC number:	236-236-8
EC name:	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid
CAS number (in the EC inventory):	13252-13-6
CAS number: **Deleted CAS numbers:**	-
CAS name:	Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-
IUPAC name:	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid
Index number in Annex VI of the CLP Regulation	-
Molecular formula:	C6HF11O3
Molecular weight range:	330.1
Synonyms:	• *2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid* • Perfluoro-2-methyl-3-oxahexanoic acid • exafluoropropylene oxide dimer acid • HFPO-DA • HFPO2 • FRD-903 (acid) • Perfluoro-2-propoxypropanoic acid • PFPrOPrA • Undecafluoro-2-methyl-3-oxahexanoic acid

Note: the individual isomers of FRD-903 are:

- (2*R*)-2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (CAS number 75579-39-4); and
- (2*S*)-2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (CAS number 75579-40-7).

Structural formula:

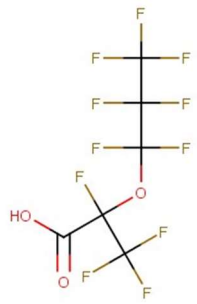

Figure 2: Chemical structure of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903)

Table 3: Substance identity Potassium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate, preregistered

EC number:	266-578-3
EC name:	Potassium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate
CAS number (in the EC inventory):	67118-55-2
CAS number: **Deleted CAS numbers:**	-
CAS name:	Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-, potassium salt (1:1)
IUPAC name:	Potassium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate
Index number in Annex VI of the CLP Regulation	-
Molecular formula:	C6F11KO3
Molecular weight range:	368.1
Synonyms:	• AC1N68UQ • SCHEMBL9173907 • CTK2F3686 • JEDKCJRGYITGMG-UHFFFAOYSA-M • Potassium perfluoro-2-methyl-3-oxahexanoate • 2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propanoic acid potassium salt • potassium 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoate

Note: the individual isomers of potassium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate are:

- potassium (2*R*)-2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate; and
- potassium (2*S*)-2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate.

Structural formula:

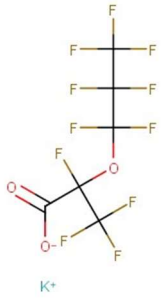

Figure 3: Chemical structure of potassium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate

Table 4: Substance identity 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoyl fluoride, preregistered, registration deadline 2010

EC number:	218-173-8
EC name:	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionyl fluoride
SMILES:	O=C(F)C(F)(OC(F)(F)C(F)(F)C(F)(F)F)C(F)(F)F
CAS number (in the EC inventory):	2062-98-8
CAS number:	2062-98-8
CAS name:	Propanoyl fluoride, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-
IUPAC name:	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoyl fluoride
Index number in Annex VI of the CLP Regulation	
Molecular formula:	C6 F12 O2
Molecular weight range:	332.05
Synonyms:	C3 dimer acid fluoride

Note: the individual isomers of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoyl fluoride are:

- (2*R*)-2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoyl fluoride; and
- (2*S*)-2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoyl fluoride

Structural formula:

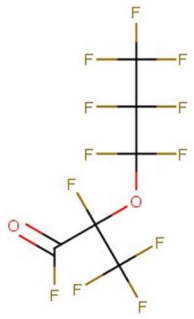

Figure 4: Chemical structure of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoyl fluoride

1.2 Composition of the substance

This Annex XV report applies to 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid, its salts and its acyl halides (covering any of their individual isomers and combinations thereof). The registered and preregistered substances that are part of this group include:

Name (1): Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902)

Name (2): 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903)

Name(3): Potassium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate

Name(4): 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoyl fluoride

Under environmental conditions, FRD-902 (ammonium salt), FRD-903 (the acid) and potassium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate and the C3 dimer acid fluoride are present in the anionic form, i.e. HFPO-DA (see Table 5). Therefore, there is no difference in the environmental fate and toxicological properties of these substances (Beekman et al. (2016)).

Table 5: HFPO-DA, the anion

EC number:	n/a
EC name:	n/a
SMILES:	FC(F)(C(F)(F)OC(F)(C([O-])=O)C(F)(F)F)C(F)(F)F
CAS number (in the EC inventory):	n/a
CAS number:	122499-17-6
CAS name:	Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-, ion(1-)
IUPAC name:	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate
Index number in Annex VI of the CLP Regulation	
Molecular formula:	C6F11O3
Molecular weight range:	329
Synonyms:	HFPO-DA

Structural formula:

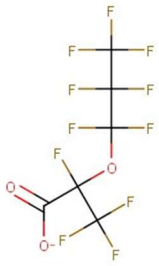

Figure 5: Chemical structure of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate

1.3 Identity and composition of structurally related substances (used in a grouping or read-across approach)

Other PFAS of interest for read-across purposes (of which some are also short-chain PFASs) are summarised in Table 6. These are extracted from Zeilmaker et al. (2018), who proposed a model for a combined toxicity assessment through so called relative potency factors (RPFs) based on similar liver effects between the substances (see Section 4 for further details). This list is not exhaustive.

Table 6: PFCAs and PFSAs (listed as acid equivalents) for which Relative Potency Factors have been derived in addition to HFPO-DA

PFAS group	PFAS (acid form)	EC number (acid form)
PFSA	Perfluorobutanesulfonic acid (PFBS)	206-793-1
	Perfluoropentanesulfonic acid (PFPeS)	220-301-2
	Perfluorohexanesulfonic acid (PFHxS)	206-587-1
	Perfluoroheptane sulfonic acid (PFHpS)	206-800-8
	Perfluorooctanesulfonic acid (PFOS)	217-179-8
	Perfluorodecane sulfonic acid (PFDS)	206-401-9
PFCA	Perfluorobutyric acid (PFBA)	206-786-3
	Perfluoropentanoic acid (PFPeA)	220-300-7
	Perfluorohexanoic acid (PFHxA)	206-196-6
	Perfluoroheptanoic acid (PFHpA)	206-798-9
	Perfluorooctanoic acid (PFOA)	206-397-9
	Perfluorononaoic acid (PFNA)	206-801-3
	Perfluorodecanoic acid (PFDA)	206-400-3
	Perfluoroundecanoic acid (PFUnDA)	218-165-4
	Perfluorododecanoic acid (PFDoDA)	206-203-2
	Perfluorotridecanoid acid (PFTrDA)	276-745-2
	Perfluorotetradecanoic acid (PFTeDA)	206-803-4
	Perfluorohexadecanoic acid (PFHxDA)	267-638-1
	Perfluorooctadecanoic acid (PFODA)	240-582-5

1.4 Physicochemical properties

Details on the studies described in this section have been obtained from study reports as published on the website Health and Environmental Research Online (HERO) of U.S. EPA on GenX (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627).

The physicochemical properties of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate have been determined in several tests with different purities and forms of the test substance. The forms most used in these tests were the ammonium salt referred to as the product H-28308 with a purity 86%, further containing 14.58% water and 7.0 ppm PFOA and the acid referred to as the product H-28307 with a purity of 98%, further containing 0.61% water and 8.6 ppm PFOA. Besides that, some tests with the dried ammonium salt are mentioned on ECHA's dissemination website with a purity of 99.4%.

The vapour pressure was determined in triplicate at a temperature of 20±0.5 °C with the static method according to OECD test guideline 104 with the product H-28308 (also referred to in the report as FRD 902). The vapour pressure at 20°C was 2910±20.8 Pa. The vapour pressure is primarily attributed to the presence of water and ammonia in the test substance (Nixon and Lezotte, 2008c). For this reasons this value is not further considered.

The same test was performed with the product H-28307 (also referred to in the report as FRD 903). The vapour pressure at 20°C was 306±13.7 Pa (Nixon and Lezotte, 2008b). Although not specified in this report, the observed vapour pressure might be affected by the presence of water as well. However, given the low percentage of the impurity this is most likely not explaining the observed vapour pressure.

On ECHA's dissemination site another value of 0.0117 ± 0.000115 Pa at 20 °C is mentioned for the dried ammonium salt. This value was obtained with the spinning rotor technique according to OECD test guideline 104. No study report is available for this study.

The QSAR program Mpbpwin v1.43 from EPI Suite estimates a vapour pressure of 63.5 Pa at 20 °C (92.1 Pa at 25 °C) for the acid form. For the ammonium form, the estimate is 0.00282 Pa at 20 °C for the solid substance and 0.0347 Pa for the subcooled liquid. HFPO-DA is present in the anionic from in the aquatic environment at environmentally relevant conditions. It is thus considered adequate to use the vapour pressure of the dried substance or to apply a fraction dissociated.

Differences were observed for the ammonium salt (solid: 0.017 Pa at 20°C) and acid (liquid: 306 Pa at 20°C). However, if the vapour pressure of the acid is multiplied for the undissociated fraction at neutral pH that is available for volatilisation, an almost identical value of 0.021 Pa is obtained (using the pKa of 2.84, see below). Therefore, the value for vapour pressure of the dried ammonium salt is further considered in the assessment.

On the ECHA dissemination website a value for the distribution between octanol and water is presented, which is a log D_{ow} of 2.58 for the ionised form estimated at environmentally relevant pHs. No further information on this value is given. KOWWIN v1.68 from EpiSuite predicts a log K_{ow} of 3.36. MarvinSketch v16.10.24 estimates a log K_{ow} of 4.00 for the neutral species and a log D_{ow} of 0.47 at neutral pHs. Bioloom v1.5 predicts a log K_{ow} of 4.98. KOAWIN v1.10 from EpiSuite predicts a logarithm of the partition coefficient between octanol and air (log K_{oa}) of 5.44. It must be noted that the relevance of the distribution between octanol and water or air is limited, because HFPO-DA does not primarily partition to lipids or fatty tissues.

The solubility of the product H-28308 (also referred to in the report as FRD 902) with a test substance content of 86% was determined with the shake-flask method at 20±0.5 °C according to OECD test guideline 105 (Nixon and Lezotte, 2008c). Analysis was performed with HPLC-MS. At a measured concentration of 739±13 g/L saturation was still not achieved. Therefore, the substance is considered infinitely soluble in water (Nixon and Lezotte, 2008c). The same test was performed with product H-28307 (also referred to in the report as FRD 903) with a test substance purity of 98%. Saturation was not achieved at a concentration of the product of 756±11.8 g/L. Also in this case the products is considered infinitely soluble in water (Nixon and Lezotte, 2008b).

On the ECHA dissemination website, two values for the solubility of the ammonium salt (82.6% purity) of 218 and 207 mg/L in Haskell well water and in AAP nutrient medium, respectively at 10 °C are reported. These values were determined from the verification by LC-MS of nominal concentrations after an equilibrium time of 25 hours. Because verified analytical concentrations were slightly in excess of the nominal concentrations, also these values seem to be unbounded (greater than) values rather than a fixed value for the aqueous solubility.

The QSAR program WSKOW v1.42 of EpiSuite predicts a solubility of 27.2 mg/L for the acid. However, the reliability of this estimate is limited, because this is based on an estimated value for the log K_{ow}. The program WATERNT v1.01 predicts a similar solubility 16.9 mg/L. This program is based on fragment additions for water solubility. It is clear that the QSAR programmes substantially underestimate the experimentally observed solubility. Following the choice on the ECHA dissemination website, 1000 g/L is used in further assessment.

The pKa of both the ammonium salt and the acid have been determined experimentally by the titration method according to OECD test guideline 121 at 20 °C. The pKa of the acid (H-28307) was 2.84 ± 0.021. The pKa of the reference substance benzoic acid was 4.28 (reference value 4.19). The study report (Murrell and Nixon, 2008) contains some results (titration curve), but the result of 2.84 cannot be reproduced from these data. Also for the ammonium salt (H-28308) a pKa value was determined (Nixon and Lezotte, 2008a). The reported pKa value was 3.82 ± 0.0589, which corresponds to a pKb values of 8.10 ± 0.0677. However, it is not clear for which transition this pKa and pKb relate to. In principle, the same pKa value is expected for 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate, and the difference cannot be explained. The reliability of these two pKa values can thus not be sufficiently assigned.

With the QSAR program MarvinSketch v16.10.24, a pKa value of -0.77 is estimated for 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate and 8.86 for ammonium. Although some uncertainties arise from the experimental data, it is clear that at environmentally relevant conditions (ambient temperature, neutral pH) HFPO-DA will be present in the anionic form. The environmental fate and toxicological properties of the ammonium salt and the acid will thus be similar in the end.

2 Harmonised classification and labelling

At present, there is no harmonised classification and labelling in Part 3 of Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation) for 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid, its salts and its acyl halides (covering any of their individual isomers and combinations thereof).

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FRD-902 is self-classified by its REACH Registrant and by an additional 30 CLP Notifiers. The 4 joint submissions self-classify FRD-902 as[2]:

- Acute Tox. 4 H302: Harmful if swallowed
- Eye Dam. 1 H318: Causes serious eye damage
- STOT RE 2 H373: May cause damage to organs through prolonged or repeated exposure:
    - for blood = 1 aggregated submission with 27 notifiers,
    - for blood and liver = 2 aggregated submissions and 3 notifiers and
    - for blood (Oral and Inhalation) = 1 aggregated submission with 1 notifier)

One notifier (1 joint submission) additionally self-classifies FRD-902 as:

- Acute Tox. 4 H312: Harmful in contact with skin
- Acute Tox. 4 H332: Harmful if inhaled

FRD-903 is self-classified by 99 CLP Notifiers. One joint submission of 66 Notifiers self-classifies FRD-903 as[3]:

- Acute Tox. 4 H302: Harmful if swallowed
- Skin Corr. 1C H314: Causes severe skin burns and eye damage
- Eye Dam. 1 H318: Causes serious eye damage
- STOT SE 3 H335: May cause Respiratory irritation

One joint submission of 30 notifiers self-classifies FRD-903 as:

- Not classified

One joint submission of 2 notifiers self-classifies FRD-903 as:

- Skin Corr. 1C H314: Causes severe skin burns and eye damage

One 1 joint submission of 1 notifier self-classify FRD-903 as:

- Skin Corr. 1B H314: Causes severe skin burns and eye damage

2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoyl fluoride is self-classified by 2 notifiers[4]. One notifier self classifies the substance as:

- Met Corr. 1 H290: May be corrosive for metals
- Skin Corr. 1C: H314: Causes severe skin burns and eye damage
- Eye Dam. 1: H318: Causes serious eye damage

One notifier self classifies the substance as:

- Skin Corr. 1B: H314: Causes severe skin burns and eye damage

[2] ECHA Dissemination website, dd. November 2018
[3] ECHA Dissemination website, dd. November 2018
[4] ECHA Dissemination website, dd. February 2019

3 Environmental fate properties

3.1 Degradation

For HFPO-DA there are no studies on its degradation potential available that follow a standardised and generally accepted study design, such as the OECD test guidelines. For the ammonium salt of HFPO-DA, ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoate (CAS 62037-80-3), only information from screening tests on ready biodegradation are available, showing the substance does not undergo primary or ultimate degradation. Further information is obtained from QSAR estimates and comparisons with structurally related compounds such as PFOA.

The stability of organic fluorine compounds has been described in detail by Siegemund et al. (2012): "When all valences of a carbon chain are satisfied by fluorine, the zigzag-shaped carbon skeleton is twisted out of its plane in the form of a helix. This situation allows the electronegative fluorine substituents to envelope the carbon skeleton completely and shield it from chemical attack. Several other properties of the carbon-fluorine bond contribute to the fact that highly fluorinated alkanes are one of the most stable organic compounds. These include polarisability and high bond energies, which increase with increasing substitution by fluorine. The influence of fluorine is greatest in highly fluorinated and perfluorinated compounds. Properties that are exploited commercially include high thermal and chemical stability".

A number of studies for the PFOA confirm that this substance is very persistent and does not undergo abiotic or biotic degradation at all under environmental conditions (ECHA, 2013b). The persistence of PFOA and its salts was recognised by the Member State Committee that identified the substances as SVHC, among other things, based on its PBT properties (ECHA, 2013a).

Also C9-C14 PFCAs as well as the ammonium and sodium salts of C9-PFCA and C10-PFCA were included on the Candidate List as substances of very high concern (SVHC). All substances meet the P and vP-criteria of REACH Annex XIII based on a weight of evidence approach (ECHA, 2015a, ECHA, 2012d, ECHA, 2012b, ECHA, 2012f, ECHA, 2012e, ECHA, 2012c). The structurally related substance PFHxS also contains this type of a stable fluorinated carbon chain. The conclusion that PFHxS fulfils the criteria for being "very persistent" was adopted by the Member State Committee in 2017 (ECHA, 2017b).

The difference between these PFCAs and HFPO-DA is the ether bond in the perfluoro chain (see also Annex II). This ether bond is not expected to lower the persistence substantially. QSARs included in BIOWIN v4.10 of EpiSuite generally have a negative fragment contribution of the ether bond on the degradation included. Further, also the length of the perfluorochain does not influence the degradability of the substances.

For some of the studies, study reports were retrieved from the Health and Environmental Research Online (HERO) website of U.S. EPA on GenX (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627). All studies and publications are considered relevant to evaluate the degradation of HFPO-DA, and are used in the weight of evidence assessment of persistence. Despite the fact that some studies are not conducted according to OECD guidelines, or may not be considered highly relevant as stand-alone studies to assess the persistence, the study results show an overall consistent pattern of degradation for HFPO-DA. Hence, the dossier submitter sees no reason to discard any study on the basis of reliability.

3.1.1 Abiotic degradation

3.1.1.1 Hydrolysis

Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (test substance H-28308,

see Section 1.4) was shown to be hydrolytically stable at pH 4, 7 and 9, with no degradation after 5 days at 50°C in a hydrolysis as a function of pH study according to OECD TG 111. Analysis was performed by HPLC-MS. Recovery of the test substance at test initiation was 105, 105, and 102% at pH 4, 7, and 9, respectively. After 5 days, recoveries were 95.4, 94.5, and 93.5% at pH 4, 7, and 9, respectively. Fortified matrix recoveries, freshly prepared and added to blank media after 5 days were 97.4, 93.1 and 91.7% at pH 4, 7, and 9, respectively. All chromatograms contained only one single peak. From these results, it is concluded that the half-life of the test substance due to hydrolysis at a relevant pH range is over 1 year (van Hoven and Nixon, 2008).

The QSAR model HYDROWIN v2.00 of the EPISuite tool (US EPA, 2002-2012) predicts rates and half-lives for hydrolysis. 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid does not contain any functional groups for which hydrolysis can be estimated by HYDROWIN v2.00.

3.1.1.2 Oxidation and reduction

The oxidation of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate was tested under laboratory conditions in quartz tubes placed in a photoreactor under ultraviolet light (3.8 µE/L/s at 253.7 nm) with 20 mM persulfate (potassium salt) at a pH of 10. The concentrations of HFPO-DA as well as from the metabolites trifluoroacetic acid (TFA) and pentafluoropropionic acid (PFA) were followed in time by HPLC-MS/MS. This method was shown to be effective to degrade PFOA and other PFCAs. In this study, the degradation of PFOA was also studied. In 180 minutes, 26% of PFOA was degraded, while this was less than 5% for HFPO-DA (Bao et al., 2018). This study shows that oxidation of HFPO-DA is even slower than that of PFOA, which has been shown to be very persistent under environmental conditions.

Under the same conditions, but with 20 mM sulphite instead of persulfate, the reduction of HFPO-DA and PFOA was tested. In this case no HFPO-DA could be detected anymore at time points later than 2 hours. PFOA was already completely degraded within 2 hours. Similar to the oxidation, the primary degradation due to reduction was faster for PFOA than for HFPO-DA (reaction rates of 0.0410 vs. 0.0338 min^{-1}). The reaction rate for HFPO-DA was also tested in artificially contaminated river water. The reaction rate was lower compared to that in pure water and decreased to 0.0296 min^{-1} (Bao et al., 2018). Also for reduction the degradation rate for HFPO-DA is slower than for PFOA. The study is reliable but the relevance for environmental conditions is limited.

3.1.1.3 Phototransformation/photolysis

3.1.1.3.1 Phototransformation in air

The QSAR model AOPWIN v1.92 of the EPISuite tool (US EPA, 2002-2012) predicts degradation rates and half-lives for direct and indirect photolytic degradation in the atmosphere. For the common preset of the model – assuming indirect degradation via OH-radicals, 12 h-day, 1.5e+06 OH radicals per m³ - the tool predicts a degradation rate constant of 0.52e-12 cm³/(molec * s) for HFPO-DA which is equal to an atmospheric half-life of 20.57 days. This estimate is the same as for PFOA and PFHxA. These results can be seen as an estimate only, because perfluorinated substances are not fully within the applicability domain of the EPISuite models.

Some experimental data is available on abiotic degradation in air of the structurally related perfluorinated carboxylic acids. Hurley et al. (2004) studied the kinetics of the reactions of OH radicals with a homologous series of perfluorinated carboxylic acids, $F(CF_2)_nCOOH$ (n = 1, 2, 3, 4), at an air pressure of 700 Torr and a temperature of 296 ± 2 K. For n > 1, the length of the $F(CF_2)_n$ group had no discernible impact on the reactivity of the molecule. Atmospheric lifetimes of $F(CF_2)_nCOOH$ with respect to reaction with OH radicals are estimated to be approximately 230 days for n = 1 and 130 days for n > 1. Reaction with OH radicals is a minor atmospheric fate of $F(CF_2)_nCOOH$ (Hurley et al., 2004).

From the data available it becomes obvious that the degradation half-life of HFPO-DA in the atmosphere is clearly above the threshold of two days which indicates that the substance has the potential for long range transport. For the assessment of the potential for long range transport the best case half-life of 20.57 days for photolytic degradation in the atmosphere was used (see Section 3.3).

3.1.1.3.2 Phototransformation in water

In the study by Bao et al. (2018) cited above, the degradation of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate and PFOA was also studied with only ultraviolet light without persulfate or sulfite. For PFOA, degradation after 180 minutes was almost 10%, while less than 5% for HFPO-DA. For HFPO-DA, the degradation under ultraviolet light only was similar to the degradation in the presence of sulfate. It follows that HFPO-DA is less degradable by phototransformation due to ultraviolet light (253.7 nm) than PFOA. The study is reliable but the relevance for environmental conditions is limited.

3.1.1.3.3 Phototransformation in soil

No information available

3.1.1.4 Summary on abiotic degradation

Experimental data for hydrolysis show that ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate is not hydrolysed. In a photoreactor under enhanced ultraviolet light only and oxidative conditions and reductive circumstances, the substance was less degradable than PFOA. QSAR estimates for hydrolysis and phototransformation in air lead to the conclusion that rapid degradation under these conditions is highly unlikely. It follows that 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate is very persistent under environmentally relevant abiotic conditions.

3.1.2 Biodegradation

3.1.2.1 Biodegradation in water

3.1.2.1.1 Estimated data

The QSAR model BIOWIN v4.10 of the EPISuite tool (US EPA, 2002-2012) includes several QSARs for estimating intrinsic substance properties and environmental fate and behaviour of chemicals, providing degradation timeframes for primary and ultimate degradation of chemicals. BIOWIN also provides an estimate whether a substance fulfils the criteria of being rated as "readily biodegradable". The outcome of the different BIOWIN QSAR estimates is provided in Table 7.

Table 7: Outcome of the different QSAR estimates on biodegradability of HFPO-DA

Model	Result (value)	Conclusions from the estimate
BIOWIN 1	-1.2769	Does Not Biodegrade Fast
BIOWIN 2	0.0000	Does Not Biodegrade Fast
BIOWIN 3	1.1634	Recalcitrant
BIOWIN 4	2.7383	Weeks-Months
BIOWIN 5	0.3069	Not Readily Degradable
BIOWIN 6	0.0000	Not Readily Degradable
BIOWIN 7	-0.2371	Does Not Biodegrade Fast
Ready biodegradability prediction	NO	Criteria of BIOWIN predictions are not fulfilled: module BIOWIN 3 should predict "degradation within weeks" or faster AND module BIOWIN5 should result in value ≥ 0.5.

The combination of these QSARS gives an estimate whether a chemical is potentially persistent or not. According to REACH guidance document R.11 (ECHA, 2017a), the screening criteria below which persistence is likely are 0.5 for BIOWIN 2 or BIOWIN 6 in combination with a value of 2.25 for BIOWIN 3. The values for HFPO-DA lie well below these screening criteria and thus the QSARs indicate that HFPO-DA is potentially (very) persistent.

For evaluation of the BIOWIN prediction it has to be kept in mind that the outcome for perfluorinated hydrocarbons has to be understood as a vague prediction. This is because the training data set is incompletely implemented for perfluorinated carbon chains. In particular, the perfluorinated carbon chain is counted as four units of "carbon with 4 single bonds & no hydrogens", while only BIOWIN 1-4 and 7 predictions include an additional fragment for a trifluoromethyl group. Considering that the perfluorinated carbon chain is expected to be very stable but not properly included in the model, this observation supports the conclusion that the persistence is underestimated by the BIOWIN predictions.

Nevertheless, the result of BIOWIN modelling provides sufficient evidence that HFPO-DA will not fulfil the criteria for being rated as "readily biodegradable". Considering the above, the screening assessment on persistence of HFPO-DA, based on BIOWIN predictions, adds to the weight-of-evidence that HFPO-DA is "potential P or vP" according to ECHA Guidance on PBT/vPvB assessment (ECHA, 2017a).

3.1.2.1.2 Screening tests

The toxicity of H-28397 (88% ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoate, 13.3% water and an impurity of 3.4 ppm perfluorooctanoic acid) to inocula was tested separately in a respiratory inhibition test according to OECD TG 209.

In an OECD 301B study for ready biodegradability 0% mineralisation (CO2 evolution) as well as 0% primary degradation was found after 28 days at a test substance concentration of 112.2-112.7 mg/L of H-28397. The carbon dioxide production in the test vessels and in the abiotic controls with the test substance was less than in the inoculum control blanks without the test

substance. The positive control substance sodium benzoate showed 72.8% mineralisation after 28 days, with more than 60% mineralisation within 14 days. The test substance had no inhibitory effect on the degradation of the positive control substance. From these results it is concluded that the substance is not readily biodegradable. The test substance was also analysed by HPLC-MS. It appeared that the recovery of the test substance was higher at the end of the test than at the beginning. Chromatograms showed a single peak. It is concluded in the report that there was no primary degradation either (Vavala and Berte, 2009).

A ready biodegradability study according to the Japanese guideline for biodegradation of chemical substances by microorganisms, which is similar to OECD TG 301C was carried out with FRD-903 (2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid; 99.6% pure; 0.4% unknown impurities). The test substance concentration was 100 mg/L. Degradation was assessed by the biological oxygen demand (BOD), dissolved organic carbon (DOC) and the analysis of the parent compound by LC-MS/MS. Degradation of the three replicates after 28 days was reported to amount to 3±4%, 1±3% and 1±3%, based on BOD, DOC, and residual test substance respectively. Based on the theoretical oxygen demand, the mineralisation of the reference substance aniline that served as positive control was 72% after 14 days. Only one peak of the test substance FRD-903 was found in the LC-MS/MS chromatograms. It is concluded in the report that the test substance is not readily biodegradable and that it is not structurally transformed under the test conditions (Kawashima, 2009).

An OECD 302C (MITI II) study for inherent biodegradability was performed with FRD-902 (ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoate, 86.9% purity). The concentrations of the test substance and the reference substance aniline were 30 mg/L and 100 mg/L, respectively. The sludge content was 100 mg/L (source activated sludge, surface soil and surface water sampled from ten sites from Nanjing city). Mineralisation was followed in time by means of the biological oxygen demand (BOD). Mineralisation calculated as percentage of theoretical oxygen demand was 82.2% after 14 days and 94.2% after 28 days for the reference substance aniline. The triplicate measurements for the test substance FRD-902 all showed <1% mineralisation. Analysis of the test substance by LC-MS/MS at the end of the 28-d day test period showed 0% degradation in all three replicates. It is concluded in the report that the test substance is not inherently biodegradable under the test conditions (Lili, 2010a).

The same OECD 302C study for inherent biodegradability was conducted with FRD-903 (2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid, 96% purity). Mineralisation of the reference substance aniline was 74.3% after 14 days and 83.8% after 28 days. The triplicate measurements for the test substance FRD-903 all showed <1% mineralisation. Degradation based on residuals measured by LC-MS/MS amounted to 1±3% in the three replicates. However, this is based on dissipation compared to the abiotic control and is not significant. Thus, no significant degradation compared to the control was observed. It is concluded in the report that the test substance is not inherently degradable (Lili, 2010b).

3.1.2.1.3 Simulation tests (water and sediments)

No data available

3.1.2.2 Biodegradation in soil

No data available

3.1.2.3 Summary and discussion on biodegradation

Based on the predictions on biodegradation by BIOWIN and the lack of any primary degradation in the screening tests available on biodegradation, it is concluded that HFPO-DA does not biodegrade.

3.1.3 Field data

HFPO-DA is monitored at locations far away from potential sources including the marine environment (see Section 3.2.5). The travelling distance is far and consequently the time to reach these areas will be very long. This is supporting the assumption that degradation of HFPO-DA under environmental conditions will be very limited.

3.1.4 Summary and discussion of degradation

PFAS compounds are resistant to degradation due to the very stable highly fluorinated alkyl chains. The high persistence is a property that is observed for other perfluorinated substances as well. A number of these structural related substances have already been shown to be very persistent. HFPO-DA is not degradable in screening tests for both abiotic degradation and biodegradation. HFPO-DA is not mineralised in these studies and does not show any primary degradation as well. The lack of biodegradation is also supported by QSAR predictions. Under highly reactive oxidative and reductive conditions, degradation of HFPO-DA occurs, but the degradation rates are lower than for PFOA, which has been shown to be very persistent. High persistency is also supported by monitoring data showing HFPO-DA in detectable concentrations in remote places where there is no indication of a possible direct emission source. Overall, degradation potential of HFPO-DA in all compartments can be concluded to be very low or negligible.

3.2 Environmental distribution

3.2.1 Adsorption/desorption

The log Koc value for H-28397 has been determined by HPLC according to OECD test guideline 121 (Bloxham, 2008). Seven reference compounds with known log Koc values for soil and sludge were used. Most important details (retention times, log Koc values for the reference compounds) are lacking from the summarising report. The obtained log Koc values were 1.08 for soil and 1.10 for sludge. The log Koc can also be determined by QSAR programmes from EpiSuite (US EPA, 2002-2012). With KOCWIN v2.00 the calculated values for HFPO-DA are 2.48 and 1.92 based on molecular connectivity indices and on estimated log Kow, respectively.

Further information is available from the study by Sun et al. (2016), who tested the adsorption of PFASs to powdered activated carbon (PAC) in amber glass bottles. The PAC concentrations used were 30, 60 and 100 mg PAC/L, and these concentrations represent the upper feasible limit used as purification for drinking water preparation. At these PAC doses, around 20%, 30% and 40% of HFPO-DA was removed from the water, respectively. These removal percentages were higher than for perfluorobutanoic acid (PFBA), but lower than for perfluorhexanoic acid (PFHxA) and perfluoro butane sulfonate (PFBS). Percentage removal was higher for the longer chain PFASs, with 90% or more at 100 mg PAC/L for PFHpA, PFOA, PFNA, PFDA, PFHxS and PFOS. This experiment thus confirms the low sorption potential of HFPO-DA.

A test with beds of regenerated subbituminous coal-based granulated activated carbon (GAC) reduced the concentrations of HFPO-DA substantially to 7% after 3500 bed volumes, but after 5000 bed volumes this increased to a value as high as 74%. HFPO-DA was even released from the GAC beds after emission reduction as the effluent concentrations were 28% higher than the influent concentrations. Initial results for anion exchange beds appeared to be more effective but also for this technique the effectiveness decreased with increasing bed volumes passed (Hopkins et al., 2018).

3.2.2 Volatilisation

No experimental data are available for Henry's Law constant. The QSAR program HenryWin

v3.20 of EpiSuite (US EPA, 2002-2012) calculates a Henry's Law constant by the bond method. This value is 20.8 Pa·m^3/mol, or 0.00893 unitless (m^3_{water}/m^3_{air}). On the ECHA dissemination website, a Henry's Law constant has been calculated for the ammonium salt amounting to $4.06 \cdot 10^{-6}$/mol Pa·m^3/mol, using the vapour pressure (0.017 Pa at 20 °C) and water solubility (1000 g/L) of the dried substance. It is mentioned that due to the ionic nature of the substance, the vapour pressure will be essentially zero. The presence in air is unlikely.

Considering the low Henry law constant and the fact that HFPO-DA is present in the anionic form in the aquatic environment at environmentally relevant conditions, volatilisation from water is expected to be a minor route.

3.2.3 Removal during drinking water treatment

A low removal capacity was demonstrated by Sun et al. (2016) by the results of their assessment of the removal of HFPO-DA in the different steps of the drinking water treatment process in a drinking water treatment plant from the Cape Fear River watershed. Water was sampled after each step in the purification process (raw water, ozonation, coagulation/flocculation/sedimentation, settled water ozonation, biological activated carbon (BAC) filtration, and disinfection by medium-pressure UV lamps and free chlorine). No significant removal was observed throughout the whole drinking water treatment process.

In a study by Hopkins et al. (2018) no removal was observed by both conventional surface water treatment processes (coagulation, flocculation, sedimentation, filtration, disinfection with free chlorine) and by several advanced water treatment processes, including raw and settled water ozonation, biofiltration, and disinfection with medium-pressure ultraviolet (UV) lamps. This was determined using of a time series of raw and finished tap water concentrations at a tap water treatment plant (WTP) located approximately 90 miles downstream of the fluorochemical plant. The hydraulic residence time in the WTP was approximately one day and the finished tap water concentrations matched the raw water concentrations from the previous days.

Similar results on the effect of water treatment techniques are reported by Dutch water producer Oasen after measurements in a WTP with GAC beds, where it is concluded that the removal of the substance using current purification techniques can be regarded as negligible (Roelandse and Timmer, 2017).

High pressure membranes are effective, but this technique is highly energy consuming and poses a problem for the management of the retentate, which has high levels of PFASs and salts (Hopkins et al., 2018).

3.2.4 Distribution modelling

On the ECHA dissemination website it is stated by the registrants that once the substance is emitted to water, it will stay in water. Further, it is mentioned that upon emission to soil the substance will partition to water and that it has a high to very high mobility to groundwater due to its low volatility and low adsorption potential. Upon emission to air, the substance will partition to water and soil due to partitioning and deposition.

This is in line with the physicochemical properties of the substance. Due to the low pKa value, the substance will be primarily in the ionic form in the environment, resulting in the above mentioned low sorption to soil and sediment and low volatilisation to air. Modelling estimates are presented in Section 3.3.

3.2.5 Field data

Recently, information coming from monitoring data has started to raise concern with regard to a possible wide spread abundance of HFPO-DA in the environment and in drinking water. These data are summarised below and an overview of monitoring locations with an indication of

concentrations detected is provided in the figure in this chapter. It should be emphasized that this may not be a complete overview of data available. Most monitoring data are from peer-reviewed publications, often with emphasis on the methodological development of the analysis of PFASs. Other data originate from laboratories with certified test protocols. The reliability of the data is thus considered high.

Rhine-Meuse delta

Since 5 years, information is being generated on the possible presence and distribution of HFPO-DA in the Rhine-Meuse delta. First information stems from 2013. At least in Europe, a combination of river currents and tidal forces and a combination of known and unknown emission sources may be responsible for the overall abundance profile.

In August 2013, water samples were taken in the Rhine-Meuse delta 2013 (Heydebreck et al., 2015, Heydebreck, 2017). About 45 km downstream (distances obtained from maps of waterways, Rijkswaterstaat (2013)) of a fluorochemical production plant near Dordrecht (NL), a HFPO-DA concentration of 91.4 ng/L was found in the river het Scheur near Rozenburg (NL). At the same time, no HFPO-DA was detected (above the method detection limit (<0.14 ng/L)) in the river de Oude Maas, which is about 10 km downstream of the fluorochemical plant. One tentative explanation for the different concentrations observed in het Scheur and de Oude Maas may be that de Oude Maas is a different confluence than het Scheur. This possible explanation is supported by further data showing that also in the river het Hollands Diep near Willemstad, which is about 15 km further downstream of de Oude Maas no HFPO-DA was detected. In Germany, in the river Rhine at Leverkusen, a concentration of HFPO-DA of 108 ng/L was found. At the other sampling stations, HFPO-DA was not detected (<0.14 ng/L) except for a low concentration of 0.75 ng/L at the sampling site Tolkamer (NL) in the river Rhine.

In a follow up study in September 2015, HFPO-DA was sampled in large transects of the river Rhine up to the Dutch-German border. The average concentration of HFPO-DA sampled in this study was 0.03±0.01 ng/L (Heydebreck, 2017). These data seem to suggest that diffuse sources of HFPO-DA are present upstream of the fluorochemical production plant at Dordrecht, and this was concluded by the authors as most likely explanation of the observed abundance profile.

In October 2016, samples were taken both upstream and downstream of the fluorochemical production plant in the Netherlands (Gebbink et al. 2017). Concentrations varying from 108 to 812 ng/L were found at sites downstream of the production plant near Dordrecht. These sites were, in order of increasing concentrations, the rivers de Noord at Papendrecht, Beneden Merwede at Papendrecht, de Noord at Alblasserdam, de Oude Maas at Dordrecht, de Nieuwe Maas at Ridderkerk, de Lek at Kinderdijk (respectively about 4, 2, 8, 4, 20, 15 and <1 km, downstream of the production plant). Concentrations of 48 to 58 ng/L were found at the rivers Breeddiep near Hoek van Holland, het Scheur at Vlaardingen, and de Nieuwe Waterweg at Maassluis (55, 40, and 45 km downstream respectively). The concentration of 58 ng/L found in the river de Nieuwe Waterweg at Maassluis is comparable to the concentration of 91.4 ng/L that was found by Heydebreck in 2013 in het Scheur near Rozenburg. Interestingly, these two locations are positioned at almost opposite sides of the same waterway. Lower concentrations were found by Gebbink et al. (2017) in the south branch of the system of waterways, with concentrations of varying from 1.7 to 14 ng/L in the rivers de Dordtsche Kil at Dordrecht, de Oude Maas at Zwijndrecht and de Oude Maas at Hoogvliet (10, 10, and 30 km downstream respectively). The concentration of 6.3 ng/L in de Oude Maas detected by Gebbink et al. (2017) in 2016 is higher than what had been detected earlier by Heydebreck in 2013, who did not detect HFPO-DA at almost the same sampling site (<0.14 ng/L). Of the sites upstream of the fluorchemical production plant, only at the sampling site Beneden Merwede at Sliedrecht, which is about 1 km upstream of the plant, a concentration of 22 ng/L was found. This observation may possibly be explained by the tidal influence in this area that may cause HFPO-DA to be found upstream of the fluorochemical plant. A similar explanation may hold for the location Kinderdijk, which is also slightly upstream of a confluence and where HFPO-DA was detected in a concentration of 433 ng/L. Concentrations in four other locations further upstream of the

waterway system of the river Rhine, were below the method limit of quantification (<0.2 ng/).

Pan et al. (2018) monitored 20 location along a trajectory from the river Mainz at Offenbach (D) along the river Rhine to the river Nederrijn at Wijk bij Duurstede (NL) and the River Waal at Zaltbommel (NL) in December 2016. HFPO-DA was detected in all samples (method detection limit 0.38 ng/L) and concentrations ranged from 0.59 to 1.98 ng/L, with rather constant concentration of 0.8 to 1.0 ng/L upstream up to Duisburg (D). The overall average and median concentrations were 0.99 and 0.90 ng/L, respectively.

In other samples from the Netherlands in 2017 (Versteegh and De Voogt, 2017), concentrations of 5.2, 12 and 16 ng/L were found in the river de Lek at Bergambacht, which is about 15 km upstream of Kinderdijk. Also here, tidal influences cannot be excluded to explain these concentrations. At Kinderdijk a concentration of 126 ng/L was found, while at Papendrecht and Ridderkerk concentrations of 60 and 90 ng/L were found, respectively. At concentrations more upstream in the river Rhine and tributaries (Lobith, Bimmen, Nieuwegein and Andijk) concentrations of HFPO-DA were 1 ng/L or less. Part of these concentrations are however higher than the concentrations found in the German part of the river Rhine in 2015. In the samples from the river Meuse and tributaries reported by Versteegh and De Voogt (2017), HFPO-DA was also detected in samples upstream of the city 's Hertogenbosch. Concentrations of HFPO-DA detected there were 1 ng/L or less. However, a small tributary appeared to contain concentrations of 42 and 47 ng/L. These concentrations point at a local source of HFPO-DA present there that is different from the fluorochemical production plant. Concentrations west of this point in the Meuse and some other (small) waterways varied from 4.3 to 47 ng/L.

In the same region, in smaller water bodies around the city of Helmond, HFPO-DA was detected in concentrations ranging from <20 to 6500 ng/L. Concentrations in Helmond could be attributed to a local source, a company that operated as a subcontractor for the production plant in Dordrecht. The highest concentration was found in a local pond around 500 m from this facility (Van Bentum et al., 2018). Earlier measurements at the same locations resulted in HFPO-DA concentrations of 65 to 7500 ng/L (Aa en Maas, 20185). At the location with the highest concentration, a local pond, also a concentration of 15000 ng/L was measured in the same period (NVWA, 2018).

Concentrations in the Rhine-Meuse delta are shown in Figure 6. It should be noted that the samples from the upstream area of the river Rhine from September/October 2015 (Heydebreck et al., 2015, Heydebreck, 2017) are remarkably lower than the concentrations of the samples from the same area in December 2016 (Pan et al., 2018). The average concentration in 2015 was 0.03 ng/L, while this was 0.99 ng/L in 2016. Also in the upstream part of the River Meuse, concentrations were in the order of 0.5-0.8 ng/L (Versteegh and De Voogt, 2017), which is comparable to the upstream concentrations in the River Rhine at the end of 2016.

[5] https://www.aaenmaas.nl/nieuws/2018/januari/hoge-waarden-genx-bij-zuiveringen-aarle-rixtel-en-eindhoven-bronnen-gevonden-op-bedrijventerreinen-helmond-en-son-en-breugel.html

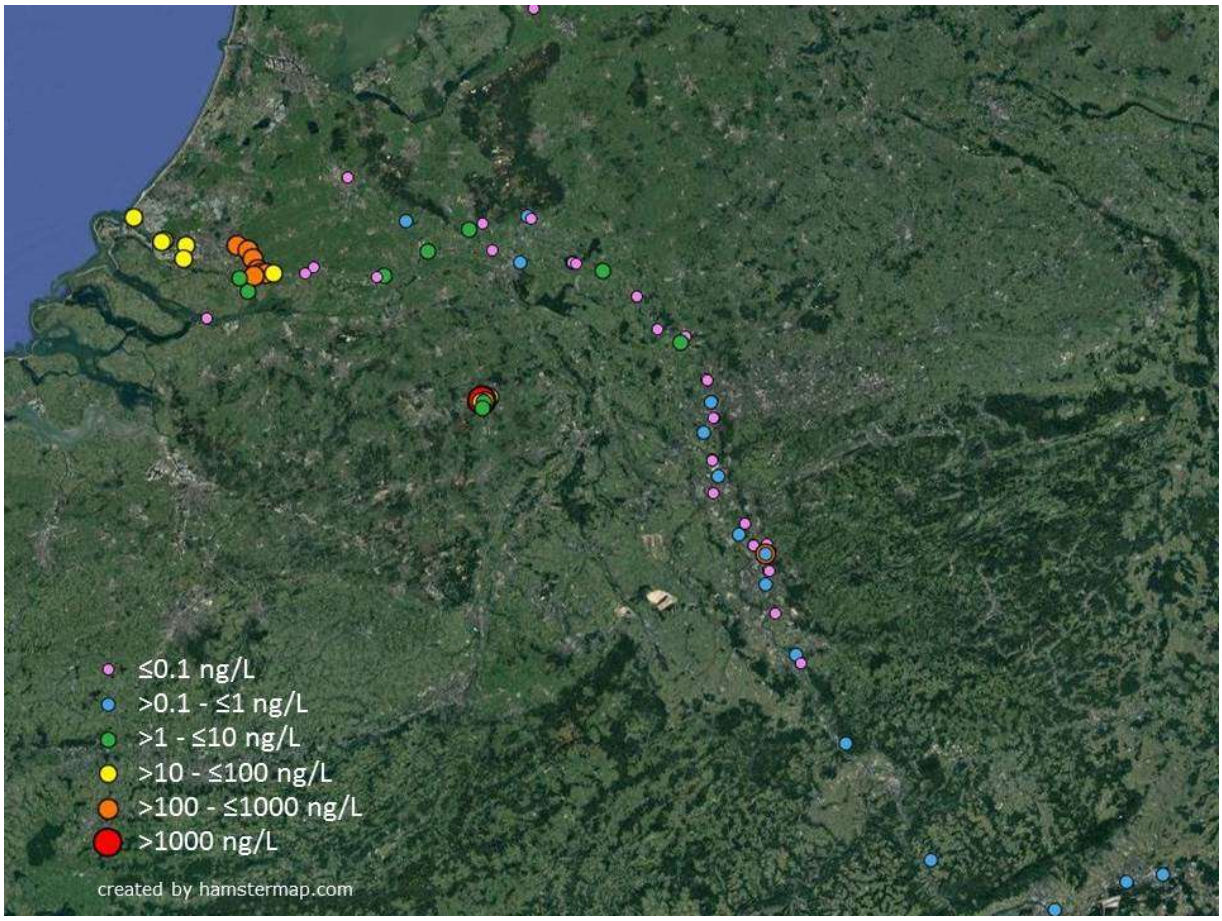

Figure 6: Concentrations in of HFPO-DA in surface waters in the Rhine-Meuse delta. Only data for which coordinates are available are included.

Other European rivers

In August 2013, three water samples were taken in the Ems delta in Germany (Heydebreck et al., 2015, Heydebreck, 2017). A concentration of 1.80 ng/L was measured in one sampling location in the Ems estuary at Wybelsum. This sampling is in the estuary and has an influence from the North Sea. In the river Ems at Gandersum and Leer concentrations were below the method detection limit (<0.14 ng/L). HFPO-DA was not detected (method detection limit 0.13 ng/L) in three samples from the river Weser (D) between Sandstedt and Bremerhaven in March 2014 (Heydebreck et al., 2015, Heydebreck, 2017). To assess the sources of HFPO-DA in the North Sea sampling was conducted in the Elbe (D) in March 2014. Between Hamburg-Altona and Bütteler Hafen HFPO-DA was not detected (method detection limit 0.13 ng/L). From Dresden to Brunsbüttel, 22 samples were taken from the river Elbe in September 2014, in which HFPO-DA was not detected (method detection limit not reported) (Heydebreck et al., 2015, Heydebreck, 2017).

In October 2016 HFPO-DA was also found in six locations in the river Thames (GB) from Oxford to Canary Wharf in London (Pan et al., 2018). All concentrations were above the method detection limit (0.38 ng/L) and ranged from 0.70 to 1.58 ng/L. The lowest concentration was observed in Oxford, the highest at Canary Wharf. The average and median concentrations were 1.12 and 1.10 ng/L, respectively.

Also in Sweden HFPO-DA was observed in the river Svartån and lake Hjälmar near Örebro, Riddarfjärden, Beckholmssundet and near Stockholm in Lake Mälaren and between Lake Mälaren and the Baltic Sea in September 2016 (Pan et al., 2018). Concentrations were above the method detection limit (0.38 g/L) and ranged between 0.88 and 2.68 ng/L, both concentrations found in

the same trench near Stockholm. The average concentration of all 10 samples was 1.47 ng/L, the median concentration was 1.38 ng/L.

Marine environment

In March and August 2014, two sampling campaigns were conducted in the North Sea and the Wadden Sea along the Dutch coast and further up to the German Bight. Further, samples were taken from the estuaries of the Ems, Weser and Elbe (Heydebreck et al., 2015, Heydebreck, 2017). HFPO-DA was detected in all samples along the coastline with an average concentration of 2.3 ± 0.9 ng/L in March and 1.5 ± 0.3 ng/L in August. In two samples from the Elbe estuary from March HFPO-DA was also detected in concentrations of 0.61 and 1.46 ng/L, but was HFPO-DA was not detected in 7 samples more upstream (method detection limit 0.13 ng/L). The sites where HFPO-DA was detected were concluded to be influenced by sea water based on the salinity of the samples. HFPO-DA was also detected in August at two sites from the Ems estuary and at the mouth of the river Ems into the estuary in concentrations of 0.62 to 1.32 ng/L, but not detected in two samples more upstream. Based on the salinity of the samples taken, it was concluded that these sites where HFPO-DA was found are influenced by seawater as well. These data show that in both the Elbe and the Ems estuary concentrations of HFPO-DA are higher in water with higher salinity. This confirms that the river water is not the source of HFPO-DA for these two rivers. Instead, the authors hypothesise that HFPO-DA is transported from the Rhine-Meuse delta along the coastline of the North Sea to the German Bight.

In March 2016, samples were taken from piers on the coast of the German Baltic Sea (Heydebreck, 2017). HFPO-DA was found at all 16 sampling sites (method detection limit 0.012 ng/L) with an average concentration of 0.14 ng/L. Similar to the observations from the Ems and Elbe, a positive correlation is observed between the concentration of HFPO-DA and the salinity of the samples. Also here, the author suggests that this indicates that HFPO-DA is not originating from direct riverine input into the Baltic Sea, but from indirect input via the North Sea, which has a higher salinity than the Baltic Sea, and for which the source may likely be the Rhine-Meuse delta.

Concentrations in the Wadden Sea, the German Bight and the German Baltic Sea, and the Weser, the Ems and the Elbe are shown in Figure 7. It should be noted that in these samples from 2014 and 2016 the concentrations in these northern German rivers are lower than in the Wadden Sea and German Bight, indicating that these are not the source of HFPO-DA.

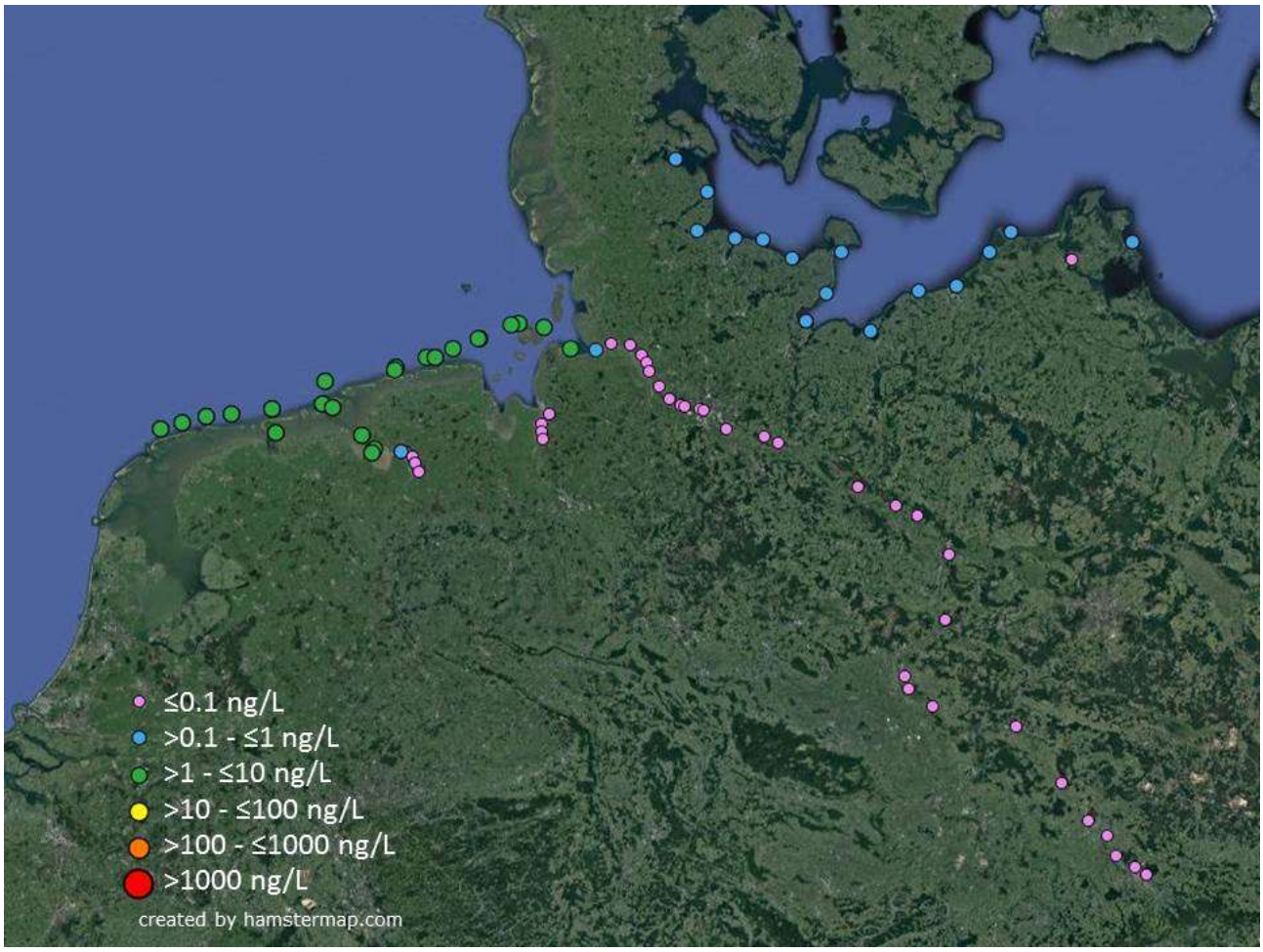

Figure 7: Concentrations of HFPO-DA in surface waters in the Baltic Sea, the Wadden Sea and the rivers Ems, Weser, and Elbe.

Although concentrations in the marine environment are low compared to concentrations of HFPO-DA detected near known point sources, monitoring data suggest that the substance is transported in water over very long distances. In June 2014, PFASs were sampled in the Norwegian Sea from a ship 11 m below the surface (Heydebreck, 2017). HFPO-DA could be measured in three sampling points in the Norwegian Sea in concentrations with an average concentration of 0.349 ng/L, ranging from 0.178 to 0.500 ng/L. Considering only negligible input from Norway and the UK, it was concluded by the author that these HFPO-DA concentrations could be explained by HFPO-DA transported over a distance of at least 1700 km after its emission from the Rhine Meuse delta into the North Sea. The substance was not detected in two samples further northwest of the three samples mentioned above (method detection limit 0.023 ng/L). This observation was explained by the authors from the fact that the first three samples are taken from water in the Norwegian Coastal Current and the latter two samples are taken from water in the East Iceland Current, which is not fed by water from the Rhine Meuse delta.

In Figure 8 the concentrations of HFPO-DA in Europe, including these marine samples, are presented.

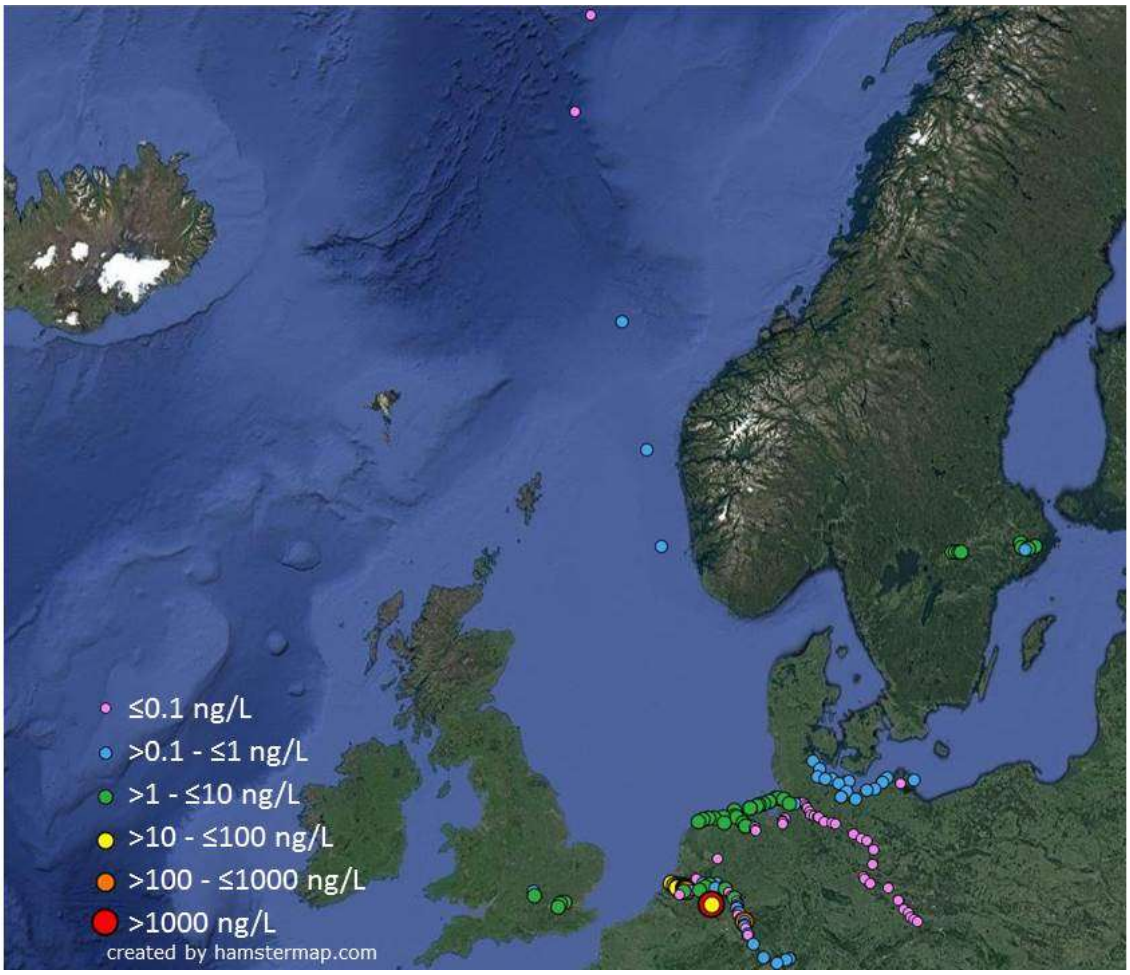

Figure 8: Concentrations in of HFPO-DA in surface waters in Europe, including the marine environment.

Other continents

HFPO-DA has been measured in river water in Asia and North America. In China, HFPO-DA was monitored in samples from April 2014 in the Xiaoqing River and some tributaries as well as in Laizhou Bay. In the upper part of the Xiaoqing River HFPO-DA was not detected but downstream concentrations of more than 100 ng/L were measured with peak concentrations of 2125 to 3825 ng/L at two sites in the Xiaoqing River and one in its tributary Dongzhulong River. These concentrations are attributed by the author to the fluoropolymer industry located in the north of Zibo. In Laizhou Bay concentrations were observed in the order of 6 to 55 ng/L. HFPO-DA could be detected at sites up to more than 30 km away from the mouth of the river. At the sites with the highest HFPO-DA concentrations, other PFAS were detected at higher concentrations (Heydebreck, 2015, 2017).

In the same area and partly the same sampling locations a second monitoring study was performed in November/December 2015 (Pan et al. 2017). The same pattern was observed with concentrations of HFPO-DA in the tributary Dongzhulong River, varying from 1750 to 2060 ng/L downstream of the fluorochemical production plant. After the inflow of the Dongzhulong River in the Xiaoqing River, HFPO-DA gradually decreased from 960 ng/L to 118 ng/L, most likely due to inflow of other tributaries. Upstream of the fluorochemical production plant in the Xiaoqing River and its tributary Dongzhulong River concentrations varied between 1.61 and 3.64 ng/L. In this study, HFPO-DA appeared to be present in relatively small concentrations compared to hexafluoropropylene oxide trimer acid (HFPO-TA) and PFOA, which together were observed to contribute to more than 90% to the total concentration of PFASs in those samples.

Several Chinese rivers were monitored in the period October to December 2016 (Pan et al., 2018). These rivers included the Liao River (6 locations), the Huai River (9 locations), the Yellow River (15 locations), the Yangtze River (35 locations), Chao Lake and surrounding rivers (13 locations), Tai Lake (15 locations) and the Pearl River (13 locations). The samples in the Yangtze River ranged in HFPO-DA concentrations from <0.18 to 1.54 ng/L with an average concentration of 0.73 ng/L and a median of 0.67 ng/L. In Chao Lake and surrounding rivers that finally end up in the Yangtze River, HFPO-DA concentrations ranged from 0.93 to 3.32 ng/L with an average concentration of 1.92 ng/L and a median of 1.81 ng/L. In a large part of Tai Lake (Lake Taihu) similar concentrations were found with a minimum of 0.38 ng/L and a median value for the whole lake of 0.77 ng/L. However, in the north-eastern part of the lake HFPO-DA concentration were elevated with a maximum concentration of 143.7 ng/L and a an average value for the whole lake of 14.0 ng/L. HFPO-DA concentrations in the Yellow River ranged from <0.18 to 1.74 ng/L with an average concentration of 1.01 ng/L and a median of 1.30 ng/L. The concentrations in the upstream western part of the Yellow River were all below the detection limit of 0.18 ng/L, while the concentrations in the downstream eastern part separated by around 600 km distance varied from 1.00 to 1.74 ng/L. Concentrations in the Pearl River, the Liao River and the Huai River were rather similar. HFPO-DA concentrations in the Pearl River ranged from 0.21 to 10.3 ng/L with an average concentration of 1.51 ng/L and a median of 0.70 ng/L. HFPO-DA concentrations in the Liao River ranged from 0.62 to 4.51 ng/L with an average concentration of 1.44 ng/L and a median of 0.88 ng/L. HFPO-DA concentrations in the Pearl River ranged from 0.83 to 3.62 ng/L with an average concentration of 1.66 ng/L and a median of 1.40 ng/L.

Water of rice paddy fields near four cities was monitored for PFASs (Cui et al., 2018). Two of the cities (Changshu and Huantai) have a large-scale fluorochemical industry, while the other two cities (Quzhou and Zhoushan) do not have such industries. The HFPO-DA concentration in Huantai (North of Zibo) was 410 ng/L, in Changsu 47.8 ng/L, in Zhoushan 2.96 ng/L and in Quzhou 1.91 ng/L. Indeed, there is a strong influence of the nearby industries.

In November 2016, 6 locations in the Han River near Seoul (South Korea) were sampled (Pan et al., 2018). HFPO-DA concentrations ranged from 0.78 to 2.49 ng/L with an average concentration of 1.38 ng/L and a median of 1.16 ng/L. A summary of the monitoring data in Asia is given in Figure 9.

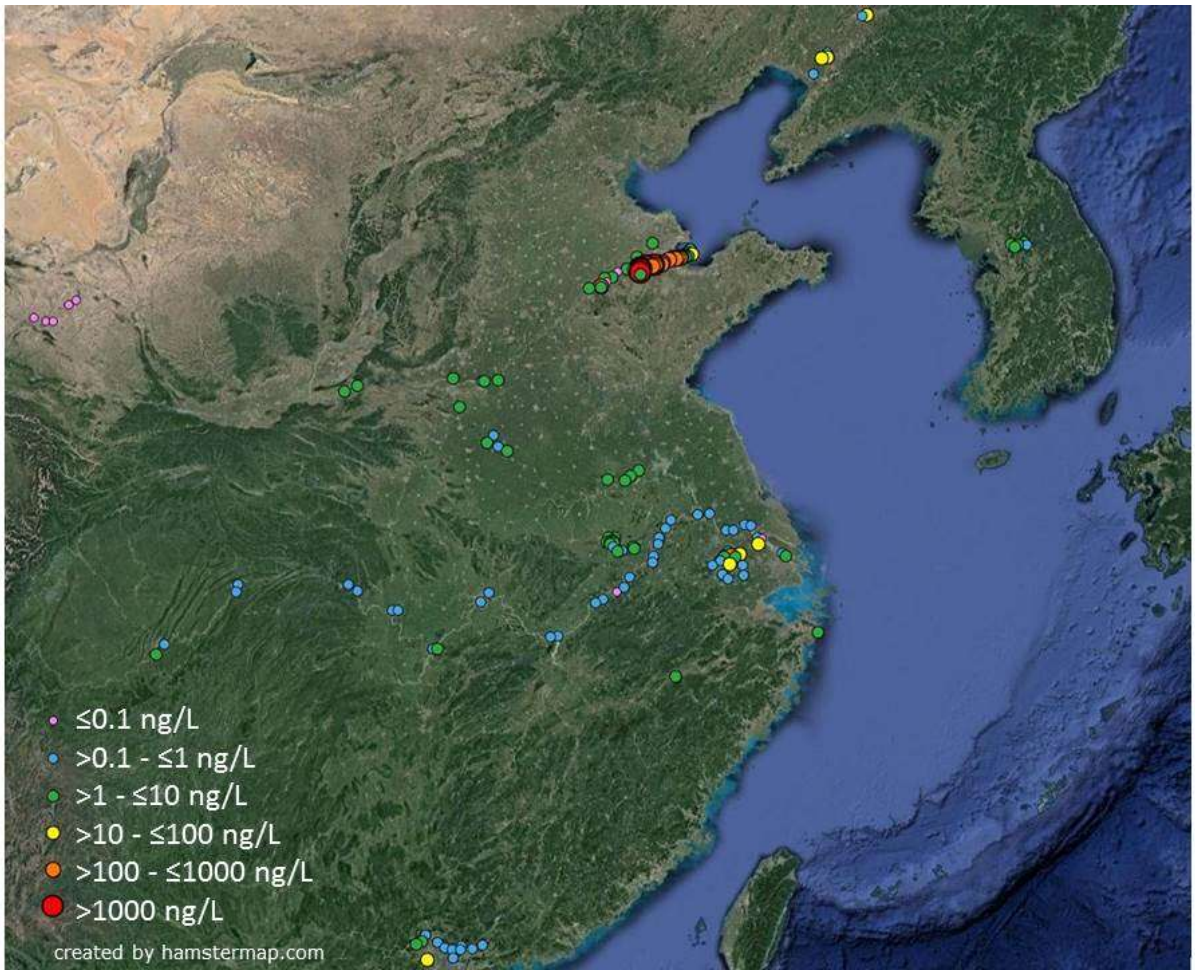

Figure 9: Monitoring data of HFPO-DA in surface waters in Asia, showing the eastern part of China, and Korea.

In the USA, HFPO-DA was monitored in the Cape Fear river (North Carolina) in summer 2012. HFPO-DA was detected in samples downstream of a fluorochemical plant (Strynar et al., 2015). In a follow-up study, the Cape Fear river was monitored at three points that are used for the abstraction of drinking water in the period June to December 2013. HFPO-DA was detected in the most downstream sampling point in concentrations varying from 55 to 4560 ng/L over the sampling period. The average concentration of HFPO-DA at this sampling point was 631 ng/L. The concentration varied with the water flux. The estimated mass flux of HFPO-DA was on average 5.9 kg/d, varying from 0.6 to 24 kg/d (Sun et al., 2016).

Further monitoring data from the Cape Fear River, mostly from June and July 2017, were retrieved from the North Carolina Environmental Quality website on GenX providing sampling data on HFPO-DA (https://deq.nc.gov/news/hot-topics/genx-investigation/genx-sampling-sites). For raw river water at the Hoffer water treatment plant, which is upstream of the fluorochemical plant, the concentrations varied between 4 and 13 ng/L. Surface waters for three downstream intake points along the river varied between 30.4 and 830 ng/L, with the highest concentrations found in the beginning of the sampling period, mid-June 2017.

Additional monitoring data were retrieved from a report on the chemical and spatial distribution of PFAS in the Cape Fear River (Geosyntec Consultants, 2018). In September 2017, concentrations nearby the factory ranged from <10 ng/L in the samples upstream of the plant to 24 to 52 ng/L in the two most downstream locations (about 3.5 and 7 km downstream). In May 2018, these concentrations ranged from <4 ng/L in the samples upstream of the plant to 16 to 26 ng/L in the two most downstream locations. Measurements along the river over 132

miles in June 2018 show concentrations that are <10 ng/L upstream of the fluorochemical plant and ranging from <10 to 17 ng/L (median value of 11 ng/L) downstream of the plant.

In June 2017, the concentrations in Georgia Branch Creek and Willis Creek were 540-690 and 230-450 ng/L, respectively. In May 2018, these concentrations were still 520 and 560-590 ng/L, respectively (Geosyntec Consultants, 2018). Concentrations of HFPO-DA were also determined in lakes nearby the fluorochemical plant (NCDEQ, 2018c, NCDEQ, 2017, NCDEQ and NCDHHS Science Advisory Board, 2018). The concentration in Marsh Wood Lake in Cumberland County (NC) was determined twice in October 2017 and March 2018, and amount to 915 and 968 ng/L, respectively. The concentration in Pages Lake near Camp Dixie in Bladen County (NC) was determined in October 2017 and was 620 ng/L. These lakes are approximately 1.5 km north and 3 km south of the fluorochemical plant in North Carolina.

The combined monitoring data show a decrease in the Cape Fear River from June 2017 to June 2018. This decrease in concentrations follows a reduction in emission sources to the Cape Fear River in 2017 (Geosyntec Consultants, 2018, Hopkins et al., 2018). However, local creeks and lakes do not show such a rapid reduction in concentrations.

Along the Delaware River and its tributary Schuykill River, HFPO-DA was measured at twelve locations in September and December 2016 (Pan et al., 2018). Concentrations ranged from 0.78 to 8.75 ng/L with an average concentration of 3.32 ng/L and a median concentration of 2.02 ng/L. The 4 locations monitored in September 2016 had all concentrations below 1 ng/L. The 8 locations monitored in December 2016 varied from 1.45 to 8.75 ng/L.

A summary of the surface water concentrations in North America is presented in Figure 10.

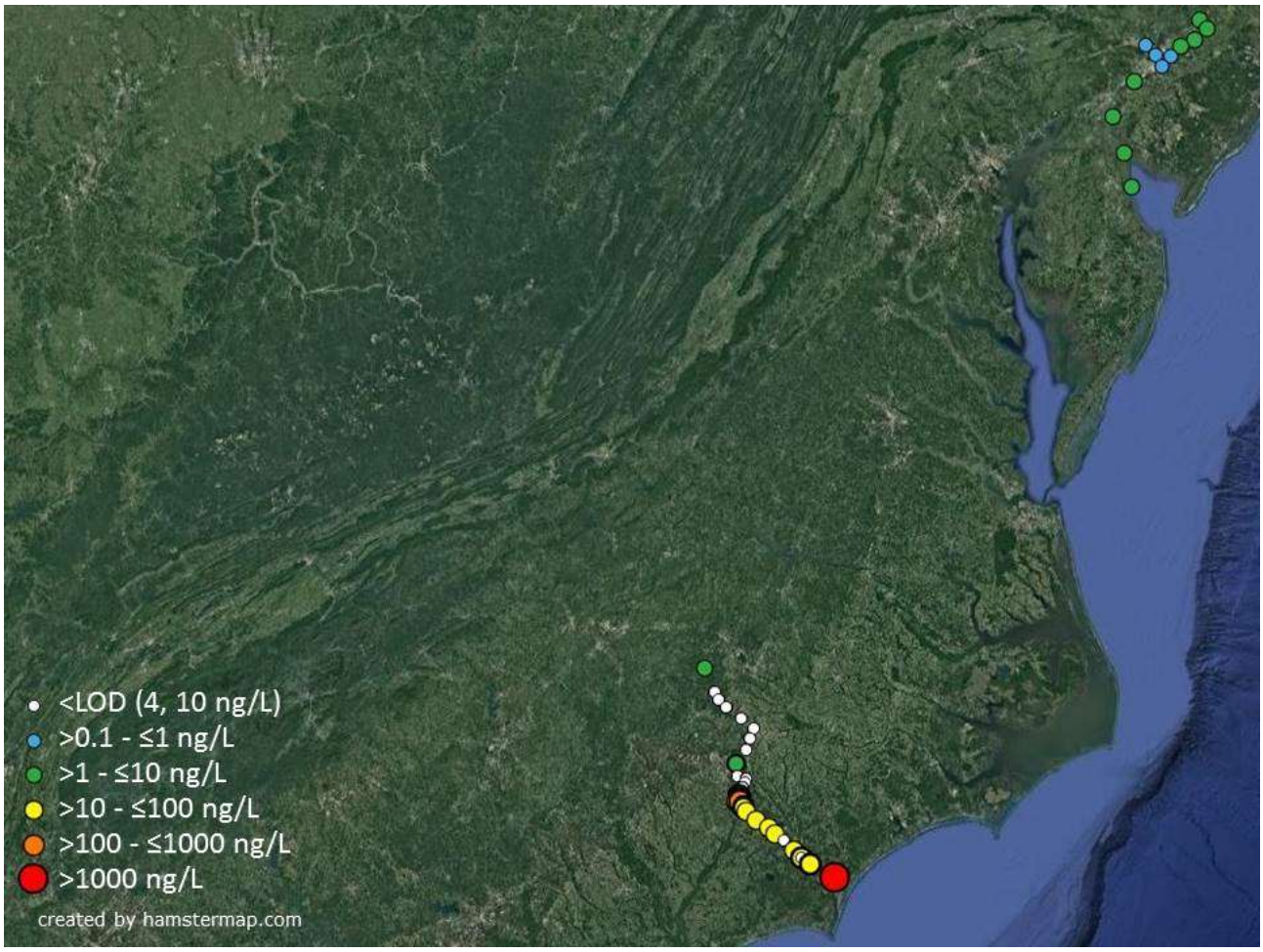

Figure 10: Monitoring data of HFPO-DA in surface waters in North America.

Air and rain water

Besides data on the presence of HFPO-DA in surface water, there is also information available on the presence of HFPO-DA in rain water and smaller water bodies used as irrigation water for vegetable gardens. Results of the measurements of soil and vegetables are reported in Section 3.5. In the vicinity of the fluorochemical plant near Dordrecht (NL) rain water that was used to irrigate vegetables, was sampled in September 2017 from rain barrels and other vessels that were connected to roofs of synthetic material. The concentrations of HFPO-DA in these water samples ranged from 11.5 to 3078 ng/L, with a geometric mean value of 102 ng/L. Because samples were not directly collected, but collected from rain barrels concentrations could be influenced by other sources e.g. from the synthetic materials. Ditches adjacent to vegetable gardens that are further disconnected from the river, had concentrations varying from 9.7 to 956.5 ng/L, with a geometric mean value 78 ng/L. Also in rain water collected in Bilthoven (NL), which is more than 50 km from the fluorochemical plant, a concentration of 12 ng/L was found for HFPO-DA. Also PFOA was detected above the LOQ in all water samples (2.3 – 4670 ng/L), with 8.4 ng/L at the reference site (Van Poll, 2018).

Also the North Carolina Division of Air Quality has sampled rain water during rain events in 28-29 January and 4-5 February 2018 at 10 sites within 3 miles around the fluorochemical plant. Concentrations varied from none detected to 630 ng/L with a median value 34 ng/L (NCDEQ, 2018b). Further information from 28 February 2018 to 2 March 2018 shows concentrations at 12 locations within 7 miles of the fluorochemical plant varying from 45.3 to 810 ng/L with a geometric mean value of 159 ng/L. The concentrations in rain water matched the concentrations found in private wells rather well (NCDEQ, 2018a). No further information is available on these measurements.

HFPO-DA was found in concentrations up to 4000 ng/L in private wells close to the fluorochemical plant in North Carolina with no direct emission of the substance and also in disconnected water bodies within 20 miles of a fluorochemical plant near Parkersburg, West Virginia (Hopkins et al., 2018). It is suggested by the authors that this is caused by air emissions of HFPO-DA and or its precursors with subsequent wet and/or dry deposition and percolation into the surficial aquifer. 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoyl fluoride (C3 dimer acid fluoride) is mentioned specifically as precursor. It is estimated that the fluorochemical plant in North Carolina emitted 230 to 300 kg to the air on a yearly basis between 2012 and 2016.

Drinking water

HFPO-DA was found in drinking water from the cities of Zwijndrecht, Dordrecht and Papendrecht in concentrations of 0.25, 0.48 and 11 ng/L, sampled in October 2016. At the same time, concentrations of HFPO-DA in drinking water of the cities of Sliedrecht, Utrecht and Wageningen (all upstream of the fluorochemical production plant) were below the method quantification limit (<0.2 ng/L) (Gebbink et al., 2017). Also in other cities in the western part of the Netherlands detectable concentrations in drinking water were reported (unpublished). In a follow up research conducted by drinking water companies, a highest concentration of 30 ng/L was found in drinking water prepared from a river bank filtrate from the river Beneden Merwede in July 2016. In drinking water prepared from water from the river Meuse in the period summer 2016 – summer 2017, HFPO-DA was found in concentrations up to 11 ng/L (Versteegh & de Voogd, 2017).

HFPO-DA was also detected in six tap water samples from six different places in the Netherlands (Brandsma et al., 2019). The tap water was collected in June 2017. The concentrations in finished drinking water ranged from 1.4 to 8.0 ng/L. Highest concentrations of 8.0 ng/L were found in drinking water from the city of Alblasserdam abstracted from the river Lek at Nieuw Lekkerland, which is close the fluorochemical plant, i.e. in 8.0 ng/L. Drinking water from the cities Rotterdam, Spijkenisse contained 5.9 ng/L, from the city Dordrecht 3.1 ng/L. The drinking water from these cities is prepared from water abstracted from the river Meuse, which confirms the presence of HFPO-DA in this river. The drinking water of the town Goedereede, which is abstracted from both Haringvliet and the river Meuse contained 1.8 ng/L. The lowest concentration of 1.4 ng/L was found in the drinking water of the city of Gouda, which is abstracted from the river Lek at Bergambacht, which is around 10 km upstream of the location at Nieuw Lekkerland.

In the Cape Fear River area (NC, USA), concentrations of 400 to 500 ng/L were observed in drinking water in August 2013 abstracted from the river 90 miles downstream of the fluorochemical plant (Sun et al., 2016). A month after an emission reduction of HFPO-DA in June 2017, concentrations in drinking water of the water treatment plant 90 miles downstream of the fluorochemical plant dropped from over 700 ng/L to 40-50 ng/L (Hopkins et al., 2018). Data obtained from the North Carolina Environmental Quality website on GenX providing sampling data on HFPO-DA (https://deq.nc.gov/news/hot-topics/genx-investigation/genx-sampling-sites) show that all water treatment plants downstream of the fluorochemical plant have similar concentrations and trends with drinking water concentrations around 40 ng/L by November 2018.

3.2.6 Summary and discussion of environmental distribution

HFPO-DA is very persistent, has a very low adsorption to organic carbon and other solids and has a low volatility. This combination of properties makes the substance very mobile in the aquatic environment, reaching areas far away from direct emissions. Monitoring data confirm this behaviour and show that HFPO-DA can be widely distributed via waterways far from the point of emission, including the marine environment. This observation is supported by monitoring data from the Netherlands, China and the USA where concentrations in surface water were studied in relation to known industrial emission sources.

However, HFPO-DA is also detected in surface water at locations that could not be explained by

any known, local emission source. This finding is also observed by Pan et al. (2018), who state that these novel PFASs, especially HFPO-DA, HFPO-TA, and 6:2 H-PFESA, show ubiquitous occurrence across the global environment. Concentrations upstream of rivers along which no known fluorochemical production facility is located suggest the presence of diffuse emission sources.

Also emission to air and transport via air (potentially over long distances) is mentioned to play a role in the widespread distribution of HFPO-DA. In this process, emissions to air of C3 dimer acid fluoride is mentioned as a possible airborne precursor of HFPO-DA. C3 dimer acid fluoride hydrolyses quickly to form HFPO-DA (Hopkins et al., 2018). HFPO-DA is measured in rain water. The observed concentrations in disconnected water bodies, soil and ground water might thus be linked to subsequent wet and dry deposition of HFPO-DA after emission to air of HFPO-DA and/or its precursors.

Monitoring data indicate that HFPO-DA is transported by sea currents over very long distances reaching the North Sea, the Wadden Sea and the German Bight. The available information demonstrates that HFPO-DA is even further transported over the North Sea to the Norwegian Sea and along the coast of Denmark to enter the Baltic Sea.

These observations for fresh water and for marine water show that HFPO-DA is very mobile and very persistent, and is subject to long range transport over vast distances by water. HFPO-DA is also detected in drinking water at locations downstream of fluorochemical production plants.

3.3 Data indicating potential for long-range transport

Section 3.2.5 gives an extensive outline of monitoring data for HFPO-DA showing that this substance is wide spread over Europe, the US and China. The monitoring data for fresh water and for marine water provide a clear indication that HFPO-DA is subject to long range transport over vast distances by water (Heydebreck, 2017). Monitoring data suggest that travelling distances from known emission sources to sites of detection can be as large as 1700 km, e.g. from the Dutch river delta all the way up to the Norwegian sea. Furthermore, HFPO-DA is detected in ground water and in drinking water (after drinking water treatments) at locations downstream of fluorochemical production plants. Consequently, the monitoring data as presented in Section 3.2.5 clearly indicate the potential for long range transport of HFPO-DA.

Also, HFPO-DA's physical-chemical properties (Section 1.4), very high persistency (Section 3.1) and its estimated atmospheric half-life indicate that the substance is capable to be transported to remote areas. Annex D, Section 1 (d) of the Stockholm Convention on Persistent Organic Pollutants (POPs) states the criterion for atmospheric half-life >2 days (48 hours), which is by far exceeded for HFPO-DA with an estimated atmospheric half-life of 20.57 days by AOPWin v1.93 of EpiSuite (see Section 3.1.1.3.1)[6]. This very long half-life is explained by the perfluorinated carbon backbone and the ether bond of HFPO-DA, which do not offer any suitable atomic site where the OH-radical, or ozone, could start oxidation/degradation. The only perceived site of oxidation is the carboxylic acid group which might be slightly vulnerable to oxidation by OH-radicals. The half-life estimate for HFPO-DA in the atmosphere is therefore identical to the estimates by AOPWin for all perfluorinated carboxylic acids like PFOA and PFHxA. For PFCAs the long atmospheric half-life has been experimentally confirmed (Section 3.1.1.3.1).

To substantiate the assumed Long Range Transport, the transport potential was modelled using the OECD tool for estimation of the Long Range Transport Potential [LRTP Tool; OECD, 2009; Wegmann et al. (2009)][7] and is evaluated against the screening criteria as they are included for the potential for long-range environmental transport in Annex D, Section 1 (d) of the Stockholm

[6] 494.4 hours, as calculated using the AOPWin v1.93 – April 2015 - software, developed by the US-EPA
[7] The OECD POV and LRTP Screening Tool, Version 2.2, http://www.oecd.org/chemicalsafety/risk-assessment/45373514.pdf

Convention on Persistent Organic Pollutants (POPs). The LRTP Tool is a spreadsheet form based on multimedia fate models. The model requires molecular mass, air-water (K_{aw}) and octanol-water (K_{ow}) partition coefficients and (estimated) half-lives in air, water and soil as input parameters for the modeling. The tool then estimates a characteristic travel distance (CTD), indicating the distance from a point source at which the chemical's concentration has dropped to 38% of its initial concentration) and an estimated overall environmental persistence (P_{ov}) in the environment (an overall half-life taking into account the estimated volumes of the emission in the different environmental compartments). Input parameters that have been used to model the long range transport potential for HFPO-DA are summarised in Table 8. This resulted in an estimated of CTD = 8682 km and a Pov = 114 days for HFPO-DA.

Table 8: Input parameters used to model the long range transport potential for HFPO-DA

Parameter	Value used in the modelling	Comments
log K_{ow}	3.36	KOWWin v1.68 estimate for the neutral carboxylic acid of HFPO-DA
log KAW	-2.077	estimated with the HenryWin v3.21 – April 2015 – software, as developed by the US-EPA
Atmospheric degradation half-life	20.6 days (494 hours)	calculated using the AOPWin v1.93 – April 2015 - software, developed by the US-EPA
Water half life	60 days	lower boundary of the vP criterion for water
Soil half life	120 days	lower boundary of the vP criterion for soil

A characteristic travel distance (CTD) of 8682 km indicates that HFPO-DA (or any other substance with similar physico-chemical properties) can reach any area in the world before any significant amount of substance degradation has occurred. The estimated overall persistency of 114 days suggests that the substance is not expected to reside in the atmospheric compartment, as this would lead to an overall persistence estimate closer to the 20 days estimated for the atmospheric half-life. Current fate modelling suggests the water compartment to be the main residence compartment.

The detailed information on the calculations from the OECD LRTP-tool estimations for HFPO-DA, given in Annex I confirm this. If emissions are only to the water compartment, 87% of the total emission volume is estimated to end up in the water compartment at equilibrium state. Also clearly visible from the detailed calculation results is that the soil compartment only becomes relevant when emissions are directly to soil (leading to 84% of the total emission volume staying in the soil, if soil is the only compartment to which emission occurs). With emissions to air and/or water, the soil compartment will not be of any significance (0.4% and 0.08% of the total emission volume respectively) for environmental concentrations of HFPO-DA. Once the substance is emitted to water, it is very likely to stay in the water compartment for a very long time.

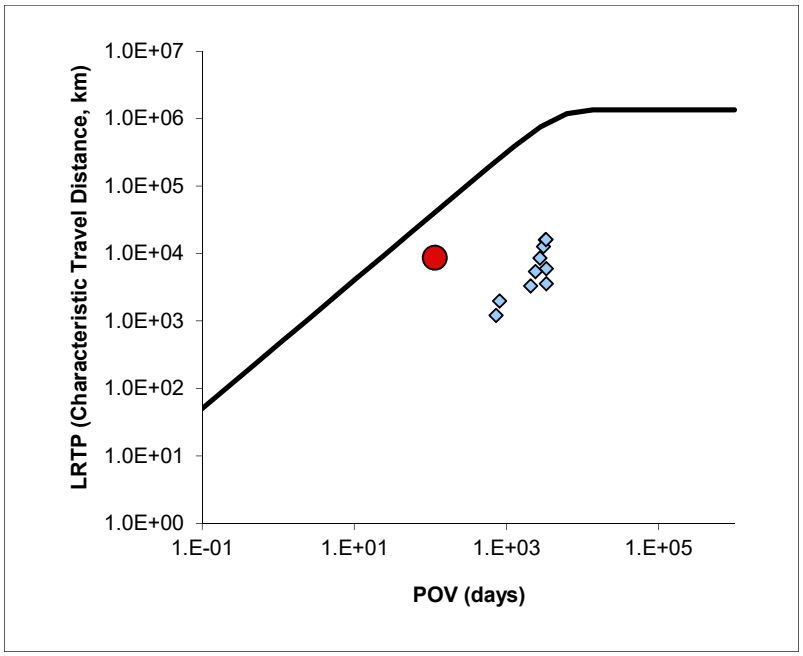

Figure 11: Characteristic Travel Distance (CTD) and overall persistence (Pov) of HFPO-DA and polychlorinated biphenyls as reference substances

Figure 11 shows the graphical output for the calculated CTD and Pov for HFPO-DA (red dot) and a set of generic Poly Chlorinated Biphenyl (PCB) homologues, which act as reference substances (blue squares). The blue dot with the highest CTD is octachlorobiphenyl, the PCBs with the same CTD estimate as HFPO-DA are the pentachlorobiphenyls. The estimated overall persistence in the environment is higher for the PCBs. This reflects the optimistic choice of environmental half-lives (water, soil) using the lower boundary of the half-life criterion for very Persistent (vP) substances. The actual half-lives for HFPO-DA in the environment are expected to well exceed this criterion, leading to longer estimated P_{ov}, and an increase in the CTD as well.

For instance, assuming a water and soil half-life of 120 and 240 days, respectively, increases the HFPO-DA estimate for P_{ov} to 163 days and the CTD estimate to 9034 km. Substances with CTD and P_{ov} values on the order of magnitude that are modelled here for HFPO-DA are expected to show very high long-range transport potential, identical to e.g. the PCBs which have been detected in remote areas like the polar regions. The solid line shown in Figure 11 represents the theoretical maximum CTD for any given P_{ov}. It shows that the estimate for HFPO-DA is closer to this theoretical maximum than any of the PCBs. Assuming that the environmental half-life for HFPO-DA is roughly identical to PCB half-life would subsequently give a higher CTD estimate for HFPO-DA than for the PCB's. Based on these modelling results it is possible that HFPO-DA has an even higher capacity for Long Range Transport in the environment than known POP-substances like PCBs.

These estimations for HFPO-DA should be considered with some reservations: the QSAR estimations of the physico-chemical properties for HFPO-DA in the OECD LRTP tool (K_{AW} and K_{OW}) are known to be difficult to predict using QSAR models. This makes these estimations less reliable. However, measuring a representative value (for fate modelling) will be difficult as well. Because of the high dissociation expected for HFPO-DA at environmental pH, one could argue that the log Kow (and Kaw) of the neutral species is not representative for the environmental fate. Using log D (the log Kow taking into account the expected dissociation behaviour) at neutral pH leads to an expected higher water solubility and lower evaporation (out of the water phase) of HFPO-DA. Such a behaviour would be in line with the monitoring data that show the water phase to be an important transport compartment. Since using log D does not change the

estimates of the persistence (half-lives), the CTD and Pov estimates in the OECD LRTP tool will continue to give rise to concern for long range transport.[8]

In a publication by Gomis et al. (2015), a comparison of the (modeled) long range transport potential of HFPO-DA, PFOA, PFOS and a number of PFOA alternatives is presented, also using the OECD LRTP tool. Although the choice of values for environmental half-lives is very different (HFPO-DA as well as PFOA and PFOS are estimated to be much more persistent than the "best case" values used in the analysis presented here), the analysis clearly concludes that the long range transport behaviour of HFPO-DA will not be significantly different than for PFOA, indicating that HFPO-DA has a very strong potential for long range transport.

3.4 Bioaccumulation

3.4.1 Bioaccumulation in aquatic organisms (pelagic & sediment organisms)

This section summarizes the bioaccumulation of HFPO-DA in fish. Data on the bioaccumulation potential of HFPO-DA in humans and mammalian species, including half-lives and toxicokinetics, is presented in Section 4.1. Similar to the accumulation in humans and mammals, accumulation in fish is not expected to be via accumulation in lipids. Few data are available on the bioaccumulation potential for HFPO-DA in fish. These data indicate that bioconcentration factors (BCF) and bioaccumulation factors (BAF) are low. Similar to the environmental monitoring data, the field bioaccumulation data are from peer- reviewed publications, often with emphasis on the methodological development of the analysis of PFASs or from laboratories with certified test protocols. The reliability of the data is thus considered high.

A laboratory BCF study was performed, in which carp were exposed to 0.2 and 0.02 mg FRD-903 (CAS# 13252-13-6)/L (Hoke et al. 2016). HFPO-DA could not be detected in fish at none of these two concentrations (<0.55 mg/kg), which resulted in BCF values <30 and <3 L/kg. It should be noted that the reporting limit is rather high in comparison with the field data described below.

In a Chinese field study (Pan et al 2017), HFPO-DA was detected in muscle of carp (n = 15) with a median concentration of 0.00153 mg/kg at a median concentration of 369 ng/L in water. This results in an average BAF of 4.1 L/kg. In the same study, a BAF for PFOA of 2.9 L/kg was found. In this study by Pan et al. (2017), the external water concentration of PFOA was much higher than that of HFPO-DA, namely 23150 ng/L for PFOA vs. 369 ng/L for HFPO-DA.

HFPO-DA was also detected in muscle of carp from a small lake in the city of Helmond (The Netherlands), known to have a local pollution. Sampling was in June 2018. The concentration HFPO-DA in carp muscle was 0.0047 mg/kg, while the concentration of PFOA was 0.0013 mg/kg. The water concentrations found in this lake were 15000 ng/L for HFPO-DA and 2300 ng/L for PFOA (NVWA, 2018). Based on these data, the BAF values are 0.31 L/kg for HFPO-DA and 0.57 L/kg for PFOA. The aqueous exposure concentrations were also determined with a sampling date 9 days earlier. The concentrations found were 6800 ng/L for HFPO-DA and 4900 ng/L for PFOA (Van Bentum et al., 2018). Based on geometric means for the water concentrations, the BAF values are 0.47 L/kg for HFPO-DA and 0.39 L/kg for PFOA.

[8] Assuming a log D value of 2.58 as given in the registration dossier (instead of the log K_{ow} of 3.36) and a log K_{aw} of 2.857 (changed the K_{aw} with the same value by using log K_{aw} = log K_{ow} – log Henry's Law constant, i.e. the value of -2.077 is lowered to -2.857), the P_{ov} half-life estimate for HFPO-DA becomes 113 days (instead of the previously derived 114 days) and a CTD of 5730 km (instead of the previously derived 8682 km). The estimate for CTD is still large enough to indicate a concern for long range transport, but is considerably lower than the first estimate (8682 km), as the partitioning to air is considerably decreased. This is also reflected by the fact that when only emission to the water compartment occurs, 94.3% of the emission volume ends up in the water compartment at equilibrium state, as compared to 87% in the first calculation.

Fish and water were monitored in Marsh Wood Lake, which is a small private-owned lake, slightly over 1 km north of the fluorchemical plant in North Carolina (NCDEQ, 2018c, NCDEQ and NCDHHS Science Advisory Board, 2018). In this study 33 PFASs were investigated. The measured water concentration for HFPO-DA was 968 ng/L. The filets of three species of fish were analysed for PFAS, which were blue catfish, redear sunfish and largemouth bass (two sizes). Only HFPO-DA, PFOS and the longer chain PFCAs (C11-C14) could be detected in fish. PFOS was detected in all species, the C11-C14 PFCAs only in largemouth bass and HFPO-DA only in redear sunfish. The concentrations of HFPO-DA in filet of redear sunfish was 0.27 µg/kg fresh weight. This results in a BAF value of 0.28 L/kg. Limits of detection/quantification (LOD/LOQ) are not given in the presented results, but presumably BAF values in the other species will be lower.

For PFOA, it has been observed that the bioaccumulation is dependent on the exposure levels (Verbruggen et al, 2017). Given its structural similarity, it is likely that the bioaccumulation of HFPO-DA follows a similar pattern. Other data for BCF and BAF of PFOA in carp (Verbruggen et al., 2017) show that the expected BAF value for PFOA is higher than what as observed for HFPO-DA at the same external water concentration. At 369 ng/L the BAF for PFOA is estimated to be in the order of 25 L/kg vs. the 4.1 L/kg for HFPO-DA observed in the study by Pan et al (2017), see Figure 12. Similarly, at 15000 ng the BAF value for PFOA would be in the order of 3 to 4 L/kg vs. the 0.31 L/kg observed for HFPO-DA. However, the BAF for PFOA for the carp from this lake is also much lower than expected from the other data for PFOA.

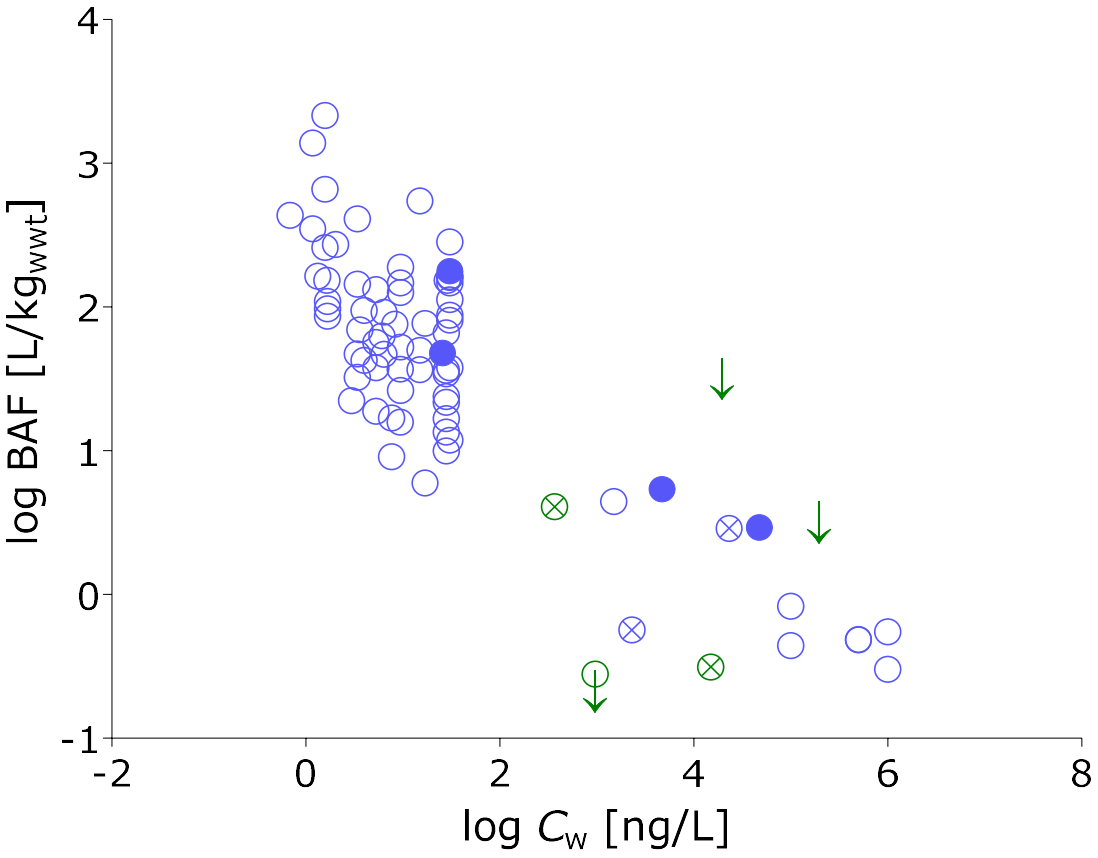

Figure 12: Comparison of BAF values for PFOA (blue) and HFPO-DA (green). Solid circles are for carp. Crossed circles are for matching data for PFOA and HFPO-DA from the same locations. Arrows denote values smaller than indicated.

The water concentrations of PFOA in the studies cited by Verbruggen et al. (2017) were always amongst the highest observed in the field studies (roughly about half of the total concentration of PFASs or more). In contrast, in the study by Pan et al. (2017), the aqueous concentration of

HFPO-DA had only a minor contribution to the sum of PFASs. If the bioaccumulation of HFPO-DA is concentration dependent, it could be possible that the accumulation of other PFASs in fish might influence the accumulation of HFPO-DA. Total PFAS concentrations in fish from the study by Pan et al. (2017) were almost 50 times higher than for HFPO-DA. Also in the lake from North Carolina, other PFASs had higher concentrations compared to HFPO-DA. The highest PFAS concentrations were found in those fish species in which HFPO-DA was not detected (NCDEQ, 2018c).

For the BAF values from the lake in the Netherlands, a similar effect occurs for PFOA. In this small lake, HFPO-DA has the higher concentrations in water and in fish, and the BAF value for PFOA is lower than expected. The bioaccumulation factors might thus be influenced by the presence of other PFASs. Taking into account the uncertainty surrounding the joint exposure of PFAS, the aquatic bioaccumulation of HFPO-DA might still be very similar to that of PFOA.

The uncertainty in this bioaccumulation potential of HFPO-DA causes uncertainty in the quantitative risk assessment, for example for exposure via fish consumption by humans, impeding the derivation of a water quality standard for HFPO-DA for this route (Smit, 2017).

3.4.2 Summary and discussion of bioaccumulation

Due to the high solubility of HFPO-DA, bioaccumulation in fish is low. Based on the structural similarities it can be expected that bioaccumulation factors are higher at low environmental concentrations, similar to PFOA. Although bioaccumulation is still low at these environmental concentrations, fish consumption could be a relevant exposure route for humans as it is for PFOA.

3.5 Enrichments in plants

Monitoring data of HFPO-DA in vegetables and fruits have been collected from kitchen gardens in the vicinity of the fluorochemical plant near Dordrecht in the Netherlands in August 2017 (Mengelers et al., 2017). The 10 locations were within 1 km of the plant (n=2), 1-2 km from the plant (n=6), 2-3 km from the plant (n=1) and 3-4 km from the plant (n=1). Further, a reference location was chosen about 50 km from the plant. The sampled vegetables and fruits included tomatoes, celery, beets, endive, zucchini, lettuce, cucumber, pumpkin, potatoes, apples, bell peppers, carrots, and pears. Vegetables and fruits were analysed both unwashed and washed or peeled. Besides HFPO-DA also PFOA was analysed. For HFPO-DA the limit of detection (LOD) was 0.5 ng/g_{fwt} and the limit of quantification (LOQ) 1.0 ng/g_{fwt}. The same LOQ was applicable to PFOA, but the LOD of PFOA was 0.4 ng/g_{fwt} for potatoes and 0.1 ng/g_{fwt} for the rest.

At the reference location neither PFOA nor HFPO-DA could be detected. Of the 74 samples originating from locations near the fluorochemical plant, HFPO-DA was below the LOD in 45 cases, between the LOD and LOQ in 29 cases and above the LOQ in 10 cases. For PFOA these numbers were 44, 27, and 3. Highest concentrations for HFPO-DA were measured in the two nearest locations, northeast of the fluorochemical plant. Washing significantly reduced the concentrations of HFPO-DA. The concentrations were significantly different for different types of vegetables with leafy vegetables having the highest concentrations, followed by tuber vegetables and fruit vegetables. The highest concentration for unwashed vegetables was 5.4 ng/g_{fwt} for lettuce, while the highest concentration for washed vegetables was 3.0 ng/g_{fwt} for tomatoes (Mengelers et al., 2017).

In a follow-up research to Mengelers et al. (2017), soil samples were taken from exactly the same locations in March 2018 (Van Poll, 2018). The HFPO-DA concentrations in soil varied between 0.1 and 1.0 ng/g_{dwt}. For PFOA the concentrations were between 0.3 and 7.7 ng/g_{dwt}. These soil samples were not taken at the time the vegetables were sampled. Other soil samples from the area of Dordrecht taken in February and March 2018 had slightly higher concentrations,

varying from 0.18 to 4.7 ng/g_{dwt} (Van Bentum et al., 2017), but in the same order of magnitude. If plant concentrations are compared to soil concentrations it appears that the ratio between plants and soil is much higher for HFPO-DA than for PFOA, due to the higher concentrations of HFPO-DA in plants and the higher concentrations of PFOA in soil.

On the basis of these data, a ratio between plants and soil can be calculated to investigate the transfer from soil to plants. For this purpose, only data for washed and/or peeled (in the case of tuber vegetables, pumpkins and fruit) were used to exclude the possible contribution of air deposition. To deal with the samples between the LOD and between the LOD and LOQ, these values were set at half the LOD (0.25 ng/g_{fwt}) and the average of LOD and LOQ (0.75 ng/g_{fwt}), respectively. For the four locations with the highest concentrations in vegetables and fruits, which all had soil concentrations above the LOQ, the following geometric mean BAF values were derived: 0.83 g_{dwt}/g_{fwt} for all vegetables and fruit, 0.57 g_{dwt}/g_{fwt} for fruit vegetables, 1.62 g_{dwt}/g_{fwt} for leaf vegetables, 0.72 g_{dwt}/g_{fwt} for tuber and root vegetables, and 0.92 g_{dwt}/g_{fwt} for fruit. If all locations were taken into account instead of the four with the highest concentrations in vegetables and fruits, values would be higher, but this is mainly due to the number of samples below the LOQ and LOD.

On another location in the Netherlands with kitchen gardens near a company that processed Teflon, vegetables and soil were sampled in 2018 (Expertisecentrum PFAS, 2018). The vegetables that were sampled included potatoes, beets, celery, kale, cucumber, bell pepper, rhubarb, lettuce, green beans, onion, and carrots. Mixed samples for both soil and vegetables were analysed. In this study the LOQ was 0.1 ng/g_{dwt} for soil and 0.1 ng/g_{fwt} for vegetables, for both HFPO-DA and PFOA. Only 4 out 21 samples for HFPO-DA in vegetables were below the LOQ. For PFOA the number of samples below the LOQ was 12. The concentration in soil was determined in three spots at three different depths: 0-20 cm, 20-50 cm and 100-150 cm. Although there were no significant differences, the concentrations of HFPO-DA tend to increase with increasing depth, 1.58 ± 1.10, 1.95± 1.30 and 2.83 ± 1.58 ng/g_{dwt}, respectively. For PFOA, the reversed situation was observed, the highest concentrations were observed in the top layer. The concentrations in the three layers were 12.3 ± 1.0, 10.8 ± 2.1 and 0.8 ± 0.17 ng/g_{dwt}, respectively. Given the fact that the HFPO-DA deposition on the soil was more recent, this is indicative of the high mobility of HFPO-DA towards the deeper soil layers. A ratio between plants and soil can be calculated in a similar way as for the other location. To deal with the samples below the LOQ, these values were set at half the LOQ (0.05 ng/g_{fwt}). For the soil concentrations, the geometric mean of concentrations for the top layer was used. The following geometric mean BAF values were derived: 0.48 g_{dwt}/g_{fwt} for all vegetables, 0.72 g_{dwt}/g_{fwt} for fruit vegetables, 0.69 g_{dwt}/g_{fwt} for leaf vegetables, 0.18 g_{dwt}/g_{fwt} for tuber and root vegetables. It can be concluded that these BAF values are in the same range as the ones determined for the other locations in the vicinity of Dordrecht.

Grass and leaves from plants were sampled on 5 locations within 3 km northeast of the fluorochemical plant in Dordrecht (NL) in August 2016 (Brandsma et al., 2019). The concentrations of HFPO-DA were the highest of the analysed PFASs, followed by PFOA. The HFPO-DA concentrations in grass varied from 1.0 to 27 ng/g_{fwt}, with a geometric mean value of 5.2 ng/g_{fwt}. Leaves from the plants hawthorn, raspberry, silver birch, ash and plane had HFPO-DA concentrations of 86, 13, 28, 16, 4,3 and <0.3 ng/g_{fwt}, respectively. Concentrations in both grass and leaves generally decreased with increasing distance to the plant. HFPO-DA concentrations were below the LOD (<0.1 ng/g_{fwt}) in grass and leaves of plane from Amsterdam, which is about 85 kilometers to the north of Dordrecht. Concentrations in this study were much higher than the concentration in the fruit and vegetables from the kitchen gardens from the same area. However, these samples were from one year earlier and were not washed. Besides that, these plants are not suitable for consumption.

BAF values for PFOA, calculated from the same studies, are much lower in both cases. Geometric BAF values were below 0.1 g_{dwt}/g_{fwt}. Uptake experiments with a series of PFCAs and PFSAs have been performed in several soils with lettuce and tomatoes (Blaine et al., 2013, Blaine et al., 2014). BAF values decreased with increasing chain length of the PFCAs and PFSAs. Moreover,

PFCAs had higher BAF values than PFSAs. As the concentrations in these studies are expressed on a dry weight basis instead of a fresh weight basis, the BAF values for HFPO-DA and PFOA from the field studies from the Netherlands have to be multiplied by approximately a factor of 20 for a direct comparison with these BAF values (water content of tomatoes and lettuce is about 95%). While BAF values for PFOA are comparable over these studies, the BAF values from HFPO-DA from the Netherlands are comparable with BAF values for the short-chain PFCAs such as PFHxA. These BAF values reflect the mobile character of these substances, also in terrestrial plants. Further, it appears that compared to PFOA, the transfer from soil to plants is much higher for HFPO-DA. It should be noted that HFPO-DA is not strongly adsorbed to soil and therefore, a BCF from pore water would be more illustrative of the bioaccumulation. However, these data are not available.

Due to the observed uptake in vegetables and fruits, consumption of these by humans can contribute significantly to the total exposure to HFPO-DA.

4 Human health hazard assessment

Availability of data sources

For HFPO-DA, a considerable number of studies is available via the REACH Registration Dossier of FRD-902. Moreover, details from the original study reports for FRD-902 (which largely contain the same studies as the REACH registration dossier) and details from the original study reports for FRD-903, published via the Health and Research Online (HERO) database of the US-EPA[9], are used in this section. Other than that, six scientific publications and two scientific reports are available in the public literature that describe the mammalian toxicity of HFPO-DA (Wang et al., 2017, Gannon et al., 2016, Caverly Rae et al., 2015, Sheng et al., 2018, Rushing et al., 2017, Li et al., 2019, US-EPA, 2018, Beekman et al., 2016).

For the toxicokinetics of the HFPO-DA, three *in vitro* and seven *in vivo* studies are available that specifically looked at the ADME (absorption, metabolism, distribution, and elimination) of HFPO-DA. Furthermore, five repeated dose studies also contain information on blood serum-, tissue-, and/or urine concentrations, and therefore are also taken into account in the ADME section. Additionally, three publications on biomonitoring of HFPO-DA in human serum are described (Pan et al., 2017, NCDHHS, 2018, Van den Berg, 2017).

For acute toxicity of FRD-902, three oral studies in the mouse and rat, one inhalatory study in the rat, and two dermal studies in the rat and rabbit are present. One study observed the oral acute toxicity of FRD-903. Furthermore, one *in vitro* corrosion study is present for FRD-903, and one *in vivo* skin irritation study for FRD-902. Two studies observed the skin sensitisation potential of FRD-902. No studies are available for respiratory sensitisation. Five *in vitro* mutagenicity studies and three *in vivo* mutagenicity studies are available for FRD-902. Except for the dermal acute toxicity test in rabbits, all studies are performed according to OECD guidelines.

With regard to repeated dose toxicity, four oral 7 days exposure studies are available, two target organ toxicity studies in mice exposed to FRD-902 and FRD-903, and two screening studies in rats exposed to FRD-902 and FRD-903. These studies are not conducted according to OECD guidelines. In general, 7 days exposure studies are not considered to be highly relevant to observe repeated organ toxicity. Moreover, two oral 28 days repeated exposure studies are available for FRD-902 in rat and mouse, two oral 90 days repeated exposure studies are available for FRD-902 in rat and mouse, and one oral combined chronic toxicity/carcinogenicity study is available for FRD-902 in the rat. Last, two oral prenatal developmental studies in rats (one full study and one conformational study), and one combined reproductive/developmental screening study in mice is available. All studies are conducted according to OECD guidelines.

One remark should be that several of the repeated-dose toxicity studies have either large dose intervals (Haas, 2008a, Haas, 2009, Craig, 2013) or may be considered not to induce sufficient toxicity at the highest dose tested[10] (Nabb, 2008a, Nabb, 2008d, MacKenzie, 2010, Edwards, 2010a). As a result, the doses used in these studies are useful to determine a NOAEL or BMD but do not serve the purpose of illustrating the toxicity of the substance within the critical window for hazard assessment (e.g. doses are either far below or far above the cut-off point for STOT RE2 classification). To overcome this issue, dose-response modelling was performed to gain information on the toxicity of the substance between the dose intervals, and to an acceptable degree above the highest dose tested.

[10]According to the latest version of OECD TG 408 and OECD TG 421, "the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering" and "a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and a NOAEL at the lowest dose level."

OECD TG 408: https://www.oecd-ilibrary.org/docserver/9789264070707-en.pdf
OECD TG 421: https://www.oecd-ilibrary.org/docserver/9789264264380-en.pdf

All studies and publications are considered relevant to evaluate the mammalian toxicity of HFPO-DA, and are used in the weight of evidence for hazard assessment. Despite the fact that some studies are not conducted according to OECD guidelines, or may not be considered highly relevant as stand-alone studies to observe repeated dose toxicity (i.e. 7 days studies), the study results show an overall consistent pattern of toxicity for HFPO-DA. Hence, the dossier submitter sees no reason to discard any study on the basis of reliability.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

4.1.1.1 Absorption

Oral

Data indicate that HFPO-DA is readily absorbed in the gastrointestinal tract (Fasano, 2011b, Fasano, 2011a). Five Crl:CD(SD) rats of each sex were administered with a single dose of 30 mg/kg/bw FRD-902 (84% purity) by gavage (water) (Fasano, 2011b). Urine was collected and pooled for the first 12 hours and 12-168 hours. Data illustrate that a mean percentage of 95% or 97% of the administered dose was excreted in the urine during the first twelve hours post-exposure for female and male rats respectively. Similarly, five Crl:CD-1(ICR) mice of each sex were administered with a single dose of 3 mg/kg/bw FRD-902 (84% purity) by gavage (water) (Fasano, 2011a). Urine was collected and pooled for the first 12 hours and 12-168 hours. Data illustrate that a mean percentage of 31% and 39% of the administered dose was excreted in the urine during the first twelve hours post-exposure for male and female mice. By 168 hours, 90% and 92% of the administered dose was retrieved in the urine respectively. These studies indicate that both mice and rats absorb almost the entire administered dose FRD-902 via the gastrointestinal tract, but mice either absorb or excrete HFPO-DA at a slower rate than rats.

Rushing et al. (2017) exposed groups (N = 6) of male and female (C57BL/6) mice to FRD-903 by gavage at doses of 1, 10, or 100 mg/kg bw/day for 28 days. Serum concentrations were measured at days 1, 5, 14, and 28 and urine concentrations were measured at days 1, 2, 3, 5, 10, and 14. Serum concentrations were significantly different from control at all time points for the 10 and 100 mg/kg bw/day groups, but not for the 1 mg/kg bw/day groups. Maximum serum concentrations were reached at day 5 for all groups, after which they dropped again with exemption of the 100 mg/kg bw/day dosed males, for which maximum concentrations remained until after 14 days of exposure. Overall, males showed higher serum concentrations as well as urine concentrations at 10 and 100 mg/kg bw/day compared to females at all time points, suggesting higher absorption in male mice.

Table 9: Plasma concentration in mice 2 hours after exposure (gavage) to FRD-902 (MacKenzie, 2010).

Dose mg/kg bw/day	Males		Females	
	ng/mL	SD	ng/mL	SD
0	<LOD	NA	<LOD	NA
0.1	736	99	824	72
0.5	3806	1175	3608	1308
5	42580	5214	35340	9362

In the oral mouse 90-day study (MacKenzie, 2010) according to OECD TG 408, groups of 10 Crl:CD-1(ICR) mice per dose and sex were exposed to FRD-902 (84% purity) by gavage (water) at dose levels of 0, 0.1, 0.5 and 5 mg/kg bw/day. Additional animals were exposed for evaluation of the plasma concentration of the substance at 2 hours after exposure on day 0, 28 and 95. LOD and LOQ were 5 ng/mL and 20 ng/mL respectively. The serum concentration 2 hours after

the first exposure (Table 9) illustrates the absorption after the first gavage exposure. Serum concentrations increased in a dose-dependent manner, illustrating differences between individual animals (indicated by a large SD). The sex difference in absorption observed by Rushing et al. (2017) is not reflected by these data.

Dermal

Skin absorption for FRD-902 was observed using human and rat skin mounted in an *in vitro* static diffusion cell at a concentration of 124 mg/mL, according to OECD TG 428 (DuPont, 2008). In rat skin, a lag time of 0.82 ± 0.77 h was observed and in human skin the observed lag time was 1.73 ± 1.01 h. Steady state penetration was 70.3± 5.3 µg/cm^2/h and 6.2 ± 5.3 µg/cm^2/h for rats and humans respectively, leading to dermal permeability coefficients (Kp) of 5.71E-4 ± 4.3E-5 cm/h and 5.02E-5 ± 4.3E-5 cm/h respectively. These results indicate that FRD-902 penetrates the skin, but on a relatively slower rate than chemicals that are well-absorbed by the skin.

4.1.1.2 Distribution

Generally, data in rats and mice indicate that HFPO-DA distributes mainly to the serum/plasma and the liver, at higher concentrations in males compared to females.

Three (Crl:CD(SD)) rats of each sex were administered a single dose of FRD-902 (84% purity) by gavage (water) at 10 or 30 mg/kg bw respectively (Gannon, 2008b). Plasma samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours post-exposure. Furthermore, liver and adipose tissue were collected after sacrifice. Plasma and tissue LOQs were approximately 20 ng/mL and 20 ng/g respectively. The results showed that at 10 mg/kg bw and 30 mg/kg bw, the mean concentrations in the liver were 73 ng/g and 38 ng/g for males and below LOQ (highest value 54 ng/g) and below LOQ for females. Mean plasma concentrations at 10 mg/kg bw and 30 mg/kg bw were 36 ng/mL and 57 ng/mL for males and below LOQ for females. This corresponded to liver tissue to plasma concentration ratios of 2.2 ± 1.1 and 0.8 ± 0.3 for males in the low and high dose groups. Ratios were not calculated for females as there were no plasma concentrations above LOQ. In adipose tissue, no HFPO-DA was found at concentrations above LOQ.

The same procedure was performed for (Crl:CD(SD)) rats administered with one single dose of FRD-903 at either 10 or 30 mg/kg bw (Gannon, 2008c). Plasma and tissue LOQs were approximately 20 ng/mL and 20 ng/g respectively. The results showed that at 10 mg/kg bw and 30 mg/kg bw, the mean concentrations in the liver were 24 ng/g and 89 ng/g for males, and below LOQ for females. Mean plasma concentrations for males were 41 ng/mL and 128 ng/mL and below LOQ for females. This corresponded to liver tissue to plasma concentration ratios of 0.6 ± 0.3 and 0.7 ± 0.2 for males in the low and high dose groups. Ratios were not calculated for females as there were no plasma nor liver tissue concentrations above LOQ. In adipose tissue, no HFPO-DA was found at concentrations above LOQ.

Also for mice the tissue distribution was evaluated. Three Crl:CD-1(ICR) mice of each sex were administered with a single dose of FRD-902 (84% purity) by gavage (water) at 10 or 30 mg/kg bw respectively (Gannon, 2008a). Plasma samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours post-exposure. Furthermore, liver and adipose tissue were collected after sacrifice. Plasma and tissue LOQs were approximately 20 ng/mL and 20 ng/g respectively. The results showed that at 10 mg/kg bw and 30 mg/kg bw, the mean concentrations in the liver were 384 ng/g and 457 ng/g for males and below LOQ for females. This corresponded to liver tissue to plasma concentration ratios of 0.5 ± 0 and 0.6 ± 0.1 for males in the low and high dose groups. Ratios were not calculated for females as there were no liver tissue concentrations above LOQ. In adipose tissue, no HFPO-DA was found at concentrations above LOQ.

Gestational transfer and transfer via lactation

In a reproductive/developmental toxicity screening study according to OECD TG 421, Crl:CD-1(ICR) mice (N = 25) were exposed by oral gavage at dose levels of 0, 0.1, 0.5 and 5 mg FRD-902/kg bw/day (purity 84%) (Edwards, 2010a). The F0 males were dosed during study days 0 to 84 (70 days prior to pairing through 1 day prior to euthanasia), for a total of 84 to 85 doses. The females that were selected for toxicokinetic evaluation were dosed through the day of euthanasia (lactation day 21) for a total of 54 to 65 doses. Plasma HFPO-DA LOD was 3 ng/mL.

The plasma levels of HFPO-DA in pups on post-natal day (PND) 4 were 2-4 fold below the PND 21 maternal levels and on PND 21 the plasma levels were 40-60 fold lower (Table 10 and Table **11**). This illustrates that HFPO-DA is transferred to the pups, either through gestation, lactation, or through both. Furthermore, plasma levels in pups retrieved on PND 21 were 10-32 times lower than the concentrations in pups on PND 4. This decline illustrates negligible transfer via lactation. On PND 40, after direct gavage exposure of the F1 for a consecutive 20 doses of 0, 0.1, 0.5 or 5 mg/kg bw/day, the plasma levels were comparable between the dams and offspring, showing relatively higher mean concentrations in F1 males compared to F1 females.

Table 10: Maternal plasma levels (ng/mL) at lactation day 21.

	LD 21	
Dose (mg/kg bw/day)	**mean**	**SD**
0	<LOD	NA
0.1	903	117
0.5	4966	768
5	36420	5545

Table 11: Plasma levels (ng/mL) in mouse pups on PND 2, 21 and 40.

	PND 4		**PND 21**				**PND 40**			
	Pooled		**male**		**female**		**male**		**female**	
Dose (mg/kg bw/day)	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
0	<LOD	NA	<LOD	NA	<LOD	NA	<LOD	NA	<LOD	NA
0.1	383	173	14	4	12	8	1352	295	946	118
0.5	1249	669	103	35	102	43	6282	2107	4074	1204
5	11265	3885	969	326	818	166	51340	6802	43340	8847

NA = not available

A developmental toxicity study (developmental toxicity/teratogenicity) was conducted in rats, according to OECD TG 414 (Munley, 2011, Edwards, 2010b). Pregnant Crl:CD(SD) rats were exposed to FRD-902 (84% purity) at 0, 5, 10, 100, or 1000 mg/kg bw/day by oral gavage during Gestation days (GD) 6-20. Dams were sacrificed on GD 21. Plasma concentrations were measured in the foetuses on GD 20, in dams on GD 20, and additionally in dams on GD 6 in the highest exposure group (1000 mg/kg bw/day). The LOD and LOQ were 0.7 and 3 ng/mL respectively.

The plasma concentration in foetuses (pooled concentration) was approximately one-third of the plasma concentration in the dam at GD20 (Table 12). These data indicate gestational transfer from dam to the fetus, in line with the elevated plasma concentrations in the mouse pups at PND 4 (as observed in the OECD TG 421 study).

Table 12: Plasma levels (ng/mL) of dams (GD 6 and GD 20) and pups (GD 20), N = 5

	Dams		**Pooled pups**
	GD 6	**GD 20**	**GD 20**

Dose (mg/kg bw/day)	mean	SD	mean	SD	mean	SD
0	NA	NA	33	16	19	23
5	NA	NA	3984	469	1134	175
10	NA	NA	9312	1710	2458	465
100	NA	NA	85560	10092	18320	9128
1000	NA	NA	338400	160168	99800	26482
1000	430600	162712	348400	130362	102240	28295

NA = not available

4.1.1.3 Metabolism

In an *in vitro* study using male and female rat hepatocytes was screened for potential formation of metabolites (Nabb, 2007). Incubated hepatocytes (1 x 10^6 cells/mL) were treated with FRD-902 at a concentration of 10 µL/mL, and samples were evaluated at 0, 30, 45, 60, 90, and 120 minutes. No metabolites were detected.

A similar *in vitro* study was carried out using trout hepatocytes (DuPont, 2007) and showed no indication of metabolism.

4.1.1.4 Excretion

Studies in rats and mice orally exposed to a single dose of FRD-902 (doses ranging from 3 – 30 mg/kg/bw) showed that the main excretion route for HFPO-DA is the urine (Fasano, 2011b, Fasano, 2011a). By 12 hours, 97% and 95% of the dose was retrieved in the urine of male and female rats respectively, which increased to values of 103% and 100% at the end of the study (168 hours post-dose). Negligible levels of HFPO-DA were found in the faeces (≤ 1%), which were likely contaminated with urine. Cage wash accounted for another 1% and 5% of the dose for males and females respectively. For mice, 31% (m) and 39% (f) was retrieved after the first twelve hours. By 168 hours, 90% and 92% of the administered dose was excreted in the urine. Negligible levels were retrieved in the faeces of male and female mice (2%). Another 10% and 6% were retrieved after cage wash.

4.1.1.5 Clearance time and half-lives

Clearance time

Two studies in rats (N = 3 per dose group) observed clearance rates following single oral exposure to FRD-902 and FRD-903 (Gannon, 2008b, Gannon, 2008c) receiving 0, 10 or 30 mg/kg/bw. Clearance time was described as the time required to remove 98.4% of HFPO-DA from the plasma. Results showed clearance times of 12h and 22 h for male rats, and of 4h and 8h for female rats following exposure to FRD-902 at 10 or 30 mg/kg bw, respectively. Furthermore, the clearance time in rats exposed to either 10 or 30 mg/kg bw FRD-903 were 28 h and 22 h in males and 8 h and 4 h in females. In mice administered with a single oral dose of 10 or 30 mg/kg bw FRD-902, the plasma clearance time was 143 h and 57 h for male and female mice, and 139 h and 62 h for male and female mice respectively (Gannon, 2008a) (see also Table 13).

Table 13: Clearance time (hours) of HFPO-DA in mice and rats from the plasma following a single oral dose.

	Oral exposure			
Chemical	**Male rat**	**Male mouse**	**Female rat**	**Female mouse**
	10 mg/kg/bw			
FRD-902	12	143	4	57
FRD-903	28	ND	8	ND
	30 mg/kg/bw			
FRD-902	22	139	8	62
FRD-903	22	ND	4	ND

One cross-species comparison study in cynomolgus monkeys and rats observed clearance times of HFPO-DA from the plasma following single intravenous dosing of FRD-902 (83% purity) (Gannon, 2009). Rats (3m/3f) received a single 10 mg/kg/bw or 50 mg/kg/bw intravenous bolus. Cynomolgus monkeys (3m/3f) received a 10 mg/kg/bw intravenous bolus. Blood was collected at multiple time points with a total study period of 7 days for rats and 21 days for primates. Clearance times were 22 h and 17 h in male rats and 3 h and 4 h in female rats following from single exposure of 10 or 50 mg/kg bw FRD-902 respectively. Clearance times were 11 h in male primates and 10 h in female primates following from single exposure of 10 mg/kg bw FRD-902 (Table 14).

Table 14: Clearance time (hours) of HFPO-DA in primates and rats from the plasma following a single intravenous bolus.

	Intravenous exposure			
Chemical	**Male primate**	**Male rat**	**Female primate**	**Female rat**
	10 mg/kg bw			
FRD-902	11	22	10	3
	50 mg/kg bw			
FRD-902	ND	17	ND	4

Half-lives

Gannon et al. (2016) described the absorption, distribution, metabolism and excretion (ADME) and kinetics of FRD-902 in rats, mice and cynomolgus monkeys. This publication was largely based on the studies presented in the section on clearance times.

Table 15: Pharmacokinetic parameters of FRD-902 as presented in Gannon et al. (2016).

		Rat, intravenous		**Rat, oral**		**Mouse, oral**		**Cynomolgus monkeys, intravenous**	
Constant	**Units**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Absorption *Rate constant (k_a)*	1/h	NA	NA	3.30	1.52	3.83	3.11	NA	NA
Time	h	NA	NA	0.21	0.46	0.18	0.22	NA	NA
Alpha phase *Elimination rate constant*	1/h	0.20	1.72	0.25	2.78	0.12	0.15	0.30	0.37
Half-life	h	3.6	0.4	2.8	0.2	5.8	4.6	2.3	1.9
Beta phase *Rate*	1/h	7.8E-03	3.1E-02	9.6E-03	1.0E-02	1.9E-02	2.9E-02	1.1E-02	8.7E-03
Half-life	h	89.1	22.6	72.2	67.4	36.9	24.2	64.1	79.6
Volume of distribution *Central*	L/kg	0.168	0.178	0.142	0.057	0.117	0.148	0.068	0.056
Peripheral	L/kg	0.155	1.508	0.161	2.462	0.130	0.078	0.029	0.021

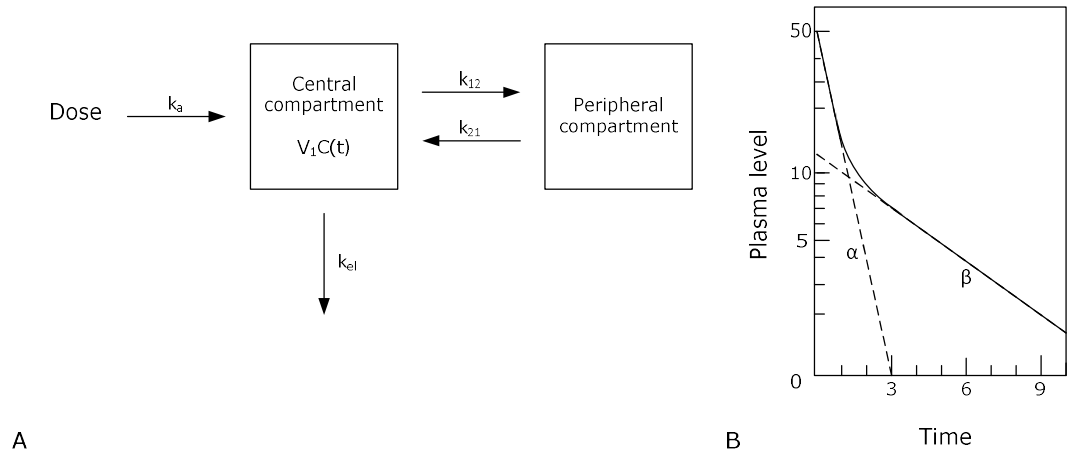

Figure 13: A (left) and B (right). Figure 13A provides a schematic overview of a two-compartment model containing a central (plasma and highly perfused body tissues) and a peripheral compartment (less perfused body tissues) with first-order absorption and elimination. The arrows represent first-order fractional rate constants for absorption (k_a), distribution (k_{12}), redistribution (k_{21}) and elimination (k_{el}). Figure 13B illustrates the alpha (distribution) and the beta (elimination) phase that together provide the best fit for a decrease in plasma levels over time.

The study authors used a one compartment and a two-compartment model to fit the plasma concentration data for rats, mice, and monkeys and concluded that the two-compartment model was the best fit (no further details are provided). From the calculations followed the pharmacokinetic parameters as presented in Table 15 (absorption rate constant, alpha elimination rate constant, beta elimination rate constant, and volume of distribution).

Additionally, alpha and beta half-lives were determined for rats (m/f) dosed orally or intravenously, mice (m/f) dosed orally, and monkeys (m/f) dosed intravenously (Table 15). In principle, the alpha phase illustrates the (rapid) distribution of the substance between the blood plasma and highly perfused body tissues, and the beta phase illustrates the elimination from the body due to metabolism and/or renal clearance from the blood (see Figure 13 A and B). Hence, the beta phase reveals the terminal half-life.

For risk assessment, the half-lives for orally administered rats and mice are considered most appropriate, as these are consistent with the route of administration used in the toxicity studies. However Gannon et al. (2016) note that the data were fitted best by a two-compartment model, this may hold true for the intravenous data, but it is doubtful whether a two-compartment model is suitable to fit the oral mouse measurements, which show a more gradual decline in serum concentrations in comparison to the oral rat measurements (as illustrated by a higher alpha phase in the mouse compared to the rat). Hence, this raises the question whether a distinction should be made between alpha and beta phases for this dataset or whether a one-compartment model would illustrate a more appropriate fit.

In addition to this, both the intravenously dosed rat (female) measurements, intravenously doses monkey measurements, and the orally dosed rat measurements are very close (or even below in the case of female monkeys data) to the limit of detection from 48-96 hours onwards[11]. This raises concern whether these data are suitable to determine the beta phase of HFPO-DA. Overall may however be concluded from the data presented in Table 15 that the terminal half-life of HFPO-DA (as revealed by the beta phase) in mice, rats, and monkeys ranges from one to several days.

[11] 20 ng/mL for the oral dataset and 1 ng/mL for the intravenous dataset

Last, Gannon et al. (2016) used the parameters in Table 15 to model repeated dosing (7 consecutive oral doses of 10 mg/kg/bw) for rats, mice, and monkeys. In this model, plasma steady state is reached after one dose in monkeys and rats. For mice, it required approximately four doses to reach steady state. However, due to the uncertainties presented above, and the results from repeated dose studies as presented below, caution should be exercised when interpreting these values.

Plasma concentration evaluation in mice upon repeated exposure

In an oral mouse 90-day study (MacKenzie, 2010) according to OECD TG 408, groups of 10 Crl:CD-1(ICR) mice per dose and sex were exposed to FRD-902 (84% purity) by gavage (water) at dose levels of 0.1, 0.5 and 5 mg/kg bw/day. Additional animals (N = 5) were exposed for evaluation of the plasma concentration of the substance at 2 hours after exposure on day 0, 28 and 95.

The test substance concentration in blood was almost similar on days 0, 28, and 95 in female mice, indicating that steady-state concentrations were almost achieved on the first day of dosing. The test substance concentration in blood from male mice was lower on day 0 than on day 28, indicating steady state may not have been achieved before day 28. Compared to female mice, for male mice it took longer to achieve steady state concentrations in blood. The plasma concentration was linear with dose, implying that absorption was not saturated over the range of doses tested in this study (Table 16).

Table 16: Plasma concentration in mice 2 hours, 28 days, and 90 days after exposure (gavage) to FRD-902 (MacKenzie, 2010).

Dose (mg/kg bw/day)	**Day 0**		**Day 28**		**Day 95**	
	ng/mL	**SD**	**ng/mL**	**SD**	**ng/mL**	**SD**
Males						
0	ND	NA	ND	NA	ND	NA
0.1	736	99	1124	238	1276	309
0.5	3806	1197	7192	3055	7068	2398
5	42580	5214	52240	16725	67980	13717
Females						
0	ND	NA	ND	NA	ND	NA
0.1	824	72	704	350	740	282
0.5	3606	1308	4198	1239	5438	1696
5	35340	9262	46580	16842	45580	5741

In an oral 28-day study in mice (C57BL/6) (Rushing et al., 2017) the immunomodulatory potential of FRD-903 was examined in groups of 12 (6m/6f) animals at 0, 1, 10, or 100 mg/kg bw/day, as well as serum concentrations were measured after 1, 5, 14 and 28 days. Serum concentration data indicated a lack of steady state for male mice after receiving 1, 10 or 100 mg/kg bw/day for 28 days. This is illustrated by the fact that serum concentrations still changed between 5, 14, and 28 days respectively. Therefore, these findings are in line with the 90-day study in mice as presented above.

A lack of steady state in male mice after 28 days of consecutive dosing is relatively long compared to the modelled point of steady state in mice in Gannon et al. (2016) (4 days with four consecutive doses). It should be noted that it is difficult to draw a conclusion with regard to a lack of steady state based on few measurements over a long period of time. To do so, it is extremely important that blood withdrawal is performed at exactly the same point in time following oral administration of the substance. Hence, the fluctuations in serum concentrations as presented in (MacKenzie, 2010, Rushing et al., 2017) could also be a reflection of the fluctuations between the times of blood withdrawal within the same animal (which is supported by the very large standard deviations). To determine whether steady state is reached requires ideally an extensive number of measurements.

4.1.2 Human information (including bioaccumulation in humans)

Half-lives

The half-life of HFPO-DA in humans is not known. Experience with PFOA shows that caution should be exercised in using animal data to estimate the half-life of perfluorinated substances in humans. For PFOA and other PFASs for which human half-lives are reported (Wang et al., 2015), values are much higher as would be expected based on allometric scaling. Overall, the data in Gannon et al. (2016) indicate that the half-lives for HFPO-DA in experimental animals vary between and one and several days. In the absence of human data for HFPO-DA, a solid conclusion on the half-life in humans cannot be drawn.

Mechanism of accumulation

Gomis et al. (2018) note that, in comparison to the classic lipophilic organic pollutants that primarily bind to fatty tissues, perfluoro carboxylic acids and perfluoro sulphonic acids primarily bind to proteins. Over 98% of the molecules are bound to serum proteins (mainly albumin) or bind to fatty acid-binding proteins in the liver. For the shorter chain perfluoro substances, the free fraction in the blood increases with increasing concentrations, suggesting saturation of the binding sites. In addition to protein binding, it is argued that the half-life of PFOA is longer in humans compared to other species since in humans there could be stronger PFOA reabsorption from ultrafiltrate in the kidney back into the blood by organic anion transporters (OATs) (Yang et al., 2010). Hence, two processes could contribute to the bioaccumulation of these substances, namely binding of the substance to proteins in the blood and the liver, and reabsorption of the free serum fraction via the ultrafiltrate in the kidney back into the blood.

A recent study showed that HFPO-DA binds to human liver fatty acid-binding protein (Sheng et al., 2018). There are no studies available investigating direct binding between HFPO-DA and albumin, and therefore it currently remains unknown whether HFPO-DA interacts with albumin directly or not. However, highest concentrations of HFPO-DA in rodents are found in the liver and the blood, providing an indication that HFPO-DA follows the same pattern of protein binding as other PFASs (Sheng et al., 2016, Chen and Guo, 2009). In addition to this, no data is available on OAT efficacy for HFPO-DA. It is therefore not known what effect HFPO-DA has on the functioning of the OATs and if resorption of HFPO-DA in the lumen of the kidney will occur in humans or not.

Human biomonitoring data

In the study by Pan et al. (2018), PFAS concentrations were determined in serum from 48 residents from the city Huantai, living close to a fluorochemical production plant. The residents had no occupational history in this plant suggesting that any observed serum concentration is of a different origin than occupational exposure. HFPO-DA was detected in 37.5% (18 residents). The median value was below the method detection limit (<0.14 ng/mL), the geometric mean was 0.13 ng/mL and the 95th percentile was 1.72 ng/mL. Following the same calculation as for HFPO-TA (a structural analogue of HFPO-DA), the estimated daily dose of HFPO-DA of these residents via fish consumption would be 0.43 ng/kg bw/d. The serum level (0.13 ng/mL) is considerably higher than the daily intake from fish consumption (0.43 ng/kg bw). The study did not specify other sources to the daily intake. It is unclear what other sources contribute to the daily intake of HFPO-DA for this specific group of people. Besides that, the distribution of HFPO-DA in the human body is unknown. This study does not provide any information on the kinetics of HFPO-DA, except that it is confirmed that HFPO-DA is absorbed by humans.

In 2017-2018, the North Carolina Department of Health and Human Services (NCDHHS) conducted a study in human volunteers to investigate the exposure to PFAS substances among people living near a fluorochemical manufacturing facility in Bladen County, NC (NCDHHS, 2018). Single blood and urine samples were obtained from 30 individuals in August 2018, and screened for 17 PFASs, including HFPO-DA. HFPO-DA was not detected in the blood or urine from any of

the 30 participants. The limit of detection (LOD) for HFPO-DA in blood and urine was 0.1 µg/L (0.1 ng/mL). All participants noted they used bottled water as their current drinking source. The average time relying on bottled water was approximately 9 months. As people, on average, stopped drinking water from private wells approximately nine months prior to sampling, the authors conclude that these data may indicate that HFPO-DA does not stay in the human body for a long period of time. It however remains unclear to what extent participants have been exposed to HFPO-DA, as individual exposure estimates are lacking. This information is essential to be able to draw any conclusions from the data presented in this study. This study does not provide any information on the kinetics of HFPO-DA.

In 2017, the blood of 24 employees from a fluorochemical production plant in the Netherlands was analysed for HFPO-DA (Van den Berg, 2017). The levels found were higher than those observed in the general population of Huantai. HFPO-DA was detected in 70.8% (17 employees). The levels in these employees varied between <1 and 169 ng/mL, with a median value of 1.55 ng/mL. These data are skewed with the highest three values being 26.6, 51.2 and 169 ng/mL, which is much higher than the other values detected. This study does not provide any information on the kinetics of HFPO-DA, except that it is confirmed that HFPO-DA is absorbed in humans.

It is not possible to derive a half-life for HFPO-DA in humans from the data presented above within a reasonable margin of certainty. Experimental data in some mammalian species show relatively short half-lives ((Gannon et al., 2016); see also Section 3.1.2). As described in Section 3.1.2 for PFOA, the half-life in humans of 3.8 years (Olsen et al., 2007a) is much higher than would be expected based on data from rodents and monkeys, in which half-lives of 2-4 hours up to 17-19 days were determined (Lau et al., 2007, Butenhoff et al., 2004). Because of this discrepancy between humans and tested mammals for PFOA, an extrapolation of half-lives of HFPO-DA in mammalian test species to humans may not be straightforward either. The ongoing Substance Evaluation on FRD-902 aims to generate further insight in the bioaccumulation potential of FRD-902 (and thereby HFPO-DA) in humans. This information will also provide more information on the extent to which bioaccumulation may occur in other air breathing animals.

4.1.3 Conclusion on toxicokinetics (and bioaccumulation in humans)

The available data indicate that FRD-902 is quickly absorbed in mammals after oral exposure, and distributes mainly to the plasma and liver. Male rats and mice showed overall higher HFPO-DA tissue and plasma concentrations compared to females upon exposure to equipotent dosages, which might be explained by more effective elimination in females compared to males. Data furthermore indicate that the substance distributes into the foetus, and that there is limited transfer of HFPO-DA via lactation. The substance is not metabolised, is eliminated almost completely within approximately 24 hours via urine in rats and monkeys, and it takes up to 7 days to be fully retrieved in the urine from mice.

The half-lives established in experimental animals vary between one and several days. Human biomonitoring data in workers and the general population illustrate detectable HFPO-DA concentrations in serum/plasma as high as 169 ng/mL in workers at a fluorochemical production site in The Netherlands. It is not possible to derive a half-life for HFPO-DA in humans from these data within reasonable certainty.

For PFASs in general it may be concluded that the half-life in humans is considerably higher compared to the half-lives for experimental animals (including primates). These variations in biological half-lives between species are suggested to be mainly due to differences in renal clearance. The human half-life of HFPO-DA currently is unknown, and in absence of human data for HFPO-DA, a solid conclusion on the half-life of HFPO-DA cannot be drawn.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Three studies in rats and one study in mice are available on acute toxicity by the oral exposure route. The studies were performed according to OECD TG 425. FRD-902 (86% purity) and FRD-903 (99% purity) were administered by oral gavage at doses of 175, 550, 1750, and 5000 mg/kg/bw in rats and FRD-902 (86% purity) was administered at 175, 550 and 1750 mg/kg/bw in mice. Animals were observed during 14 days and then necropsied.

Female Crl:CD(SD) rats in the highest dose group (administered with 5000 mg/kg/bw FRD-902) died; one at the day of dosing, one the following day, and one 2 days after dosing. In these rats, lung discoloration, discoloration of the mandibular lymph nodes, and liver were found. Hair loss, high posture, stained fur/skin, wet fur, lethargy, clear ocular discharge, prostrate posture, partially closed eyes, and/or salivation were observed in all female rats. However, with the exception of hair loss, these clinical symptoms had reversed after day 2. No body weight loss was observed. The oral LD50 for female rats was 3129 mg/kg/bw (Carpenter, 2007f).

All male Crl:CD(SD) rats in the highest dose group (administered with 5000 mg/kg/bw FRD-902) and one male rat in the 1750 mg/kg/bw dose group died. The remaining rats showed lethargy, skin stain, expanded lungs, eye discoloration and stomach discoloration. One rat in the 175 mg/kg/bw dose group also showed lethargy. Other clinical findings in the 550 and 1750 mg/kg/bw dose groups were wet fur and stained fur or skin, which reversed after 2 days post-dosing. No body weight loss was observed. The oral LD50 for male rats was 1750 mg/kg/bw, with 95% profile likelihood confidence interval 1239 – 4450 mg/kg/bw (Carpenter, 2007g).

In male Crl:CD(SD) rats administered with FRD-903, 1/4 male rats in the 550 mg/kg/bw dose group, 2/6 male rats in the 1750 mg/kg/bw dose group, and 3/3 male rats in the 5000 mg/kg/bw dose group died. Gross findings included stomach discoloration, stomach thick, skin stain, stomach distended with gas, and/or small testes and epididymides. The remaining rats showed lung noise, absent faeces, lethargy, not eating, stained fur/skin, wet fur, laboured breathing, lethargy, decreased muscle tone, prostrate posture, tremors, clear oral discharge, diarrhoea, ataxia, and/or high posture. No biologically relevant body weight losses occurred in surviving male rats. The oral LD50 was 1730 mg/kg/bw (Carpenter, 2008).

In female Crl:CD(SD) rats administered with FRD-903, 3/4 females in the 1750 mg/kg/bw dose group, and 1/1 females dosed at 5000 mg/kg/bw died. Gross findings included stomach discoloration, stomach thick, oesophagus fluid, skin wet, and/or mesenteric lymph nodes discoloration. The remaining rats, except a single rat dosed at 175 mg/kg/bw, showed wet fur, stained fur/skin, ataxia, laboured breathing, cold to touch, clear ocular or oral discharge, lethargy, lung noise, absent faeces, not eating, and/or rubbing face on bottom of cage. No body weight losses occurred in female rats. The oral LD50 was 1750 mg/kg/bw (Carpenter, 2008).

In mice, all animals in the highest dose group (1750 mg/kg/bw) died. These mice exhibited lethargy and low posture. One mouse in the 550 mg/kg/bw dose group exhibited wet fur. No effects on body weight were observed. A number of gross lesions was observed, including discoloration of the lungs, cyst in ovaries of one mouse, and skin stain in two mice, but these lesions were considered nonspecific by the authors. The oral LD50 for mice was 1030 mg/kg/bw (Carpenter, 2007d).

4.2.1.2 Acute toxicity: inhalation

One study is available on acute inhalation toxicity for FRD-902, performed according to OECD TG 403 (Kegelman, 2009). Rats were nose-only exposed to aerosol concentrations of 13, 100, and 5200 mg/m^3 for 4 hours. Animals were observed for 2 – 14 days after exposure and necropsy and microscopic evaluation of the respiratory tract tissues were performed, except in the highest

dose group. No mortality was observed. Rats in the highest dose group (5200 mg/m^3) showed red discharge around the eyes, nose and mouth, and red stained faces that lasted for 2 days. Rats in the 100 mg/m^3 dose group also showed red nasal discharge immediately after exposure. No mortality, other clinical signs of toxicity or substance-related microscopic findings were observed in any dose group in this study (albeit microscopic analysis was not performed in the 5200 mg/m^3 dose group). Body weight loss between 2.5% - 6.8% was observed in rats in the highest dose group as compared to controls. Rats in the other dose groups also showed minor decreases in body weight, however, a similar minor decrease in body weight was also observed in the control group. The LC50 for acute inhalation toxicity in male rats was reported as >5200 mg/m^3.

4.2.1.3 Acute toxicity: dermal

Two acute toxicity studies for the dermal exposure route are available studying FRD-902; one in rabbits and one in rats.

Rabbits were exposed by occlusive patch for 24 hours to a dose of 5000 mg/kg/bw (no guideline cited). No mortality was observed (2 rabbits were used in the study). Moderate to mild erythema was observed that lasted for 10 days after exposure and then decreased. Epidermal scaling and sloughing was observed in both rabbits from 6 to 13 days after application and one rabbit showed a small area of necrosis outside the test area (attributed to test substance running out of the test site) between 2 – 6 days after exposure. An approximate lethal dose (ALD) of >5000 mg/kg/bw was reported (Filliben, 1996).

The study in rats was performed according to OECD TG 402 and included semi-occlusive application for 24 hours, followed by wash-off, post-exposure observation for 14 days and necropsy. The applied dose was 5000 mg/kg/bw (Carpenter, 2007b). No mortality was observed. Reversible local effects were observed on the treated skin. The dermal LD50 was >5000 mg/kg/bw.

4.2.1.4 Acute toxicity: other routes

No data available.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

The oral LD50s for FRD-902 are 3129 mg/kg/bw, 1750 mg/kg/bw, and 1030 mg/kg/bw for female rats, male rats, and mice respectively. The LC50 for acute inhalation toxicity is >5200 mg/m^3 in male rats. For dermal acute toxicity, an ALD of >5000 mg/kg/bw is established in rabbits, and a dermal LD50 of > 5000 mg/kg/bw in rats. The oral LD50s for FRD-903 are 1730 mg/kg/bw and 1750 mg/kg/bw for male and female rats respectively, no information for other exposure routes is available for this substance.

These values indicate that FRD-902 and FRD-903 cause mortality in experimental animals upon acute exposure via the oral route. FRD-902 caused toxicity at lower doses via the oral route compared to the inhalatory or dermal route. FRD-902 causes mortality at lower doses in mice compared to rats upon acute exposure via the oral route, and also appears to be more toxic to male rats than to female rats. Furthermore, FRD-903 and FRD-902 show acute toxicity at equipotent dosages in female rats, whereas the FRD-902 oral LD50 for male rats was higher compared to the FRD-903 oral LD50 in male rats. These findings may be explained by

toxicokinetic differences between exposure routes, species and sexes as has been illustrated in Section 4.1.

4.3 Irritation/ Corrosivity

In an *in vitro* Corrositex assay according to OECD TG 435, FRD-903 was evaluated for skin corrosion potential (Carpenter, 2007e). 500 µL FRD-903 was applied to 4 membrane discs to study penetration through an artificial membrane barrier. The substance passed though all membranes, with a mean breakthrough time of 68 minutes. Based on these results, FRD-903 was determined to be corrosive and was assigned to corrosive subcategory 1C.

The available skin irritation study according to OECD TG 404 showed limited and reversible erythema (score 1 or 2) at 1 hour after removal of FRD-902 (86% purity) (Carpenter, 2007a). Additionally, the available eye irritation study according to OECD TG 405 showed irreversible effects in 1 young adult New Zealand White rabbit treated with FRD-902 (86% purity) including cornea opacity, iritis and conjunctival chemosis and discharge (Carpenter, 2007c). The rabbit was euthanised the day after treatment for humane reasons.

4.3.1 Summary and discussion of irritation/corrosivity

Based on the available data, FRD-902 causes serious eye damage. Furthermore, FRD-902 does not result in skin irritation. In the *in vitro* Corrositex assay, FRD-903 was graded as corrosive to the skin in corrosive subcategory 1C.

4.4 Sensitisation

4.4.1 Skin

4.4.1.1 Non-human information

In a LLNA test according to OECD TG 429 (Hoban, 2007), FRD-902 dissolved in dimethylformamide at 0, 5, 25, 50 and 100% induced no increase in the stimulation index above 3 (EC3). Therefore, this test was considered negative and does not warrant classification as skin sensitiser.

A second LLNA test was available in the registration dossier in which a crude industrial grade HFPO-DA was tested positive with an EC3 of 37%. However, the relation of the tested substance with the marketed substance was questioned (Hoban, 2006).

4.4.1.2 Human information

No data available.

4.4.2 Respiratory system

4.4.2.1 Non-human information

No data available.

4.4.2.2 Human information

No data available.

4.4.3 Summary and discussion of sensitisation

Based on the first test the assumption is that FRD-902 does not induce skin sensitisation.

4.5 Repeated dose toxicity

4.5.1 Non-human information

4.5.1.1 Repeated dose toxicity: oral

7-day toxicity studies

In a screening study not according to OECD and GLP, 5 Crl:CD(SD) rats per dose and sex were exposed by gavage (water) to FRD-902 (86.6% purity) for 7 days to 0, 30, 300 and 1000 mg/kg bw/day (Nabb, 2008b).

Body weight was significantly decreased at the high dose level in male rats (-8%). Statistically significant decreases in red cell mass parameters (red blood cell, haemoglobin, and haematocrit were observed in male rats at 300 and 1000 mg/kg bw/day and in females at 1000 mg/kg bw/day. Statistically significant increases in red cell distribution width, reticulocytes and neutrophils were also present in 1000 mg/kg bw/day females. Decreases in serum lipids (triglycerides and/or cholesterol) and globulin were present in all dosed male groups and in females at 300 and/or 1000 mg/kg bw/day. Other changes in clinical chemistry parameters occurred at 300 and/or 1000 mg/kg bw/day and included increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BUN), and glucose; and decreased sorbitol dehydrogenase (SDH), creatinine, and calcium (Table 17).

Increased liver weight parameters were present in males at all dose levels and in females in the 1000 mg/kg bw/day group, up to 45%-63% increase relative to brain weight in males exposed at 300 and 1000 mg/kg bw/day. These livers, changes were correlative to microscopic hepatocellular hypertrophy were observed. Other organ weight changes included decreases in heart weight parameters (1000 mg/kg bw/day males; around -12% absolute and relative weight) and increases in some kidney weight parameters (up to 17% increased kidney to brain weight in 1000 mg/kg bw/day females). There were no corresponding microscopic changes in these organs. Test substance-related microscopic changes were limited to hepatocellular hypertrophy in the liver. Minimal to mild hypertrophy was present in male rats at all doses and in females administered with 1000 mg/kg bw/day. Microscopic and organ weight changes in the liver were associated with increases in beta-oxidation and/or increases in total cytochrome P-450 enzyme activity.

Table 17: Selected haematology and clinical chemistry parameters presented in percentage change compared to control in rats after 7 days exposure.

	Dose (mg/kg bw/day)							
	Male				**Female**			
	0	**30**	**300**	**1000**	**0**	**30**	**300**	**1000**
Haematological effects								
Red blood cells	0	-5	-12	-17*	0	1	-4	-18*
Haemoglobin	0	-3	-11*	-18*	0	3	-3	-16*
Haematocrit	0	-3	-11*	-16*	0	3	-3	-15*

RBC distribution	0	5	5	13	0	-1	4	15*
Reticulocytes	0	-10	0	-3	0	4	33	158*
Neutrophils	0	0	-7	54	0	68	-7	115*
Serum clinical chemistry								
Albumin	0	9	6	-6	0	0	-5	-10
Globulin	0	-16*	-28*	-36*	0	0	-12	-23*
Total protein	0	-3	-10*	-21*	0	0	-8	-15*
Total bilirubin	0	-15	0	15	0	-8	-8	-23*
BUN	0	0	27	36	0	-20	-17	-27
Creatinine	0	-9	-9	-16*	0	-5	-5	-19*
Cholesterol	0	-48*	-45*	-46*	0	-19	-25*	-29*
Triglycerides	0	-54*	-53	-57*	0	-39	-46	-42
Glucose	0	15	27*	26*	0	-2	-8	0
Calcium	0	0	-3*	-8*	0	-1	-2	-5
Sodium	0	-1	0	-2*	0	0	0	0
Liver enzymes								
AST	0	3	5	37	0	5	0	16
ALT	0	12	23	85	0	10	30	45
SDH	0	-1	-31	-70*	0	-34	6	-29

*P < 0.05

A statistically significant increase in peroxisomal beta-oxidation activity was present in the 30, 300, and 1000 mg/kg bw/day male groups and in the 1000 mg/kg bw/day female group at the 7-day sacrifice. A statistically significant increase in total microsomal cytochrome P-450 content was present in the 300 and 1000 mg/kg bw/day male groups and in the 1000 mg/kg bw/day female group at the sacrifice on day 7.

In a study not according to OECD, 5 Crl:CD(SD) rats per dose and sex were exposed by gavage (water) to FRD-903 (99% purity) for 7 days to 0, 30, 100, and 300 mg/kg bw/day (Nabb, 2008c). No deaths occurred, and no significant body weight effects were observed at any dose level. Statistically significant decreases in red cell mass parameters (haemoglobin and haematocrit) were observed in males dosed at 300 mg/kg bw/day and in females (red blood cell count) at 300 mg/kg bw/day. Furthermore, an increase in red cell distribution width was observed in females at this dose. Decreases in cholesterol were observed in males at all dose groups, triglyceride and globulin decreases at the mid- and top dose, and creatinine, total protein, and calcium were decreased in males at 300 mg/kg bw/day. Females illustrated significantly decreased bilirubin at 300 mg/kg bw/day (Table 18).

Gross pathological observations include increased relative (about 12% to body) kidney weight and increased absolute and relative (53-88% to body and brain) liver weight in males at all dose groups. Females illustrated increased absolute and relative (18-21% to body and brain) liver weight at 300 mg/kg bw/day, but this effect was more apparent in males than in females. Microscopic findings were limited to hepatocellular hypertrophy in all treated male and female dose groups. Lesions were graded as mild in all male groups and as minimal in all female groups.

A statistically significant increase in peroxisomal beta-oxidation activity was present in the 30, 100, and 300 mg/kg bw/day male groups and in the 300 mg/kg/day female group at the 7-day sacrifice. A statistically significant increase in total microsomal cytochrome P-450 content was present in the 30, 100 and 300 mg/kg bw/day male groups at the sacrifice on day 7.

Table 18: Selected haematology and clinical chemistry parameters presented in percentage change compared to control in rats after 7 days exposure.

	Dose (mg/kg bw/day)							
	Male				**Female**			
	0	**30**	**100**	**300**	**0**	**30**	**100**	**300**
Haematological effects								
Red blood cells	0	-4	-6	-6	0	4	-4	-11*
Haemoglobin	0	0	-5	-8*	0	3	-2	-10
Haematocrit	0	0	-6	-8*	0	4	-2	-9
RBC distribution	0	2	0	3	0	2	2	20*

Reticulocytes	0	0	-1	3	0	-1	-4	146
MCV	0	5*	0	2	0	0	2	4
Serum clinical chemistry								
Albumin	0	12*	12*	3	0	3	0	0
Globulin	0	-8	-12*	-31*	0	0	-4	-8
Total protein	0	2	2	-12*	0	3	-2	-3
Total bilirubin	0	-15	0	15	0	0	-8	-23*
BUN	0	-6	0	25*	0	-12	-12	-12
Creatinine	0	-3	-9	-15*	0	-3	-6	-11
Cholesterol	0	-49*	-34*	-41*	0	33*	7	8
Triglycerides	0	-18	-46*	-34*	0	6	0	15
Glucose	0	34	20	26*	0	1	-3	-2
Calcium	0	-2	-2	-6*	0	2	-2	1
Liver enzymes								
ALP	0	48*	22	48*	0	4	29	5

*P < 0.05

In a study to determine target organ toxicity in mice (Crl:CD1(ICR)), not performed according to OECD and GLP, male mice (N = 5) were exposed to FRD-902 (86.6% purity) by gavage (water) for seven consecutive days at either 0 or 30 mg/kg bw/day. Body (105%) and liver weight (217%) were increased. Histopathology showed an increase in moderate liver hypotrophy, minimal single cell necrosis and a moderate increase mitotic figures (Nabb, 2008a).

Also FRD-903 was studied in mice (Crl:CD1(ICR)) to determine target organ toxicity (Nabb, 2008d). Male mice (N = 5) were exposed to FRD-903 (99% purity) by gavage for seven consecutive days at either 0 or 30 mg/kg bw/day. Liver weights were increased (223%) compared to controls. Microscopic changes included minimal single cell necrosis of hepatocytes, moderate hepatocellular hypertrophy, and moderate increases in mitotic figures. Also minimal vacuolation of hepatocytes was present in 2/5 animals but this effect was of uncertain relevance according to the authors.

28-days toxicity study in rats

In the 28-day repeated dose toxicity study in rats performed according to OECD TG 407, groups of 10 Crl:CD(SD) rats per dose and sex were exposed to FRD-902 (purity 88%) by gavage (water) (Haas, 2008a). Males were exposed to 0, 0.3, 3 and 30 mg/kg bw/day whereas females were exposed to 3, 30 and 300 mg/kg bw/day. Additional animals were used to determine the recovery within 4-weeks.

At 30 mg/kg bw/day, decreased RBC count, hemoglobin, and hematocrit were observed in males. These effects were also visible at 3 mg/kg bw/day (Table 19). The decreases in red cell mass parameters were ≤7.9% below the control mean, but increased with dose. Results returned to control values after a four-week recovery period. No haematological effects were observed in females.

Alterations in serum clinical chemistry (Table 19) included decreased total globulin and increased A/G ratio at the 3 and 30 mg/kg bw/day dosed males and the 300 mg/kg bw/day dosed females respectively. Additionally, males illustrated increased albumin at 30 mg/kg

Table 19: Selected haematology and clinical chemistry parameters presented in percentage change compared to control in rats measured at week 4 (primary necropsy).

	Dose (mg/kg bw/day)							
	Male				**Female**			
	0	**0.3**	**3**	**30**	**0**	**3**	**30**	**300**
Haematological effects								
Red blood cells	0	-2.0	-3.8*	-5.6**	0	-2.1	-3.0	-0.8
Haemoglobin	0	0	-3.1*	-6.7**	0	-1.2	-1.2	-1.2
Haematocrit	0	-1.5	-4.8**	-7.9**	0	-2.1	-0.2	0.2
Serum clinical								

chemistry								
Albumin	0	0	4.9	14.6**	0	2.2	2.2	4.4
Globulin	0	-8.7	-13.0*	-21.7**	0	4.3	4.3	-8.7*
A/G ratio	0	4.9	15.8**	40.8**	0	2.1	2.1	20.2**
BUN	0	0.7	1.3	23.5**	0	-7.6	1.7	8.7
Glucose	0	-9.5	0	15.2**	0	-4.8	-28.6	-19.0
Cholesterol	0	-21.6*	-19.6*	-27.5*	0	-2.4	-16.9	-6.0
Triglycerides	0	-18.1	-22.2*	-18.1	0	-11.5	-3.3	3.3
SDH	0	-35.7**	-7.1	-21.4*	0	-7.7	15.4	30.8

*P < 0.05; **P < 0.01

bw/day. Furthermore, males illustrated decreases in cholesterol and triglycerides at all doses, but the decrease in triglycerides (22%) was only statistically significant from control at 3 mg/kg bw/day. Other observations included increases in blood urea nitrogen (BUN) and glucose in the 30 mg/kg bw/day dosed males. Results returned to control values after a four-week recovery period, apart from increased cholesterol levels in males at 30 mg/kg bw/day (35.6%).

In 30 mg/kg bw/day dosed males, a relative increased kidney weight was reported of 15%, as well as minimal mineralization of the kidneys in 1/10 males. No changes in kidney weight were observed in females. Relative liver weights were increased (18.6% and 55.5% respectively) in males at 3 and 30 mg/kg bw/day and in females a relative liver increase (12.1%) was reported at 300 mg/kg bw/day. In males, SDH was decreased at 0.3 and 30 mg/kg bw/day.

Test substance-related changes of multifocal centrilobular hypertrophy were observed in the liver of 3 and 30 mg/kg bw/day group males and the 300 mg/kg bw/day group females. 4/10 and 7/10 male rats illustrated this effect at 3 and 30 mg/kg bw/day. For females, this effect was visible in 4/10 animals at 300 mg/kg bw/day. Single cell necrosis (multifocal) and hepatocellular necrosis was observed in 1/10 and 3/10 males at 30 mg/kg bw/day and hepatocellular necrosis was observed in 1/10 and 1/10 females at 30 and 300 mg/kg bw/day (Table 20). No statistical analysis was performed for these effects. Lastly, male and female rats illustrated β-oxidation activity at the middle and high doses.

The NOAEL for this study was set at 0.3 mg/kg bw/day, based on the changes in blood parameters, increases in albumin and A/G ratio, reduction in cholesterol and globulin, and incidences of liver hypertrophy observed in male rats at 3 mg/kg bw/day.

Table 20: Incidences of selected histopathological findings in rats measured at week 4 (primary necropsy).

	Dose (mg/kg bw/day)							
	Male				**Female**			
	0	**0.3**	**3**	**30**	**0**	**3**	**30**	**300**
Liver hypertrophy	0/10	0/10	4/10	7/10	0/10	0/10	0/10	4/10
Liver Single Cell Necrosis								
Multifocal-Mild	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10
Liver Necrosis Hepatocellular								
Focal-Minimal Focal-Mild	0/10 0/10	0/10 0/10	0/10 0/10	3/10 0/10	0/10 1/10	0/10 0/10	1/10 0/10	1/10 0/10

28-days toxicity study in mice

In the 28-day study with mice according to OECD TG 407, groups of 10 or 20 Crl:CD-1(ICR) mice per dose and sex were exposed to FRD-902 (88% purity) by gavage (water) at dose levels of 0, 0.1, 3 and 30 mg/kg bw/day (Haas, 2008b). The reversibility of the effects in the high dose mice was determined after a 4-week recovery period.

Decreases in haemoglobin and haematocrit were observed at 3 and 30 mg/kg bw/day in male mice, accompanied by a decrease in RBC count at 30 mg/kg bw/day. The changes in red cell mass parameters were decreased >10% compared to controls (Table 21). Effects were reversible upon recovery. No effects were observed in female mice.

In addition, alterations in serum clinical chemistry were observed. In both sexes, A/G ratio was increased at 3 mg/kg bw/day and above. Albumin was increased in both sexes at 30 mg/kg bw/day, and globulin was decreased at 3 mg/kg bw/day and above for both sexes. Moreover, the serum liver enzymes (AST, ALT, ALP, and SDH) were increased at 3 and 30 mg/kg bw/day in males, and at 30 mg/kg bw/day in females (ALP and SDH). These liver enzyme level changes were consistent with hepatocellular injury. BUN was also slightly increased in the 30 mg/kg/day group males at the end of exposure (Table 21). Effects were reversible upon recovery.

Test substance-related gross necropsy findings included enlarged liver in the 30 mg/kg bw/day group males at the primary necropsy. Both sexes illustrated increased relative liver weights at 3 and 30 mg/kg bw/day (78% and 163%, and 32% and 103% respectively). Liver weights were mostly, but not completely, reversible in the 30 mg/kg bw/day males and females. Test substance-related changes of multifocal centrilobular hypertrophy were observed in the liver of 3 and 30 mg/kg bw/day group males and the 300 mg/kg bw/day group females. Adrenal gland weights (absolute and relative to body and brain weights) were increased in the 3 and 30 mg/kg bw/day group males at the end of the exposure period.

Females illustrated increased absolute (11% and 20.6%) and relative (8.3% and 17.4%) kidney weights at the 0.1 and 30 mg/kg bw/day dosed groups. Furthermore, females had increased absolute (27.3%) and relative (23.6%) spleen weight at 0.1 mg/kg bw/day (however regarded as non-treatment related by the study authors). Minimal hypertrophy of the adrenal cortex was observed in 8/10 males at 30 mg/kg bw/day, whereas females illustrated mild or minimal adrenal cortex congestion at 30 mg/kg bw/day. Decreased uterus weights (absolute and relative to body weights; 39.1% and 40.5% respectively) were present

Table 21: Selected haematology and clinical chemistry parameters presented in percentage change compared to control in mice measured at week 4 (primary necropsy).

	Dose (mg/kg bw/day)							
	Male				**Female**			
	0	**0.1**	**3**	**30**	**0**	**0.1**	**3**	**30**
Haematological effects								
Red blood cells	0	-4.1	-5.9	-7.6*	0	4.8	-0.8	-0.7
Haemoglobin	0	-2.1	-5.0*	-7.1**	0	5.8	0.7	1.5
Haematocrit	0	-3.2	-5.0*	-6.5**	0	-0.2	-4.8	-3.8
Serum clinical chemistry								
Albumin	0	0	3.1	31.3**	0	-5.6	-2.8	5.6*
Globulin	0	0	-19.0**	-14.3**	0	-5.3	-15.8**	-21.1**
A/G ratio	0	1.3	24.7**	50.6**	0	2.6	14.0**	27.5**
BUN	0	-4.0	10.9	21.9*	0	-11.1	13.4	1.4
Cholesterol	0	-13.6	-25.2**	-10.9	0	-11.2	16.8	-0.9
Liver enzymes								
AST	0	0	25.0	477.8**	0	-1.7	-3.5	1.7
ALT	0	-26.9	57.7	1253.8**	0	-26.9	21.2	59.6
ALP	0	-17.0	63.6	1221.6**	0	7.8	6.7	14.0**
SDH	0	-8.3	91.7	1800.0**	0	14.3	14.3	185.7**

*P < 0.05; **P < 0.01

Table 22: Incidences of selected histopathological findings in mice measured at week 4 (primary necropsy).

	Dose (mg/kg bw/day)							
	Male				**Female**			
	0	**0.1**	**3**	**30**	**0**	**0.1**	**3**	**30**
Liver hypertrophy	0/10	0/10	10/10	10/10	0/10	0/10	10/10	10/10
Liver Necrosis Single Cell								
Multifocal – minimal	0/10	0/10	4/10	10/10	0/10	0/10	0/10	4/10
Liver Necrosis Hepatocellular								
Focal – minimal Focal - mild	0/10 0/10	0/10 0/10	1/10 0/10	0/10 1/10	0/10	1/10	0/10	0/10
Increased mitosis	0/10	0/10	0/10	9/10	0/10	0/10	0/10	5/10

in the 30 mg/kg bw/day group females at the end of the exposure period. There were no histopathological changes in the uterus that were correlative to the uterine weight changes.

There was multifocal single cell liver necrosis (minimal) in the 3 (4/10) and 30 (10/10) mg/kg bw/day group males and 30 (4/10) mg/kg/day group females at the primary necropsy (Table 22). These effects were not present anymore following the 4-week recovery period, but single cell hepatocellular necrosis was observed at 30 mg/kg bw/day in 1/10 males and 2/10 females. Lastly, male mice illustrated β-oxidation activity at all doses, and females at the middle and high doses.

There was an increased number of animals in the diestrus stage of the oestrous cycle in the 30 mg/kg/day group females compared to control group females at the primary necropsy. However, ovarian morphology, including number and maturational stages of corpora lutea were similar between treated and control groups, suggesting normal oestrous cycling. The significance of the differences in oestrous stage distribution between the 30 mg/kg bw/day group females and control group females is uncertain. The number of animals in the diestrus stage of the oestrous cycle was equal in the control and 30 mg/kg bw/day group females at the recovery necropsy.

The NOAEL for this study was set at 0.1 mg/kg bw/day, based on a decrease in globulin and an increase in A/G ratio in both sexes, and reduced haemoglobin, haematocrit, an increase of markers for liver damage (AST, ALT, ALP, SDH) and liver cell necrosis (during primary necropsy) in male mice at 3 mg/kg bw/day.

Other 28-days studies in mice

In addition to the studies included in the REACH Registration Dossier, two 28-day mouse studies were published in 2017.

Rushing et al. (2017)

In an oral 28-day study in mice (C57BL/6) (Rushing et al., 2017) the immunomodulatory potential of FRD-903 was examined in groups of 12 (6m/6f) animals at 0, 1, 10, or 100 mg/kg bw/day. Two replicates of this study were performed, temporised 8 weeks apart. In one replicate of the study, serum concentrations of FRD-903 were measured after 1, 5, 14 and 28 days. At day 24 all mice (both replicates) were immunised using SRBC (sheep red blood cells). SRBC-specific IgM antibody titres were determined in serum at test end (T-cell antibody response, TDAR). Splenic lymphocyte subpopulations were analysed at test end. One day after the final gavage dose the animals were killed and the weights of thymus, spleen and liver were determined. Livers were analysed for peroxisome proliferation (peroxisomal fatty acid oxidation, hepatic acyl CoA oxidase).

Observed effects at 100 mg/kg bw/day include suppression of T cell-dependent antibody responses (7.3%) and increased T lymphocyte numbers (74% on average). Relative liver weights were increased at 10 mg/kg bw/day and 100 mg/kg bw/day for both sexes (40%-160%) and liver peroxisome proliferation (measurement of hepatic acyl CoA oxidase) was found at 10 and 100 mg/kg bw/day (males; 122% and 222%) or at 100 mg/kg bw/day only (females; 100%). No effect on spleen weight was found in males but in females absolute and relative spleen weights were decreased at 100 mg/kg bw/day (11%).

The NOAEL in this study was 10 mg/kg bw/day based on immune effects (suppression of the T cell-dependent antibody response in females and increased T lymphocyte numbers in males) at 100 mg/kg bw/day.

Wang et al. (2017)

Wang et al. (2017) carried out an oral 28-days study in mice focused on the induction of liver effects by two test chemicals, i.e. FRD-903 and the tetramer [HFPO-TA]. A single dose level of 1 mg/kg bw/day was tested for both compounds in groups of 12 male ICR mice. Liver weights were increased in both groups, most markedly so in the tetramer group. Furthermore, ALP was increased in both compounds. AST and ALT were increased in the tetramer group only. Moreover, there was an increase in low-density lipoprotein cholesterol and decreases in total and direct bilirubin. Liver histopathology showed damage in both groups (hepatocellular hypertrophy, lipid droplet accumulation, swollen hepatocytes, and nuclei, steatosis, karyolysis) with the tetramer showing additional adverse effects such as focal cell necrosis, infiltration of inflammatory cells and vacuolar degeneration. The severity of these effects was not indicated. A NOAEL/BMDL was not derived in this study.

90-days study in rats

In a 90-day repeated dose toxicity study according to OECD TG 408, groups of 10 Crl:CD(SD) rats per dose and sex were exposed to FRD-902 (purity 84%) by gavage (water) (Haas, 2009). Males were exposed to 0, 0.1, 10 and 100 mg/kg bw/day whereas females were exposed to 0, 10, 100 and 1000 mg/kg bw/day. Additional animals were used to determine the recovery within 4-weeks. Three high-dosed females died before the end of the study, of which two deaths were treatment related.

Table 23: Selected haematology and clinical chemistry parameters presented in percentage change compared to control in rats measured at week 12 (primary necropsy).

	Dose (mg/kg bw/day)							
	Male				**Female**			
	0	**0.1**	**10**	**100**	**0**	**10**	**100**	**1000**
Red blood cells	0	-1.5	-7.0**	-11.0**	0	-0.8	-3.1	-28.4**
Haemoglobin	0	-0.6	-6.7**	-12.8**	0	1.3	-1.3	-20.9**
Haematocrit	0	-1.1	-7.1**	-12.0**	0	0.9	-2.7	-18.2**
MCV	0	0.6	-0.2	-1.0	0	1.7	0.6	15.3**
MCH	0	1.1	0.0	-1.7	0	2.1	2.1	11.2**
MCHC	0	0.3	0.0	-0.9	0	0.3	1.1	-3.9**
Reticulocytes	0	0.0	25.0	66.7**	0	15.4	-15.4	392.3**
Platelets	0	-9.1	-1.0	16.8*	0	7.6	1.8	29.5**
Basophils	0	-25.0	-25.0*	-50.0**	0	0	-33.3	-33.3*
Serum clinical chemistry								
Albumin	0	2.4	9.5*	11.9**	0	6.0	0.0	2.0
Globulin	0	-3.8	-11.5*	-15.4**	0	0.0	-4.2	-33.3*
A/G ratio	0	5.5	25.6**	35.4**	0	3.3	2.8	58.2**
Total protein	0	0	0	1.5	0	2.7	0	-9.5**
Total bilirubin	0	-9.1	-27.3	-18.2	0	0	-25**	-50.0**
BUN	0	3.4	4.1	37.7**	0	-1.8	3.0	6.0
Cholesterol	0	-7.4	-13.2	-30.9**	0	0	-19.8*	-30.9**
GGT	0	20.0	60.0	-40.0	0	-43.3	-43.3	-68.8**
Liver enzymes								
AST	0	10.8	21.6	17.6	0	-19.2	-25.8	-32.5
ALT	0	2.8	27.8	36.1	0	-23.8	-30.2	-42.9
ALP	0	-1.3	47.5*	106.3**	0	-8.6	-20.7	65.5**

*P < 0.05; **P < 0.01

Both males and females showed decreases in haemoglobin, haematocrit, and RBC count at 10 and 100 mg/kg bw/day (males) and 1000 mg/kg bw/day (females) respectively. These parameters were approximately 7%-13% lower in males and 18%-28% lower in females when compared to the respective control group (Table 23). In addition, reticulocytes and platelet count were increased in males at 100 mg/kg bw/day, and basophils were decreased in males at 10 and 100 mg/kg bw/day. At 1000 mg/kg bw/day, females illustrated increased mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), platelet count, and reticulocytes, and decreased mean corpuscular hemoglobulin concentration (MCHC) and basophils (Table 23). After the recovery period, RBC count, haemoglobin, haematocrit, and reticulocytes were still statistically significant different from control for males at 100 mg/kg and haemoglobin,

haematocrit, MCV, and reticulocytes were still statistically significant from control for females in the 1000 mg/kg bw/day group.

In both sexes, changes in serum clinical chemistry were observed. Males showed increases in albumin and A/G ratio and decreases in globulin at 10 and 100 mg/kg bw/day. Females showed increased A/G ratio and decreased globulin at 1000 mg/kg bw/day. Furthermore, serum cholesterol was decreased in males and females at 100 mg/kg bw/day and 100 and 1000 mg/kg bw/day respectively. In males, BUN was increased at 100 mg/kg bw/day. ALP levels and serum phosphorus levels were increased in males and females at 10 and 100 mg/kg bw/day and 1000 mg/kg bw/day respectively. Total bilirubin was decreased in females at 100 and 1000 mg/kg bw/day, and total protein and γ-glutamyl transferase (GGT) were decreased at 1000 mg/kg bw/day. Last, a decrease in urine pH and a large increase in total urine volume were observed in females at 1000 mg/kg bw/day.

Absolute kidney weights were increased in the highest dose group for both sexes (11% (m) and 18% (f)). Relative kidney weights were increased at 10 and 100 mg/kg bw/day in males (12% and 16%) and in females at all dose groups (9%-23%). One female at the highest dose group illustrated tubular and papillary necrosis of the kidney, as did one of the preliminary died females in the same dose group. 1/10 males dosed at 10 mg/kg bw/day exhibited transitional hyperplasia and mild acute inflammation of the kidney. Absolute and relative kidney weights were still increased upon recovery in males dosed at 100 mg/kg bw/day.

Absolute liver weights were increased in males at 10 and 100 mg/kg bw/day for males (23% and 59%) and at 1000 mg/kg bw/day for females (77%). Similarly, also relative liver weights were increased for these dose groups. In the 100 mg/kg/day group males, relative liver weight increase was mostly, but not completely reversible. In the 1000 mg/kg bw/day group females, liver weight changes showed partial recovery, but were not completely reversible following the 4-week recovery period. 3/10 and 10/10 males showed hepatocellular hypertrophy at 10 and 100 mg/kg bw/day, as well as 10/10 females dosed at 1000 mg/kg bw/day. Hypertrophy was not associated with microscopic changes indicative of liver injury (such as degeneration or necrosis) or with changes in serum chemistry indicative of liver injury, nor was hypertrophy observed in animals at the recovery necropsy.

The NOAEL for this study was set at 0.1 mg/kg bw/day, based on and an increased relative kidney weight in both sexes at 10 mg/kg bw/day and changes in blood parameters, an increase in albumin and A/G ratio, a reduction in globulin and cholesterol, and an increased liver weight in male rats at 10 mg/kg bw/day.

90-days study in mice

In an oral mouse 90-day study (MacKenzie, 2010) according to OECD TG 408, groups of 10 Crl:CD-1(ICR) mice per dose and sex were exposed to FRD-902 (84% purity) by gavage (water) at dose levels of 0, 0.1, 0.5 and 5 mg/kg bw/day. Additional animals were exposed for evaluation of the plasma concentration of the substance at 2 hours after exposure on day 0, 28 and 95.

Table 24: Selected haematology and clinical chemistry parameters presented in percentage change compared to control in mice measured at week 12 (primary necropsy).

	Dose (mg/kg bw/day)							
	Male				**Female**			
	0	**0.1**	**0.5**	**5**	**0**	**0.1**	**0.5**	**5**
Red blood cells	0	-1.0	0.5	-3.3	0	1.8	-0.2	3.1
Haemoglobin	0	-4.0	-3.3	-4.0	0	4.8	2.0	1.4
Haematocrit	0	-2.8	-1.7	-1.5	0	3.7	2.6	2.9
MCV	0	-1.8	-2.0	2.2	0	1.8	2.8	0.0
MCH	0	-2.5	-3.1	-0.6	0	3.1	2.5	-1.9
MCHC	0	-1.2	-1.5	-2.8**	0	1.2	-0.6	-1.5
Reticulocytes	0	-9.6	0.3	8.2	0	-11.3	-16.4	-13.8
Platelets	0	12.6	26.6**	26.0**	0	3.5	-8.2	3.5

Serum clinical chemistry								
Albumin	0	0.0	3.6	14.3**	0	0.0	3.6	3.6**
Globulin	0	4.8	4.8	4.8	0	5.3	0.0	-5.3
A/G ratio	0	0.0	0.0	15.4	0	-6.7	6.7	13.3
Total protein	0	2.0	2.0	10.2**	0	0.0	2.1	0.0
Total bilirubin	0	-5.9	-23.5	0.0	0	0.0	0.0	-14.3
BUN	0	0.0	7.1	3.6	0	-9.5	-4.8	0.0
Cholesterol	0	-6.0	0.8	-26.3**	0	17.3	8.6	13.6
Liver enzymes								
AST	0	8.1	35.5	106.5**	0	4.4	1.5	8.8
ALT	0	26.5	34.7	420.4**	0	0.0	-11.1	41.7**
ALP	0	10.0	40.0	1134.0**	0	18.5	10.8	143.1**
SDH	0	-2.3	-3.0	307.9**	0	-9.5	-6.7	32.4**

*P < 0.05; **P < 0.01

Statistically significant increases in mean final body weight (test day 91) and overall body weight gain (test days 0-91) were observed in the male 5 mg/kg bw/day group, relative to control. Mean final body weight and overall body weight gain were 108% and 136% of control, respectively. The difference in body weight and body weight gain in the high dose males was attributed primarily to increased liver weight.

Increased platelet count was observed for males dosed at 0.5 and 5 mg/kg bw/day. Furthermore, a small decrease in corpuscular haemoglobin concentration was observed in males at 5 mg/kg bw/day (Table 24). Also serum clinical chemistry changes were observed, however these changes were more evident in males compared to females. Cholesterol was decreased in male mice dosed with 5 mg/kg bw/day. Serum liver enzymes (AST, ALT, and ALP) were increased in males and females (ALT and ALP) at 5 mg/kg bw/day. Furthermore, SDH and albumin were increased in both sexes, and total serum protein was increased in males at 5 mg/kg bw/day. Moreover, there was an increase in total bile acid at the highest dose.

Changes in serum liver enzymes were consistent with hepatocellular damage and/or cholestasis. A test substance related increase in mean liver weight parameters was observed in mice exposed to ≥ 0.5 mg/kg bw/day (males) and 5 mg/kg bw/day (females). The increase in liver weight parameters in both sexes correlated with a treatment-related enlarged liver and microscopic hepatic changes. Additionally, mean relative (to brain) kidney weight was increased in males dosed at 5 mg/kg bw/day, but this was not associated with an increase in absolute or relative (% body weight) kidney weight. Other observations include lower mean weights of brain and epididymides relative to body weight, a higher mean weight of heart relative to brain weight in male mice given 5 mg/kg bw/day of test substance as compared to controls, and decreased mean spleen weight relative to brain and body weight at 0.5 and 5 mg/kg bw/day dosed females respectively.

Histopathological findings in the liver include increased single-cell necrosis (10/10), minimal hypertrophy (10/10), Kupffer cell pigments (10/10), and mitotic figures (9/10) in male mice, and mild hypertrophy (10/10), minimal focal necrosis (3/10), and single-cell necrosis (1/10) in female mice at 5 mg/kg bw/day (Table 25). In 8/10 male mice minimal hypertrophy was

Table 25: Incidences of selected histopathological findings in mice measured at week 12 (primary necropsy).

	Dose (mg/kg bw/day)							
	Male				**Female**			
	0	**0.1**	**0.5**	**5**	**0**	**0.1**	**0.5**	**5**
Focal necrosis								
minimal	0/10	0/10	0/10	0/10	1/10	0/10	2/10	3/10
Hypertrophy								
mild	0/10	0/10	8/10	0/10	0/10	0/10	0/10	10/10
minimal	0/10	0/10	0/10	10/10	0/10	0/10	0/10	0/10
Single cell necrosis; hepatocellular								
minimal	0/10	0/10	0/10	10/10	0/10	0/10	0/10	1/10

Mitotic figures, increased	0/10	0/10	0/10	9/10	0/10	0/10	0/10	0/10

observed at 0.5 mg/kg bw/day. Additionally, minimal bile duct hyperplasia was present in the liver of one male mouse in the 5 mg/kg/day group. Furthermore, minimal renal tubular epithelial hypertrophy was present in 5 mg/kg bw/day dosed males (9/10).

The NOAEL for this study was set at 0.5 mg/kg bw/day, based on the observed increases in liver weight and liver hypertrophy in both sexes accompanied by changes in liver serum enzymes, and single-cell necrosis in male mice at 5 mg/kg bw/day.

2-year study in rats

In the 2-year oral rat study according to OECD TG 453 (Caverly Rae et al., 2015, Craig, 2013) Crl:CD(SD) rats were exposed to FRD-902 (84% purity) by gavage (water) at 0, 0.1, 1 and 50 mg/kg bw/day (males) or 0, 1, 50 and 500 mg/kg bw/day (females).

One test substance-associated cause of death/morbidity was inflammation/necrosis of the kidneys, which occurred in seven 500 mg/kg bw/day females and was characterised by

Table 26: Selected haematology parameters presented in percentage change compared to control, measured in rats at 3, 6 and 12 months time-interval.

		Dose (mg/kg bw/day)							
		Male				**Female**			
	Month	**0**	**0.1**	**1**	**50**	**0**	**1**	**50**	**500**
Red blood cells	3	0	-3.8	-1.3	-8.6*	0	-1.8	-0.1	-12.7**
	6	0	-1.8	1.5	-6.8	0	0.3	-2.0	-17.4**
	12	0	-1.3	1.9	-2.0	0	-2.9	-6.3**	-28.0*
Haemoglobin	3	0	-3.5	-2.4	-8.8*	0	-3.8**	-0.4	-12.7**
	6	0	-1.4	1.4	-7.1**	0	-1.5	-1.7	-15.7*
	12	0	-0.5	0.1	-3.8	0	-4.7	-5.1	-23.9*
Haematocrit	3	0	-3.7	-1.5	-8.2*	0	-2.8	-0.7	-11.7**
	6	0	-1.1	1.7	-6.9**	0	-1.9	-3.0	-13.4**
	12	0	0.5	2.1	-2.1	0	-4.3	-5.2	-19.8*
MCV	3	0	0.2	-0.2	0.5	0	-1.1	-0.7	1.0
	6	0	0.7	0.1	0.0	0	-2.1	-1.0	6.8
	12	0	1.8	0.0	0.0	0	-1.3	1.1	12.0**
MCH	3	0	0.4	-1.2	-0.3	0	-2.0	-0.6	0.1
	6	0	0.5	-0.2	-0.4	0	-1.7	0.3	3.2
	12	0	0.6	-2.1	-1.8	0	-1.4	1.2	6.6
MCHC	3	0	0.1	-0.9	-0.7	0	-0.9	0.2	-1.0
	6	0	-0.3	-0.2	-0.4	0	0.4	1.4	-3.0
	12	0	-1.2	-2.0	-1.8	0	-0.2	0.2	-4.8*
Platelets	3	0	10.9	3.8	18.5	0	10.1	4.8	17.3
	6	0	6.2	9.5	15.5	0	2.5	-1.6	23.1
	12	0	-5.5	-3.2	-2.4	0	-0.7	0.9	28.0
Reticulocytes	3	0	5.2	1.2	14.8	0	1.0	-2.0	33.4
	6	0	-9.1	2.0	27.2	0	-18.1	-17.7	105.9
	12	0	-3.3	3.0	12.5	0	-16.9	-3.1	106.3

*P < 0.05; **P < 0.01

papillary necrosis. Females were terminated during Week 101, prior to scheduled termination, due to low survival in all female dose groups, especially control and 50 mg/kg bw/day groups. However, this did not impact the study as this was approximately 2 years of test article exposure. Even though survival among all female groups was low there were no statistically significant differences and survival was comparable among all groups.

Mean body weight in 50 mg/kg bw/day males was statistically significantly below control (-4% at week 52) over most of the first year, and exposure to 500 mg/kg bw/day substance produced adverse reductions in body weight and body weight gain in females (-13% reduction at week 52 and -20% mean body weight gain between week 1-52). During the 3 and 6 months time-interval, RBC count, haemoglobin, and haematocrit were decreased in male rats, but not at 12 months

(Table 26). Females dosed at 500 mg/kg bw/day exhibited decreases in these parameters at 3, 6 and 12 months, as well as decreases in the RBC count for 50 mg/kg bw/day dosed females at 12 months. Additionally, MCV was increased and MCHC was decreased in females dosed at 500 mg/kg bw/day at 12 months.

Table 27: Selected clinical chemistry parameters presented in percentage change compared to control, measured in rats at 3, 6 and 12 months time-interval.

		Dose (mg/kg bw/day)							
		Male				**Female**			
	Month	**0**	**0.1**	**1**	**50**	**0**	**1**	**50**	**500**
Serum clinical chemistry									
Albumin	3	0	3.1	1.7	10.6*	0	3.3	5.6	10.4**
	6	0	2.6	2.6	9.1*	0	-5.0	1.4	-1.8
	12	0	6.7	8.3**	16.3*	0	-0.7	0.0	4.9
Globulin	3	0	-3.4	-7.9	-9.0**	0	5.2	-1.9	-7.2**
	6	0	-0.5	-3.3	-6.0	0	0.0	-6.5**	-17.4*
	12	0	1.1	-4.8	-8.2	0	-1.6	-3.3	-14.9*
A/G ratio	3	0	7.6	10.9**	23.9*	0	-0.9	7.3**	20.2*
	6	0	8.4	9.5	17.9*	0	-5.2	8.7**	20.0*
	12	0	6.8	15.9**	28.4*	0	-89.3	3.6	23.2*
Total protein	3	0	-0.3	-3.3	0.4	0	4.2	2.0	2.0
	6	0	1.0	-0.4	1.4	0	-2.7	-2.3	-9.0*
	12	0	3.8	1.4	3.3	0	-1.2	-1.5	-4.5
Total bilirubin	3	0	11.1	0.0	-22.2	0	-5.6	-27.8**	-33.3**
	6	0	0.0	-27.8	-11.1	0	-10.5	-21.1	-47.4*
	12	0	16.7	0.0	8.3	0	-12.5	-31.3**	-37.5*
BUN	3	0	2.5	3.8	16.4**	0	-4.5	-9.1	-5.7
	6	0	10.8	5.8	16.7**	0	-4.4	4.4	4.4
	12	0	7.2	15.3	5.4	0	-2.5	3.4	35.3**
Cholesterol	3	0	19.2	9.6	5.3	0	-8.5	-21.8	-10.0
	6	0	16.9	7.8	-6.6	0	-17.9	-17.8	-23.9**
	12	0	0.2	-4.9	-20.2	0	-18.4	-17.5	-24.1**
GGT	3	0	20.0	10.0	0.0	0	-14.3	-14.3	0.0
	6	0	23.5	29.4	17.6	0	-23.3	-20.0	-40.0**
	12	0	-9.1	18.2	-9.1	0	-23.1	-15.4	-23.1
Liver enzymes									
AST	3	0	-1.2	-12.2	-8.6	0	-0.6	-6.7	-13.3
	6	0	9.4	-2.1	18.5	0	-33.3	-38.4	-50.6
	12	0	-10.0	-12.2	93.9	0	-10.9	-1.0	3.3
ALT	3	0	-15.7	-14.3	-1.4	0	9.4	3.7	-2.2
	6	0	-3.8	-12.6	70.4	0	-35.3	-48.5	-65.5**
	12	0	-12.3	-5.8	228.2**	0	-9.6	-0.8	-3.8
ALP	3	0	9.8	4.4	52.5*	0	-13.0	13.6	-2.0
	6	0	29.2	12.9	110.9*	0	0.3	10.1	-17.7
	12	0	28.1	46.6	180.4*	0	-11.6	27.0	35.4
SDH	3	0	15.3	12.7	35.1	0	42.1	73.8	44.8
	6	0	23.5	-7.9	11.4	0	-51.0	-64.1	-78.1
	12	0	8.6	17.9	140.8**	0	-7.6	5.4	-16.0

*P < 0.05; **P < 0.01

Table 28: Incidences of selected histopathological kidney findings for the chronic study in female rats at final sacrifice.

	Dose (mg/kg bw/day)			
	0	**1**	**50**	**500**
Dilatation, tubular	4/70	2/70	5/70	28/70*
Oedema, papilla	4/70	1/70	2/70	43/70*
Hyperplasia, transitional cell	6/70	3/70	12/70	33/70*
Mineralisation, tubular	25/70	32/70	28/70	42/70*
Necrosis, papillary	0/70	0/70	0/70	16/70*
Nephropathy, chronic progressive	39/70	40/70	41/70	64/70*

*Statistically significant from control (P < 0.05)

At 12 months, serum albumin levels increased in males at 1 mg/kg bw/day (Table 27). Serum globulin was increased in females at 50 mg/kg bw/day during the 6 months interval. The changes in albumin and globulin in the mid- and high-dose male and female groups resulted in statistically significant increases in A/G ratio in these groups at all intervals, apart from the 1 mg/kg bw/day dose group at 6 months. Bilirubin levels were statistically significant reduced in females at the mid- and high dose groups at almost all intervals. Furthermore, serum liver enzymes (ALP, ALT, and SDH) were increased in males at 50 mg/kg bw/day. Other observations included decreases in total protein and GGT for females in the high dose group, and increases in BUN for males and females in the high dose group. Also, phosphorus levels were increased for males and females in the high dose groups, as well as chloride, and potassium levels were increased in females in the high dose group.

In females receiving 500 mg/kg bw/day, minimal, statistically significant increases in urine volume and pH and decreases in urine specific gravity (suggestive of a minimal diuresis) were present at both the 6- and 12-month intervals. Although minimal, these changes may be correlative to increased incidences and severity of chronic progressive nephropathy observed microscopically in this dose group at the 1-year interim sacrifice. Females dosed at 500 mg/kg bw/day illustrated increased kidney weights and changes in the kidney, such as increased incidence of tubular dilation, oedema of the renal papilla, transitional cell hyperplasia, tubular and pelvic mineralisation, renal papillary necrosis, and chronic progressive nephropathy (Table 28). A test article-related macroscopic observation included "irregular surface" of the kidneys at interim sacrifice in one of the 500 mg/kg bw/day dosed females. At terminal sacrifice, this effect was noted in 16/70 females dosed at 500 mg/kg bw/day.

In high-dosed animals of both sexes, increases in relative liver weight were observed at interim sacrifice. Three males in the highest dose group illustrated minimal focal cystic degeneration and five minimal to mild focal necrosis. For all females, centrilobular hypertrophy was observed at 500 mg/kg bw/day at the 12 month sacrifice. Additional microscopic changes at final sacrifice include increased centrilobular hepatocellular hypertrophy in 7/70 males and 65/70 females and increased centrilobular hepatocellular necrosis in 5/70 males and 7/70 females at 50 and 500 mg/kg bw/day respectively (Table 29). Furthermore, males showed a decrease in focal and periportal vacuolisation at 50 mg/kg bw/day. In females, a decrease in

Table 29: Incidences of selected histopathological liver findings for the chronic study in rats at final sacrifice.

	Dose (mg/kg bw/day)							
	Male				**Female**			
	0	**0.1**	**1**	**50**	**0**	**1**	**50**	**500**
Degeneration, cystic, focal	24/70	24/70	19/70	42/70*	2/70	2/70	2/70	14/70*
Hypertrophy, hepatocyte, centrilobular	0/70	0/70	0/70	7/70*	0/70	0/70	3/70	65/70*
Hypertrophy, hepatocyte, panlobular	NA	NA	NA	NA	0/70	0/70	0/70	3/70*
Necrosis, hepatocyte, centrilobular	1/70	0/70	1/70	5/70*	1/70	1/70	4/70	7/70*
Necrosis, individual hepatocyte	NA	NA	NA	NA	0/70	0/70	0/70	3/70*

*Statistically significant from control (P < 0.05)

centrilobular vacuolisation, panlobular hepatocellular hypertrophy, individual cell hepatocellular necrosis, and angiectasis (i.e. blood- or lymph vessel dilation) were observed at 500 mg/kg bw/day.

Non-neoplastic lesions include hyperplasia of the limited ridge of the nonglandular stomach in 9/70 females and of the squamous cell in the tongue in 13/70 females, as well as an increased incidence of inflammation of the tongue in 13/70 females, dosed at 500 mg/kg bw/day.

The NOAEL for this study was set at 0.1 mg/kg bw/day, based on an increase in A/G ratio in male rats at 1 mg/kg bw/day.

Reproduction/developmental toxicity screening study in mice

In a reproduction/developmental toxicity screening study (OECD TG 421) Crl:CD1(ICR) mice (N = 25) were exposure by oral gavage at dose levels of 0, 0.1, 0.5 and 5 mg FRD-902/kg bw/day (purity 84%) (Edwards, 2010a). The F0 males were dosed during study days 0 to 84 (70 days prior to pairing through 1 day prior to euthanasia), for a total of 84 to 85 doses. The females that delivered (with the exception of those females selected for toxicokinetic evaluation) were dosed from study day 56 through the day prior to euthanasia (14 days prior to pairing through lactation day 20) for a total of 53 to 64 doses. The females that were selected for toxicokinetic evaluation were dosed until the day of euthanasia (lactation day 21) for a total of 54 to 65 doses.

No effect on mortality was observed in the parental animals. In females dosed at 5 mg/kg bw/day, an increased kidney weight was noted. In males dosed at 0.5 and 5 mg/kg bw/day, increased kidney tubular cell hypertrophy was observed. In both sexes increases in liver weight and liver hypertrophy were observed. Furthermore, incidences of single cell necrosis were observed in males and females in all dose groups, with 24/24 males and 21/24 females exhibiting single cell necrosis at 5 mg/kg bw/day. Females also illustrated increased incidences of focal/multifocal necrosis at the middle- (3/24) and high dose group (5/24) respectively. Hypertrophy and necrosis were observed in males and females at 0.5 mg/kg bw/day (Table 30).

In males, kidney weights increased with 8% at 5 mg/kg bw/day, which correlated with increased kidney tubular cell hypertrophy at doses 0.5 mg/kg bw/day (6/24) and 5 mg/kg bw/day (18/24) (Table 30). In females, absolute and relative kidney weights were increases with 21% and 10% at 5 mg/kg bw/day respectively.

The observed effects related to reproduction are noted in Section 4.8.

The NOAEL for systemic toxicity in parental animals was 0.1 mg/kg/day based on the incidences of single cell necrosis observed in the liver of males at 0.5 mg/kg/day.

Table 30: Liver and kidney histopathological findings in male and female mice.

	Dose (mg/kg bw/day)								
	Males					**Females**			
Liver	**0**	**0.1**	**0.5**	**5**		**0**	**0.1**	**0.5**	**5**
Hypertrophy, hepatocellular	0/25	0/24	12/24	24/24		0/24	0/22	14/24	24/24
Necrosis, single cell	1/25	1/24	5/24	24/24		1/24	3/22	2/24	21/24
Necrosis, focal/multifocal	0/25	0/24	1/24	1/24		1/24	0/22	3/24	5/24
Pigment, increased	0/25	0/24	0/25	21/24		0/24	0/22	0/24	5/25
Mitotic figures, increased	0/25	0/24	0/25	18/24		0/24	0/22	0/24	5/24
Kidney									
Hypertrophy, tubular cell	1/25	0/24	6/24	18/24		0/24	0/22	0/24	0/24

Table 31: Liver histopathological findings in female rats.

	Dose (mg/kg bw/day)			
Liver	**0**	**10**	**100**	**1000**
Focal necrosis, minimal	0	0	2/22	4/22
Focal necrosis, moderate	0	0	0	1/22
Hypertrophy, hepatocellular, mild	0	0	0	18/22
Hypertrophy, hepatocellular, moderate	0	0	0	1/22

Developmental toxicity study in rats

A study on developmental toxicity (developmental toxicity/teratogenicity) was conducted in rats, according to OECD TG 414 (Edwards, 2010b). Pregnant Crl:CD(SD) rats (N = 22) were

exposed to FRD-902 (84% purity) at 0, 10, 100, or 1000 mg/kg bw/day by oral gavage during Gestation days 6-20. Dams were sacrificed on Gestation day 21. Organs including the ovaries and uterus, and foetuses were examined.

One female in the highest dose group died on GD 20, due to liver and kidney damage (moderate coagulative necrosis in the liver and fibrin thrombi in the glomerular capillaries). Test-substance related clinical findings (yellow material on various body surfaces, salivation), higher mean kidney weight, and reduction in maternal body weight gains were observed at 1000 mg/kg bw/day. Decreased gravid uterine weights were found in the 100 and 1000 mg/kg/day groups. Furthermore, an increase in liver weight was reported in the animals dosed at 100 and 1000 mg/kg bw/day, accompanied by focal necrosis in some animals at these dose groups (Table 31). Also liver hypertrophy occurred at 1000 mg/kg bw/day, and an oedematous pancreas was noted in two females (that delivered early) in this dose group at necropsy.

The observed effects related to development are noted in Section 4.8.2.

The NOAEL for maternal toxicity is considered to be 10 mg/kg bw/day, based on mortality, lower mean body weight gains and food consumption at 1000 mg/kg bw/day, and early deliveries, and microscopic findings in the liver (focal necrosis) at 100 and 1000 mg/kg bw/day.

4.5.1.2 Repeated dose toxicity: inhalation

No data available.

4.5.1.3 Repeated dose toxicity: dermal

No data available.

4.5.1.4 Repeated dose toxicity: other routes

No data available.

4.5.2 Dose-response modelling

To provide an overview of the dose-responses and equipotent effect doses resulting from the available repeated-dose toxicity studies presented above, a dose-response analysis was performed using PROAST software version 66.16 and 66.24 (www.proast.nl).

4.5.2.1 Methods

Two 90-day studies (mouse and rat) and one chronic toxicity/carcinogenicity study (rat) were included for analysis (Haas, 2009, MacKenzie, 2010, Craig, 2013). Information from 90 days, interim sacrifice (365 days), as well as from the final sacrifice (707 days (f) and 728 days (m)) was included from the chronic toxicity/carcinogenicity study. In addition, also data from the males in a mouse reproduction/developmental screening study were included in the analysis (Edwards, 2010a), as these animals were exposed to HFPO-DA under a exposure regimen (84-85 days) comparable to the 90-day studies.

Information was assembled on absolute and relative (to body) organ weight (brain, heart, kidney, liver, spleen, testes, thymus, adrenal glands, epididymis, ovaries, uterus), serum clinical chemistry parameters (AST, ALT, SDH, ALP, GGT, bilirubin, bile acids, BUN, creatinine, cholesterol, triglyceride, glucose, total protein, albumin, globulin, and A/G ratio), haematology parameters (red blood cell count, haemoglobin, haematocrit, MCV, MCH, MCHC, platelet count, and absolute number of reticulocytes), liver histopathology data (hypertrophy, mitotic figures,

increased pigment, mononuclear infiltration, focal necrosis, single cell necrosis, cystic focal degeneration, liver adenoma, liver carcinoma), kidney histopathology data (hypertrophy, tubular dilation, papilla oedema, transitional cell hyperplasia, tubular mineralization, papillar necrosis, chronic progressive nephropathy), and other lesions (pancreatic acinar adenoma and carcinoma, interstitial cell hyperplasia testes, interstitial cell adenoma testes) (Haas, 2009, MacKenzie, 2010, Craig, 2013, Edwards, 2010a).

Firstly, a dose-response function was fitted to the available toxicity data. In concordance with EFSA guidelines (EFSA, 2017), (continuous) organ weight data, serum chemistry parameter data, and haematology parameter data were analysed using an exponential and Hill model. For the (quantal) histopathology data, a suit of eight models (two stage, log-logistic, Weibull, log-probit, gamma, logistic, exponential LVM and Hill LVM) was used. This resulted in dose-response curves for each end-point and exposure duration (90/365/707 & 728 days). For each end-point and duration combination the data were tested using covariates, on possible differences in dose-responses between species (rat/mouse), sex (male/female), and study, when various studies with data on the same end-point, duration, species and sex were available.

Secondly, the fitted dose-response curves were used to calculate effect doses (ED) corresponding to pre-defined effect sizes and their confidence intervals (90% upper and lower bounds). As an effect size, 5% and 10% increase or decrease in organ weight was calculated. For liver histopathology, 10% extra risk was chosen as effect size. Values for serum clinical chemistry and haematology parameters were, as far as available, based on biologically relevant effect sizes mentioned in literature (WHO, 2015, Muller et al., 2006). These effect doses are provided as lower and upper bound limits (EDL; EDU), reflecting the dose range in which the effect occurs, taking into account model uncertainty and the uncertainty in the underlying experimental data.

Calculated effect doses have as advantage that they are equipotent, and serve as a good starting point to compare between species, sexes, and study durations. Furthermore, they are not limited to the applied doses in the experiments, and therefore provide useful information when, e.g. large dose intervals are used and effect of interest falls between or just outside the applied doses. The effect doses of continuous endpoints are derived as a range from the lowest EDL from the exponential model and the Hill model, to the highest EDU of both models. The effect dose range (EDL to EDU) of quantal endpoints is derived by model averaging the results of the eight quantal models (see EFSA 2017 for details). Some of the upper dose limits reached infinity, indicating that the effect dose could possibly lie (far) outside the experimental dose range.

4.5.2.2 Results

An overview of all effect dose ranges (EDL and EDU) is provided in Annex III of this report. The effect sizes that are considered most relevant for hazard assessment are discussed below.

4.5.2.2.1 Liver

Subchronic exposure in mice

Relevant liver effects observed in male mice include 50% increase in AST (0.298-7.03 mg/kg bw/day) and 10% increase in albumin (0.376-5.41 mg/kg bw/day). In both males and females, 50% increase in ALT (0.512-3.62 mg/kg bw/day and 3.77-12.3 mg/kg bw/day respectively), 50% increase in ALP (Figure 15; 0.286-1.15 mg/kg bw/day and 1.35-2.96 mg/kg bw/day respectively), 50% increase in SDH (1.58-3.47 mg/kg bw/day and 4.47-7.91 mg/kg bw/day respectively), 10% increase in absolute liver weight (0.0945-0.322 mg/kg bw/day and 0.211-0.74 mg/kg bw/day respectively), and 10% increase in relative liver weight (Figure 14; 0.0856-0.224 mg/kg bw/day and 0.177-0.525 mg/kg bw/day respectively) were noted. Relevant histopathological findings include 10% extra risk on minimal liver hypertrophy (0.389-0.438 mg/kg bw/day and 0.995-1.72 mg/kg bw/day for males and females respectively), 10% extra risk on minimal focal necrosis (1.13-4.51 mg/kg bw/day and 0.475-9.21 mg/kg bw/day for males

and females respectively), and 10% extra risk on single cell necrosis (1.55-1.79 mg/kg bw/day) in males. Other effect dose ranges may be observed in Figure 16, with corresponding values in Annex III.

In MacKenzie (2010), the LOAEL for most liver effects was 5 mg/kg bw/day, with effects being most apparent in males (with the exemption of focal necrosis in females (2/10 and 3/10 at 0.5 and 5 mg/kg bw/day)). For instance, in males the LOAEL for single cell necrosis and statistically significant increases in liver enzymes was 5 mg/kg bw/day. At 0.5 mg/kg bw/day (middle dose) some histopathology was observed, but single cell necrosis was observed in 0/10 animals. In Edwards (2010a), the LOAEL for liver histopathology in male mice was 0.5 mg/kg bw/day, based on an increase in single cell necrosis in 5/24 animals. In this study no clinical serum parameters were analysed and hence no accompanying increases in liver enzymes are available. By means of dose response modelling, a better overall image is provided of the effects occurring in male mice, showing that histopathological effects and increases in liver enzymes occur with EDLs in the range 0.3-1.6 mg/kg bw/day.

Subchronic exposure in rats

Relevant liver effects observed in male rats include 50% increase in ALT (109-1350 mg/kg bw/day), and 50% increase in ALP (41.2-73.1 mg/kg bw/day). In males and females, 10% increase in absolute liver weight (5.94-16.9 mg/kg bw/day and 36.8-106 mg/kg bw/day respectively) and 10% increase in relative liver weight (Figure 14; 3.9-9.63 mg/kg bw/day and 27.8-66.9 mg/kg bw/day respectively) was noted. Relevant histopathological findings include minimal hypertrophy (8.41-10.5 mg/kg bw/day and 172-347 mg/kg bw/day for males and females respectively). Other effect dose ranges may be observed in Figure 16, with corresponding values in Annex III.

In Haas (2009), the LOAEL for most of the observed liver effects in females was 1000 mg/kg bw/day (the highest dose tested), whereas at 100 mg/kg bw/day almost no statistically significant increased effects were observed. For males, the LOAEL was 10 mg/kg bw/day for ALP and increased liver weight, but other liver enzymes were not statistically significant from control up to the high dose (100 mg/kg bw/day), apart from ALP which was increased at 10 mg/kg bw/day. In (Craig), liver enzymes were not significantly changed from control up to the highest dose tested (50 mg/kg bw/day (m) – 500 mg/kg bw/day (f)). Dose response modelling illustrates that also the EDL of 50% increase in ALT lies around 100 mg/kg bw/day, and that various toxicologically relevant effects on liver end-points, e.g. 50% increase in ALP, 10% increase in liver weight and 10% extra risk in minimal hypertrophy, occur well below 100 mg/kg bw/day.

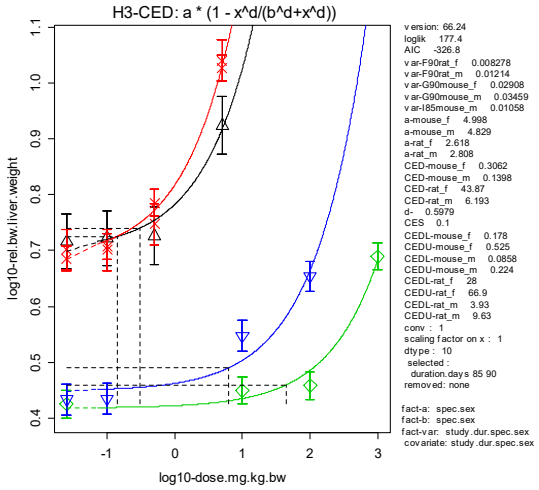

Figure 14: Increasing trends in relative liver weight for male mice (red), female mice (black), male rats 90-days in Haas (2009) (blue), and female rats 90-days in Haas (2009) (green) exposed to FRD-902 for 85-90 days, fitted by a Hill model. Dotted horizontal lines indicate 10% increase in relative liver weight, whiskers indicate 95% confidence interval of the median response. Corresponding EDL and EDU are 0.0856-0.224 mg/kg bw/day (male mice), 0.177-0.525 mg/kg bw/day (female mice), 3.9-9.63 mg/kg bw/day (male rats), and 27.8-66.9 mg/kg bw/day (female rats).

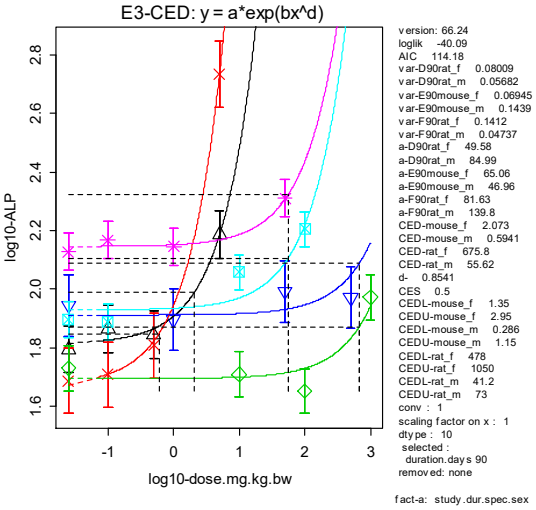

Figure 15: Increasing trends in ALP for male mice (dark blue), female mice (green), male rats 90-days in Haas (2009) (red), female rats 90-days in Haas (2009) (black), male rats 90-days in Craig (2013) (pink), and female rats 90-days in Craig (2013) (light blue), fitted by an exponential model. Dotted horizontal lines correspond to 50% increase in ALP, whiskers indicate 95% confidence interval of the median response. Corresponding EDL and EDU are 0.286-1.15 mg/kg bw/day (male mice), 1.35-2.96 mg/kg bw/day (female mice), 41.2-73.1 mg/kg bw/day (male rats), and 478-1050 mg/kg bw/day (female rats).

Chronic exposure in rats

Relevant effects observed in male rats at interim sacrifice include 50% increase in SDH (1.26-51.5 mg/kg bw/day), 50% increase in ALT (3.01-41.3 mg/kg bw/day), 50% increase in AST (17.7-44.9 mg/kg bw/day), 10% increase in albumin (3.84-44.5 mg/kg bw/day), and 10% increase in relative liver weight (3.22-99.8 mg/kg bw/day). Additionally, relevant effects observed in both sexes at interim sacrifice include 50% increase in ALP (Figure 15; 0.645-8.35 mg/kg bw/day and 38.1-61000 mg/kg bw/day respectively) and 10% increase in bile acids (0.0152-210 mg/kg bw/day for both sexes).

At final sacrifice, 10% increase in absolute liver weight (0.244-37.4 mg/kg bw/day and 2.74-331 mg/kg bw/day respectively) and 10% increase in relative liver weight (0.956-31.8 mg/kg bw/day and 16.9-345 mg/kg bw/day respectively) were noted in male and female rats. Furthermore, a 10% extra risk for cystic focal degeneration (0.674-37.3 mg/kg bw/day) was observed in male rats. An interesting observation is that for males, 10% extra risk for hepatocellular hypertrophy occurs at higher doses (45.2-76.4 mg/kg bw/day) compared to the increases in absolute and relative liver weight, cystic focal degeneration, and increases in liver enzymes. Other effect dose ranges may be observed in Figure 16, with corresponding values in Annex III.

In Craig (2013), the LOAEL for most of the observed liver effects in females was 500 mg/kg bw/day (the highest dose), whereas at 50 mg/kg bw/day (middle dose) almost no statistically significant increases were observed. The same holds for males, for which the LOAEL for most observed liver effects was 50 mg/kg bw/day (the highest dose), whereas there were almost no statistically significant effects at 1 mg/kg bw/day (middle dose). Dose response modelling

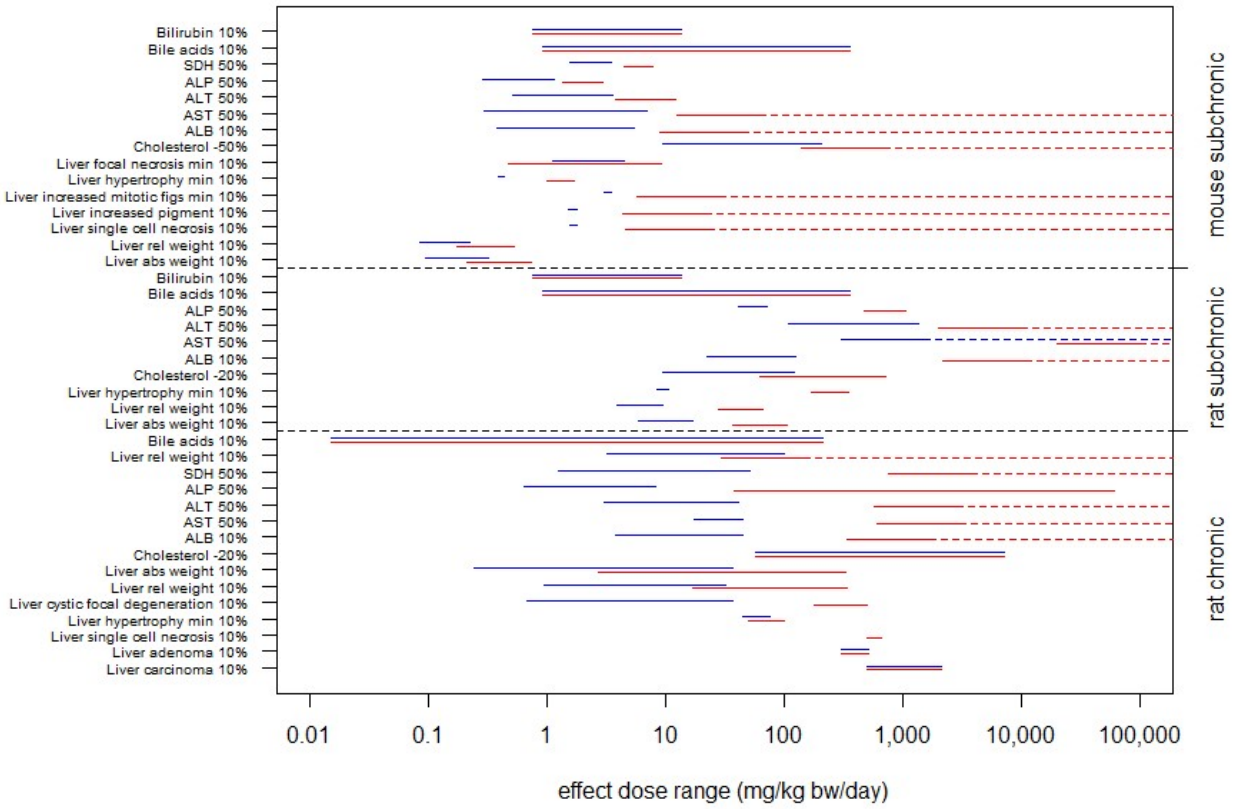

Figure 16: Overview of relevant effect dose ranges (EDL - EDU) for end-points related to liver (left y—axis), expressed in mg/kg bw/day on a log (10) scale (x-axis). Male effect dose ranges are plotted in blue and female effect dose ranges are plotted in red. Effect dose ranges for which the EDU reached up to infinity, are provided as partly dotted lines.

illustrates that for males and females, the EDLs for relevant liver effects are substantially lower than 50 mg/kg bw/day.

4.5.2.2.2 Kidney

Subchronic exposure in mice

Relevant kidney effects include in a 10% increased risk of kidney tubular cell hypertrophy in males (1.59-3.19 mg/kg bw/day). Additionally, for male and female mice a 10% increase in absolute kidney weight (2.53-13.6 mg/kg bw/day and 1.33-17.3 mg/kg bw/day respectively) was observed. Other effect dose ranges may be observed in Figure 17, with corresponding values in Annex III.

In MacKenzie (2010), no statistically significant kidney effects were observed, apart from increased kidney to brain weights at 5 mg/kg bw/day. In Edwards (2010a), male mice exhibited tubular cell hypertrophy in 6/24 animals at a LOAEL of 0.5 mg/kg bw/day. Additionally, absolute kidney weight was significantly increased at 5 mg/kg bw/day in females. Dose response modelling indicates that 10% increased absolute kidney weight is observed with EDLs being 1.33 mg/kg bw/day and 2.53 mg/kg bw/day for males and females respectively. The EDL for kidney tubular cell hypertrophy is also within this range (1.59 mg/kg bw/day).

Subchronic exposure in rats

Relevant kidney effects for male and female rats include 10% increase in absolute kidney weight (40.3-287 mg/kg bw/day and 170-1060 mg/kg bw/day respectively) and 10% increase

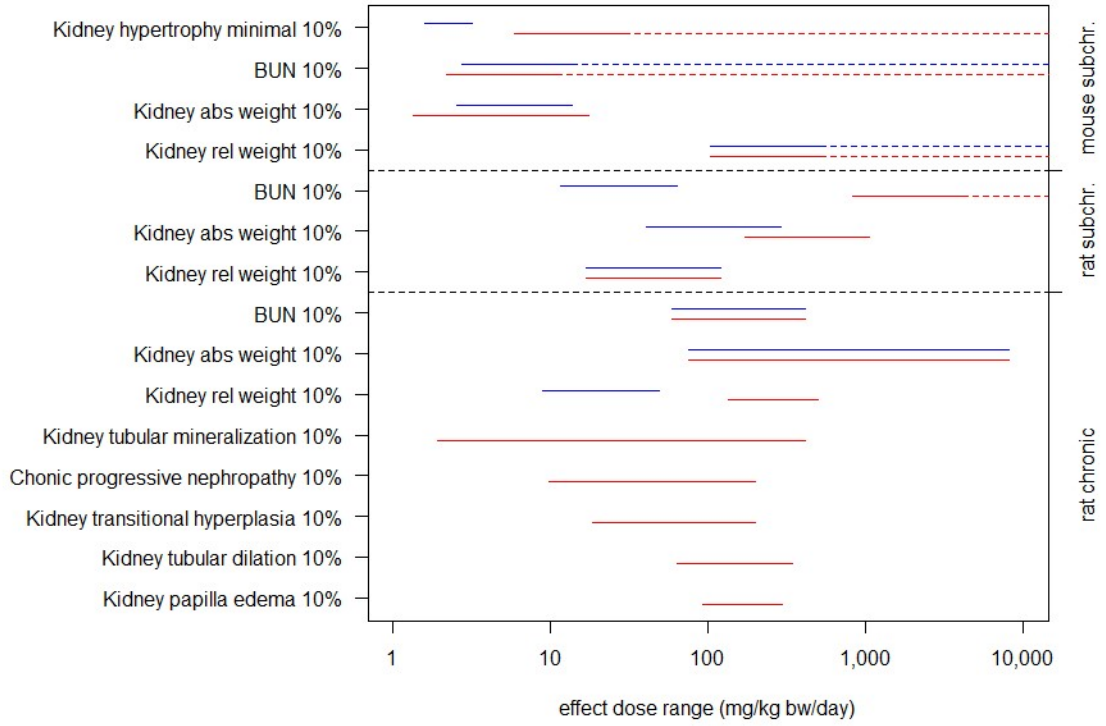

Figure 17: Overview of relevant effect dose ranges (EDL - EDU) for end-points related to kidney (left y—axis), expressed in mg/kg bw/day on a log (10) scale (x-axis). Male effect dose ranges are plotted in blue and female effect dose ranges are plotted in red. Effect dose ranges for which the EDU reached up to infinity, are provided as partly dotted lines.

in relative kidney weight (16.7-121 mg/kg bw/day for both sexes). For males, a 10% increase in BUN was established (11.6-63.4 mg/kg bw/day). Other effect dose ranges may be observed in Figure 17, with corresponding values in Annex III.

In Haas (2009), the LOAEL for kidney effects was 0.1 mg/kg bw/day and 10 mg/kg bw/day for increased relative kidney weight in females and males respectively, 100 mg/kg bw/day for increased absolute kidney weight and increased BUN in males, and 1000 mg/kg bw/day for increased absolute kidney weight in females (no statistically significant increase in BUN observed). In Craig (2013), the LOAEL for increased BUN was 50 mg/kg bw/day in males, whereas in females this parameter was not significantly changed from control. Dose response modelling illustrates that EDLs for increases in BUN and absolute and relative kidney weights are in the range 11.6-40.3 mg/kg bw/day.

Chronic exposure in rats

Relevant kidney effects in male and female rats include 10% increase in BUN at interim sacrifice (Figure 18; 58.5-413 mg/kg bw/day for both sexes), 10% increase in absolute kidney weight at final sacrifice (Figure 19; 74.4-8100 mg/kg bw/day for both sexes), and 10% increase in relative kidney weight at final sacrifice (8.97-49.2 mg/kg bw/day and 133-502 mg/kg bw/day for male and female rats, respectively). Note that the data from males (alone) do not indicate a significant trend, however, due to the lack of a dose group above 5 mg/kg bw/day it cannot be excluded that FRD-902 is equally potent in males and females, and thus has the same ED (range) in both sexes. Additionally, a 10% extra risk of progressive nephropathy (9.8-200 mg/kg bw/day) and a 10% extra risk of tubular mineralisation (1.9-415 mg/kg bw/day) were determined for female rats at final sacrifice. Other effect dose ranges may be observed in Figure 17, with corresponding values in Annex III.

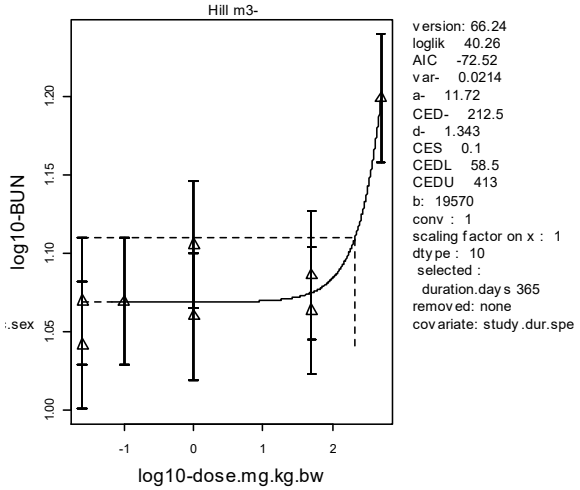

Figure 18: Increasing trend in BUN for male and female rats (covariates show no differences in dose-response between sexes) upon treatment with FRD-902 for one year (interim sacrifice), fitted by a Hill model. Dotted horizontal line indicates an increase of 10% BUN, with corresponding EDL and EDU of 58.5-413 mg/kg bw/day. Whiskers indicate 95% confidence interval of the median response.

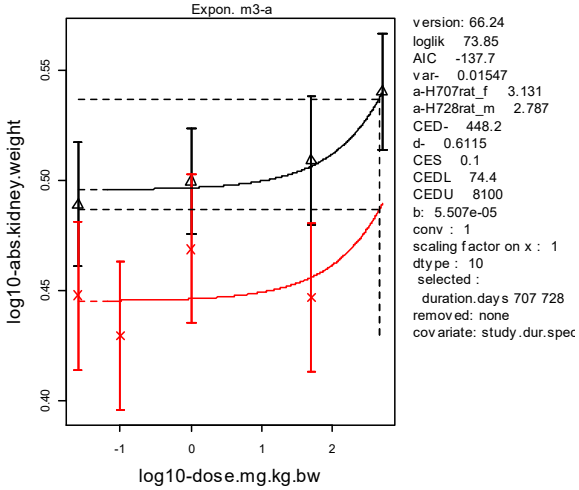

Figure 19: Increasing trends in absolute kidney weight in male (red) and female (black) rats upon treatment with FRD-902 for two years (final sacrifice), fitted by an exponential model. Dotted horizontal lines indicate a 10% increase in absolute kidney weight. Corresponding EDL and EDU are 74.4-8100 mg/kg bw/day for both sexes. Whiskers indicate 95% confidence interval of the median response.

In Craig (2013), the LOAEL for kidney effects was 500 mg/kg bw/day in female rats, whereasin males no kidney effects were observed. Based on dose-response modelling may however be concluded that the EDL for kidney effects in male and female rats ranges between 1.9 and 133 mg/kg bw/day.

4.5.2.2.3 Haematological system

Subchronic exposure in mice

In males, 10% decrease in red blood cell count (8.28-299 mg/kg bw/day), 10% increase in MCV (5.8-10.3 mg/kg bw/day), 10% increase in total reticulocytes (3.32-7.47 mg/kg bw/day), and 10% increase in platelets (0.085-19.9 mg/kg bw/day) were observed. Additionally, 10% increase in MCHC was observed for both sexes (6.9-258 mg/kg bw/day and 6.65-827 mg/kg bw/day for males and females respectively). Effect dose ranges may also be observed in Figure 22 and Annex III.

In MacKenzie (2010), LOAEL for an increase in platelets was 0.5 mg/kg bw/day, and LOAEL for decreased MCHC was 5 mg/kg bw/day. For females, these parameters were not significantly changes from control up to the highest dose tested (5 mg/kg bw/day). Other parameters (red blood cell count, haemoglobin, haematocrit, MCV, MCH, MCHC, reticulocytes) were not changed from control in both sexes up to the highest dose tested (5 mg/kg bw/day) for both sexes.

Subchronic exposure in rats

Females illustrated a 10% increase in MCH (938-1180 mg/kg bw/day) and 10% increase in MCV (818-968 mg/kg bw/day). Additionally, both sexes illustrated 10% decrease in red blood cell count (75.4-135 mg/kg bw/day and 171-433 mg/kg bw/day for males and females respectively), 10% decrease in haemoglobin (54-103 mg/kg bw/day and 158-454 mg/kg bw/day for males and females respectively), 10% decrease in haematocrit (56.5-132 mg/kg bw/day and 135-492

mg/kg bw/day for males and females respectively), 10% increase in total reticulocytes (30.6-67.1 mg/kg bw/day and 139-476 mg/kg bw/day for males and females respectively), and 10% increase in platelets (3.84-80.1 mg/kg bw/day and 28-719 mg/kg bw/day). Effect dose ranges may also be observed in Figure 22 and Annex III.

In Haas (2009), a LOAEL of 10 mg/kg bw/day was determined for red blood cell count, haemoglobin, and haematrocrit changes, and a LOAEL of 100 mg/kg bw/day for increases in reticulocytes. Other parameters (MCV, MCH, and MCHC) showed no statistically significant effects up to the highest dose tested (100 mg/kg bw/day). For females, the LOAEL for changes in all parameters (red blood cell count, haemoglobin, haematocrit, MCV, MCH, MCHC, reticulocytes, platelets) was 1000 mg/kg bw/day. In (Craig), the LOAEL for males was 50 mg/kg bw/day for changes in red blood cell count, haemoglobin, and haematocrit. Other parameters (MCV, MCH, MCHC, platelets, total reticulocytes) were not significantly changed from control. In females, the LOAEL for red blood cell count and haematocrit were 500 mg/kg bw/day, whereas haemoglobin was significantly changed at 1 mg/kg bw/day (although not in a dose-dependent manner). Dose response modelling illustrates that the EDL for relevant effects overall occur in the dose range 50-200 mg/kg bw/day.

Chronic exposure in rats

At interim sacrifice, 10% decrease in red blood cell count (Figure 20; 106-317 mg/kg bw/day for both sexes), 10% increase in total reticulocytes (10.2-339 mg/kg bw/day for both sexes), 10% decrease in haemoglobin (Figure 21; 108-296 mg/kg bw/day for both sexes), 10% decrease in haematocrit (140-348 mg/kg bw/day for both sexes), 10% increase in MVC (331-584 mg/kg bw/day), and 10% increase in MCHC (579-1690 mg/kg bw/day) were determined for male and female rats. Effect dose ranges may also be observed in Figure 22 and Annex III.

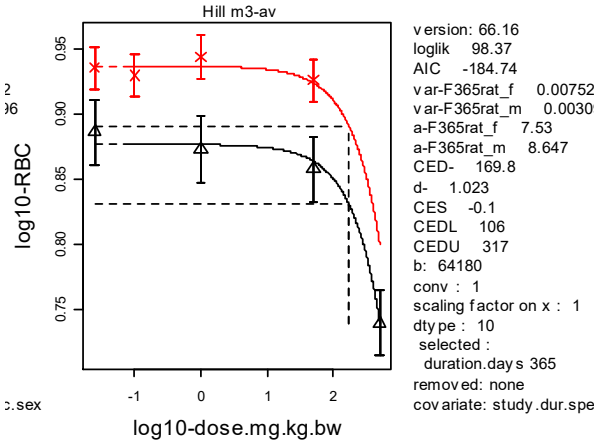

Figure 20: Decreasing trends in red blood cell count for male (red) and female (black) rats exposed to FRD-902 for one year (interim sacrifice), fitted by a Hill model. Dotted horizontal lines indicate 10% decrease in red blood cell count, with corresponding EDL and EDU of 106-317 mg/kg bw/day for both sexes. Whiskers indicate 95% confidence interval of the median response.

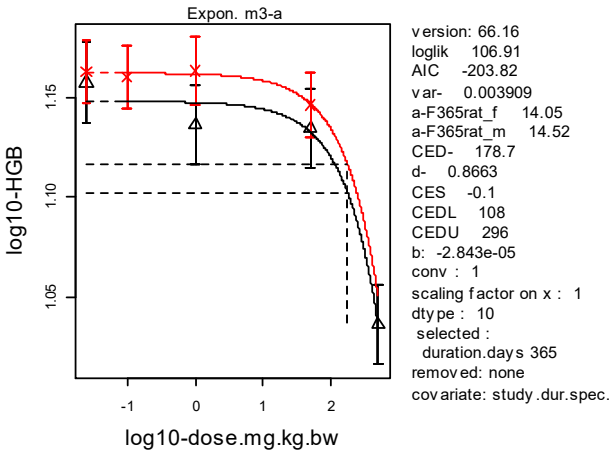

Figure 21: Decreasing trends in haemoglobin for male (red) and female (black) rats exposed to FRD-902 for one year (interim sacrifice), fitted by an exponential model. Dotted horizontal lines indicate 10% decrease in haemoglobin, with corresponding EDL and EDU of 108-296 mg/kg bw/day for both sexes. Whiskers indicate 95% confidence interval of the median response.

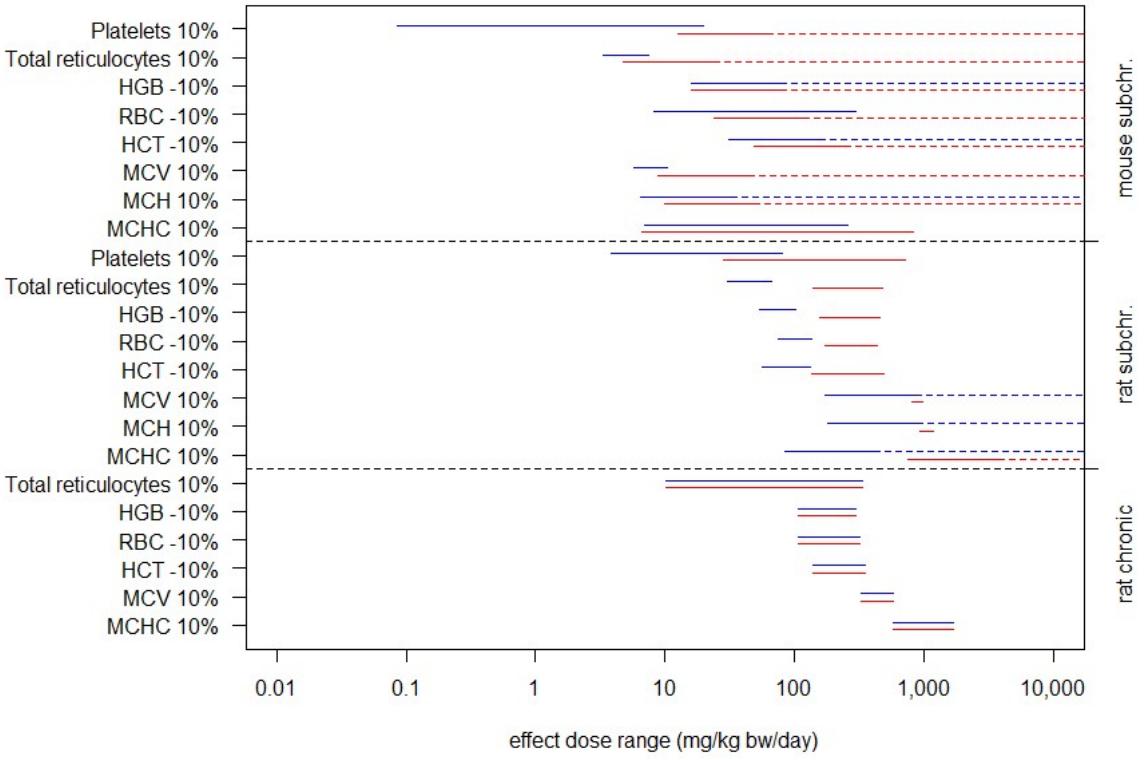

Figure 22: Overview of relevant effect dose ranges (EDL - EDU) for end-points related to the haematological system (left y—axis), expressed in mg/kg bw/day on a log (10) scale (x-axis). Male effect dose ranges are plotted in blue and female effect dose ranges are plotted in red. Effect dose ranges for which the EDU reached up to infinity, are provided as partly dotted lines.

In Craig (2013), females illustrated statistically significant changes in blood parameters at a LOAEL of 500 mg/kg bw/day (highest dose tested), apart from decreases in red blood cell count with a LOAEL of 50 mg/kg bw/day. For males, no significant increases were observed up to the highest dose tested (50 mg/kg bw/day). Dose response modelling illustrates that the EDL for relevant haematological effects lies within the dose interval 50-500 mg/kg bw/day. Due to the lack of a dose group above 5 mg/kg bw/day in males it cannot be excluded that FRD-902 is equally potent in males and females, and thus has the same ED (range) in both sexes.

4.5.2.2.4 Immune system

Subchronic exposure in mice

Relevant effects include 10% increase in albumin in males (0.376-5.41 mg/kg bw/day), 10% decrease in globulin in females (4.46-13.3 mg/kg bw/day), and 10% increase in A/G ratio in both sexes (0.713-7.26 mg/kg bw/day and 0.584-12.7 mg/kg bw/day for males and females respectively. Effect dose ranges may also be observed in Figure 25 and Annex III. In MacKenzie (2010), the LOAEL for increased albumin was 5 mg/kg bw/day for both sexes, no statistically significant increases were observed for globulin and A/G ratio.

Subchronic exposure in rats

Relevant effects include 10% increase in albumin in males (22.5-124 mg/kg bw/day), 10% decrease in globulin in both sexes (74-125 mg/kg bw/day and 419-631 mg/kg bw/day for males and females respectively), and 10% increase in A/G ratio for both sexes (5.46-37.2 mg/kg bw/day and 41.8-238 mg/kg bw/day for males and females respectively). Effect dose

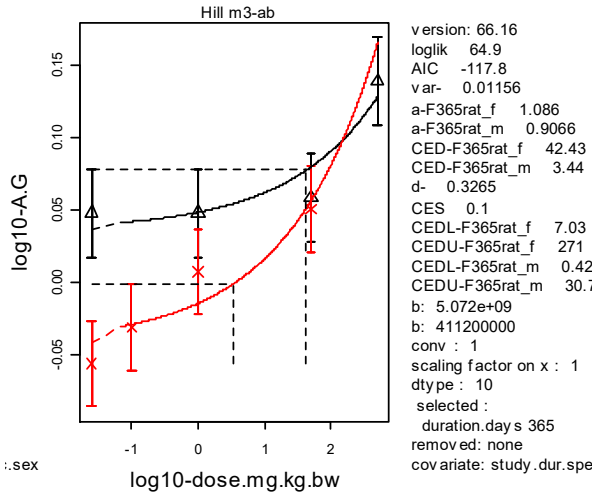

Figure 23: Increasing trend in A/G ratio in male (red) and female (black) rats exposed to FRD-902 for one year (at interim sacrifice), fitted by a Hill model. Dotted horizontal lines indicate a 10% increase in A/G ratio, whiskers indicate 95% confidence interval of the median response. Corresponding EDL and EDU are 0.426-30.8 mg/kg bw/day and 7.03-271 mg/kg bw/day for male and female rats respectively.

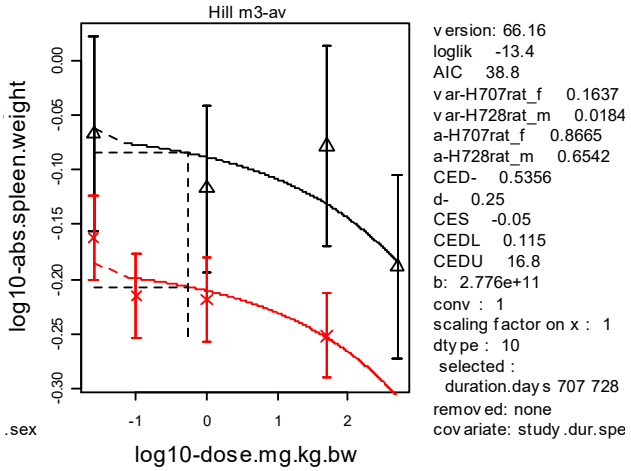

Figure 24: Decreasing trend in absolute spleen weight in male (red) and female (black) rats exposed to FRD-902 for two years (at final sacrifice), fitted by a Hill model. Dotted horizontal lines indicate a 5% decrease in absolute spleen weight, whiskers indicate 95% confidence interval of the median response. Corresponding EDL and EDU are 0.115 mg/kg bw/day and 16.8 mg/kg bw/day for both sexes.

ranges may also be observed in Figure 25 and Annex III.

In Haas (2009), the LOAEL for changes in albumin, globulin, and A/G ratio was 10 mg/kg bw/day, whereas for females the LOAEL for globulin and A/G ratio was 1000 mg/kg bw/day (no significant changes in albumin detected). In (Craig), the LOAEL for changes in albumin and globulin was 50 mg/kg bw/day in males and 500 mg/kg bw/day in females, whereas the LOAEL for A/G ratio was 1 mg/kg bw/day and 50 mg/kg bw/day for males and females respectively.

Chronic exposure in rats

Relevant effects at interim sacrifice include 10% increase in albumin (3.84-44.5 mg/kg bw/day). Furthermore, both males and females illustrated 10% decrease in globulin (36.7-442 mg/kg bw/day for both sexes, and 10% increase in A/G ratio (0.426-30.8 mg/kg bw/day and 7.03-271 mg/kg bw/day respectively) at interim sacrifice (Figure 23). Last, for male and female rats, 5% decrease in absolute spleen weight (0.115-16.8 mg/kg bw/day for both sexes) was observed at final sacrifice (Figure 24). Effect dose ranges may also be observed in Figure 25 and Annex III.

In Craig (2013), the LOAEL for decreased globulin and increased A/G ratio was 1 mg/kg bw/day for male rats (no significant increase in albumin observed up to the highest dose tested). For females, the LOAEL for increased albumin, decreased globulin, and increased A/G ratio was 500 mg/kg bw/day. No statistically significant changes in spleen weight were observed.

4.5.2.2.5 Other effects

Chronic exposure in rats

A 10% increase in testes hyperplasia in male rats (7.27-64.8 mg/kg bw/day) was observed at final sacrifice.

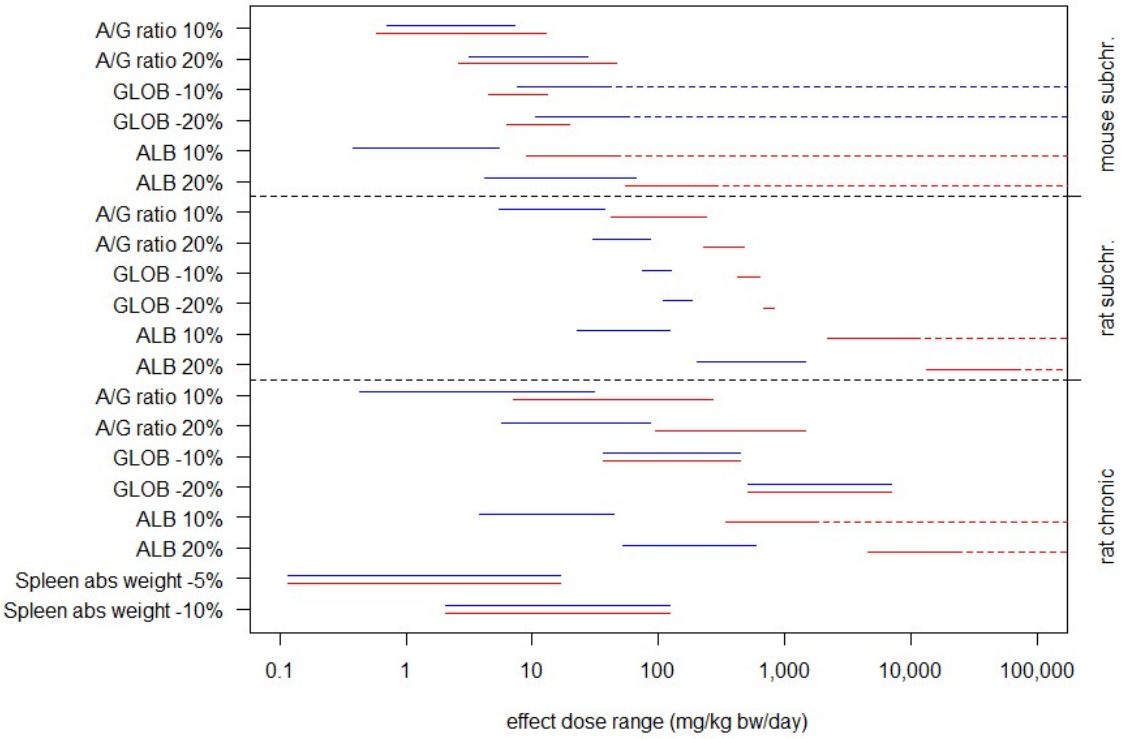

Figure 25: Overview of relevant effect dose ranges (EDL - EDU) for end-points related to the immune system (left y—axis), expressed in mg/kg bw/day on a log (10) scale (x-axis). Male effect dose ranges are plotted in blue and female effect dose ranges are plotted in red. Effect dose ranges for which the EDU reached up to infinity, are provided as partly dotted lines.

4.5.3 Human information

No data available.

4.5.4 Summary and discussion of repeated dose toxicity

Based on the available data, it may be concluded that the main target organs of FRD-902 include the liver, the kidney, the haematological system, and the immune system. The overall NOAEL resulting from the studies is 0.1 mg/kg bw/day, based on an increase in A/G ratio observed in male rats dosed 1 mg/kg bw/day for two years

Liver

The liver is the main target organ following from exposure to FRD-902 via the oral route. It is suggested that the observed effects in the liver could be explained by peroxisome proliferation. Stimulation of peroxisome proliferation via the PPARα receptor, especially occurring in the liver, is known to cause liver toxicity and neoplastic lesions in rodents, with unknown relevance for human health. Further consideration on the mechanisms by which HFPO-DA could induce these effects is provided in the section on the carcinogenicity of the substance.

Mice and rats showed increased (relative) liver weight upon exposure to the substance varying from 0.5 to 1000 mg/kg bw/day under a subchronic exposure regimen. Also in a chronic study relative increases in liver weight were observed during interim sacrifice in males dosed at 50 mg/kg bw/day, but these changes were less severe than observed in the subchronic studies

(15% compared to 163% in males dosed with 30 mg/kg bw/day for 28 days) (Craig, 2013, Caverly Rae et al., 2015). At terminal sacrifice, liver weights of the high-dosed males in the chronic study did not significantly increase from control. Conversely, in the high-dosed females in the chronic study, relative liver weights were significantly increased from control.

Clinical chemistry findings confirm the observed liver damage. Significant increases in serum liver enzymes were observed in mice and in rats. Changes in male mice include ALT (420%-1254%), AST (106%-478%), ALP (1134%-1221%) and SDH (1134-1221%) in the 28 and 90 day studies respectively (Haas, 2008b, MacKenzie, 2010). Increases in some of these parameters (ALT, ALP, SDH) were less elevated in female mice, and remained below 200%. Rats show overall smaller increases for these parameters in the subchronic studies. Notably, in the chronic study, the 50 mg/kg bw/day dosed males illustrated increases in ALT (228%), ALP (180%) and SDH (140%) at interim sacrifice (1 year) (Craig, 2013, Caverly Rae et al., 2015). Furthermore, a structural decrease in cholesterol serum concentrations was observed upon exposure to FRD-902 in all studies, as much as 30.9% in male and female rats exposed for 90 days at 100 and 1000 mg/kg bw/day respectively (Haas, 2009).

Microscopical changes include increases in liver hypertrophy in rats and mice of both sexes in almost all the studies, as low as 3 mg/kg bw/day and 0.5 mg/kg bw/day in male rats and mice under subchronic exposure regimen respectively (Haas, 2008a, Edwards, 2010a). These effects were repeatedly accompanied by single-cell (multifocal) and/or hepatocellular necrosis at doses between 0.5-30 mg/kg bw/day for mice (Edwards, 2010a, Haas, 2008b) and 30-1000 mg/kg bw/day for rats (Haas, 2008a, Haas, 2009, Edwards, 2010b, Craig, 2013). The lowest observed treatment-related effects for the liver therefore include liver hypertrophy and single-cell necrosis in male mice in the reproduction/developmental screening study and the 28-day study at 0.5-3 mg/kg bw/day (Edwards, 2010a, Haas, 2008b). In addition, Wang et al. (2017) observed steatosis of the liver in mice dosed at 1 mg/kg bw/day for 28-days. The severity of this effect was not indicated. It is not clear whether steatosis occurred in the other studies as well, as steatosis was not taken into consideration in these studies.

By means of dose response modelling, an overview of equipotent effect doses is provided. In the subchronic mouse study, male mice show that histopathological effects and increases in liver enzymes occur with relevant effect doses starting from 0.3-1.6 mg/kg bw/day. In the subchronic rat study dose-response modelling illustrates that also the lower limit of the 50% effect dose in ALT lies around 100 mg/kg bw/day, and that various toxicologically relevant effects on liver end-points, e.g. 50% increase in ALP, 10% increase in liver weight and 10% extra risk in minimal hyperthropy, occur well below 100 mg/kg bw/day. In the chronic rat study, the LOAEL for most of the observed liver effects in females was 500 mg/kg bw/day (the highest dose), whereas at 50 mg/kg bw/day (middle dose) almost no statistically significant increases were observed. The same holds for males, for which the LOAEL for most observed liver effects was 50 mg/kg bw/day (the highest dose), whereas there were almost no statistically significant effects at 1 mg/kg bw/day (middle dose). Dose response modelling illustrates that for males and females, the EDLs for relevant liver effects are substantially lower than 50 mg/kg bw/day.

Kidney

Increased kidney weight was observed in mice and rats of both sexes treated with doses varying from 5 to 1000 mg/kg bw/day FRD-902 under a subchronic dosing regimen (Edwards, 2010a, Edwards, 2010b, Haas, 2008a, Haas, 2009). In the 28-day study in mice, kidney hypertrophy was observed in males at 30 mg/kg bw/day (Haas, 2008a). Furthermore, in male mice dosed for 85 days in the reproduction/developmental screening study, increased incidence of kidney tubular cell hypertrophy was observed at 0.5 mg/kg bw/day and 5 mg/kg bw/day. Microscopically observed kidney damage was present at 1000 and 500 mg/kg bw/day in female rats in the 90-day and 2-year study respectively. Additionally, in several rat studies, increases in BUN were observed in 30-100 mg/kg bw/day dosed males (Haas, 2008a, Haas, 2009, Craig, 2013).

Although kidney effects are observed upon exposure to FRD-902 (i.e. increased kidney weight, kidney hypertrophy, increases in BUN, microscopically observed kidney damage), these effects generally occur at higher dosages than the observed liver effects. Increased kidney weight was accompanied with increased BUN in several studies, but in the cases where kidney hypertrophy was observed, this was not accompanied by histopathological changes in the kidney, apart from the 500 mg/kg bw/day dosed females in the chronic study (Craig, 2013). Therefore, the biological relevance of the increases in BUN and the kidney hypertrophy without any microscopically observed kidney damage is unclear.

In the subchronic mouse study, dose response modelling indicates that 10% increased absolute kidney weight is observed with EDLs being 1.33 mg/kg bw/day and 2.53 mg/kg bw/day for males and females respectively. The EDL for kidney tubular cell hypertrophy is also within this range (1.59 mg/kg bw/day). Dose response modelling of the data from the subchronic rat studies illustrate that EDLs for increases in BUN and absolute and relative kidney weights are in the range 11.6-40.3 mg/kg bw/day. In Craig (2013), the chronic rat study, the LOAEL for kidney effects was 500 mg/kg bw/day in female rats, whereas in males no kidney effects were observed, apart from increases in BUN, for which the LOAEL was 50 mg/kg bw/day. Based on dose-response modelling may however be concluded that the EDL for kidney effects in male and female rats ranges between 1.9 and 133 mg/kg bw/day.

Haematological system

Changes in the haematological system include red cell mass reduction (4% - 28%), decreased haemoglobin (5% - 21%), and decreased haematocrit (5% - 18%) in mice and rats dosed with 3-1000 mg/kg bw/day FRD-902. Furthermore, increases in absolute reticulocytes (67% and 390%) were observed for male and female rats exposed to FRD-902 for 90 days at 100 mg/kg bw/day and 1000 mg/kg bw/day respectively (Haas, 2008a). Other observations include increased platelets in male rats (17%) and male mice (26%) exposed at 100 mg/kg bw/day and 0.5 mg/kg bw/day (Haas, 2009, MacKenzie, 2010), and decreases in basophils in male rats (25%) and female rats (33%) exposed to FRD-902 for 90 days at 10 mg/kg bw/day and 1000 mg/kg bw/day respectively (Haas, 2009). These effects suggest that exposure to FRD-902 may promote anaemia.

These changes were overall relatively mild, with parameters not exceeding 10% change from control up to dosages of 50 mg/kg bw/day in a chronic study in rats. However, data from female rats dosed at 1000 mg/kg bw/day under a subchronic exposure regimen illustrate that FRD-902 may promote severe anaemic conditions (Haas, 2009).

In the subchronic mouse studyMacKenzie (2010), dose–response analysis of the male results showed 10% decrease in red blood cell count (8.28-299 mg/kg bw/day), 10% increase in MCV (5.8-10.3 mg/kg bw/day), 10% increase in total reticulocytes (3.32-7.47 mg/kg bw/day), and 10% increase in platelets (0.085-19.9 mg/kg bw/day). Additionally, 10% increase in MCHC was observed for both sexes (6.9-258 mg/kg bw/day and 6.65-827 mg/kg bw/day for males and females respectively). In the subchronic rat studies dose response modelling illustrates that the EDL for relevant effects overall occur in the dose range 50-200 mg/kg bw/day. In the chronic rat study, females illustrated statistically significant changes in blood parameters at a LOAEL of 500 mg/kg bw/day (highest dose tested), apart from decreases in red blood cell count with a LOAEL of 50 mg/kg bw/day. For males, no significant increases were observed up to the highest dose tested (50 mg/kg bw/day). Dose response modelling illustrates that the EDL for relevant haematological effects lies within the dose interval 50-500 mg/kg bw/day. Due to the lack of a dose group above 5 mg/kg bw/day in males it cannot be excluded that FRD-902 is equally potent in males and females, and thus has the same ED (range) in both sexes.

Immune system

In many studies, increased albumin and/or decreased globulin, and associated increases in A/G ratio occurred in mice and rats of both sexes administered with 1-500 mg/kg bw/day FRD-902

for 12 months or less. Decreased globulin and corresponding increases in A/G ratio are considered early signs of potentially reduced immune function. Furthermore, Rushing et al. (2017) observed suppression of the T cell-dependent antibody response (TDAR) in females and increased T lymphocyte numbers (but no suppression of TDAR) in males exposed to the substance at a dose of 100 mg/kg bw/day for 28 days.

In addition to this, in the mouse 90-day study relative spleen weight was reduced at 0.5 and 5 mg/kg bw/day (21% and 18%), but this finding was reported not to be treatment-related (MacKenzie, 2010). However, a recently conducted study by Rushing et al. (2017) also reports relative decreased spleen weight (11.3%) at 100 mg/kg bw/day in female mice, and considers this a treatment related effect. Additionally, dose response modelling also illustrated a decrease in absolute spleen weight in male and female rats exposed to FRD-902 under a chronic exposure regimen (Craig, 2013).

Dose-response analysis of the subchronic mouse data shows that relevant effects include 10% increase in albumin in males (0.376-5.41 mg/kg bw/day), 10% decrease in globulin in females (4.46-13.3 mg/kg bw/day), and 10% increase in A/G ratio in both sexes (0.713-7.26 mg/kg bw/day and 0.584-12.7 mg/kg bw/day for males and females, respectively. Relevant effects from the subchronic rat studies include 10% increase in albumin in males (22.5-124 mg/kg bw/day), 10% decrease in globulin in both sexes (74-125 mg/kg bw/day and 419-631 mg/kg bw/day for males and females respectively), and 10% increase in A/G ratio for both sexes (5.46-37.2 mg/kg bw/day and 41.8-238 mg/kg bw/day for males and females, respectively).
In the chronic rat study effects at interim sacrifice include 10% increase in albumin (3.84-44.5 mg/kg bw/day). Furthermore, both males and females illustrated 10% decrease in globulin (36.7-442 mg/kg bw/day for both sexes, and 10% increase in A/G ratio (0.426-30.8 mg/kg bw/day and 7.03-271 mg/kg bw/day respectively) at interim sacrifice. Last, for male and female rats, 5% decrease in absolute spleen weight (0.115-16.8 mg/kg bw/day for both sexes) was observed at final sacrifice.

4.6 Mutagenicity

4.6.1 Non-human information

4.6.1.1 In vitro data

In two Ames tests with different species of prokaryotes according to OECD TG 471 (Donner, 2008, Myhre, 2008) dosed up to 5000 ug/plate using plate incorporation, FRD-902 was negative with and without metabolic activation. In a mammalian cell gene mutation assay according to OECD TG 476 (Clarke, 2008) in which the pH was adjusted to neutral, FRD-902 was negative with and without metabolic activation. In two in vitro mammalian chromosome aberration tests according to OECD TG 473, FRD-902 was negative after 4 and 20 hour exposure without metabolic activation but positive after 4 hour exposure with metabolic activation at the highest exposure level of 3471 ug/ml (Glover, 2008, Glatt, 2009).

4.6.1.2 In vivo data

In a mouse micronucleus test according to OECD TG 474 (Gudi and Krsmanovic, 2007) at dose levels up to 1300 mg FRD-902/kg bw by gavage, a reduction in PCE/EC was observed in the bone marrow, showing that the substance reached the bone marrow, but no increase in micronucleated PCE. Some mortality was observed at the highest dose. In a mouse chromosome aberration test according to OECD TG 475 (Gudi and Krsmanovic, 2007) at dose levels up to 1300 mg FRD-902/kg bw by gavage, a decrease in the mitotic index of bone marrow cells was observed but no increase in structural or numerical chromosome aberrations. Some mortality was observed at the highest dose. In a rat unscheduled DNA synthesis test according to OECD TG 486 at dose levels up to 2000 mg FRD-902/kg bw by gavage, no increase in net grains per nucleus was observed (Pant and Sly, 2007).

4.6.2 Human information

No data available.

4.6.3 Summary and discussion of mutagenicity

The available in vitro and in vivo genetic toxicity and mutagenicity studies show that FRD-902 is not mutagenic. EFSA (2009) concluded that FRD-902 is non-genotoxic based on the same dataset.

4.7 Carcinogenicity

4.7.1 Non-human information

4.7.1.1 Carcinogenicity: oral

In a combined chronic and carcinogenicity study performed according to OECD TG 453, 80 rats per dose and sex were exposed to FRD-902 (purity 84%) by gavage (water). The dose levels were males: 0, 0.1, 1, 50 mg/kg bw/day and females: 0, 1, 50, 500 mg/kg bw/day. Interim necropsy was performed on 10 animals after 12 months. The remaining animals were necropsied after 101 weeks (females) or 104 weeks (males) (Caverly Rae et al., 2015, Craig, 2013).

Table 32: Tumour incidences and related histological changes in the OECD 453 study in rats.

Tumour type	Sex	Hist control	Control	Low	Mid	High
Hepatocellular adenoma	Females	0-5%	0	0	0	11 (15.7%)*
	Males		1	2	1	1
Hepatocellular carcinoma	Females	0-1.7%	0	0	0	4 (5.7%)*
	Males		1	0	0	2
Pancreatic acinar cell adenoma	Males	0-5%	0	1	0	3 (4.3%)
Pancreatic acinar cell carcinoma	Males	0-1.7%	0	0	0	2 (2.8%)
Combined acinar cell tumours	Males		0	1	0	5*
Interstitial cell adenoma testes	Males	0-8.3%	4	4	1	8 (11.43%)
Interstitial cell hyperplasia	Males	0-8.3%	7	7	3	15 (21.4%)
Uterine stromal polyps	Females	0-13.8%	1	2	1	7 (10%)*

* Statistically significant (p = 0.05) in at least 2/3 statistical tests.

The study demonstrated statistically significant induction of adenomas/carcinomas in the pancreas in males at 50 mg/kg bw/day, statistically significant induction of hepatocellular adenomas and carcinomas in females at 500 mg/kg bw/day, and increased incidence of Leydig cell tumours in the testes at 50 mg/kg bw/day (Table 32). The incidence of Leydig cell tumours was not statistically significant, amongst others due to a relatively high incidence of these lesions in the controls. The incidence of interstitial cell hyperplasia was increased at 50 mg/kg bw/day and outside the historical control range. One interstitial cell adenoma was also present in one male in this group at the interim necropsy. The increase in uterus stromal polyps was within the range of the historical controls, and therefore it is uncertain whether this statistical significant increase in polyps is substance related.

The NOAEL for carcinogenicity is 1 mg/kg bw/day in males based on an increase in combined adenoma and carcinoma of the pancreas and 50 mg/kg bw/day in females based on an increase in liver tumours at 500 mg/kg bw/day.

4.7.1.2 Carcinogenicity: inhalation

No data available.

4.7.1.3 Carcinogenicity: dermal

No data available.

4.7.1.4 Carcinogenicity: other routes

No data available.

4.7.2 Human information

No data available.

4.7.3 Summary and discussion of carcinogenicity

The study demonstrated statistically significant induction of adenomas/carcinomas in the pancreas in males at 50 mg/kg bw/day, statistically significant induction of hepatocellular adenomas and carcinomas in females at 500 mg/kg bw/day, and increased incidence of Leydig cell tumours in the testes at 50 mg/kg bw/day.

It was suggested by the study authors (Caverly Rae et al., 2015) that the observed increase in tumours was induced via the non-genotoxic PPARα mode of action, which is specific for rodents. Although the available data do indicate a non-genotoxic mechanism, it cannot be excluded that another mechanism may be responsible for the observed liver tumours. Furthermore, it is uncertain whether PPARα activation is responsible for the Leydig cell- and pancreatic acinar tumours, as the underlying mode of action for these tumour types remains unclear. Therefore the substance is considered to be potentially carcinogenic to humans.

PPARα is the most extensively studied signal pathway behind PFOA induced carcinogenicity, and it therefore is one of the primary suggested modes of action underlying the observed liver carcinogenicity in rats (US-EPA, 2016). However, studies report that PFOA may induce tumours via mechanisms other than PPARα in the liver, pancreas, and/or testes (Benninghoff et al., 2011, Benninghoff et al., 2012, Buhrke et al., 2015, Rosen et al., 2017, Cheng and Klaassen, 2008, Rosen et al., 2008b, Ren et al., 2009, Abe et al., 2017, Scharmach et al., 2012).

PFOA and HFPO-DA show similar neoplastic changes upon exposure (Caverly Rae et al., 2015, Butenhoff et al., 2012, Biegel et al., 2001). In 2011, ECHA's Risk Assessment Committee (RAC) concluded that, for APFO[12], the contribution to PFOA-induced liver tumours of molecular pathways other than PPARα cannot be fully excluded, the mode of action of pancreatic cell adenomas was unknown, and evidence was insufficient to link Leydig cell tumours to PPARα (ECHA, 2011). Consequently, APFO was classified as Carc. 2 under CLP Regulation (EC) No. 1272/2008. Also IARC concluded in 2016 that there was moderate evidence for the mechanisms underlying PFOA-induced carcinogenicity, among which some evidence with relevance to humans. Therefore, PFOA was evaluated by IARC as possibly carcinogenic to humans (IARC, 2016).

Based on the given, there is a concern that HFPO-DA is a human carcinogen as well. Data are currently insufficient to conclude on the substance its full carcinogenic potential. From the data presented in Section 4.5 can be concluded that overall, the registered substance shows a higher

[12] Ammonium pentadecafluorooctanoate; EC number 223-320-4; CAS number 3825-26-1

toxic potency in mice compared to rats. Consequently, there is a lack of data with regard to the carcinogenic potential of this substance in species other than the rat, and therefore the requested carcinogenicity study in mice is warranted. This is under investigation in an ongoing Substance Evaluation.

Liver

It is well established that PFOA and other PFASs are able to change the PPRAα-induced expression of genes associated with fatty acid oxidation, lipid transport, glucose metabolism, ketogenesis, cholesterol biosynthesis, steroid metabolism, bile acid synthesis, and inflammation (Rosen et al., 2008a). One of the modes of action proposed for the observed hepatic tumours therefore is activation of PPARα in the rodent liver (Corton et al., 2018). Upon PPARα stimulation by the agonist, several genes would be upregulated that are involved in cell cycle control and apoptosis, resulting in cell proliferation. Subsequently, formation of preneoplastic cells and new focal lesions would result in selective clonal growth of foci, which ultimately would develop in liver tumours (Klaunig et al., 2012). Exposure of PFOA, PFOS, PFHxS and PFNA in PPARα knockout mice illustrated that a large part of the biological response is regulated via PPARα (activation of PPARα-controlled genes varied between ~75% and ~90% for the latter mentioned chemicals) (Rosen et al., 2008b, Rosen et al., 2017).

PPARα induction as a relevant mechanism underlying human hepato carcinogenicity is still under debate (Corton et al., 2018, Guyton et al., 2009, Felter et al., 2018). It is known that the expression level of PPARα is about ten-fold lower in the human liver compared to that in rodents (Palmer et al., 1998). Additionally, PPARα-dependent mechanisms are illustrated to be less pronounced in non-human primates (Hoivik et al., 2004). However, research indicates that exposure to PFOA leads to liver toxicity in PPARα insensitive species and the liver effects observed are not confined to PPARα activation. This might also hold true for HFPO-DA. PFOA-induced liver toxicity was seen after 6-months oral administration in cynomolgus monkeys (Butenhoff et al., 2002), and PPARα knockout mice still exhibited PFOA-mediated effects in the liver after oral administration for 7 consecutive days, such as accumulation of cytoplasmic vacuoles in hepatocytes and hepatocyte proliferation (Wolf et al., 2008). RAC concluded in 2011 that contribution to PFOA-induced liver tumours of molecular mechanisms other than PPARα cannot be fully excluded (ECHA, 2011). That is to say, it is doubted whether PPARα is the only mode of action by which PFOA may cause liver cancer.

Gene expression patterns of PPARα knockout mice treated with PFOA, PFOS, PFHxS and PFNA showed that about 11-24% of the regulated genes activated by PFASs act in a PPARα-independent manner (Rosen et al., 2008b, Rosen et al., 2017). In comparison, only 6% of the genes activated by the PPARα agonist DEHP were independent of PPARα regulation, and for the PPARα agonist WY-14,643 this was only 2% (Ren et al., 2010, Rosen et al., 2017). Thus, among PPARα agonists, there is difference in how many, and the degree by which they are able to activate PPARα-independent genes. This complicates comparison of biological effects induced by chemicals that are known to activate PPARα, such as fibrates, phthalate esters, and per- and polyfluorinated substances (PFASs).

Alternative mechanisms have been suggested by which PFASs could induce liver cancer. Studies have shown that PFASs affect the estrogen receptor α (ERα) (Benninghoff et al., 2011, Benninghoff et al., 2012, Buhrke et al., 2015, Rosen et al., 2017), that PFOA, HFPO-TA and HFPO-DA activate PPARγ (Li et al., 2019, Vanden Heuvel et al., 2006), that PFOA activates constitutive androstane receptor (CAR), and pregnane X receptor (PXR) (Cheng and Klaassen, 2008, Rosen et al., 2008b, Ren et al., 2009, Abe et al., 2017), and that PFOA inhibits the function of hepatocyte nuclear factor 4α (HNF4α) (Scharmach et al., 2012). These findings indicate that tumour formation upon exposure to PFOA via other MoAs than PPARα cannot be excluded, and a similar conclusion might hold true for HFPO-DA. Therefore, the formation of liver tumours upon exposure to HFPO-DA in the rat should be considered relevant for humans.

Research has suggested a role for CAR in the observed PFOA-induced liver tumours. *Cyp2b10*

mRNA levels were not increased in CAR knockout mice after exposure to PFOA but were increased in wild-type mice, and CAR nuclear translocation was stimulated in these wild-type mice as well (Abe et al., 2017). Activation of CAR generally leads to liver tumours in rodents (Elcombe et al., 2014). Upon CAR activation, altered gene expression is observed in a large number of genes responsible for phase I and II xenobiotic metabolism, cell proliferation, apoptosis, and energy metabolism in the rodent liver. Activation of CAR would result in enhanced cell proliferation in altered hepatic foci, subsequently followed by formation of liver adenomas and carcinomas (Elcombe et al., 2014). Studies in *Pparα-null* mice suggest that CAR is expressed to a higher extent in these animals compared to wild-type mice after treatment with PFOA, PFOS, and PFNA. The increased activation of CAR in the absence of PPARα suggests competition between these two receptors for shared co-activators (Corton et al., 2018). It therefore cannot be excluded that in species where PPARα is less activated, the CAR pathway may be more pronounced, and hence the substance could lead to liver tumorigenesis in that way.

Recently, (Li et al., 2019) illustrated that HFPO-TA, PFOA, and HFPO-DA are able to bind the nuclear receptor PPARγ and show agonistic activity in the order HFPO-TA > PFOA > HFPO-DA. Additionally, PPARγ is stimulated upon exposure to PFASs in *Pparα-null* mice, and PFASs furthermore are reported to activate human PPARγ in the US-EPA ToxCast program (Rosen et al., 2017). This receptor is primarily expressed in brown and white adipose tissue, the spleen, and the large intestine. It regulates a large variety of cell processes and functioning, such as insulin sensitivity, glucose and lipid metabolism, fat distribution, cell proliferation, and cell differentiation. A tumorigenic role for PPARγ has been assigned in bladder tumours, renal pelvic tumours, hemangioma, lipoma, skin fibrosarcoma, mammary adenocarcinoma, and hepatic tumours. The mechanisms by which PPARγ could induce carcinogenicity have not been elucidated, and some studies even suggest that PPARγ-ligands function as anti-tumorigenic agents in PPARγ-expressing tumours. PPARγ and its ligands behave ambivalently, either inhibiting or promoting cell apoptosis and proliferation in different cell types, cell lines, and concentrations of PPARγ ligands (Yousefnia et al., 2018). HFPO-TA, HFPO-DA and PFOA showed agonistic effects towards both human and mouse PPARγ *in vitro*, with HFPO-TA showing higher transcriptional activity in human PPARγ compared to mouse PPARγ (Li et al., 2019). Additionally, PPARγ showed the same sensitivity to phthalate monoesters in humans and mice (Bility et al., 2004). This evidence suggests activation of PPARγ upon exposure to PFASs, without large interspecies differences in terms of receptor sensitivity.

PFASs have also been associated with disruption of the endocrine system *in vivo*, as PFOA has been reported to cause decreased testosterone and increased estradiol levels in mice (Biegel et al., 1995, Biegel et al., 2001). This is suggested to be facilitated either via interaction with the estrogen or androgen receptor directly, or via disruption of steroid hormone biosynthesis (Behr et al., 2018). Benninghoff et al. (2011) studied exposure of PFASs in rainbow trout. The rainbow trout is considered a useful animal model to study the PPARα independent mechanisms underlying the potential carcinogenicity of PFASs, as rainbow trout mimic the human insensitivity to PPARα. Benninghoff and co-workers illustrated that the PFASs had weak to very weak affinity to bind to ERα in humans, mice, and rats *in vitro*. Additionally, they illustrated that PFASs (PFOA, PFNA, PFDA, and PFOS) enhanced liver carcinogenesis in rainbow trout *in vivo* via interaction with the hepatic oestrogen receptor, whereas the PPARα agonist clofibrate failed to enhance such a response (Benninghoff et al., 2011). However, conflicting results have been reported concerning the interaction of PFASs with ERα, as a recent study illustrated endocrine effects of PFASs in human cells *in vitro,* but not at concentrations relevant for human exposure (Behr et al., 2018). Still, PFASs were shown to impact cholesterol biosynthesis, potentially via a PPARα-mediated mechanism, which also indirectly may affect steroidogenesis and hormone homeostasis (Buhrke et al., 2015).

PFOA showed to have an inhibitory effect on HNF4α in human HepG2 cells (Scharmach et al., 2012). This nuclear receptor is involved in embryogenesis and liver development, energy homeostasis, and glucose-, lipids-, and amino acids metabolism, and is expressed in the kidney, liver, small intestine, colon, and pancreatic β cells. HNF4α is constitutively active, and does not require ligand-binding to be activated. Cancer is associated with decreased expression of HNF4α

in organs in which the receptor normally is expressed. For instance, suppression of HNF4α has been reported to transform slow-growing hepato carcinoma into fast-growing de-differentiated hepato carcinoma, indicating that inhibition of HNF4α affects cell differentiation and stimulates cell proliferation in the liver (Walesky and Apte, 2015). Therefore, this receptor could potentially have a role in PFOA-induced (and thus also potentially HFPO-DA-induced) liver cancer as well.

Pancreas

For PFOA as well as HFPO-DA, the mechanism behind the induced pancreatic acinar cell tumours is not understood, and available data are limited. A proposed mode of action involves activation of PPARα in the liver causing decreased bile acid flow and/or altered bile acid composition, which subsequently would result in hepatic cholestasis. Secondary to cholestasis, decreased bile acid synthesis would lead to an increase in serum levels of the growth factor cholecystokinin (CCK, cholecystokinin-33 in humans), causing acinar cell proliferation with development of acinar cell tumours as a result (Klaunig et al., 2012). However, altered testosterone and estradiol levels, CCK expression, CCKA receptor overexpression, and a high fat diet are also mentioned as factors having impact on the pancreatic acinar cell hypertrophy, hyperplasia, and tumours observed in the rat. Data supporting this mode of action for PFOA remain confined to the observations that PFOA increased biliary excretion (Minata et al., 2010) and altered expression of bile transporters (OATPs and MRPs) in mice (Cheng and Klaassen, 2008, Maher et al., 2008).

Furthermore, as explained in the above text, PFASs are able to stimulate various other molecular pathways that could lead to tumorigenesis in the liver. The importance these molecular pathways for the development of pancreatic acinar cell adenoma and carcinoma is not known, but at least expression of the receptors CAR, PPARγ, ERα, and HPF4α is not confined to the liver. However, the limitations in available data on the mechanism(s) underlying the pancreatic acinar cell tumours preclude any conclusion concerning the interspecies differences for the observed HFPO-DA-induced tumours in the pancreas, and therefore the observations should be considered relevant for human health.

Testes

The link between PPARα and Leydig cell tumours remains uncertain (Klaunig et al., 2012), but the modulation of testosterone levels is suggested to play an important role in promotion of cell proliferation and testicular Leydig cell tumour development (ECHA, 2011, Klaunig et al., 2012, Sun et al., 2018). Studies have shown that PFASs affect the oestrogen receptor ERα (Benninghoff et al., 2011, Benninghoff et al., 2012, Buhrke et al., 2015, Rosen et al., 2017), and have reported that increases in testicular interstitial fluid estradiol and TGFα as well as affected Leydig cell functioning potentially contribute to Leydig cell adenomas (Biegel et al., 1995). These results suggest a hormone interference mode of action for PFOA, based on potential effects of steroid hormone synthesis. Based on the given, exposure to HFPO-DA could lead to testicular cancer via a non-PPARα related mechanism, and should be considered relevant to the human situation.

In conclusion, beyond the question on whether the biological responses observed in rodents related to the activation of PPARα are relevant to assess the carcinogenic potential in humans, contribution of other pathways to tumour development after exposure to HFPO-DA cannot be ruled out. Consequently, it is not certain whether PPARα is required for tumorigenesis in either rodents or humans.

4.8 Toxicity for reproduction

4.8.1 Effects on fertility

4.8.1.1 Non-human information

In a reproduction/developmental screening test according to OECD TG 421, Crl:CD1(ICR) mice (N = 25) were exposed by oral gavage at dose levels of 0, 0.1, 0.5 and 5 mg FRD-902/kg bw/day (purity 84%) (Edwards, 2010a). The F0 males were dosed during study days 0 to 84 (70 days prior to pairing through 1 day prior to euthanasia), for a total of 84 to 85 doses. The females that delivered (with the exception of those females selected for toxicokinetic evaluation) were dosed from study day 56 through the day prior to euthanasia (14 days prior to pairing through lactation day 20) for a total of 53 to 64 doses. The females that were selected for toxicokinetic evaluation were dosed through the day of euthanasia (lactation day 21) for a total of 54 to 65 doses.

No effect on mortality was observed for the parental animals. In both sexes increases in liver weight and liver hypertrophy were observed. Furthermore, incidences of single cell necrosis were observed in males and females in all dose groups, with 24/24 males and 21/24 females exhibiting single cell necrosis at 5 mg/kg bw/day. Females also illustrated increased incidences of focal/multifocal necrosis at the middle- (3/24) and high dose group (5/24) respectively. Additionally, in males, kidney weights increased 8% at 5 mg/kg bw/day, which correlated with increased kidney tubular cell hypertrophy at doses 0.5 mg/kg bw/day (6/24) and 5 mg/kg bw/day (18/24). In females, absolute and relative kidney weights were increases with 21% and 10% at 5 mg/kg bw/day.

No effect on reproductive performance was reported. Sex ratio, survival, and physical condition of the F1 pups was unaffected at all dose levels. However, F1 animals of both sexes showed decreased mean body weight during the pre-weaning period. At 5 mg/kg bw/day, a reduction in mean body weight gain of the male pups was observed during weaning, but not for the female pups (only a decreased mean body weight was observed in the pre-weaning period). Serum concentrations of parental animals and pups indicate limited transfer of FRD-902 via lactation.

Based on these results, the NOAEL for reproductive toxicity was 5 mg/kg bw/day, as no effects on reproduction were observed at any of the doses levels tested. The NOAEL for systemic toxicity in parental animals was 0.1 mg/kg bw/day based on the incidences of hepatic single cell necrosis observed in males at 0.5 mg/kg bw/day. The NOAEL for systemic toxicity in the offspring was 0.5 mg/kg bw/day based on body weight decrements in the F1 males and females in the 5 mg/kg bw/day group during the pre-weaning period.

However, the dossier submitter is of the opinion that the results from this study in mice do not allow for final conclusions regarding the reproductive effects because the highest dose level tested only exerted minimal effects in the parental animals. Therefore, information is regarded inconclusive with respect to potential effects to the reproductive system.

4.8.1.2 Human information

No data available.

4.8.2 Developmental toxicity

4.8.2.1 Non-human information

A study on developmental toxicity (developmental toxicity/teratogenicity) was conducted in rats, according to OECD Guideline 414 (Edwards, 2010b). Pregnant rats were exposed to FRD-902 (84% purity) at 0, 10, 100, or 1000 mg/kg bw/day by oral gavage during Gestation days 6-20.

Dams were sacrificed on Gestation day 21. Organs including the ovaries and uterus, and foetuses were examined.

One female in the highest dose group died on GD 20, due to liver and kidney damage. Four and 9 females in the 100 and 1000 mg/kg bw/day groups, respectively, delivered early on gestation day 21. The mortality in the 1000 mg/kg bw/day group and early deliveries in the 100 and 1000 mg/kg/day groups were considered test substance-related.

Test-substance related clinical findings (yellow material on various body surfaces, salivation), higher mean kidney weight, and reduction in maternal body weight gains were observed at 1000 mg/kg bw/day. Decreased gravid uterine weights were found in the 100 and 1000 mg/kg bw/day groups. Furthermore, an increase in liver weight was reported in the animals dosed at 100 and 1000 mg/kg bw/day, accompanied by focal necrosis in some animals at these dose groups. Also liver hypertrophy occurred at 1000 mg/kg bw/day, and two females showed an oedematous pancreas in this dose group.

At 100 and 1000 mg/kg bw/day, mean foetal weight was reduced by 8.8% and 28.1% respectively. No effects were found on foetal survival, on malformations or on variations, besides a higher incidence of 14th rudimentary ribs at 1000 mg/kg bw/day.

A second study was conducted to verify the apparent dose-related early deliveries in the dams on gestation day 21. This study used the same experimental design but was confined to a control group and a group dosed at 1000 mg/kg bw/day. The increase in early deliveries was confirmed this study, as three early deliveries were observed in an unknown number of dams versus none in the controls. Also the foetal weight was decreased. In addition, comparable maternal effects were observed as in the above study.

The NOAEL for maternal toxicity is considered to be 10 mg/kg bw/day, based on mortality, lower mean body weight gains and food consumption at 1000 mg/kg bw/day, and early deliveries, and microscopic findings in the liver (focal necrosis) at 100 and 1000 mg/kg bw/day. The NOAEL for developmental toxicity is 10 mg/kg bw/day, based on early deliveries and lower mean foetal weights at 100 and 1000 mg/kg bw/day.

4.8.2.2 Human information

No data available.

4.8.3 Summary and discussion of reproductive toxicity

In the available reproduction/developmental screening study, FRD-902 did not cause any reproductive effects. The NOAEL for reproductive toxicity is set at 5 mg/kg bw/day, as no effects on reproduction were observed at any of the doses levels tested. In the parental animals, liver single-cell necrosis was observed in males at 0.5 mg/kg bw/day, in concordance with the effects observed in the subchronic and chronic toxicity studies. Furthermore, F1 animals of both sexes showed decreased mean body weight during the pre-weaning period. However, the dossier submitter is of the opinion that the results from the reproduction/developmental screening study in mice do not allow for final conclusions regarding the reproductive effects because the highest dose level tested only exerted minimal effects in the parental animals. Therefore, information is regarded inconclusive with respect to potential effects to the reproductive system.

With regard to developmental toxicity, FRD-902 induced effects at 100 and 1000 mg/kg bw/day (i.e. early deliveries and lower mean foetal body weight). Maternal toxicity included mortality, lower mean body weight gains and decreased food consumption, decreased gravid uterine weight, higher mean kidney weight, liver hypertrophy, and microscopic changes in the liver at 1000 mg/kg bw/day. Also in the group exposed to 100 mg/kg bw/day, focal necrosis was

observed in 2/22 animals, and a mean decreased gravid uterine weight was reported.

4.9 Other effects

4.9.1 Non-human information

4.9.1.1 Neurotoxicity

Richards et al. (2018) reports an abstract of an ex vivo neurotoxicity study for FRD-902 using rat brain capillaries. The effect on the blood brain barrier (BBB) was examined by measuring the effect at FRD-902 test concentrations of 1, 10, 100 1000 nM on 3 well-established efflux transporters (P-gp, BCRP, MRP2). A concentration-related decrease in P-gp and BCRP activity was found, an effect which may reduce the protective function of the BBB. The authors state that future studies will investigate the mechanism underlying the decreased P-pg and BCRP transporter activity following in vivo exposure and the disposition and pharmacokinetics of FRD-902 at environmentally relevant doses.

4.9.1.2 Immunotoxicity

Rushing et al. (2017) studied the immune effects of FRD-903 in a subacute study in mice. Groups of 12 (6 m, 6 f) mice (C57BL/6) were given oral doses of 0, 1, 10 or 100 mg/kg bw/day via gavage for 28 days. Two replicates of this study were done, temporised 8 weeks apart. In one replicate of the study serum concentrations of FRD-903 were measured after 1, 5, 14 and 28 days. At day 24 all mice (both replicates) were immunised using SRBC (sheep red blood cells). SRBC-specific IgM antibody titres were determined in serum at test end (T-cell antibody response, TDAR). Splenic lymphocyte subpopulations were analysed at test end. One day after the final gavage dose the animals were killed and the weights of thymus, spleen and liver were determined. Livers were analysed for peroxisome proliferation (peroxisomal fatty acid oxidation, hepatic acyl CoA oxidase).

Relative liver weights were increased at 10 and 100 mg/kg bw/day (both sexes) and liver peroxisome proliferation (measurement of hepatic acyl CoA oxidase) was found at 10 and 100 mg/kg bw/day (males) or at 100 mg/kg bw/day only (females). A suppression of TDAR was found at 100 mg/kg bw/day (females only). T-lymphocyte numbers were increased (B-lymphocytes unchanged) at 100 mg/kg bw/day (males only). No effect on spleen weight was found in males but in females absolute and relative spleen weights were decreased at 100 mg/kg bw/day. The NOAEL for immunotoxicity in this study was 10 mg/kg bw/day. The authors of the study conclude that these observations are in line with parameters affected by PFOA, albeit FRD-902 appears to be less potent, and further studies are required to determine the full immunomodulatory profile of FRD-902 and possible synergism with other PFASs.

4.9.1.3 Endocrine disruption

No studies are available providing insight into potential endocrine (disrupting) mode of action for HFPO-DA, such as *in vivo* modulation of thyroid hormone (T3, T4, TSH), androgenic/estrogenic effects, or in *vitro* receptor binding studies.

The studies in Section 4.5 show that HFPO-DA caused decreased uterus weight in female rats (Haas, 2008a, Edwards, 2010b) and resulted in an increased number of females that were in the diestrus stage of the oestrous cycle (Haas, 2008a). However, ovarian morphology was similar in these animals compared to controls. Additionally, male mice had decreased mean weight of epididymides, but without any morphological changes observed (MacKenzie, 2010). These findings may provide some indications that HFPO-DA potentially could possess endocrine disrupting properties. However, it must be noted that these were incidental findings that were

not seen consistently throughout the conducted studies.

Despite the lack of information for HFPO-DA on endocrine mode of action, studies repeatedly have shown that PFASs affect the oestrogen receptor ERα (Benninghoff et al., 2011, Benninghoff et al., 2012, Buhrke et al., 2015, Rosen et al., 2017). Also, PFOA, PFNA, PFDA and their salts have been classified as either Toxic to Reproduction 1B or 2 by ECHA's Risk Assessment Committee (RAC) (ECHA, 2011, ECHA, 2014, ECHA, 2015b).

Both PFOA and HFPO-DA induced Leydig cell tumours in male rats (Cavalry Rae et al. 2015). The modulation of testosterone levels is suggested to play an important role in promotion of cell proliferation and testicular Leydig cell tumour development (ECHA, 2011, Klaunig et al., 2012, Sun et al., 2018). However, as stated above, there is no data available that either confirms or rejects that HFPO-DA could modulate testosterone levels.

No significant thyroid weight changes were observed in rodents upon treatment with HFPO-DA, consistent with findings for other PFAS, as thyroid morphology alterations do not seem to be a sensitive end-point. However, no final conclusion can be drawn with respect to treatment-affected thyroid functioning, since inconsistent results are reported in epidemiological and toxicity studies (ATSDR, 2018).

4.9.1.4 Specific investigations: other studies

Mechanistic studies

Wang et al. (2017) carried out an oral 28-days study in mice focused on the induction of liver effects by two test chemicals, i.e. FRD-903 and the tetramer [HFPO-TA]. A single dose level of 1 mg/kg bw/day was tested for both compounds in groups of 12 male ICR mice. Liver weights were increased in both groups, most markedly so in the tetramer group. Furthermore, ALP was increased in both compounds. AST and ALT were increased in the tetramer group only. Moreover, there was an increase in low-density lipoprotein cholesterol and decreases in total and direct bilirubin. Liver histopathology showed damage in both groups (hepatocellular hypertrophy, lipid droplet accumulation, swollen hepatocytes, and nuclei, steatosis, karyolysis) with the tetramer showing additional adverse effects such as focal cell necrosis, infiltration of inflammatory cells and vacuolar degeneration. The severity of these effects was not indicated. High throughput RNA-sequence data from liver tissues were generated to study the mechanism of the liver damage. In the dimer and tetramer groups 146 and 1295 transcripts, respectively, were changed, with lipid metabolism associated genes being dominant. Many genes of the PPAR-pathway were induced. The authors suggest that the fact that the effect by the tetramer was larger than that for the dimer, endorses the notion that PFAS chain length is an important variable for their hepatotoxicity.

Sheng et al. (2018) studied the in vitro cytotoxicity of FRD-903, the acid trimer of HFPO, and the acid tetramer of HFPO in human liver HL-7702 cell line and compared the results with those for PFOA and PFOS. In addition, the binding mode and affinity to human liver fatty acid binding protein (hL-FABP) was determined for each of these test compounds. The acid trimer and tetramer of HFPO showed greater cytotoxicity compared to PFOA and PFOS (no result presented for the acid dimer). Binding affinity to hL-FABP was lower than that for PFOS and PFOA for the acid dimer of HFPO, higher than PFOA (and equal to PFOS) for the acid trimer of HFPO and higher than both PFOS and PFOA for the acid tetramer of HFPO. The binding to hL-FABP in a 15-Å gorge was predicted with molecular docking, indicating that the HFPO-derived chemicals had greater binding activity. According to the study authors, their results suggest that the oxygen atom inserted in the molecule and the longer chain length are variables linked with a stronger effect on hepatic fatty acid metabolism.

Li et al. (2019) studied the binding affinity, agonistic activity, and adipogenesis activity of HFPO-DA, HFPO-TA and PFOA for the PPARγ receptor *in vitro*. In a competitive binding assay, all three chemicals showed binding affinity towards human and mouse PPARγ ligand binding domains

(LBDs) in a dose-dependent manner. HFPO-TA illustrated 4.8-7.5 fold higher binding affinity compared to PFOA (mouse and human PPARγ respectively), whereas HFPO-DA illustrated lower binding capacity compared to PFOA (IC50 of HFPO-DA was beyond detection). Furthermore, the binding of HFPO-DA, HFPO-TA, and PFOA was predicted with molecular docking. This analysis illustrated that all three compounds could form hydrogen bonds with human and mouse PPARγ, but HFPO-TA and PFOA could form more hydrogen bonds with human PPARγ compared to mouse PPARγ, whereas HFPO-DA could bind equally to human and mouse PPARγ. The authors conclude that this may explain the higher binding affinity of the chemicals towards human PPARγ *in vitro*.

Additionally, Li et al. (2019) note that HFPO-DA, HFPO-TA, and PFOA enhanced human and mouse PPARγ-mediated luciferase transcription activity in HEK 293 cells in a dose-dependent manner, with highest human PPARγ transcriptional activity of 1.2-, 2.7-, and 1.4-fold at 50 μM for HFPO-DA, HFPO-TA, and PFOA respectively. For all three chemicals, human PPARγ agonistic activity was higher compared to the mouse PPARγ agonistic activity. Lastly, HFPO-DA, HFPO-TA, and PFOA caused increased lipid accumulation and adipogenesis activity in primary human preadipocytes (HPA-s) and mouse 3T3-L1 preadipocytes *in vitro* upon 10 days of exposure in the order of HFPO-TA > PFOA > HFPO-DA, and caused significantly increased expression level of adipogenic genes in HPA-s and 3T3-L1 cells. All in all, these results show that all three chemicals bind to PPARγ, show agonistic activity, and cause increased adipogenesis activity, in the order HFPO-TA > PFOA > HFPO-DA, with adipogenesis being a more sensitive end-point in human preadipocytes compared to mouse preadipocytes.

Mixture toxicity

PFASs often occur together as contamination in soil, groundwater or drinking water. To be able to better assess the risks of this type of contamination, the RIVM investigated the extent to which it is possible to express the harmfulness of a number of PFASs in relation to PFOA. In Zeilmaker et al. (2018), so-called Relative Potency Factors (RPFs) were derived for several perfluorocarboxylic acids (PFCAs), perfluorosulphonic acids (PFSAs) and HFPO-DA, to allow for risk assessment of combined toxicity to a mixture of PFASs based on the principle of dose-addition (EFSA, 2008, EFSA, 2013). This concept is developed for several PFASs, assuming that they act in a similar manner, with the same mechanism/mode of action, resulting in dose-responses with the same shape but with different potencies for each of the individual substances (see Figure 26). Based on available subacute and subchronic oral toxicity studies in rodents, relative potency factors (RPFs) were derived for 20 individual PFASs, including HFPO-DA.

In principle, the RPF method scales the dose of each substance, according to its potency, to a dose of the Index (reference) Compound (IC), with the IC having a RPF equal to 1 (Table 33). In this specific case, the IC is PFOA. Combining the occurrence of each mixture component with its specific RPF value then expresses each of the mixture components in terms of IC equivalents. Summing over all mixture components then leads to mixture exposure expressed in term of IC equivalents. Subsequently, the latter can be compared with IC Health Based Guidance Value (HBGV), i.e. the HBGV of PFOA. Note that the methodology presented in this report is based on effects on the liver as observed in animal studies.

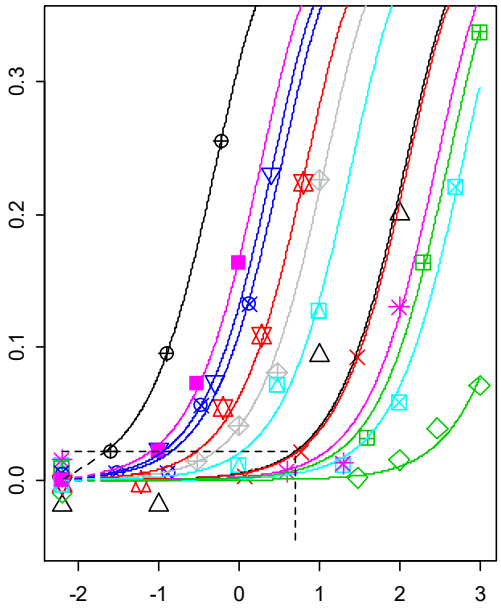

Figure 26: Dose responses of relative liver weight (in male rat) of a series of individual PFASs after normalising to background. The dose responses have the same shape, i.e. the curves are parallel on the log-dose scale, but each PFAS has a different potency: different doses are required to result in the same increase in response.

Hence, based on the proposed RPFs for 20 PFASs in Zeilmaker et al. (2018), the combined effect on the liver can be estimated. It must be noted that many more PFASs exist that might contribute as well. However, derivation of RPFs for these substances is not possible at this moment, as insufficient data are available.

Gomis et al. (2018) investigated to what extent distribution and elimination kinetics influence toxicological effect thresholds (which are expressed as administered dose) established for fluorinated alternatives and their predecessors (i.e. PFBA, PFBS, PFHxA, HFPO-DA, PFOA, PFOS). A male rat dynamic one compartment toxicokinetic (TK) model was used to calculate internal doses of these substances in serum and in liver, based on equipotent external doses derived from dose-response curves of liver enlargement in subchronic oral rat toxicity studies. The authors observed that converting external doses to internal serum and liver concentrations resulted in reduced variability in the dose-response curves of PFBA, PFHxA, PFOA and HFPO-DA. They concluded that HFPO-DA interacts more strongly with its target (i.e. has a higher toxic potency) than its predecessor PFOA when correcting for differences in toxicokinetics. More precisely, ranking the substances based on toxic potency resulted in HFPO-DA > PFOA> PFHxA > PFBA and HFPO-DA > PFOA ≈ PFHxA ≈ PFBA for internal concentrations in serum and liver respectively. For PFOS and PFBS, no differences in potency were observed based on internal and external doses.

Table 33: Relative Potency Factors (RPFs) derived for several PFCAs, PFSAs and HFPO-DA from relative liver weight data. RPFs values using PFOA as the Index Compound, obtained from Zeilmaker et al. (2018).

PFAS group	**PFAS**	**RPF**
PFSA	Perfluorobutanesulfonate (PFBS)	0.001
	Perfluoropentane sulfonic acid (PFPeS)	0.001 ≤ RPF ≤ 0.6
	Perfluorohexanesulfonate (PFHxS)	0.6
	Perfluoroheptane sulfonic acid (PFHpS)	0.6 ≤ RPF ≤ 2
	Perfluorooctanesulfonate (PFOS)	2
	Perfluorodecane sulfonic acid (PFDS)	2
PFCA	Perfluorobutyrate (PFBA)	0.05

PFAS group	PFAS	RPF
	Perfluoropentanoic acid (PFPeA)	0.01 ≤ RPF ≤ 0.05
	Perfluorohexanoate (PFHxA)	0.01
	Perfluoroheptanoic acid (PFHpA)	0.01 ≤ RPF ≤ 1
	Perfluorooctanoic acid (PFOA)	1
	Perfluorononaoic acid (PFNA)	10
	Perfluorodecanoic acid (PFDA)	4 ≤ RPF ≤ 10
	Perfluoroundecanoic acid (PFUnDA)	4
	Perfluorododecanoic acid (PFDoDA)	3
	Perfluorotridecanoid acid (PFTrDA)	0.3 ≤ RPF ≤ 3
	Perfluorotertadecanoic acid (PFTeDA)	0.3
	Perfluorohexadecanoic acid (PFHxDA)	0.02
	Perfluorooctadecanoic acid (PFODA)	0.02
PFECA	Hexafluoropropylene oxide-dimer acid (HFPO-DA)	0.06

4.9.2 Summary and discussion of other effects – human health

Richards et al. (2018) report an abstract of an ex vivo neurotoxicity study for FRD-902 using rat brain capillaries. The effect on the blood brain barrier (BBB) was examined by measuring the effect at FRD-902 on efflux transporters (P-gp, BCRP, MRP2). A concentration-related decrease in P-gp and BCRP activity was found, indicative of potential reduction of the protective function of the BBB.

Rushing et al. (2017) observed suppression of the T cell-dependent antibody response in females and increased T lymphocyte numbers in males exposed to the substance at a dose of 100 mg/kg bw/day for 28 days. The authors of the study conclude that these observations are in line with parameters affected by PFOA, albeit FRD-903 appears to be less potent, and further studies are required to determine the full immunomodulatory profile of FRD-903 and possible synergism with other PFAS compounds.

Wang et al. (2017) illustrated the effect of FRD-903 (HFPO dimer acid) upon the liver in mice, and furthermore conducted high-throughput RNA sequencing on these liver tissues. Liver weights were increased in both groups and liver histopathology showed damage (i.e. hepatocellular hypertrophy, lipid droplet accumulation, swollen hepatocytes, and nuclei, steatosis, karyolysis). In the liver tissue, 146 transcripts were changed, with lipid metabolism associated genes being dominant, and many genes of the PPAR-pathway were induced. The authors suggest that PFAS chain length is an important variable for hepatotoxicity.

Sheng et al. (2018) report that the in vitro cytotoxicity of FRD-903 and related compounds in human liver HL-7702 cell line, and the binding mode and affinity to human liver fatty acid binding protein (hL-FABP) was studied. However, in the study no cytotoxicity result was presented for FRD-903. Binding affinity to hL-FABP was lower for FRD-903 than that for PFOS and PFOA. According to the study authors their results suggest that the oxygen atom inserted in the molecule and the longer chain length are variables linked with a stronger effect on hepatic fatty acid metabolism.

Zeilmaker et al. (2018) established relative potency factors (RPFs) to be able to evaluate combined exposure to mixtures of PFASs. As these substances (perfluoro carboxylic acids, perfluoro sulphonic acids, and perfluoro ether carboxylic acids) are assumed to cause liver toxicity by a similar mechanism/mode of action, dose-addition is justified. With the proposed RPFs, the combined effect on the liver for 20 PFASs can be estimated, using IC equivalents. Due to current absence of toxicity data for many PFASs, no RPF can be calculated for these substances.

Gomis et al. (2018) investigated to that extent the toxicokinetics of PFASs influence the toxicological effect threshold. They concluded that HFPO-DA interacts more strongly with its target than its predecessor PFOA (i.e. HFPO-DA has a higher toxic potency) when correcting for

toxicokinetics. Ranking the substances based on toxic potency resulted in HFPO-DA > PFOA > PFHxA > PFBA and HFPO-DA > PFOA ≈ PFHxA ≈ PFBA for internal concentrations in serum and liver respectively. For PFOS and PFBS, no differences in potency were observed based on internal and external doses.

4.10 Reference values

An overview of the existing reference values for HFPO-DA is provided in Table 34. The registrant derived worker DNELs of 0.02 mg/kg bw/day for dermal exposure and 0.14 mg/m^3 for inhalation, based on a NOAEL of 1 mg/kg bw/day for liver toxicity obtained from a 2-year chronic toxicity study in rats. For consumers, the registrant derived a DNEL of 0.04 mg/m^3 for inhalation and 0.01 mg/kg bw/day for oral exposure. The registrant DNEL derivation is in line with the ECHA Guidance for derivation of DNELs (ECHA, 2012a). However, the derivation of limit values below which exposure to HFPO-DA or its salts/acid can be considered safe is hampered by the uncertainty on bioaccumulation in humans which, according to the dossier submitter, should be taken into account when deriving a DNEL for these type of substances. In the ECHA Guidance Ch. R.8 no specific guidance is given on how to derive a DNEL that covers this specific type of uncertainty.

Table 34: Reference values for HFPO-DA.

Type	Reference	NOAEL	NOAEC	Assessment factors (AFs)	Value
tTDI inhalation general population	(Beekman et al., 2016)	0.1 mg/kg bw/day	0.087 mg/m^3	Additional tokicokinetics AF: 66 Interspecies remaining toxicokinetics AF: 1.8 Intraspecies AF: 10 Total AF: 1188	73 ng/m^3
tTDI oral general population	(Janssen, 2017)	0.1 mg/kg bw/day	Not applicable	Additional tokicokinetics AF: 66 Interspecies remaining toxicokinetics AF: 1.8 Intraspecies AF: 10 Total AF: 1188	21 ng/kg bw/day
DNEL inhalation general population	REACH Registration Dossier	1 mg/kg b/day	0.87 mg/m^3	Interspecies AF other: 2.5 Intraspecies AF: 10 Total AF: 25	0.04 mg/m^3
DNEL oral general population	REACH Registration Dossier	1 mg/kg b/day	Not applicable	Interspecies AF: 4 Interspecies AF other: 2.5 Intraspecies AF: 10 Total AF: 100	0.01 mg/kg bw/day
DNEL worker inhalation	REACH Registration Dossier	1 mg/kg b/day	1.76 mg/m^3	Interspecies AF other: 2.5 Intraspecies AF: 5 Total AF: 12.5	0.14 mg/m^3
DNEL worker dermal	REACH Registration Dossier	1 mg/kg b/day	Not applicable	Interspecies AF: 4 Interspecies AF other: 2.5 Intraspecies AF: 5 Total AF: 50	0.02 mg/kg bw/day

Janssen and Beekman started from the more sensitive endpoint of immune effects observed with a NOAEL of 0.1 mg/kg bw/day. They did not derive DNELs but instead derived tentative Tolerable Daily Intake limit values (TDIs) for the general population of 21 ng/kg bw/day for oral exposure and 73 ng/m^3 for inhalation. In addition to the uncertainty factors as recommended in the ECHA Guidance Ch. 8 (ECHA, 2012a), they applied an uncertainty factor of 66 specifically covering uncertainty between bioaccumulation in humans and experimental animals based on the ratio of PFOA half-lives in humans and monkeys (1378/20.9 = 66 ; (Olsen et al., 2007a, Butenhoff et al., 2004)).

The 2 to 3 orders of magnitude difference in DNELs/TDIs can mainly be attributed to the use of different NOAELs and the use of an additional safety factor in Beekman et al. (2016) and Janssen (2017) to correct for the uncertainty in bioaccumulation potential between humans and

experimental animals.

4.11 Summary and discussion of human health hazard assessment

In summary, the data illustrate that HFPO-DA induces tumours in the liver, pancreas, and testes in rats upon chronic exposure. Main target organs upon subchronic and chronic exposure to FRD-902 in mice and rats include the liver, the kidney, the haematologic system, and the immune system. Furthermore, exposure to HFPO-DA resulted in early deliveries, and decreased birth weight of pups. Observed differences in HFPO-DA clearance between males and females could explain higher sensitivity of males compared to females.

Information is inconclusive with respect to potential effects to the reproductive system. Additionally, whereas the immune system seems to be affected upon treatment with HFPO-DA, there is little information available, which hampers full assessment of the immunomodulatory effects of HFPO-DA. The same applies to potential endocrine disrupting effects (e.g. changes in testosterone levels), as well as mechanistic studies targeting protein binding and toxicogenomics. Lastly, in the absence of appropriate human data for HFPO-DA, a solid conclusion on the half-life in humans cannot be drawn.

5 Environmental hazard assessment

The environmental health hazards of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid, its salts and its acyl halides (covering any of their individual isomers and combinations thereof) are considered not relevant in the context of the present Annex XV proposal to motivate the very high concern for these substances that lead to propose these substances as SVHC according to art. 57(f) of REACH. Subsequently, no environmental hazard assessment has been performed.

6 Conclusions on the SVHC Properties

6.1 CMR assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57 (f) REACH.

6.2 PBT and vPvB assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57 (f) REACH.

6.3 Assessment under Article 57(f)

An assessment is made in this dossier in order to conclude whether 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid, its salts and its acyl halides (covering any of their individual isomers and combinations thereof), further denoted as HFPO-DA, should be regarded as a "substance for which there is scientific evidence of probable serious effects to human health or the environment which give rise to an equivalent level of concern to those of other substances listed in Article 57 points (a) to (e) REACH".

This should be a case-by-case assessment. The assessment is therefore done on the basis of the information available on the different substances of the group applying a weight of evidence approach. All publicly available information was taken into account and no information was given additional weight in relation to other information. It will be demonstrated in this section and its subsections 6.3.1 - 6.3.4, that these substances are of equivalent level of concern because of their chemical properties of:

- very high persistence,
- very high mobility, and
- adverse effects on human health,

and the fact that these substances:

- show a widespread occurrence in the Netherlands and in other EU Member States, in drinking water, fish and in home grown vegetables,
- show an unpredictable and uncontrollable occurrence in the environment,
- are ubiquitously present in the global environment at low background concentrations,
- are irreversibly present in the environment due to the absence of degradation and the impossibility to remove the substance from the environment after release, and
- are found to be present in the serum of workers occupationally exposed to these substances and of citizens living in the proximity of an facility where these substances are produced, used or processed,

which will be shown to lead to a:

- societal concern for irreversible and intergenerational effects on humans and the environment for which as of yet no safe limit of exposure can be derived and which exposure cannot be predicted, controlled nor reversed.

As has been outlined in Section 1, salts and acyl halides of HFPO-DA rapidly dissociate (i.e. salts) or react (i.e. acyl halides) in water to form the anionic form of HFPO-DA under environmental conditions. Consequently, monitoring and experimental studies only measure HFPO-DA and do not allow any distinction of the HFPO-DA observed originating from one of the salts or acid halides. Therefore, the Equivalent Level of Concern assessment provided below therefore also applies to e.g. ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902), potassium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903), and 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoyl fluoride, for which there is information available in the ECHA dissemination site.

FRD-902 is also being assessed under Substance Evaluation. A decision has been issued requesting the following studies:

- Carcinogenicity study in mice via oral route; test method OECD 451.
- Human biomonitoring study in volunteering workers at the manufacturing site.

The Substance Evaluation decision addresses concerns of carcinogenicity which could lead to a potential classification and the half-life in humans (bioaccumulation potential) which could lead to identification of the substance as PBT/vPvB and/or a different DNEL for workers and consumers. Conclusions of this equivalent level of concern assessment do not undermine the need for further information to improve safe management of the substances.

6.3.1 Summary of the data on the hazardous properties

6.3.1.1 Degradation – abiotic and biotic

QSAR modelling and the different tests exploring ready or inherent biodegradability show that HFPO-DA does not exhibit any primary biodegradation, is not readily or inherently biodegradable and is not structurally transformed under the experimental test conditions. Experimental data suggest that under abiotic conditions HFPO-DA is more persistent than PFOA. The very high persistence is a property that is also observed for other perfluorinated substances and can be attributed to the full fluorination of the carbon chain (see Section 3.1). High persistency is also supported by the presence of HFPO-DA in remote places where there is no indication of a possible direct emission source (see Section 3.2.5).

6.3.1.2 Bioaccumulation

Bioaccumulation of HFPO-DA in humans and the environment is as of yet uncertain. The ongoing Substance Evaluation on FRD-902 aims to generate further information on the bioaccumulation potential of FRD-902 (and thereby HFPO-DA) in humans and therefore clarify the concern for risks to workers and suspected PBT/vPvB properties.

Information from environmental data

Few data are available on the bioaccumulation potential for HFPO-DA in the aquatic environment. Laboratory bioconcentration factors (BCF) and field bioaccumulation factors are low (Hoke et al., 2016, Pan et al., 2017, NVWA, 2018, NCDEQ, 2018c). Based on the structural similarities it is concluded that bioaccumulation may be dependent on the external water concentration, like is observed for PFOA (Verbruggen et al., 2017). Furthermore, the data available suggest that bioaccumulation of HFPO-DA is influenced by the presence of other PFASs in the same environment. The BCF of HFPO-DA may therefore be higher than is currently observed and that, depending on the environmental conditions (concentration of HFPO-DA in the water and co-

exposure of environmental organisms to HFPO-DA and other PFAS), the BCF might be similar to that of PFOA (see also Section 1.3 and Annex II for a further motivation of the similarities in properties between different PFAS). The observed concentrations of HFPO-DA in wild fish provides evidence that humans can be exposed to HFPO-DA via the food chain through fish consumption, which is reason for concern. The uncertainty in the bioaccumulation potential of HFPO-DA is a complicating factor in the quantitative risk assessment, and hampers e.g. the risk from fish consumption to humans, which is impeding the derivation of a water quality standard for HFPO-DA for this route (Smit, 2017).

Information from human data

HFDO-DA has been found in the serum of residents living close to a fluorochemical plant in China (Pan et al., 2017), and in the blood of employees from a fluorochemical production plant in the Netherlands (Van den Berg, 2017). Concentrations in the Chinese residents were in the order of 0.13 ng/ml (geometric mean) with a 95th percentile at 1.72 ng/ml. HFPO-DA levels in the blood of employees from a Dutch fluorochemical plant varied between <1 and 169 ng/ml, with a median value of 1.55 ng/ml. It is not possible to derive a half-life for HFPO-DA in humans from these data within a reasonable certainty. Experimental data in some mammalian species show half-lives between hours and several days (Gannon et al. (2016); see also Section 3.2.1). As described in Section 4.1.2 for PFOA, the half-life in humans of 3.8 years (Olsen et al., 2007b) is much higher than would be expected based on data from rodents and monkeys, in which half-lives of 2-4 hours up to 17-19 days were determined (Lau et al., 2007, Butenhoff et al., 2004). Because of this discrepancy between humans and other mammalian species for PFOA, an extrapolation of half-lives of HFPO-DA in mammalian test species to humans may not be straightforward either.

6.3.1.3 HFPO-DA removal from the environment and from drinking water

The removal of HFPO-DA from the different environmental media is important for e.g. the production of drinking water from raw water and the remediation of contaminated area's to prevent for continuous exposure of the environment and humans via the environment.

HFPO-DA has a low sorption potential. The registration dossier of FRD-902 reports a log K_{oc} of 1.1. Sun et al. (2016) studied the removal of HFPO-DA by powdered activated carbon (PAC) under laboratory conditions, simulating maximum efficiency water treatment processes, and its removal at the different steps of the actual drinking water treatment process. They found that PAC was to some extent able to remove HFPO-DA from water. The removal efficiency was higher than for PFBA, but lower than for PFHxA, PFBS, PFOA and PFOS. Study of the actual drinking water treatment process nevertheless indicated no significant removal of HFPO-DA in any of the water treatment steps, suggesting that in practice the possibility to remove HFPO-DA from drinking water is poor at best (Sun et al. (2016)). Hopkins et al. (2018) and Roelandse and Timmer (2017) confirmed this observation for current possibilities for surface water treatment, noting that HFPO-DA is very difficult to remove from the water phase and that methods available to possibly remove HFPO-DA from the water phase may not be effective in practice and may be highly energy demanding.

6.3.1.4 Measured levels of HFPO-DA in surface, sea, rain and ground water

Monitoring data from Europe indicate that HFPO-DA is transported by sea currents over very long distances reaching the North Sea, the Wadden Sea and the German Bight and is further transported to the Norwegian Sea and along the coast of Denmark to enter the Baltic Sea. Concentrations are found to range from 10 - 100 ng/l in the Dutch estuaries to 1 – 10 ng/l in the North Sea and Wadden Sea, and 0.1 - 1 ng/l in the Norwegian Sea, along the coast of Denmark and in the Baltic Sea. For these waters, a clear correlation was found between possible emission sources and the detection of HFPO-DA. HFPO-DA is also detected in surface water at locations for which observed concentrations cannot be explained by any known, local emission source. Concentrations upstream of rivers along which no known fluorochemical production

facility is located suggest the presence of diffuse emission sources. Observed concentrations in the Netherlands, the UK, Sweden, China and the US without an apparent emission source present range between 0.1 – 10 ng/l. Pan et al. (2018) and Hopkins et al. (2018) furthermore report the ubiquitous occurrence of low level background concentrations of HFPO-DA (together with other PFAS like HFPO-TA, and 6:2 H-PFESA) across the global environment in the low ng/l range.

Waste treatment and waste transport are identified as possible sources of diffuse emissions. Another source that could lead to diffuse pollution is emission through air. Monitoring data show the possible dispersion of airborn HFPO-DA in the environment via rain water, and also airborne C3 dimer acid fluoride is mentioned as a possible diffuse source of HFPO-DA. For the plant in Fayetteville NC in the US this acid fluoride was proposed as a significant source leading to the widespread diffuse pollution observed and it was estimated that 225 kg or more was emitted into the air between 2012 and 2016 (Hopkins et al., 2018). For the fluorochemical plant in the Netherlands, recorded air emissions of FRD-902/FRD-903 that could give rise to a diffuse spread of HFPO-DA for 2012 – 2015 lies between 200 – 420 kg/year (Beekman et al., 2016). This emission was used to model the possible exposure in close proximity of the plant at 15 ng/m3; exposure reduces with longer distance from the plant. Residual HFPO-DA that can be present in low concentrations in PTFE and FEP containing products and articles, as is indicated in the registration dossier, may be another possible source that could give rise to diffuse emissions.

6.3.1.5 Measured levels of HFPO-DA in drinking water

HFPO-DA is detected in finished drinking water at several locations downstream of the fluorochemical production plant in the Netherlands in concentrations up to 30 ng/l. The raw water for drinking water preparation was extracted from local surface water and a clear correlation was found between possible emission sources and the detection of HFPO-DA in finished drinking water. HFPO-DA was also found in tap water from some other Dutch cities, not located downstream of a fluorochemical plant (see Section 3.2.5). In the Cape Fear River area (NC, USA), concentrations of 400 to 500 ng/L were observed in drinking water in August 2013 abstracted from the river 90 miles downstream of the fluorochemical plant. A month after an emission reduction of HFPO-DA in June 2017, concentrations in drinking water of the water treatment plant 90 miles downstream of the fluorochemical plant dropped from over 700 ng/L to 40-50 ng/L (Hopkins et al., 2018). Data obtained from the North Carolina Environmental Quality website on GenX (HFPO-DA) sampling[13] show that all water treatment plants downstream of the fluorochemical plant have similar concentrations and trends with drinking water concentrations around 40 ng/L by November 2018.

6.3.1.6 Long range transport

The potential for long range transport of HFPO-DA is modelled and evaluated against the screening criteria as they are included for the potential for long-range environmental transport in Annex D, Section 1 (d) of the Stockholm Convention on Persistent Organic Pollutants (POPs). HFPO-DA is modelled to have a characteristic travel distance (CTD) of 8682 km, which indicates that HFPO-DA can reach any area of the world before any significant degradation has occurred. Long range transport is found to occur predominantly via the water compartment. In air, HFPO-DA is modelled to have an atmospheric half-life of 20.57 days, which by far exceeds the criterion in the Stockholm Convention of >2 days. Hence, the modelling of HFPO-DA suggests that this substance has the potential for long range transport and can be transported to remote areas.

The monitoring data for rain water, fresh water and for marine water support the modelling results that HFPO-DA is subject to long range transport over vast distances by air and water. The travelling distances of HFPO-DA from known emission sources to the site of detection can be large (e.g. from the Dutch river delta all the way up to the Norwegian sea). Consequently the monitoring data clearly indicate the potential for long range transport of HFPO-DA.

[13] https://deq.nc.gov/news/hot-topics/genx-investigation

6.3.1.7 Levels in biota

There is only little information available on HFPO-DA in biota. HFPO-DA is found in fish in three field studies, one in China, one in the US (North Carolina) and one in the Netherlands. These field studies do not exclude that the concentration in fish depends on the water concentration, similar to PFOA, and seems to be dependent on any co-exposure to other fluorochemicals, like for example PFOA. In the presence of other fluorochemicals, competition for uptake seems to occur. Taking into account the uncertainty surrounding the joint exposure of PFAS, these observations also warrant caution with regard to any conclusion concerning the aquatic bioaccumulation potential of HFPO-DA and suggest that this bioaccumulation might still be very similar to that of PFOA. The uncertainty in assessing the bioaccumulation potential of HFPO-DA is a complicating factor for quantitative risk assessment, for estimation of exposure of man via environment and secondary poisoning.

6.3.1.8 Enrichment in plants

Monitoring data of HFPO-DA in plants have been collected from vegetables and fruits from kitchen gardens in the vicinity of the fluorochemical plant near Dordrecht in the Netherlands in August 2017 (see Section 3.5). A total of 10 locations were sampled at various distances from the fluorochemical plant. The detected concentrations were significantly different for different types of vegetables with leafy vegetables having the highest concentrations, followed by tuber vegetables and fruit vegetables. Further research hints that HFPO-DA may enrich more strongly in plants than PFOA does and may be more comparable to short-chain PFCAs. The BCF values reflect their mobile character, also in terrestrial plants. Further, it appears that compared to PFOA, the transfer from soil to plants is much higher for HFPO-DA. Due to the considerable uptake in vegetables and fruits, consumption of these by humans contributes significantly to the total exposure to HFPO-DA.

6.3.1.9 Toxicokinetics

The available data indicate that HFPO-DA is quickly absorbed in mammals after oral exposure, and distributes mainly to the plasma and liver. Male rats and mice showed overall higher HFPO-DA tissue and plasma concentrations compared to females upon exposure to equipotent dosages, which might be explained by more effective elimination in females compared to males. Data furthermore indicate that the substance distributes into the foetus, and that there is limited transfer of HFPO-DA via lactation. The substance is not metabolised, and is eliminated almost completely within approximately 24 hours via urine in rats and monkeys, and it takes up to 7 days to be fully retrieved in the urine from mice.

The half-lives established in experimental animals vary between one and several days. As described in Section 4.1.1.5 for PFOA, the half-life in humans of 3.8 years ((Olsen et al., 2007a)) is much higher than would be expected based on data from rodents and monkeys ((Lau et al., 2007);(Butenhoff et al., 2004)). Because of the observed discrepancy between humans and tested mammals for PFOA, an extrapolation of half-lives of HFPO-DA in mammalian test species to humans may not be straightforward either. These limitations in the available knowledge means that the half-life for HFPO-DA in humans remains currently unknown. In order to resolve part of these limitations, the potential for bioaccumulation in humans is under investigation in the ongoing Substance Evaluation on HFPO-DA, where a human biomonitoring study in volunteering workers at the manufacturing site is being requested.

Protein binding

The limited data available suggest that HFPO-DA binds to proteins. The substance is mainly distributed to the plasma and the liver. More general observations for PFASs indicating chain length dependent binding to proteins, with higher chain-length leading to better protein-binding

efficacy. In comparison to the classic lipophilic organic pollutants that primarily bind to fatty tissues, perfluoro carboxylic acids and perfluoro sulphonic acids primarily bind to proteins. Over 98% of the molecules are bound to serum proteins (mainly albumin) or bind to fatty acid-binding proteins in the liver. For the shorter chain substances, the free fraction in the blood increases with increasing concentrations, suggesting saturation of the binding sites. There are no studies available investigating direct binding between HFPO-DA and albumin, and therefore it currently remains unknown whether HFPO-DA interacts with albumin directly or not.

Organic Anion Transporter binding

It is argued that the half-life of PFOA is longer in humans compared to other species, since in humans there could be stronger PFOA reabsorption from ultrafiltrate in the kidney back into the blood by organic anion transporters (OATs) (Yang et al., 2010). No data is available on OAT efficacy for HFPO-DA in humans. It is therefore not known what effect HFPO-DA has on the functioning of the OATs and if resorption of HFPO-DA in the lumen of the kidney will occur in humans or not.

6.3.1.10 Effects on human health - toxicity

As lined out in Section 4.11, the toxicity data available illustrate that HFPO-DA induces tumours in the liver, pancreas, and testes in rats upon chronic exposure. Main target organs upon subchronic and chronic exposure to HFPO-DA in mice and rats include the liver, the kidney, the haematologic system, and the immune system. Furthermore, HFPO-DA crosses the placenta and distributes into the foetus, causes early deliveries, and results in decreased birth weight in pups.

FRD-902 does not have a harmonised classification (see Section 2). Below a summary is provided of the adverse health effects of highest concern.

Effects on human health – repeated dose toxicity

The information available shows that the main target organs of HFPO-DA after repeated exposure are the liver, the kidney, the haematological system and the immune system. All these effects are considered of concern. The overall NOAEL resulting from these studies is 0.1 mg/kg bw/day, based on an increase in A/G ratio observed in males dosed 1 mg/kg bw/day for two years.

Liver

The liver is the main target organ following from exposure to FRD-902 via the oral route. A detailed description of the effects observed and possible underlying mechanisms can be found in Section 4.5.1.1. It is unclear whether solely peroxisome proliferation via the PPARα receptor is responsible for the observed liver effects observed, giving rise to concern for human health. Effects observed are liver weight increases, serum liver enzyme increases up to 1000 fold in male mice, and microscopical changes of which some remained after 4 weeks post-exposure. These microscopical changes include increases in liver hypertrophy in rats and mice of both sexes, repeatedly accompanied by single-cell-, (multi)focal-, and/or hepatocellular necrosis. In addition, one study observed steatosis.

Kidney

Kidney effects were observed upon exposure to HFPO-DA (i.e. increased kidney weight, kidney hypertrophy, increases in BUN, microscopically observed kidney damage). These effects generally occur at higher dosages than the observed liver effects. Increased kidney weight was accompanied with increased BUN in several studies, but in the cases where kidney hypertrophy was observed, this was not accompanied by histopathological changes in the kidney, apart from the 500 mg/kg bw/day dosed females in the chronic study (Craig, 2013). Furthermore, in male mice dosed for 85 days in the reproduction/developmental screening study, increased incidence of kidney tubular cell hypertrophy was observed at 0.5 mg/kg bw/day and 5 mg/kg bw/day. The biological relevance of the increases in BUN and the kidney hypertrophy without any

microscopically observed kidney damage is unclear.

Haematological system

Changes in the haematological system include red cell mass reduction and decreased haemoglobin and haematocrit. Other observations include increases in absolute reticulocytes, platelets and decreases in basophils. These changes suggest that exposure to HFPO-DA may promote anaemia.

These changes were overall relatively mild, with parameters not exceeding 10% change from control up to dosages of 50 mg/kg bw/day in a chronic study in rats. However, data from female rats dosed at 1000 mg/kg bw/day under a subchronic exposure regimen illustrate that FRD-902 may promote severe anemic conditions.

Immune system

Effects on the immune system include increases in albumin and decreases in globulin (accompanied increases in A/G ratio). These changes are an early sign of potentially reduced immune function. At higher doses, suppression of the T cell-dependent antibody response and increased T lymphocyte numbers were observed. Also, two studies observed relative decreased spleen weight. Whereas the immune system seems to be affected upon treatment with HFPO-DA, there is little information available, which hampers full assessment of the immunomodulatory effects of HFPO-DA.

Effects on human health – carcinogenicity

The information available demonstrates statistically significant induction of adenomas/carcinomas in the pancreas in males at 50 mg/kg bw/day, statistically significant induction of hepatocellular adenomas and carcinomas in females at 500 mg/kg bw/day, and increased incidence of Leydig cell tumours in the testes at 50 mg/kg bw/day.

The information available does indicate a non-genotoxic mechanism. HFPO-DA induced liver carcinogenicity may be caused via the PPARα mode of action. This mode of action would be specific for rodents. However, it cannot be excluded that another mechanism may be responsible for the observed liver tumours, nor is there a direct link between PPARα and the observed pancreatic and testes tumours established. More specific, for HFPO-DA, it is uncertain whether PPARα activation is required for the liver tumours observed to develop, and the underlying mode of action for the Leydig cell- and pancreatic acinar tumour types remains unclear. Therefore, HFPO-DA is considered potentially carcinogenic to humans and the information available is concluded by the dossier submitter to possibly meet the CLP criteria for a category 2 carcinogen. Data are however currently insufficient to conclude on the full carcinogenic potential of HFPO-DA. The undergoing Substance Evaluation requires more information to elucidate this concern.

Effects on human health – fertility and developmental toxicity

No effects on reproduction were observed at any of the doses levels tested. In the parental animals, liver single-cell necrosis was observed in males, in concordance with the effects observed in the subchronic and chronic toxicity studies. Furthermore, F1 animals of both sexes showed decreased mean body weight during the pre-weaning period. However, the results from the developmental/reproduction screening study in mice do not allow for final conclusions regarding the reproductive effects because the highest dose level tested only exerted minimal effects in the parental animals. Therefore, information is regarded inconclusive with respect to potential effects to the reproductive system.

With regard to developmental toxicity, HFPO-DA crosses the placenta and distributes into the fetus, causes early deliveries, and results in decreased birth weight in pups without causing severe parental toxicity at 100 mg/kg bw/day. Maternal toxicity included mortality, lower mean

body weight gains and decreased food consumption, decreased gravid uterine weight, higher mean kidney weight, liver hypertrophy, and microscopic changes in the liver at 1000 mg/kg bw/day. Also in the group exposed to 100 mg/kg bw/day, focal necrosis was observed in 2/22 animals, and a mean decreased gravid uterine weight was reported.

6.3.1.11 Human health – reference values

An overview of and discussion on the derivation of tTDI's and DNELs is provided in Section 4.10. The dossier submitter is of the opinion that the DNELs need further consideration. Derivation of limit values below which exposure to HFPO-DA or its salts/acid can be considered safe is hampered by the uncertainty on bioaccumulation in humans, and as a consequence currently only a tentative TDI can be determined. In the absence of appropriate human data for HFPO-DA, a solid conclusion on the half-life in humans cannot be drawn. Therefore, the tTDI requires the inclusion of a safety factor to account for the uncertainty in the bioaccumulation potential of HFPO-DA in humans, until the half-life for humans is available. This leads to oral and inhalation tentative Tolerable Daily Intake (tTDI) values for the general population are derived at 21 ng/kg bw/day and 73 mg/m^3.

6.3.1.12 Human health - Co-exposure with other PFECAs and PFAS

As is described in Section 4.9.1.4, PFASs often occur together as contamination in soil, groundwater or drinking water. Zeilmaker et al. (2018) derived so-called Relative Potency Factors (RPFs) for several perfluorocarboxylic acids (PFCAs), perfluorosulphonic acids (PFSAs) and HFPO-DA, to allow for risk assessment of combined toxicity to a mixture of PFASs based on the principle of dose-addition. This concept is developed assuming that the PFAS act in a similar manner, with the same mechanism/mode of action, resulting in dose-responses with the same shape but with different potencies for each of the individual substances. In principle, the RPF method scales the dose of each substance, according to its potency. Based on available subacute and subchronic oral toxicity studies in rodents, relative potency factors (RPFs) were derived for 20 individual PFASs, including HFPO-DA. From this work is derived that the potency of e.g. PFOA > PFTeDA > HFPO-DA > PFHxA > PFHxDA > PFBS. Hence, based on the proposed RPFs for 20 PFASs in Zeilmaker et al. (2018), the combined effect on the liver can be estimated. Rushing et al. (2017) suggests that a similar exercise could be conducted for immune effects of PFCAs.

Gomis et al. (2018) investigated to what extent distribution and elimination kinetics influence toxicological effect thresholds (which are expressed as administered dose). It was observed that converting external doses to internal serum and liver concentrations resulted in reduced variability in the dose-response curves of PFBA, PFHxA, PFOA and HFPO-DA. They concluded that HFPO-DA interacts more strongly with its target (i.e. has a higher toxic potency) than its predecessor PFOA when correcting for differences in toxicokinetics. More precisely, ranking the substances based on toxic potency resulted in HFPO-DA > PFOA > PFHxA > PFBA and HFPO-DA > PFOA ≈ PFHxA ≈ PFBA for internal concentrations in serum and liver respectively. For PFOS and PFBS, no differences in potency were observed based on internal and external doses.

To conclude, HFPO-DA contributes to the overall combined (additive) toxicity of the related PFASs.

6.3.2 Equivalent level of concern assessment

In 2015, ECHA and Member States prepared a discussion paper on the identification of substances as SVHC under art. 57(f) with sensitisers as an example[14]. Some years later the Joint Research Centre repeated this exercise for the example of neurotoxic and immunotoxic substances[15]. These papers outline the following factors for being of importance to consider in

[14] https://echa.europa.eu/documents/10162/13657/svhc_art_57f_sensitisers_en.pdf
[15] Identification of Substances of Very High Concern (SVHC) under the 'equivalent level of concern' route (REACH Article 57(f)) – neurotoxicants and immunotoxicants as examples;

the case-by-case assessment of an equivalent level of concern for substances according to art 57(f):

- With regard to health effects, it was suggested to consider:
    - Type of possible health effects
    - Irreversibility of health effects
    - Delay of health effects
- Other factors suggested to consider were:
    - Quality of life affected
    - Societal concern
    - Is derivation of a 'safe concentration' possible?

In 2018, further discussion among ECHA, Member States and the Commission in the context of the RiME meeting resulted in the identification of several additional elements that could be considered, for example in the context of evaluating an equivalent level of concern for the environment:

- Inter-generational effects;
- Impact on migratory species (spatial effects);
- Impact of short-term exposure (long-term effects);
- Potential to impair population level structure and recruitment or ecosystem function and stability.

HFPO-DA is proposed to be of equivalent level of concern according to article 57(f) of the REACH Regulation. Arguments that lead to this conclusion are lined out below. Because salts of HFPO-DA and the C3 dimer acid fluoride rapidly hydrolyse or react to HFPO-DA under environmental conditions, the equivalent level of concern assessment presented here equally applies to these substances. In this assessment, hazard related arguments and arguments related to environmental abundance and fate play an important role in concluding on the weight of evidence for an equivalent level of concern for this substance. This is in line with a recent judgement of the Court (Case C-323/15 P) where ELoC provision "encompasses the possibility of taking into consideration, for the purposes of comparison, material going beyond merely the hazards arising from the intrinsic properties of the substances concerned" (paragraph 34).

These considerations for a possible equivalent level of concern are further elaborated on in the sections below.

6.3.2.1 *HFPO-DA is very persistent and mobile, efficiently distributes throughout the aquatic environment and contaminates remote regions*

In Section 3.1 it is shown that HFPO-DA is so very persistent that the substance practically does not degrade in the environment. Based on the available experimental and QSAR information on HFPO-DA and on the information of structurally related substances, it is expected that HFPO-DA will meet the P and vP-criteria of REACH Annex XIII. Consequently, once HFPO-DA has entered the environment, it will not be removed via any natural process such as e.g. bio- or photodegradation. The substance will remain in the water phase due to its high solubility (see Sections 1.4 and 3.3) and its very low sorption potential (see Section 3.2.1). Because the

http://publications.jrc.ec.europa.eu/repository/bitstream/JRC96572/jrc96572-identification%20svhc%20reach%20article%2057f.pdf

substances stays in the water phase and will not adsorb to soil, sediment or organic matter, the substance will remain bioavailable and its presence, and therefore also its environmental exposure can be considered irreversible.

Due to the very high persistence of the substance, the exposure will remain over multiple generations and hence will lead over time to inter-generational effects. Its' very high persistence and inability to adsorb to soil, sediment and organic matter also mean that as long as emissions continue, environmental water concentrations will increase.

Its' physicochemical properties (see Section 1.4) make HFPO-DA very mobile in the aquatic environment. Because HFPO-DA does not adsorb to soil, sediment or active coal and dissolves very well in water, there is no natural barrier to prevent the substance from being distributed to remote areas and to groundwater once HFPO-DA has entered the environment, e.g. in a surface water body. If HFPO-DA enters soil, it will be further transported to surface water or ground water. With time and continuous emissions, environmental concentrations of HFPO-DA will increase in the aquatic environment, including ground water. The ubiquitous presence of HFPO-DA at background concentrations in the low ng/l range in the global environment is already being reported and support this concern.

Due to the global water cycle and the fact that the aqueous compartments are all well connected, the very high persistency and the high mobility of HFPO-DA lead to long distance transport processes in the environment. Transport is mainly taking place via river and ocean currents. This is clearly demonstrated by the monitoring data presented, showing the occurrence of HFPO-DA in remote regions of the North Sea and the Baltic and further north in the Norwegian Sea (see Section 3.2.5). However, distribution of the substance may also take place via air, e.g. when emitted as acid fluoride. When HFPO-DA is emitted to air, both modelling and monitoring data indicate that the substance has a high potential for long range transport. Monitoring data furthermore show that long range distribution of the substance from its source may occur via rain water. This process has been described as an important route for HFPO-DA pollution of the environment in the US. The relevance of this contamination process for Europe is unknown as there is no information available on the use of the C3 dimer acid fluoride, or its possible formation e.g. as part of the use of FRD-902 as processing agent. The wide spread occurrence in the environment and the low background concentrations in water observed world-wide raise a concern for impact on migratory species and show that the substance has the potential to impair population level structure and recruitment or ecosystem function and stability at remote and pristine areas.

The fact that today's environmental distribution observed for Europe developed over a period of only 5 years' time with the only REACH-registered import and use site in the Netherlands adds to the concern for rapid long distance transport and the societal concern for this substance being ubiquitously and irreversibly present in the environment.

In summary, HFPO-DA releases to the environment are of relevance on a great spatial scale even when emission sources are geographically limited. Ubiquitous presence of HFPO-DA at low background concentrations in the global environment is already being reported. Due to its very high persistence, once released any amount will persist and environmental occurrence will only increase with continued production, use and emission of HFPO-DA and any of its precursors. Hence, environmental concentrations of HFPO-DA should be considered irreversible. As HFPO-DA migrates to remote regions, it has the potential to affect humans and the environment far away from its point of emission into the environment. Vulnerable populations and ecosystems in remote regions will be affected by this. Irreversible concentrations in the environment will furthermore lead to inter-generational effects. This is considered to be of high societal concern and affect the quality of life of both humans and the environment.

6.3.2.2 *HFPO-DA causes adverse effects on human health*

In Section 4 is argued that HFPO-DA induces tumours in the liver, pancreas, and testes in rats upon chronic exposure. Main target organs upon subchronic and chronic exposure include the

liver, the kidney, the haematologic system, and the immune system. Furthermore, the substance is observed to cross the placenta and to distribute into the foetus, to cause early deliveries and to result in decreased birth weight in pups.

None of the substances addressed in this report has a harmonised classification. HFPO-DA causes a tumorigenic response in rats in multiple organs. At two tumour sites both adenomas and carcinomas were observed, illustrating potential progression of benign tumours into malignant tumours. The ongoing substance evaluation for FRD 902 notes that the current information is not sufficient to conclude on the carcinogenic potential and requests further information to address the concern for carcinogenicity. Additionally, HFPO-DA induces a consistent pattern of effects resulting in severe liver damage in rodents, partly being irreversible. The absence of effects observed for reproductive toxicity are interpreted by the dossier submitter as inconclusive due to the specific top dose used in toxicity testing. Tentative Tolerable Daily Intake limit values (TDIs) for the general population may be as low as 21 ng/kg bw/day for oral exposure and 73 ng/m^3 for inhalation. Based on the effects on carcinogenicity it is concluded that HFPO-DA causes adverse effects on human health that can be considered irreversible. The effect on human health may come with a possible delay between the moment of exposure and the onset of any observable adverse effect (i.e. cancer), but may also already be relevant upon short-term exposure (i.e. immune effects, haematological effects, liver effects, kidney effects).

The current concern for carcinogenicity and the effects observed in the liver together with the present uncertainties around the potential for bioaccumulation, the observation that HFPO-DA does bind to human liver proteins and the fact that the current *in vivo* test methods do not allow a straight forward translation between the toxicity observed in mammals and the toxicity to be expected in humans (due to the uncertainty in bioaccumulation; see Section 4.1), suggest that the effects of HFPO-DA on human health may be more severe than can be concluded on the basis of the currently available toxicity data alone. As a consequence of the very high persistence and chronic background concentrations in the environment, continuous exposure may lead to the irreversibility of adverse effects that are normally considered reversible upon the removal of exposure in standard toxicity studies. Based on the information available, it is concluded that the human health related effects of HFPO-DA are severe and the dossier submitter concludes that the effects observed can be considered severe in the context of the assessment of an equivalent level of concern for this substance.

The concern for severe effects for HFPO-DA is further supported when compared to other PFAS. Based on available subacute and subchronic oral toxicity studies in rodents, relative potency factors were derived as a function of the external exposure ranking the toxicity as PFOA > PFTeDA > HFPO-DA > PFHxA > PFHxDA > PFBS. Ranking different PFAS substances based on toxic potency for internal concentrations in serum and liver resulted in HFPO-DA > PFOA > PFHxA > PFBA and HFPO-DA > PFOA ≈ PFHxA ≈ PFBA, respectively. Hence, it is concluded that HFPO-DA has a higher internal toxic potency than PFOA.

The very high persistency of the substance and the current uncertainties in bioaccumulation potential furthermore hamper the derivation of a possible threshold to effects for humans. Its' mobility in the environment and unpredictable environmental occurrence (see Section 3.2.5) furthermore complicate the setting of a safe exposure level. Consequently, the dossier submitter concludes that on the basis of the current data, a derivation of safe exposure levels is highly uncertain.

6.3.2.3 *HFPO-DA enters the biosphere and humans via several routes*

HFPO-DA enters the biosphere as result of industrial emissions, waste transport and waste treatment activities and to a minor extent through the use of mixtures and articles containing residual amounts of HFPO-DA as impurities remaining from the production process. Residual concentrations in polymer formulations are stated by the registrant to be <1 – 50 ppm and in articles <1 ppm. Biota can be exposed to HFPO-DA via the food web (e.g. plants and fish) and via surface water. Exposure via air can be an important route in the vicinity of (industrial) air-

emission sources, and possibly when there is emission of the C3 dimer acid fluoride. Airborne emissions are considered a less important route once HFPO-DA has entered the water environment due to the low volatility of the substance, but emission of HFPO-DA (and its acyl fluoride) in air can lead to detectable concentrations of HFPO-DA in rain water. This can result in detectable concentrations in rain water far away from the source, which contributes to a wide-spread exposure of biota.

Few studies are available that report HFPO-DA concentrations in biota or in food and feedstuff. HFPO-DA is detected in fish, at concentrations that seem to depend on the water concentration and co-exposure with other PFAS, at concentrations of *0.27 µg/kg* in redear sunfish and *1.53 µg/kg and 4.7 µg/kg* in carp. HFPO-DA is also recently found in home-grown vegetables at concentrations in the low ng/g range in the Netherlands. Over the last year, more and more information is being generated from monitoring studies suggesting that food may act as a source of HFPO-DA to humans. These findings trigger the concern that HFPO-DA is taken up by plants and can enter the food-chain. This is of relevance for example when agricultural soil or surface water is contaminated with HFPO-DA, leading to the contamination of agricultural plants. Given that HFPO-DA is only recently introduced at the EU market (i.e. in 2012 through its ammonium salt FRD-902), it may be expected that with time and continued emissions, HFPO-DA concentrations in food and feedstuff will rise with rising background concentrations of the substance in the environment. Something similar is observed for other short-chain-PFAAs for which concentrations in biota rose with increasing use from 2002 to 2014, for example, PFBS as an alternative to PFOS (Lam et al., 2016). Similarly, HFPO-DA concentrations in biota may increase in the future if its use is continued or further increased. This is of high societal concern.

Drinking water may also be a source of direct HFPO-DA exposure of humans. Monitoring data from several locations in the Netherlands show that drinking water (tap water) may contain HFPO-DA. At present, concentrations of up to 30 ng/L are already found (see Section 3.2.5). Current data, also from the US, suggest that the presence in drinking water is typically related to industrial activities that involve HFPO-DA or its salts. However, when environmental concentrations of HFPO-DA rise with increasing or continued use, one may expect that also other drinking water locations may become contaminated as a consequence of the very mobile character of the substance and its potential for long range transport.

Industrial activities are another important source of direct exposure of humans. HFPO-DA is detected in workers of a fluorochemical plant in the Netherlands and in citizens living in the proximity of a fluorochemical plant in China.

Human biomonitoring data suggest that workers and the general population are already exposed to low levels of HFPO-DA at a daily basis. Especially those that live in close proximity to production or use (industrial) sites of HFPO-DA precursors or are exposed to HFPO-DA in an occupational setting. Blood/serum levels provide evidence that HFPO-DA is taken up by the human body and, even though rapid elimination via the urine is observed in test animals, bioaccumulation in humans cannot be ruled out due to the absence of data in humans. The ubiquitous environmental presence found in fresh water and marine environment between 0.1 ng/l and 1 ng/l, away from known sources and after only a few years of use, and much higher concentrations closer to known sources, suggest chronic exposure of humans and environmental organisms, which is of high societal concern. The presence of HFPO-DA found in drinking water, fish and home grown vegetables further raises societal concern that humans can be exposed via food and water intake on a daily basis. In addition to HFPO-DA, humans are also exposed to several other per- and polyfluorinated substances with (at least partly) similar health effects at different potencies. Effects resulting from combined exposure are of concern but are as of yet unknown.

Independent of the half-lives in humans, irreversible background concentrations of HFPO-DA in water, which may lead to the contamination of drinking water and food and feedstuff, lead to long-term continuous human exposure, that can be expected to be inter-generational. Together with the potential of the substance to adversely affect human health, the continuous long term exposure of humans may impact the quality of life and gives rise to high societal concern.

6.3.2.4 *Exposures and emissions are difficult to regulate with end-of-pipe solutions*

Because HFPO-DA does not adsorb to sediment, soil and active coal, and does not show any (bio) degradation under environmental conditions, it is very difficult to remove HFPO-DA from water (see also Section 3.2). Even advanced water purification techniques that are in place in Waste Water Treatment Plants and in plants that produce drinking water are mostly not able to remove HFPO-DA, or only to a very limited extent. Techniques may become available in the future for industrial use. However, their practical and economical applicability beyond targeted industrial uses are as of yet uncertain. This is of relevance for the production of drinking water, for the treatment of wastewater and for the remediation of contaminated sites. Emissions to the environment are therefore considered irreversible, leading to an irreversible exposure of the environment and of man via the environment including secondary poisoning. Monitoring data from the Netherlands, Sweden and the UK furthermore point to an unpredictable and uncontrollable spread of HFPO-DA in water and soil. Recent research from the Dutch Enforcement Authority points to significant emission sources due to waste treatment and waste transport activities in the Netherlands, Italy, Belgium and the United Kingdom. Lack of information and communication through the value chain, including the waste phase of the substance makes these sources uncontrollable at the end-of-pipe, which is of particular concern for HFPO-DA due to its very persistent nature. The uncertainty in spatial distribution of the substance and possible emission sources highly complicates the possibility for taking timely action to remediate contaminated sites before HFPO-DA may find its way to surface or ground water. Therefore, when a human activity causes a release of HFPO-DA, the exposure of the environment and humans via the environment is considered to be impossible to prevent and reverse in practice.

6.3.2.5 *Comparison of above concerns of HFPO-DA with concerns of PBT/vPvB substances*

The ECHA Guidance for PBT/vPvB assessment (Chapter R.11) (ECHA (2017a) states:

"Experience with PBT/vPvB substances has shown that they can give rise to specific concerns that may arise due to their potential to accumulate in parts of the environment and

- that the effects of such accumulation are unpredictable in the long-term;
- such accumulation is in practice difficult to reverse as cessation of emission will not necessarily result in a reduction in substance concentration."

"Furthermore, PBT or vPvB substances may have the potential to contaminate remote areas that should be protected from further contamination by hazardous substances resulting from human activity because the intrinsic value of pristine environments should be protected" (ECHA Guidance R.11).

As outlined above, the environmental concentrations of HFPO-DA will increase in the aquatic environment when the use (and hence emission of the substance) continues. Because of its very persistent nature and high mobility in water, this is also the case for remote and pristine environments. The environmental abundance of HFPO-DA is considered to be impossible to reverse in practice, and hence exposure of humans and the environment will continue after cessation of use.

Furthermore, Section 4.0.1 of REACH Annex I explains, that a hazard assessment addressing all the long-term effects and the estimation of the long-term exposure of humans and the environment cannot be carried out with sufficient reliability for PBT/vPvB-substances. With respect to long-term exposure estimations this is also the case for HFPO-DA, because of the very high persistency of HFPO-DA, the adverse effects observed and the uncertainty of effects arising at longer time scales.

6.3.3 HFPO-DA is of equivalent level of concern

HFPO-DA is very persistent, very mobile in the water environment and shows adverse effects in humans. The available information on toxicity shows that some of these effects can be considered irreversible (i.e. acting as a potential human carcinogen). The current uncertainty regarding bioaccumulation adds to the conclusion that adverse effects of HFPO-DA may occur at lower concentrations than the available toxicity data currently suggests. Further concern also arises as a consequence of the very high persistence and chronic background concentrations in the environment, resulting in continuous exposure that may lead to the irreversibility of adverse effects that are normally considered reversible upon the removal of exposure in standard toxicity studies. These observations lead to conclude that the effects on human health can be considered severe. These present uncertainties also mean that a threshold to effect cannot be derived. Monitoring data suggest that the presence of HFPO-DA in the environment cannot be predicted.

The combination of very persistent and very mobile characteristics means that with continuous emission into the environment, the concentrations of HFPO-DA in the environment will increase, the substance will be distributed world-wide and pristine areas and groundwater will become irreversibly affected. Monitoring data in water, soil, vegetables, fish and humans show that the emission of the last 5 years in Europe already has led to a wide spread of HFPO-DA in the environment and the possible daily exposure of humans. Worldwide, background concentrations are already observed in the low ng/l range. Monitoring data point at the presence of unpredictable and uncontrollable emission sources from human activities. Current purification and remediation techniques are not effective to prevent this wide spread environmental distribution and more effective techniques may be costly. This may lead to continuous exposure to both the environment and humans, through the food chain and through drinking water, that will last over very long times. Cessation of emission will not necessarily result in a reduction in substance concentration. As HFPO-DA adversely affects human health, continuous exposure over very long times can lead to inter-generational effects that will impact the quality of life and are of high societal concern. The co-exposure of HFPO-DA and other very persistent fluorochemicals such as PFOA, PFHxA and PFBS present in the environment lead to combination effects on human health, which add further onto the societal concern for this substance. This all may lead to serious risks for humans and the environment, and leads to the conclusion that HFPO-DA is of equivalent level of concern according to article 57(f) of the REACH Regulation.

6.3.4 Conclusion on the hazard properties and equivalent level of concern assessment

2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid, its salts and its acyl halides (covering any of their individual isomers and combinations thereof), further denoted as HFPO-DA, are identified as substances of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) because in water under environmental conditions these substances exist in the form of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate, for which there is scientific evidence of probable serious effects to the environment and human health which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of REACH.

As HFPO-DA is very persistent and very mobile in the aquatic environment, continuous emission into the environment will lead to increasing concentrations of HFPO-DA in the environment and to a world-wide distribution, also to pristine areas and groundwater, which will become irreversibly affected. Available information already provides evidence that HFPO-DA emissions have resulted in a ubiquitous presence in the environment in the low ng/l range. Environmental monitoring data show the presence of local emission sources that can be attributed to (industrial) human activities involving HFPO-DA and diffuse and uncertain emissions without any identifiable source.

HFPO-DA adversely impacts human health at a daily intake that could be as low as 21 ng/kg bw/day (tTDI). Key target organs are the liver, the kidney, the blood and the immune system. HFPO-DA may also be a human carcinogen, but data are currently insufficient to fully determine its carcinogenic potential. The carcinogenicity of FRD-902 is currently under investigation in an ongoing Substance Evaluation. Based on the information available, it is concluded that the effects of HFPO-DA are severe. The effect on human health may come with a possible delay between the moment of exposure and the onset of any observable adverse effect (i.e. cancer), but may also already be relevant upon short-term exposure (i.e. immune effects, haematological effects, liver effects, kidney effects). Further concern arises as a consequence of the very high persistence and chronic background concentrations in the environment resulting in continuous exposure that may lead to the irreversibility of adverse effects that are normally considered reversible upon the removal of exposure in standard toxicity studies.

Based on the hazard and fate properties there is a serious concern that effects in the general human population are unavoidable in the future and, when occurring, are irreversible. Cessation of emissions will not necessarily result in a reduction in substance concentration and continuous exposure over very long times can lead to inter-generational effects that will impact the quality of life and are of high societal concern. Furthermore, due to the potential for wide spread distribution and long range transport of HFPO-DA, effects will not only occur at the point of release but also far away from its point of release and will affect a very large number of people. The substance will be distributed world-wide and pristine areas and groundwater will become irreversibly affected. The demonstrated presence of HFPO-DA in drinking water in several areas across the globe, in fish and in vegetables indicate the daily exposure of humans, which is of high societal concern. The co-exposure of HFPO-DA and other very persistent fluorochemicals such as PFOA, PFHxA and PFBS present in the environment lead to combination effects on human health, which add further onto the societal concern for this substance.

The above given arguments demonstrate that there is scientific evidence of probable serious effects to the environment and humans, which gives rise to an equivalent level of concern according to article 57(f) of REACH.

Part II

7 Registration and C&L notification status

Of the different substances under consideration in the present Annex XV dossier, only one substance is registered, namely ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoate (FRD-902; CAS 62037-80-3). The other substances have a preregistration under REACH. Hence the information on production and use of these latter substances is limited.

7.1 Registration status

Table 35: Registration status for ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902; CAS 62037-80-3)

From the ECHA dissemination site[16]	
Registrations	☒ Full registration(s) (Art. 10)

The substance is registered by one registrant. The substance is not produced but instead the substance is imported into Europe.

7.2 CLP notification status

None of the substances subject to this Annex XV dossier have a harmonised classification. Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902; CAS 62037-80-3) and 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903; EC 236-236-8) have a notified self-classification.

Table 36: CLP notifications for ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902; CAS 62037-80-3)

	CLP Notifications[17]
Number of aggregated notifications	4
Total number of notifiers	31

Table 37: CLP notifications for potassium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (EC 266-578-3)

	CLP Notifications[18]
Number of aggregated notifications	1
Total number of notifiers	1

[16] *https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/2679/1 (dd. 19.12.2018)*
[17] C&L Inventory database, http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database (accessed 31 January 2018)
[18] C&L Inventory database, http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database (accessed 31 January 2018)

Table 38: CLP notifications for 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903; EC 236-236-8)

	CLP Notifications[19]
Number of aggregated notifications	4
Total number of notifiers	99

8 Total tonnage of the substance

2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903; EC 236-236-8) was preregistered for the registration deadline May 2013, concerning substances manufactured or imported in the EU between 100 – 1000 tonnes per year. This substance has not been registered yet.

Potassium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (EC 266-578-3) was preregistered for the registration deadline November 2003, concerning substances manufactured or imported in the EU in amounts over 1000 tonnes per year, CMR substances Cat. 1 or 2, or very toxic to aquatic organisms. This substance has not been registered yet.

Table 39: Tonnage status of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902; CAS 62037-80-3)

Total tonnage band for the registered substance (excluding the volume registered under Art 17 or Art 18)[20]	10 - 100 t/pa

9 Information on uses of the substance

The registrant has replaced the use of PFOA by FRD-902 in the so-called GenX technology. FRD-902 is used as processing aid in PTFE (polytetrafluorethylene) and FEP (fluorinated ethylene propylene) plants of the registrant. Other uses of FRD-902 are not described in the registration dossier or in the literature. Fluoropolymer resins and finished goods are used in many applications like wire cables and PTFE coating. During the resin manufacturing process, FRD-902 is transformed into the hydrophobic water-insoluble hydride (also known as E1) and to FRD-903. During the process, FRD-903 and E1 are emitted to air from the PTFE and from the FEP plants. Furthermore, FRD-902 and FRD-903 are emitted to wastewater in the form of HFPO-DA, which is sent to the local municipal sewage treatment plant.

Monitoring data suggest wide spread abundance of HFPO-DA in the environment. Environmental abundances are due to the mobile nature of the substance. Part of the monitoring data can be explained by specific emission sources due to industrial activities. Also, emissions occur during waste treatment and transport activities. However, as has been described in Section 3.2.5 there are also monitoring data that cannot be explained by known use activities. Possible sources that could contribute to a wide spread abundance are air-emissions, transportation via water droplets in air and residual concentrations in ready-for-use products (articles and formulations).

In 2018, research by the Dutch Enforcement Agency on Chemicals showed unexpectedly high concentrations between <20 ng/l and 3.0 mg/l of HFPO-DA in waste that was handled by a Dutch Waste Treatment Facility. This facility treats amongst other waste streams, waste with very high

[19] C&L Inventory database, http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database (accessed 31 January 2018)
[20] https://echa.europa.eu/nl/substance-information/-/substanceinfo/100.124.803 (dd.19.12.2018)

and very low pHs (strongly basic and strongly acidic, respectively) (de Kort et al *in prep.*[21]). The respective waste seemed to have no apparent relation to the Dutch fluorochemical plant where FRD-902 is used, and hence suggested the possibility of unknown diffuse sources. The Dutch enforcement agency is currently following-up on this finding to find the possible origin of the HFPO-DA found in this waste. This follow-up research aims to provide insight in possible yet unknown diffuse sources of HFPO-DA that may lead to exposure of humans and the environment.

Table 40: Uses for FRD-902

	Use(s)	**Registered use** *(If not, specify the source of the information)*	**Use likely to be in the scope of Authorisation**
Uses as intermediate			
Formulation or repacking			
Uses at industrial sites	Processing aid for polymerisation Unloading and storage	Yes Yes	Yes Yes
Uses by professional workers			
Consumer uses	None reported		
Article service life			

10 Information on structure of the supply chain

FRD-902 is manufactured by mixing the acid form of HFPO-DA, FRD-903 (EC 236-236-8; CAS 13252-13-6), with an ammonium hydroxide solution. (Beekman et al., 2016). Manufacturing takes place outside the EU. FRD-902 is being imported into the EU, under a REACH registration with one registrant. The substance is notified by 30 other notifiers, suggesting that the substance is used on its own or in preparations also by others. FRD-903 is not registered under REACH and hence is suggested that FRD-903 is not imported as such or in mixtures into the EU. The substance is notified by 99 notifiers under the CLP-regulation. It is unknown how these notifiers use or handle FRD-903 and to what extent.

FRD-902 is primarily used as processing aid for the production of fluoro-polymers. Some is being emitted as HFPO-DA as part of the industrial process via air or water emissions. The major part of all used FRD-902 is being recovered as part of the industrial process or send to waste treatment (recovery, recycling or incineration) at external location, across the EU. The Waste Framework Directive is leading for how this waste is treated.

[21] M.J. de Kort, A. Verschoor, C. de Jong, 2019. Verspreiding van GenX-stoffen in het milieu – Metingen 2013-2018. RIVM report (*in prep.*)

11 Additional information

Exposure potential for workers

Workers may be exposed to FRD-902 during processing. Process categories reported in the registration dossier include PROC1 (use in closed process, no likelihood of exposure), PROC 8b (transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities), PROC3 (use in closed batch process (synthesis or formulation)) and PROC 15 (use as laboratory reagent) (ECHA dissemination website[22]).

The substance is a solid powder and workers could potentially be exposed via the inhalation and dermal routes. DNELs as derived by the registrant are:

- 0.14 mg/m3 inhalation, systemic effects long-term exposure for workers
- 0.02 mg/kg bw/day dermal, systemic effects long-term exposure for workers

In addition, biomonitoring data are available showing that HFPO-DA can be detected in blood of workers from a fluorochemical production plant in the Netherlands in concentrations varying from <1 to 169 ng/ml (Van den Berg, 2017).

Exposure potential for consumers

FRD-902 is used as a processing aid in the production of fluoropolymers. It is understood that it has no function in the final product and may only be present as an impurity. The process in the plant in Dordrecht is designed to remove as much of the processing aid from the polymer as possible. Therefore in the registration dossier no exposure to humans or the environment is anticipated after polymerisation. Though the registration dossier indicates that exposure to the general population is unlikely, inhalation and oral exposure are considered plausible by the dossier submitter, as detectable concentrations of HFPO-DA have been observed in drinking water and vegetables/fruits.

DNELs used in the registration dossier:

- 0.04 mg/m3 inhalation, systemic effects long-term exposure for the general population
- 0.01 mg/kg bw/day, oral, systemic effects long-term exposure for the general population

Much lower provisional limit values were derived by the RIVM for the general population for assessment of local exposures near the factory. In air, a limit value of 73 ng/m3 was derived (Beekman et al., 2016). For oral uptake, a provisional limit value of 21 ng/kg bw/day was derived (Janssen, 2017).

Exposure potential for the environment

Environmental release categories (ERCs) reported in the registration dossier include: ERC4 (Use of non-reactive processing aid at industrial site (no inclusion into or onto article)).

For the environmental assessment, the registration dossier indicates that the monitored releases are the anion since FRD-902 readily dissociates into the ammonium cation and the C3 dimer anion in water. The site is working diligently to reduce the use of FRD-902. All wastewater first enters the onsite waste water treatment plant before going into the local municipal sewage treatment plant (STP) after all the C3 dimer anion and acid removal steps on site. No sludge

[22] Information extracted from the ECHA dissemination website dd. August 2018

(WWTP and STP) is applied to soil. PNECs used in the registration dossier are:

- PNEC aqua (freshwater) = 0.108 mg/L
- PNEC sediment (freshwater) = 0.518 mg/kg sediment dw
- PNEC aqua (marine water) = 0.011 mg/L
- PNEC sediment (marine water) = 0.052 mg/kg sediment dw
- PNEC STP = 100 mg/L
- PNEC soil = 0.066 mg/kg soil dw
- Air: No hazard identified

A study by the Dutch Enforcement Authority nevertheless point at wide spread emissions in the Netherlands of which at least part can be linked to waste transport and waste treatment activities of waste containing HFPO-DA. The study will be published beginning of 2019.

Exposure potential for man via environment

There is evidence of exposure of the general population to the registered substance via several routes including drinking water (Gebbink et al., 2017; Versteegh & de Voogd, 2017; Sun et al., 2016; Roelandse and Timmer, 2017), local emitted air (Beekman et al., 2016), home grown fruits and vegetables (Mengelers et al., 2017) and fish (Pan et al, 2017). Concentrations of HFPO-DA in drinking water in the Netherlands were up to 30 ng/L (Gebbink et al., 2017; Versteegh & de Voogd, 2017) and up to almost 500 ng/L in drinking water from the Cape Fear River area in the USA (Sun et al., 2016). Concentrations of HFPO–DA in air were not measured, but were calculated based on the permitted emission. This concentration was up to 20 ng/m3 in populated areas closest to the fluorochemical production plant in the Netherlands (Beekman et al., 2016). Vegetables and fruit were monitored in the same area. Here, concentrations of HFPO-DA of up to 5.9 ng/g at a location within one kilometer of the fluorochemical production plant were measured (Mengelers et al. 2017). Fish was sampled in China in a river that was impacted by a fluorchemical production plant. The concentrations in muscle of carp ranged up to 6.46 ng/g (95th percentile).

Local environmental and probably worker exposures were also detected in situations where fluoropolymers containing HFPO-DA as an impurity were processed (Personal communication). It is not excluded that cross-contamination of other materials could occur at sites where both HFPO-DA, fluoropolymers containing HFPO-DA and other materials are treated.

11.1 Substances with similar hazard and use profiles on the Candidate List

FRD-902 was introduced by the registrant in 2012 as an alternative to PFOA. PFOA is identified as SVHC and its use profile is at least partly similar to FRD-902. As is being summarised in Section 1.3 also the hazard profile may be similar. Other long chain PFCAs that are already on the Candidate List include PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTDA, C11-C14-PFCAs and PFHxS. There are no short chain PFCAs yet included in the Candidate List.

11.2 Alternatives

There are currently no known alternatives to FRD-902 other than alternative perfluoro-chemicals. Perfluorinated substances that may be considered as alternatives for FRD-902, and also for PFCAs and PFSAs, are for example 3H-perfluoro-3-[(3-methoxy-propoxy)propanoic acid]

(ADONA) and hexafluoropropylene oxide trimer acid (HFPO-TA). These compounds will most likely be similar in their properties with regard to persistence, mobility and possibly toxicity to HFPO-DA and other PFCAs and PFSAs and are therefore considered as undesirable substitutes. Upon personal communication, the registrant did inform the dossier submitter about ongoing development of a fluoro-free alternative. To date however, no other information has been shared to shed further light on this development.

Annex I – OECD LRTP Tool calculation outcomes

OECD Pov & LRTP Screening Tool — HFPO-DA

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HFPO-DA

Overall Persistence POV (days)	Transport Potential CTD (km)	Transport Potential Transfer Efficiency (%)
114	8683	8.87E+00

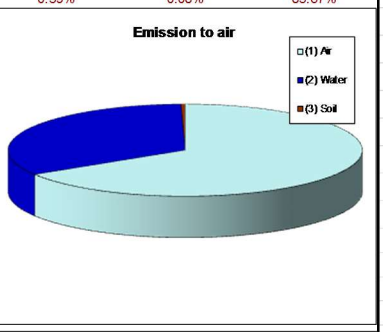
	POV (days)	CTD in air (km)	CTD in water (km)	Surface Transfer Efficiency (%)
Emission to air	38	8683		8.87E+00
Emission to water	69		104	1.13E+00
Emission to soil	114			1.56E+00

Partition Coefficients:
LogKow = 3.36
LogKaw = -2.077

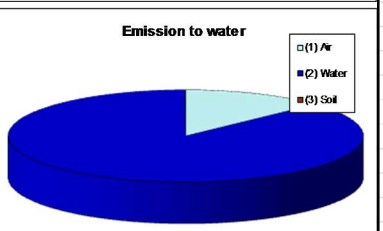
Half Lives (hours):
Air: 494.4
Water: 1440
Soil: 2880

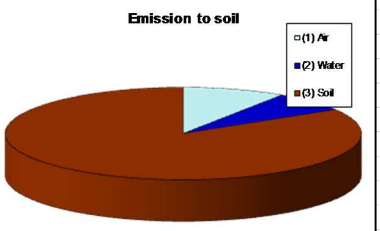
Bulk Compartment Properties	Volume (m3)	Depth (m)	Area (m2)	Density (kg/m3)	Z (mol/Pa.m3)	Equilibrium	Emission to air	Emission to water	Emission to soil
(1) Air	3.06E+18	6000	5.10E+14	1.185	4.03E-04	41.25%	66.12%	12.98%	9.33%
(2) Water	3.62E+16	100	3.62E+14	1000	4.82E-02	58.29%	33.49%	86.94%	7.00%
(3) Soil	1.48E+13	0.1	1.48E+14	1500	9.41E-01	0.47%	0.39%	0.08%	83.67%

Sub-compartment Properties	Volume (m3)	Vol Fraction	Fraction OC	Density (kg/m3)	Z (mol/Pa.m3)	Comp. Partitioning
(1,1) Air	3.06E+18	1	0	1.185	4.03E-04	100.00%
(3,1) Aerosols	6.12E+07	2E-11	0	2400	4.63E+01	0.00%
(2,2) Water	3.62E+16	0.9999995	0	1000	4.82E-02	99.99%
(3,2) Suspended sediment	1.81E+10	0.0000005	0.1	2400	9.27E+00	0.01%
(1,3) Soil air	2.96E+12	0.2	0	1.185	4.03E-04	0.01%
(2,3) Pore water	4.44E+12	0.3	0	1000	4.82E-02	1.53%
(3,3) Soil solids	7.40E+12	0.5	0.02	2400	1.85E+00	98.46%



Degrading Reactions	D (mol/Pa.h)	k (h-1)	t1/2 (h)	Eair Rate (mol/h)	water Rate (mol/h	Esoil Rate (mol/h)
(1,5) Air	1.73E+12	1.40E-03	4.94E+02	84.53	30.18	35.49
(2,5) Water	8.40E+11	4.81E-04	1.44E+03	14.70	69.39	9.14
(3,5) Soil	3.35E+09	2.41E-04	2.88E+03	0.08	0.03	54.63
Physical Removal						
(1,4,1) Air-Stratosphere	1.32E+10	1.07E-05	6.50E+04	0.64	0.23	0.27
(2,4,1) Water particle sinking	7.27E+07	4.17E-08	1.66E+07	0.00	0.01	0.00
(2,4,2) Water deep mixing	1.99E+09	1.14E-06	6.08E+05	0.03	0.16	0.02
				0.00	0.00	0.10
(3,4,2) Soil water leaching	2.14E+07	1.53E-06	4.52E+05	0.00	0.00	0.35
Inter-compartment Exchange						
(1,2) Total air-water	4.69E+11	3.80E-04	1.82E+03	22.91	8.18	9.62
(1,3) Total air-soil	3.17E+09	2.57E-06	2.70E+05	0.15	0.06	0.07
(2,1) Total water-air	4.67E+11	2.68E-04	2.59E+03	8.18	38.63	5.09
(3,1) Total soil-air	2.48E+09	1.78E-04	3.90E+03	0.06	0.02	40.35
(1,2,1) Air-water diffusion	4.67E+11	3.79E-04	1.83E+03	22.83	8.15	9.58
(1,2,2) Air-water dry deposition	3.62E+06	2.94E-09	2.36E+08	0.00	0.00	0.00
(1,2,3) Air-water rain dissolution	1.69E+09	1.37E-06	5.06E+05	0.08	0.03	0.03
(1,2,4) Air-water wet deposition	6.51E+06	5.27E-09	1.31E+08	0.00	0.00	0.00
(1,3,1) Air-soil diffusion	2.48E+09	2.01E-06	3.46E+05	0.12	0.04	0.05
(1,3,2) Air-soil dry deposition	1.48E+06	1.20E-09	5.78E+08	0.00	0.00	0.00
(1,3,3) Air-soil rain dissolution	6.91E+08	5.60E-07	1.24E+06	0.03	0.01	0.01
(1,3,4) Air-soil wet deposition	2.66E+06	2.15E-09	3.22E+08	0.00	0.00	0.00
(2,1,1) Water-air diffusion	4.67E+11	2.68E-04	2.59E+03	8.18	38.63	5.09
(3,1,1) Soil-air diffusion	2.48E+09	1.78E-04	3.90E+03	0.06	0.02	40.35
(3,2,1) Soil-water water runoff	2.78E+08	2.00E-05	3.47E+04	0.01	0.00	4.53



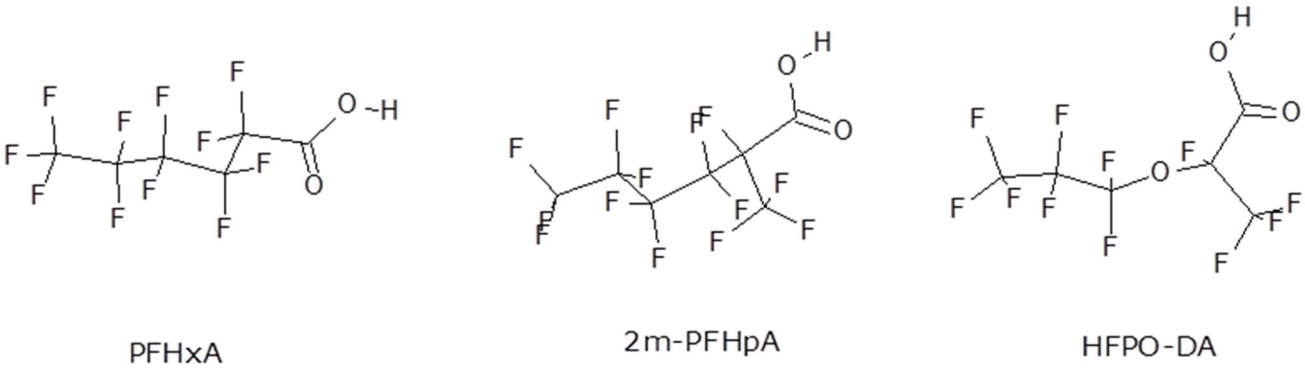


Annex II - Additional information on read across approach

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

Structural similarities of HFPO-DA to PFCAs

The difference between HFPO-DA and PFCAs is the ether bond in the perfluoro chain. The ether bond does not change the steric conformation of the (perfluoro)carbon-chain as compared to (perfluoro)alkyl carboxylic acids, The length of the C-O bond in the ether group is not very different from the length of the C-C bond in an (perfluoro)alkyl chain. Also the angle of the C-O-C bond (~120 degrees) is close to the C-C-C bond in an alkyl chain of 109 degrees. The backbone of HFPO-DA consists of a perfluoropropylene group, ether bond and a perfluoro acetic acid group, and will therefore have approximately the same length as linear PFHxA and in structure resemble the branched form of PFHpA (2m-PFHpA). Linear PFHxA, branched 2m-PFHpA and HFPO-DA are depicted in structure optimized 3D representation (ACDLabs, ChemSketch) to show the similar steric conformations and similar chain length.



PFHxA 2m-PFHpA HFPO-DA

The difference is that HFPO-DA and 2m-PFHpA have an additional trifluoromethyl-substituent in the position next to the carboxylic acid group, where PfHxA only has a fluoro atom. PFHxA and m-PFHpA have a fluorinated carbon atom in the third position, where HFPO-DA has an oxygen of the ether in the third position.

The trifluoromethyl group, which adds branching to the alkyl-chain backbone, is considered to lead to higher persistence when considering non-fluorinated hydrocarbons. For the perfluorinated hydrocarbons branching will probably not change the already very high persistence of the unbranched perfluoro acid like PFHxA.

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

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

Under environmental conditions in aqueous media the free perfluorinated carboxylic acids stay in equilibrium with their conjugate bases, the perfluorinated carboxylates. The fraction of each species depends on the acid dissociation constant (pKa) and the pH of the environmental compartment. Salts of PFCAs and HFPO-DA, which are sometimes used in laboratory experiments, will be in equilibrium with the corresponding acid in aqueous phases as well. Currently used techniques for analysis and quantification of PFCAs in i.e. environmental samples are not able to distinguish between both of the species. Therefore, reported concentrations always include the acids as well as the bases. If reported concentrations are used for the

determination of bioaccumulation factors or for experiments determining the persistency, aqueous phase concentrations include both acid and base. Experimental determination of pKa is difficult for PFCAs, i.e. because of the surface active properties. Calculated values should be taken with care, because for most of the models it is unclear whether PFCAs are within their applicability domain. For assessing the intrinsic properties of HFPO-DA within this dossier the exact knowledge of the fraction of each species is not required, because both of the species will be available independently from the starting conditions.
Physicochemical properties and partition coefficients of HFPO-DA and C4 to C8-PFCAs are presented in the table below. The estimates have to be considered as rough indications because of the high degree of dissociation of the substances in water. It can be concluded that HFPO-DA has estimated properties that are comparable to the C4 to C8-PFCAs.

Table A: Basic substance information and physical chemical properties relevant to justify grouping

Acronym	HFPO-DA	PFBA	PFPeA	PFHxA	PFHpA	PFOA
IUPAC Name	2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propanoic acid	Butanoic acid, heptafluoro-	Pentanoic acid, nonafluoro-	Hexanoic acid, undecafluoro-	Heptanoic acid, tridecafluoro-	Octanoic acid, pentadecafluoro-
Chemical Structure	$CF_3(CF_2)_2O$ $CF(CF_3)COOH$	$CF_3(CF_2)_2$-COOH	$CF_3(CF_2)_3$-COOH	$CF_3(CF_2)_4$-COOH	$CF_3(CF_2)_5$-COOH	$CF_3(CF_2)_6$-COOH
CAS No	13252−13−6	375-22-4	2706-90-3	307-24-4	375-85-9	335-67-1
Molecular Weight g/mol	330.06	214.04	264.05	314.05	364.06	414.07
Partitioning Coefficient log K_{OW}	3.36	2.14	2.81	3.48	4.15	4.81
log K_{OA}	5.44	4.45	4.40	4.35	4.30	4.24
log K_{AW}	-2.08	-2.31	-1.59	-0.87	-0.15	0.57
Dissociation constant pKa	-0.77	-1.07	0.34	-0.78	-2.24	-4.20
Log K_{OC}	2.48/1.92	1.81/1.34	2.46/1.71	3.12/2.08	3.77/2.45	4.42/2.82
Water solubility	17/27	316/1373	17/197	0.85/27	0.042/3.6	0.0020/0.48
Vapour pressure	92	2000	688	263	149	19
Boiling point	187	123	145	165	185	204

All data are estimated by EpiSuite v 4.11, except the pKa, which was estimated by MarvinSketch v16.10.24. For log K_{oc} and solubility two values are estimated by EpiSuite. The first value denotes the fragment method, the second value the value based on log K_{ow}.

Annex III – Overview of dose-response modelling results

Table 41: Effect dose lower limits and upper limits for parameters observed in Craig (2013) (both sexes), Haas (2009) (both sexes), MacKenzie (2010) (both sexes), and Edwards (2010a) (males).

			Chronic rat, final sacrifice				Chronic rat, interim sacrifice				Subchronic rat				Subchronic mouse			
			Female		Male		Female		Male		Female		Male		Female		Male	
Organ	Parameter/ effect	Effect size	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU
Liver	Carcinoma	10	502	2120	502	2120	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Liver	Adenoma	10	301	522	301	522	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Liver	Cystic focal degeneration	10	181	501	0.674	37.3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Liver	Single cell necrosis	10	509	672	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4.58	inf	1.55	1.79
Liver	Focal necrosis minimal	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.475	9.21	1.13	4.51
Liver	Focal necrosis mild	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.684	6.59	NA	NA
Liver	Hypertrophy minimal	10	50.6	100	45.2	76.4	NA	NA	NA	NA	172	347	8.41	10.5	0.995	1.72	0.389	0.438
Liver	Hypertrophy mild	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3.29	4.77	1.51	3.29
Liver	Increased pigment	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4.39	Inf	1.51	1.79
Liver	Mitotic figures minimal	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5.82	inf	3.08	3.47
Liver	Mitotic figures mild	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5.32	inf	3.83	5.32
Liver	Relative weight	10	16.9	345	0.956	31.8	29.5	Inf	3.22	99.8	27.8	66.9	3.9	9.63	0.177	0.525	0.0856	0.224
Liver	Relative weight	5	3.64	314	0.201	26.4	2.01	Inf	0.22	50.7	7.9	24.9	1.12	3.53	0.0515	0.186	0.0243	0.0835
Liver	Absolute weight	10	2.74	331	0.244	37.4	NA	NA	NA	NA	36.8	106	5.94	16.9	0.211	0.74	0.0945	0.322
Liver	Absolute weight	5	0.239	293	0.0266	28.4	NA	NA	NA	NA	11.4	45.8	1.86	7.15	0.0668	0.305	0.029	0.14
	Cholesterol	-50	NA	NA	NA	NA	3510	700000	3510	700000	2000	14600	226	3390	142	Inf	9.6	208
	Cholesterol	-20	NA	NA	NA	NA	58.2	7170	58.2	7170	62.1	714	9.6	121	16.7	Inf	0.403	6.19
	AST	50	NA	NA	NA	NA	612	Inf	17.7	44.9	20400	Inf	305	Inf	12.6	Inf	0.298	7.03
	AST	20	NA	NA	NA	NA	496	Inf	5.14	36.7	8630	Inf	150	Inf	2.16	Inf	0.0121	2.78
	ALT	50	NA	NA	NA	NA	578	Inf	3.01	41.3	2010	Inf	109	1350	3.77	12.3	0.512	3.62
	ALT	20	NA	NA	NA	NA	379	Inf	0.406	33.8	1150	Inf	63.1	583	1.29	5.37	0.142	2.91
	ALP	50	NA	NA	NA	NA	38.1	61000	0.645	8.35	478	1050	41.2	73.1	1.35	2.96	0.286	1.15
	ALP	20	NA	NA	NA	NA	1.52	2460	0.0258	1.26	156	423	12.7	35.3	0.408	1.47	0.0836	0.593
	SDH	50	NA	NA	NA	NA	764	Inf	1.26	51.5	NA	NA	NA	NA	4.47	7.91	1.58	3.47
	SDH	20	NA	NA	NA	NA	1110	Inf	0.0506	36.6	NA	NA	NA	NA	2.41	5.03	0.754	2.74

			Chronic rat, final sacrifice				Chronic rat, interim sacrifice				Subchronic rat				Subchronic mouse			
			Female		Male		Female		Male		Female		Male		Female		Male	
Organ	Parameter/ effect	Effect size	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU
	Bile acids	10	NA	NA	NA	NA	0.0152	210	0.0152	210	0.928	356	0.928	356	0.928	356	0.928	356
	Bile acids	5	NA	NA	NA	NA	0.00106	168	0.00106	168	0.138	311	0.138	311	0.138	311	0.138	311
	Bilirubin	10	NA	NA	NA	NA	NA	NA	NA	NA	0.757	13.8	0.757	13.8	0.757	13.8	0.757	13.8
	Bilirubin	5	NA	NA	NA	NA	NA	NA	NA	NA	0.0483	2.76	0.0483	2.76	0.0483	2.76	0.0483	2.76
Kidney	Papilla oedema	10	93.1	295	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kidney	Tubular dilation	10	64	344	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kidney	Transitional hyperplasia	10	18.4	200	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kidney	Progressive nephropathy	10	9.8	200	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kidney	Tubular mineralisation	10	1.9	415	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kidney	Tubular hypertrophy	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	5.86	inf	1.59	3.19
Kidney	Relative weight	10	133	502	8.97	49.2	NA	NA	NA	NA	16.7	121	16.7	121	104	Inf	104	Inf
Kidney	Relative weight	5	30.8	424	2	41.6	NA	NA	NA	NA	1.14	15.5	1.14	15.5	7.55	Inf	7.55	Inf
Kidney	Absolute weight	10	74.4	8100	74.4	8100	NA	NA	NA	NA	170	1060	40.3	287	1.33	17.3	2.53	13.6
Kidney	Absolute weight	5	5.07	637	5.07	637	NA	NA	NA	NA	32.5	866	8.66	129	0.296	Inf	0.579	5.11
	BUN	10	NA	NA	NA	NA	58.5	413	58.5	413	825	Inf	11.6	63.4	2.2	Inf	2.71	Inf
	BUN	5	NA	NA	NA	NA	17.7	340	17.7	340	362	Inf	2.97	47.5	0.826	Inf	0.984	Inf
	Total protein	20	NA	NA	NA	NA	NA	NA	NA	NA	2340	Inf	154	Inf	9.42	Inf	5.68	75.1
	Total protein	10	NA	NA	NA	NA	NA	NA	NA	NA	2360	Inf	131	inf	7.87	Inf	2.4	12.3
	Albumin	50	NA	NA	NA	NA	64200	Inf	987	14900	NA	NA	NA	NA	NA	NA	NA	NA
	Albumin	20	NA	NA	NA	NA	4560	Inf	51.7	598	13400	Inf	203	1450	54.2	Inf	4.24	67
	Albumin	10	NA	NA	NA	NA	339	Inf	3.84	44.5	2170	inf	22.5	124	9.05	Inf	0.376	5.41
	Globulin	50	NA	NA	NA	NA	2360	700000	2360	700000	NA	NA	NA	NA	NA	NA	NA	NA
	Globulin	20	NA	NA	NA	NA	513	7050	513	7050	678	831	108	185	6.31	19.5	10.6	Inf
	Globulin	10	NA	NA	NA	NA	36.7	442	36.7	442	419	631	74	125	4.46	13.3	7.69	inf
	A/G ratio	50	NA	NA	NA	NA	847	36900	94.8	1530	NA	NA	NA	NA	NA	NA	NA	NA
	A/G ratio	20	NA	NA	NA	NA	95	1470	5.75	86.3	228	482	30.1	86.3	2.61	46.7	3.18	27.2
	A/G ratio	10	NA	NA	NA	NA	7.03	271	0.426	30.8	41.8	238	5.46	37.2	0.584	12.7	0.713	7.26
Spleen	Absolute weight	10	2.05	124	2.05	124	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Spleen	Absolute weight	5	0.115	16.8	0.115	16.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MCHC	10	NA	NA	NA	NA	579	1690	579	1690	756	Inf	83.6	Inf	6.65	827	6.9	258
	MCH	10	NA	NA	NA	NA	NA	NA	NA	NA	938	1180	179	inf	9.83	Inf	6.52	inf

			Chronic rat, final sacrifice				Chronic rat, interim sacrifice				Subchronic rat				Subchronic mouse			
			Female		Male		Female		Male		Female		Male		Female		Male	
Organ	Parameter/ effect	Effect size	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU	EDL	EDU
	MCV	10	NA	NA	NA	NA	331	584	331	584	818	968	173	Inf	8.83	Inf	5.8	10.3
	HCT	-10	NA	NA	NA	NA	140	348	140	348	135	492	56.5	132	49.2	Inf	31.2	inf
	RBC	-10	NA	NA	NA	NA	106	317	106	317	171	433	75.4	135	23.7	Inf	8.28	299
	HGB	-10	NA	NA	NA	NA	108	296	108	296	158	454	54	103	15.9	Inf	12.5	Inf
	Total reticulocytes	10	NA	NA	NA	NA	10.2	339	10.2	339	139	476	30.6	67.1	4.79	Inf	3.32	7.47
	Platelets	10	NA	NA	NA	NA	NA	NA	NA	NA	28	719	3.84	80.1	12.5	Inf	0.085	19.9
Testes	Hyperplasia	10	NA	NA	7.27	64.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

NA = not available; Inf = infinity

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