National Institute for Public Health
and the Environment
*Ministry of Health, Welfare and Sport*

# Evaluation of substances used in the GenX technology by Chemours, Dordrecht

RIVM Letter report 2016-0174
M. Beekman et al.

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# Colophon

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# Synopsis


**Evaluation of substances used in the GenX technology by Chemours, Dordrecht**

Since 2012, Chemours (Dordrecht) is using the GenX technology to produce plastics (fluoropolymers). In this technology, the substances FRD-902, FRD-903 and E1 replace the controversial PFOA substances. No health risk is expected for people living in the vicinity of the plant due to the emissions of these substances.

This is the finding of the RIVM. Commissioned by the Ministry of Infrastructure and the Environment (IenM), it is investigated to what extent the three substances are harmful to people living near the factory. For this, the scientific literature and the information in the European chemicals legislation REACH are examined on the properties of the listed substances. In addition, based on both the maximum authorised quantity and the recorded emission data that Chemours has provided, it is calculated to what extent they are released.

FRD-903 is used to manufacture FRD-902. E1 is formed during the manufacturing process. FRD-903 and E1 are emitted to the air. Like PFOA, FRD-903, FRD-902 and E1 are perfluorinated hydrocarbons and poorly degradable in the environment. Also, FRD-902 and FRD-903 are causing similar harmful effects as PFOA (such as carcinogenic and effects on the liver). These substances are, however, less harmful to reproduction than PFOA; reproduction toxicity is the reason to regard PFOA as substance of very high concern. In contrast to PFOA, FRD-902 and FRD-903 seem not to bioaccumulate in humans.

A safe limit value for the general population is derived based on a worst-case scenario. The concentration FRD-903 in air stays below this limit value. For E1, information is missing to derive a limit value. Based on the limited available information, this substance is probably less harmful than PFOA.


Keywords: GenX, PFOA alternative, PBT assessment, risk assessment, REACH



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# Publiekssamenvatting


## Beoordeling van de stoffen die door Chemours (Dordrecht) bij de GenX technologie worden gebruikt

Sinds 2012 gebruikt fabrikant Chemours (Dordrecht) de GenX-technologie om plastics (fluorpolymeren) te maken. Bij deze technologie zijn de omstreden PFOA-verbindingen vervangen door de stoffen FRD-902 en FRD-903 en E1. Naar verwachting vormt de uitstoot van deze stoffen door de fabriek via de lucht geen risico voor de gezondheid van omwonenden.

Dit blijkt uit onderzoek van het RIVM. In opdracht van het ministerie van Infrastructuur en Milieu (IenM) is onderzocht in hoeverre de drie stoffen schadelijk zijn voor omwonenden van de fabriek. Hiervoor is in de wetenschappelijke literatuur en de informatie in de Europese stoffenwetgeving REACH onderzocht wat bekend is over de eigenschappen van de genoemde stoffen. Daarnaast is op basis van zowel de maximaal vergunde hoeveelheid als de emissiegegevens die Chemours heeft verstrekt, berekend in welke mate ze zijn vrijgekomen.

FRD-903 wordt gebruikt om FRD-902 te maken. E1 ontstaat tijdens het productieproces. FRD-903 en E1 worden via de fabrieksschoorsteen naar de lucht uitgestoten. Net als PFOA zijn geperfluorideerde koolwaterstoffen FRD-902 en FRD-903 en E1 slecht afbreekbaar in het milieu. Ook veroorzaken FRD-903 en FRD-902 vergelijkbare schadelijke effecten als PFOA (zoals kankerverwekkend en effecten op de lever). Deze stoffen zijn wel minder schadelijk voor de voortplanting dan PFOA; bij PFOA is dit aspect juist de reden om deze stof als zeer zorgwekkend te beschouwen. In tegenstelling tot PFOA lijken FRD-903 en FRD-902 zich niet in de mens op te hopen.

Voor FRD-903 en FRD-902 heeft het RIVM een veilige grenswaarde voor de algemene bevolking afgeleid op basis van een worst-case scenario. De concentratie FRD-903 in lucht blijft onder deze grenswaarde. Voor E1 ontbreekt informatie om een grenswaarde te kunnen bepalen. Op basis van de beperkt beschikbare informatie wordt verondersteld dat deze stof waarschijnlijk minder schadelijk is dan PFOA.


Kernwoorden: GenX, PFOA alternatief, PBT beoordeling, risicobeoordeling, REACH



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# Samenvatting

In dit rapport worden de perfluorverbindingen (FRD-903, FRD-902 en E1) geëvalueerd. Deze verbindingen worden gebruikt of ontstaan bij de GenX technologie voor het maken van fluorpolymeren bij Chemours in Dordrecht. Bij deze technologie wordt geen gebruik gemaakt van de omstreden PFOA-verbindingen die eerder werden toegepast. Hierbij worden de volgende vragen beantwoord:

1. Wat is bekend over de PBT[1] eigenschappen van FRD-903, FRD-902 en E1?
2. Wat is bekend over de eventuele CMR[2] en STOT RE[3] eigenschappen (met name lever- en niertoxiciteit) van FRD-903, FRD-902 en E1?
3. Wat is bekend over de emissie van FRD-903 en E1 bij Chemours in Dordrecht?
4. Wat is er te zeggen over de gezondheidseffecten (nu en in de toekomst) voor de omwonenden als gevolg van blootstelling aan FRD-903 en E1?

Aangezien alle beschikbare toxiciteitsstudies zijn uitgevoerd met het ammoniumzout (FRD-902) en niet met het zuur (FRD-903), is de beoordeling van FRD-903 in dit rapport gebaseerd op de gegevens van FRD-902. Het is gerechtvaardigd om de gegevens van FRD-902 te gebruiken voor FRD-903 omdat de effecten in het lichaam bij beide stoffen veroorzaakt worden door het anion (2,3,3,-tetrafluoro-2-(heptafluoropropoxy)propanoate).

Bij de eerste vraag concludeert het RIVM dat het niet is uitgesloten dat de aan de GenX technologie gerelateerde stoffen (FRD-903, FRD-902 en E1) voldoen aan de PBT of vPvB[4] criteria. Alle drie de stoffen zijn perfluorverbindingen en hiervan kan worden gesteld dat ze vrijwel zeker zeer slecht in het milieu worden afgebroken. Aangezien FRD-903 en FRD-902 sneller dan PFOA het lichaam verlaten, wordt verwacht dat beide stoffen een geringere bioaccumulatie vertonen. Er kan echter geen definitieve conclusie worden getrokken omdat data over de eliminatiesnelheid bij de mens ontbreken. Voor de stof E1 is er onvoldoende informatie om een conclusie te trekken over de mogelijke bioaccumulatie. Aangezien E1 geen hydrofiele groep bevat, is de verwachting dat de eliminatie van E1 trager is en daarmee een hogere potentie voor bioaccumulatie heeft dan PFOA. Aan de andere kant wordt E1 waarschijnlijk weer gemakkelijk uitgeademd. FRD-903 en FRD-902 zijn naar verwachting minder gevaarlijk dan PFOA, maar ook hiervoor kunnen geen definitieve conclusies ten aanzien van het T criterium worden getrokken. E1 voldoet waarschijnlijk niet aan het T criterium van de PBT analyse.

[1] Persistent, Bioaccumulative and Toxic
[2] Carcinogenic, mutagenic or toxic for the reproduction
[3] Specific target organ toxicity after repeated exposure
[4] Very Persistent and very Bioaccumulative



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Bij de beoordeling van de CMR en STOT RE eigenschappen, wordt geconcludeerd dat FRD-903 en FRD-902 geclassificeerd zouden moeten worden als kankerverwekkend categorie 2 (mogelijk kankerverwekkend voor de mens). Verder laten de beschikbare studies zien dat beide stoffen niet mutageen zijn. De beperkte reproductie-toxische effecten die gevonden worden, leiden normaal gesproken niet tot een classificatie op dit onderdeel. Dit is in tegenstelling tot PFOA, welke geclassificeerd is als schadelijk voor de voortplanting (categorie 1B). Ten slotte is het lastig om de toxiciteit voor organen (zoals lever en nier) te beoordelen omdat de testen die bij muizen zijn gedaan, zijn uitgevoerd bij doseringen lager dan de voorgeschreven doseringen in de guidance documenten. Dit kan een indicatie zijn dat classificatie als STOT RE categorie 2 noodzakelijk is. De effecten die bij de rat zijn waargenomen, zijn marginaal en eveneens moeilijk te beoordelen vanwege de grote stappen in de doseringsniveaus die zijn gehanteerd. De effecten op de lever zijn bij FRD-902 en PFOA waargenomen bij ongeveer vergelijkbare doseringen.

De beschikbare informatie over de toxiciteit van E1 is beperkt, maar de informatie die beschikbaar is, wijst op een lage tot zeer lage toxiciteit. Deze conclusie wordt ondersteund door informatie over de toxiciteit van vergelijkbare verbindingen. Wel dient opgemerkt te worden dat alle beschikbare studies enkel zijn uitgevoerd met mannetjes proefdieren en slechts van beperkte blootstellingsduur waren. De beschikbare in vitro en in vivo mutageniteitsdata, gecombineerd met de data van vergelijkbare verbindingen, tonen aan dat het onwaarschijnlijk is dat E1 mutageen is. Verder laten de beschikbare gegevens zien dat het waarschijnlijk niet nodig is om E1 te classificeren voor acute toxiciteit en voor STOT RE door inademing. De beoordeling van E1 voor classificatie op andere eindpunten, waaronder carcinogeniteit, reproductietoxiciteit en STOT RE door orale blootstelling, is niet mogelijk op basis van de nu beschikbare informatie.

Voor FRD-903 en FRD-902 wordt in dit rapport – rekening houdend met een worst-case aanpak - een chronische inhalatieblootstellingslimiet van 73 ng/m$^3$ afgeleid. Hierbij is een extra veiligheidsmarge gehanteerd vanwege de onzekerheid over de mogelijke bioaccumulatie van deze stoffen. De jaargemiddelde concentraties van FRD-903 in de lucht zijn berekend op basis van de maximaal vergunde hoeveelheden. Deze berekening laat zien dat de concentratie FRD-903 in lucht 20 ng/m$^3$ is bij de dichtstbijzijnde bewoonde gebieden (de dijk aan de overkant van de rivier) en lagere concentraties verder van de fabriek. De berekening op basis van de gerapporteerde emissies in 2014, komt uit op 15 ng/m$^3$ voor de dichtstbijzijnde bewoonde gebieden. Het vergelijken van deze berekende concentraties met de afgeleide grenswaarde van 73 ng/m$^3$ leidt tot de conclusie dat op basis van de beschikbare informatie er geen gezondheidsrisico voor de omwonenden van de Chemours fabriek door blootstelling aan FRD-903 te verwachten is.

De informatie over de toxiciteit van E1 is beperkt. De gegevens over de toxiciteit van E1 zijn onvoldoende om een inhalatieblootstellingslimiet voor E1 af te leiden. De jaargemiddelde concentraties van E1 in de lucht zijn berekend op basis van de maximaal vergunde hoeveelheden. Deze berekening laat zien dat de concentratie E1 in lucht 40 ng/m$^3$ is bij de



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dichtstbijzijnde bewoonde gebieden (de dijk aan de overkant van de rivier) en lagere concentraties verder van de fabriek. De berekening op basis van de gerapporteerde emissies in 2014 komt uit op 20 ng/$m^3$ voor de dichtstbijzijnde bewoonde gebieden. Vanwege de ontbrekende informatie over de toxiciteit van E1, kan er geen conclusie worden getrokken over een mogelijk gezondheidsrisico voor de omwonenden van de Chemours fabriek door blootstelling aan E1.



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# Summary

In this report, the GenX related perfluorinated substances (FRD-903, FRD-902 and E1) are evaluated. These substances are used or are formed during the production of fluoropolymers by Chemours (Dordrecht) applying the GenX technology. In this technology, the controversial PFOA substances are replaced. The following questions are addressed in the evaluation:

1. What is known about the PBT[5]-properties of FRD-903, FRD-902 and E1?
2. What is known about the possible CMR[6]-properties and STOT RE-properties[7] (especially the toxicity to kidney and liver) of FRD-903, FRD-902 and E1?
3. What is known about the emission of FRD-903 and E1 by Chemours (Dordrecht)?
4. What are the possible health effects (now and in the future) for people living in the vicinity of the Chemours Dordrecht plant due to exposure to FRD-903 and E1?

For FRD-903 the evaluation is based on read across from FRD-902, since all available toxicological studies were performed with the ammonium salt (FRD-902). Read-across of the toxicological properties of the ammonium salt to the acid (FRD-903) is considered justified for systemic effects as after dissolution and dissociation of the acid and the salt the absorption in the intestinal tract and the lungs and distribution over the body of the anion (2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate) will be the same.

As to the first question above, it is concluded that RIVM cannot exclude that the GenX related substances meet the PBT/vPvB[8] criteria. All evaluated substances (FRD-903, FRD-902 and E1) are perfluorinated compounds and can be regarded as certainly very persistent. Since FRD-903 and FRD-902 are more rapidly eliminated than PFOA, it is expected that both substances bioaccumulate to a lesser degree than PFOA does. However, it is not possible to reach a conclusion on the human bioaccumulation potential in absence of data on the human clearance time. For the substance E1, insufficient information is available to draw a conclusion about the bioaccumulation potential. Since E1 contains no hydrophilic group, the human clearance time of the substance and the bioaccumulation potential are expected to be higher than for PFOA, although E1 has the potential to be excreted via exhalation. FRD-903 and FRD-902 are considered less hazardous compared to PFOA. However, no definitive conclusion can be reached whether they meet the T criteria. E1 will most likely not meet the T criteria.

For the CMR and STOT RE properties, it is concluded that classification as carcinogenic category 2 (suspected human carcinogen) is justified for

[5] Persistent, Bioaccumulative and Toxic
[6] Carcinogenic, mutagenic or toxic for the reproduction
[7] Specific Target Organ Toxicity, Repeated Exposure
[8] Very Persistent and very Bioaccumulative



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FRD-903 and FRD-902. The available studies show that both substances are not mutagenic. On reproductive toxicity the limited effects observed in presence of maternal toxicity do not normally result in classification, whereas PFOA is classified as toxic for the reproduction (category 1B). The requirement of STOT RE 2 (like liver and kidney) is difficult to assess due to dose levels tested in mice clearly below the guidance values, which may be taken as an indication that STOT RE 2 is needed. The effects in the rat are borderline and difficult to assess due to the large steps in the dose levels. Effects on the liver are observed at the similar dose levels for FRD-902 and PFOA.

The available information on the toxicity of E1 is limited but that information indicates that E1 has a low to very low toxicity. This is supported by the repeated dose toxicity information on some structural analogues. However, all available studies were performed in male animals only and were of limited duration only. The available in vitro and in vivo data on mutagenicity combined with the read-across data show that E1 is unlikely to be mutagenic. In addition, the available data indicate no requirement for classification for acute toxicity nor probably for STOT RE via inhalation. The requirement for classification for other hazard classes including carcinogenicity, reproductive toxicity and STOT RE via oral exposure, however, is unknown.

A chronic inhalation exposure limit of 73 ng/m$^3$ for FRD-903 and FRD-902 is derived in the present report in a worst-case approach, taking into account an extra safety margin due to uncertainty in the accumulation potential. The year-average air concentrations of FRD-903 were calculated based on the permitted emissions. This led to estimated concentrations in air of about 20 ng/m$^3$ for the nearest populated areas (along the dike opposite side of the river) and lower concentrations at greater distances from the plant. Based on the recorded emissions for 2014 the estimated concentrations for the nearest populated areas are about 15 ng/m$^3$. Comparing these concentrations with the limit value of 73 ng/m$^3$ leads to the conclusion that based on the available data, no health risk is expected for people living in the vicinity of the Chemours Dordrecht plant due to exposure to FRD-903.

The information on the toxicity of E1 is limited. The data are insufficient for deriving an inhalation exposure limit for the general population. The year-average air concentrations for E1 were calculated based on the permitted emissions. This led to estimated concentrations in air of about 40 ng /m$^3$ for the nearest populated areas (along the dike at the opposite side of the river) and lower concentrations at greater distances from the plant. Based on recorded emissions for 2014 the estimated concentrations for the nearest populated areas are about 20 ng/m$^3$. Due to the insufficient health effects information available for E1, these concentrations cannot be evaluated as to the possible health risk they might pose for people living in the vicinity of the Chemours plant in Dordrecht.



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# 1 Introduction

DuPont has developed the GenX technology as a polymerization aid to make fluoropolymers like teflon without the use of perfluorooctanoic acid (PFOA)[9]. PFOA is an important representative of the substance group of per- and polyfluorinated substances (PFASs). PFASs consist of carbon chains of different chain length, where the hydrogen atoms are completely (perfluorinated) or partly (polyfluorinated) substituted by fluorine atoms. The very stable bond between carbon and fluorine is only breakable with high energy input. Therefore, perfluorinated acids, like PFOA, are not degradable in the environment. The hazard profile of PFOA is well known: PFOA is a persistent, bioaccumulative, and toxic substance (PBT), which may cause severe and irreversible adverse effects on the environment and human health. Due to its PBT properties and toxicity to the reproduction, PFOA and its ammonium salt (APFO) have been identified as substances of very high concern (SVHC) under REACH[10]. Further, a proposal for restricting the manufacture and use of PFOA is under discussion within the context of the REACH regulation[11].

Chemours Dordrecht has started to replace the use of PFOA by the GenX technology from 2005 (in the USA) onwards and has completely ceased the use of PFOA since 2012 at the plant in Dordrecht. This technology is also based on perfluorinated substances. According to the manufacturer, the resin manufacturing process includes the thermal transformation of the GenX processing aid (FRD-902) into the hydrophobic water-insoluble hydride (E1). The present assessment focuses on the GenX related substances:

- the precursor 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoic acid (FRD-903),
- the processing agent ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902) and
- the transformation product heptafluoropropyl 1,2,2,2-tetrafluorethyl ether (E1).

Another substance, perfluorisobutene, a by-product emitted during the production of fluoropolymers is cause for concern due to its highly toxic properties. This substance is not covered by the current assessment because this substance is not specific to the GenX technology. This assessment compares the specific substances used in the GenX technology with APFO.

Concerns have been raised about the hazard and risk properties of the GenX technology used by Chemours (Dordrecht) and therefore the Ministry of Infrastructure and Environment has requested RIVM to

[9] https://www.chemours.com/Dordrecht-Plant/nl_NL/assets/downloads/pdf/2016-0909-met-behulp-van-genx-fact-sheet.pdf
[10] Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
[11] Annex XV restriction dossier, https://echa.europa.eu/previous-consultations-on-restriction-proposals/-/substance-rev/1908/term.



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evaluate the substances used in this GenX technology. More specifically the Ministry asks RIVM to answer the following questions:

1. What is known about the PBT-properties of FRD-903 and E1[12]?
2. What is known about the possible CMR-properties and STOT RE-properties (especially the toxicity to kidney and liver) of FRD-903 and E1?
3. What is known about the emission of FRD-903 and E1 by Chemours (Dordrecht)?
4. What are the possible health effects (now and in the future) for people living in the vicinity of the Chemours Dordrecht plant due to exposure to FRD-903 and E1?

The assessment by RIVM is based on available literature, which mainly originates from REACH. For FRD-902 one REACH registration dossier is available (10-100 tonnes per year). FRD-902 is on the REACH Community Rolling Action Plan (CoRAP) for 2017 (for substance evaluation on the potential PBT/vPvB properties, which will be conducted by Germany). The acid and the hydride are not registered. There is no harmonised classification available for any of the substances. FRD-902 is described in the REACH Annex XV restriction dossier of PFOA under the chapter on alternatives. The comparison made between PFOA and FRD-902 in this restriction dossier is used for the present assessment and is supplemented with data from the registration dossier, studies provided by Chemours and publications in the scientific literature.

No additional information was retrieved on the human toxicological and environmental properties of FRD-903. Therefore, assessment of these properties in chapter 3 and 4 is based on read-across with the ammonium-salt (FRD-902). For E1 available data proved to be limited only and for this chemical the current assessment is therefore limited to a screening and is mainly based on QSAR estimations and mainly old data provided by Chemours.

It has to be noted that in accordance to the request from the Ministry, the possible health effects for people living near the Chemours plant is assessed. Exposure to these substances by inhalation is considered the most relevant route for people living near the Chemours plant. Further information is needed to assess the possibility of exposure by contaminated drinking water.

Report structure
Some general information on the substances used in the GenX technology is given in chapter 2. In chapter 3 and 4 of this report the PBT and human health (CMR, STOT-RE) properties, respectively, are evaluated. In chapter 4, exposure limits for the general population are derived for both FRD-903 and E1. Chapter 5 presents the known emissions of the substances by Chemours. In chapter 6 the possible health effects are described. And finally, concluding remarks are made in chapter 7.

[12] Including possible other relevant substances related to the GenX technology.



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# 2 General information

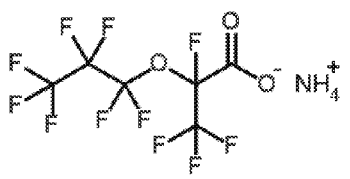
## 2.1 Description GenX technology

FRD-902 is used as processing aid in the Teflon PTFE and Teflon FEP plants of Chemours. Other uses of FRD-902 are not described in the registration dossier or in the literature. FRD-902 is manufactured by mixing FRD-903 with an ammonium hydroxide solution. FRD-903 is imported.

FRD-902 controls the polymerization to make fluoropolymers. Fluoroploymer resins and finished goods are used in many applications like wire cables and Teflon coating. During the resin manufacturing process, FRD-902 is transformed into the hydrophobic water-insoluble hydride (E1). During the process, FRD-903 and E1 are emitted to air from the Teflon PTFE and from the Teflon FEP plants. Furthermore, FRD-902 and FRD-903 are emitted to wastewater. After removal of these compounds, the wastewater is sent to the local municipal sewage treatment plant. Exposure of people living in the vicinity of the Chemours is expected to be primarily through the emission to air.

## 2.2 Substance identity and status of FRD-902

| | |
|---|---|
| Name: | ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate |
| CAS-number: | 62037-80-3 |
| EC-number: | 700-242-3 |
| Synonyms: | FRD-902, C3-dimer salt |
| IUPAC name: | ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate |
| Structure: | C6H4NF11O3 |



| | |
|---|---|
| REACH: | registered by Chemours Netherlands BV: 10 – 100 TPA, full registration |
| CLP[13]: | no harmonised classification, 28 notifiers to the CLP inventory (19 September 2016) (Acute Tox. 4; H302, Eye Dam. 1; H318, STOT RE 2; H373 (blood)), see table 1. |

[13] Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP Regulation)



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*Table 1 Notifications to the CLP inventory for FRD-902 (19 September 2016).*

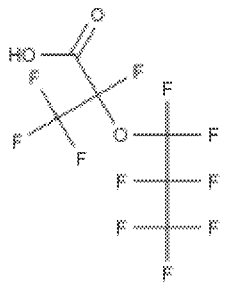
| Classification | | | Labelling | | Specific Concentration limits, M-Factors | Notes | Classification affected by Impurities / Additives | Additional Notified Information | Number of Notifiers | Joint Entries |
|---|---|---|---|---|---|---|---|---|---|---|
| Hazard Class and Category Code(s) | Hazard Statement Code(s) | Hazard Statement Code(s) | Supplementary Hazard Statement Code(s) | Pictograms, Signal Word Code(s) | | | | | | |
| Acute Tox. 4 | H302 | H302 | | GHS07<br>GHS05<br>GHS08<br>Dgr | | | | State/Form | 27 | |
| Eye Dam. 1 | H318 | H318 | | | | | | | | |
| STOT RE 2 | H373 (Blood) | | | | | | | | | |
| Acute Tox. 4 | H302 | H302 | | GHS07<br>GHS05<br>GHS08<br>Dgr | | | | IUPAC Names | 1 | |
| Eye Dam. 1 | H318 | H318 | | | | | | | | |
| STOT RE 2 | H373 (blood anaemia) | H373 | | | | | | | | |

### *Physical chemical properties*[14]

| | |
|---|---|
| Melting point: | 208 °C (99.4% purity) |
| Freezing point: | -21 °C (86% purity) |
| Vapour pressure: | 0.012 Pa (99.4% purity) |
| Solubility in water: | >1000 g/L (99.4 % purity) |
| Form: | liquid (86% purity, marketed form), solid (dried substance, 99.4% purity) |
| Color: | colourless liquid |
| Density: | 1118 g/L (99.4% purity) |
| Dissociation constant: | pKa: 3.82 (86% purity) |

## 2.3 Substance identity and status of FRD-903

| | |
|---|---|
| Name: | 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid |
| CAS-number: | 13252-13-6 |
| EC-number: | 236-236-8 |
| Synonyms: | FRD-903, C3-dimer |
| IUPAC name: | 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid, perfluoro-2-methyl-3-oxahexanoic acid |
| Structure: | C6HF11O3 |



| | |
|---|---|
| REACH: | not registered |
| CLP: | no harmonised classification, 99 notifiers to the CLP inventory (including Acute Tox. 4; H302, Skin Corr. 1B or 1C; H314, Eye Dam. 1; H318, STOT SE 3; H335 (Respiratory) and no classification), see table 2. |

[14] REACH registration data, 19 September 2016



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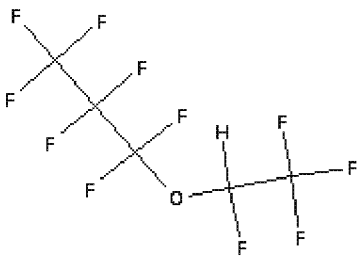
*Table 2. Notifications to the CLP inventory for FRD-903 (19 September 2016).*

| Classification | | | Labelling | | Specific Concentration limits, M-Factors | Notes | Classification affected by Impurities / Additives | Additional Notified Information | Number of Notifiers | Joint Entries |
|---|---|---|---|---|---|---|---|---|---|---|
| Hazard Class and Category Code(s) | Hazard Statement Code(s) | Hazard Statement Code(s) | Supplementary Hazard Statement Code(s) | Pictograms, Signal Word Code(s) | | | | | | |
| Acute Tox. 4 | H302 | H302 | | GHS07<br>GHS05<br>Dgr | | | | State/Form | 66 | |
| Skin Corr. 1C | H314 | H314 | | | | | | | | |
| Eye Dam. 1 | H318 | | | | | | | | | |
| STOT SE 3 | H335 (Respiratory sys…) | H335 | | | | | | | | |
| | | | | | | | | | 30 | |
| | | H314 | | GHS05<br>Dgr | | | | | 2 | |
| Skin Corr. 18 | H314 | H314 | | GHS05<br>Dgr | | | | State/Form IUPAC Names | 1 | |

## 2.4 Substance identity and status of E1

Name: heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether
CAS-number: 3330-15-2
EC-number: 671-353-1
Synonyms: propane, 1,1,1,2,2,3,3-heptafluoro-3-(1,2,2,2-tetrafluoroethoxy)- E1
IUPAC name: heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether
Structure: C5HF11O




REACH: not registered
CLP: no harmonised classification, 3 notifiers to the CLP inventory (29 August 2016)

*Table 3. Notifications to the CLP inventory for E1 (19 September 2016).*

| Classification | | | Labelling | | Specific Concentration limits, M-Factors | Notes | Classification affected by Impurities / Additives | Additional Notified Information | Number of Notifiers | Joint Entries |
|---|---|---|---|---|---|---|---|---|---|---|
| Hazard Class and Category Code(s) | Hazard Statement Code(s) | Hazard Statement Code(s) | Supplementary Hazard Statement Code(s) | Pictograms, Signal Word Code(s) | | | | | | |
| Skin Irrit. 2 | H315 | H315 | | GHS07 Wng | | | | State/Form IUPAC Names | 2 | |
| Eye Irrit. 2 | H319 | H319 | | | | | | | | |
| STOT SE 3 | H335 (Lungs) (Inhalation) | H335 | | | | | | | | |
| Skin Irrit. 2 | H315 | H315 | | GHS07 Wng | | | | | 1 | |
| Eye Irrit. 2 | H319 | H319 | | | | | | | | |
| STOT SE 3 | H335 (Not Specified) | H335 | | | | | | | | |

***Physical chemical properties (MSDS, 2007)***

| | |
|---|---|
| Vapour pressure: | 30 kPa |
| Solubility in Water: | 7 mg/L |
| Henry's law constant: | $5.54 * 10^2$ $Pa.m^3/mol$ (calculated) |
| Odor: | no Distinct Odor |

| | |
|---|---|
| Form: | liquid |
| Color: | clear, colorless |
| Density: | 1.54 g/mL |
| Relative density: | 1.59 |
| Viscosity: | 0.5 cp |
| Pour point: | -155 °C (-247 F) |
| Log10Pow: | 3.83±0.04 |
| Freezing point: | -54.9°C |
| Boiling point: | 49°C (measurement 1) 40.6°C (measurement 2) |

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# 3 PBT properties

In this chapter a PBT/vPvB assessment according to the criteria for the identification of PBT substances and vPvB substances in Annex XIII of the REACH regulation is made[15].

## 3.1 Persistence FRD-902

FRD-902 is hydrolytically stable, has surface-active properties and is not readily biodegradable. In the ready biodegradation test (OECD 301B16) 0% degradation was observed after 28 days. In addition, in an inherent biodegradation test (OECD 302C) no biodegradation was observed after 28 days. Simulation tests (which are performed to establish half-life values) have not been conducted for FRD-902. As a result, no definitive conclusion on the P and vP criteria can be drawn. However, as FRD-902 is a perfluorinated ether-compound, it is almost certain that FRD-902 will be P and vP. This is strongly supported by all QSAR predictions (especially the Biowin QSAR models).

Given the log $K_{oc}$ values of respectively 1.1 and 1.08, the low Henry's law constant of 4.06E-06 Pa-$m^3$/mol and a water solubility of 207 mg/L, FRD-902 is expected to have low potential to bind to sludge and soil. On the other hand, surface-active properties tend to increase the binding potential. In water FRD-902 will be dissociated at ambient temperature at neutral pH (pKa=3.82; pKb=8.10; OECD 112 at 20°C).

## 3.2 Bioaccumulation FRD-902

As the evaluation of PFOA pointed out, accumulation in fat tissue is not relevant for assessing the bioaccumulation potential of perfluorinated compounds. Perfluorinated compounds bind to proteins, in particular in blood and liver. The log $K_{ow}$ is only indicative of binding to lipids, not for binding to proteins and does not provide an indication on bioaccumulation potential of perfluorinated compounds. To illustrate, the log $K_{ow}$ of PFOA (2.69) is far below the screening criterion for bioaccumulation. Still, elevated levels of PFOA in human blood and excretion via breastmilk are observed widely. In addition, biomagnification factors in the terrestrial food chain exceed the value of 1. Although such data are not available for FRD-902, based on the perfluoration and analogy with PFOA, it is expected that FRD-902 will bioaccumulate via protein binding.

It is unclear which substance properties determine the protein binding potential, but possibly the number of perfluorinated carbon atoms is crucial for protein binding. FRD-902 has 4.5 perfluorinated carbon atoms (one carbon atom contains a carboxyl group and is therefore not completely perfluorinated), whereas PFOA (which is concluded to be bioaccumulative (B)) has seven perfluorinated carbon atoms. Another

[15] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:069:0007:0012:EN:PDF
[16] OECD guideline, see http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm



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perfluorinated compound, PFHxA (which is concluded to be not B) has five perfluorinated carbon atoms, but does not contain ether bonds. Within this comparison, the effect of the ether bond on the protein-binding potential is unknown.

A bioconcentration test with the acid FRD-903 shows limited bioconcentration in carps (<30; Hoke et al., 2016), which is expected given the high water solubility and is in agreement with PFOA.

Oral toxic kinetic studies (Gannon et al., 2016) with mice and rats indicate that FRD-902 is easily absorbed and fully excreted via the urine without metabolism within hours up to seven days. The clearance time of FRD-902 in mice, rats and monkeys is an order of a magnitude lower compared to PFOA.
In view of this, FRD-902 may be expected to bioaccumulate to a lesser extent compared to PFOA. However, the human clearance time for PFOA is an order of magnitude higher (2-4 yrs.) in comparison to all tested animal species (up to 60 days). It is not possible to draw a conclusion on the bioaccumulation potential of FRD-902 in absence of data on the human clearance time.

## 3.3 Toxicity FRD-902

As indicated 2.2, there is no harmonized classification available for this substance. The self-classification notifications are: Acute Tox. 4, Eye Dam. 1 and STOT RE 2 (substances presumed to have the potential to be harmful to human health following repeated exposure).

In paragraphs 4.1 and 4.2 an assessment of the human health toxicity is given. It is concluded that for FRD-902 it is difficult to assess the requirement for STOT RE 2. Furthermore, it is concluded that FRD-902 will normally not result in classification for mutagenicity and toxic for the reproduction. For carcinogenicity, classification as category 2 is justified.

For aquatic organisms, this substance is not acutely toxic (LC/EC50> 100 mg/L) or chronically toxic (NOEC > 1 mg/L; lowest NOEC 1.08 mg/L). Therefore, for ecotoxicity, this substance does not meet the T criterion (a factor 100 above the criteria).

Given the available toxicity data it can be concluded that FRD-902 is less toxic compared to PFOA. No conclusion can be drawn whether the effects observed after repeated exposure are sufficient proof of chronic toxicity to meet the T-criterion. Based on the data used for this report, the substance should be considered borderline T.

## 3.4 Conclusion on PBT/vPvB status for FRD-902

- P/vP: Since FRD-902 is a perfluorinated compound, the substance is almost certain P/vP. All data and QSAR model predictions point in this direction.
- B: FRD-902 is more rapidly eliminated than PFOA. Consequently, FRD-902 is expected to bioaccumulate less than PFOA. However, it is not possible to reach a conclusion on the human bioaccumulation potential of FRD-902 in absence of data on the human clearance time.



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- T: FRD-902 is less toxic compared to PFOA; however, no definitive conclusion on the T criteria can be reached since the substance is considered borderline T for STOT RE.

Overall, it cannot be excluded that FRD-902 meets the PBT/vPvB criteria.

## 3.5 Persistence, bioaccumulation and toxicity FRD-903

No additional information was retrieved on the human toxicological and environmental properties of FRD-903. Therefore, no separate PBT/vPvB assessment for FRD-903 is made, the conclusions on FRD-902 are valid for FRD-903 as well.

The self-classification notifications for the acid are also comparable to FRD-902 (Acute Tox. 4, Skin Corr. 1C/1B, Eye Dam. 1 and STOT SE 3).

## 3.6 Persistence E1

E1 is potentially persistent based on the biodegradation QSARs Biowin2&3 (0.00 en 1.11) and Biowin6&3 (0.00 en 1.11). In addition, the PB score tool, as developed by the RIVM, characterizes E1 as persistent. Due to the perfluoration, it is almost certain that E1 is persistent and meets the P and vP-criteria.

## 3.7 Bioaccumulation E1

E1 does not dissociate; estimated log $K_{ow}$ values are 3.44 (KOWWIN v1.68) and 4.25 (Bioloom). The available bioaccumulation QSARs are based on lipid-binding accumulation and are not suitable for perfluoro compounds (such as E1), which are expected to accumulate via protein binding (like PFOA). In comparison to PFOA and FRD-902, it is expected that E1 has a higher bioaccumulation potential as it does not contain any hydrophilic groups (presumably resulting in a lower water solubility and slower excretion rate). However, the high vapour pressure may indicate that the substance is excreted via exhalation.

## 3.8 Toxicity E1

The information on classification and labeling of E1 (no harmonized classification and the following self-classification notifications: Skin Irrit 2, Eye irrit 2 and STOT SE 3) gives no indication that E1 potentially meets the T criteria for human toxicity. In paragraph 4.5 it is concluded that although the available information on E1 is limited, it indicates that E1 has a low to very low human toxicity. No information on ecotoxicity is provided in the MSDS (2007).

The ecotoxicity QSAR ECOSAR estimates a chronic toxicity NOEC for E1 of 0.68 mg/L for daphnids. Based on this estimate, E1 does not meet the T criteria for ecotoxicity.

## 3.9 Conclusion on PBT/vPvB status for E1

- P/vP: Since E1 is a perfluorinated compound, the substance is almost certain P/vP. All QSAR model predictions point in this direction.
- B/vB: Insufficient information is available to draw a conclusion

about the bioaccumulation potential of E1. Since E1 contains no hydrophilic group, the human clearance time of the substance and the bioaccumulation potential are expected to be higher than for PFOA (which meets the criteria for bioaccumulation), although E1 has the potential to be excreted via exhalation.
- T: E1 will most likely not meet the T criteria.

It cannot be excluded that E1 meets the vPvB criteria.



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# 4 Human health properties

The toxicological information as used in the present evaluation is mainly based on the data as summarised by the registrant within the REACH registration dossier. In addition, Chemours provided some of the study reports on request of the RIVM. Further, two publications are available on kinetics and chronic toxicity and carcinogenicity, respectively, reporting studies also present in the registration dossier. Detailed summaries of the individual studies are provided in Annex I.

## 4.1 Human health hazards FRD-902

FRD-902 is classified as follows by the registrant:

- Acute Tox. 4 H302: Harmful if swallowed
- Eye Damage 1 H318: Causes serious eye damage
- STOT RE 2 H373: May cause damage to organs <or state all organs affected, if known> through prolonged or repeated exposure <state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard>. Affected organs: Liver, Blood

Based on the data available in the registration dossier, the RIVM agrees with the classification as Acute Tox. 4; H302 and Eye Damage 1; H318. The classification with STOT RE 2 is based on the liver and red blood cell effects, as indicated by the affected organs in the available repeated dose toxicity studies. In table 4, a comparison is made of the effects at or around the guidance values for STOT RE 2 for the respective study duration with the effects which may support classification. Classification for STOT RE is based on a defined level of adverse effects occurring below specified dose levels depending on the study duration.

*Table 4. Comparison of the effects at or around the guidance values for STOT RE 2.*

| Study | STOT RE 2 guidance value | Effects observed (dose in mg/kg bw/day) | RIVM remark | Reference [17] |
|---|---|---|---|---|
| Oral, 28-day rat Males: 0.3, 3 and 30 mg/kg bw/day Females: 3, 30 and 300 mg/kg bw/day | 300 mg/kg bw/day | 30 mg/kg bw/day males/300 mg/kg bw/day females: increased liver beta-oxidation activity, increased liver and kidney weights, minimal hepatocellular hypertrophy, changes in serum lipids and proteins, and minimal decreases in red cell mass parameters (<7.9%) | Effects which may require classification (almost no information on effect size) as STOT RE including single cell necrosis and changes in serum lipids and proteins were observed at dose levels clearly below (males) or at (females) the guidance value for STOT RE 2. | Exp supporting repeated dose toxicity: oral.001 |

[17] This table refers to the literature references as included in the REACH registration dossier. According to REACH, the reference details are considered confidential.



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| Study | STOT RE 2 guidance value | Effects observed (dose in mg/kg bw/day) | RIVM remark | Reference [17] |
|---|---|---|---|---|
| Oral, 90-day rat<br>Males: 0.1, 10 and 100 mg/kg bw/day and females 10, 100 and 1000 mg/kg bw/day | 100 mg/kg bw/day | 100 mg/kg bw/day (males): red cell mass reduction (11-13%), decrease cholesterol (-31%), increased albumin (+12%) and A/G ratio (+35%), decreased globulin (-15%), increased liver weights and hypertrophy (males)(abs 59%, rel 67%, increased kidney weights (abs 11%, rel 16%) (females: rel 9.5%), no liver necrosis | The observed effects do not indicate a requirement for classification for STOT RE 2. | Exp supporting repeated dose toxicity: oral.002 |
| Oral, 28-day mouse 0.1, 3 and 30 mg/kg bw/day | 300 mg/kg bw/day | 30 mg/kg bw/day: adverse effects including increased liver weights, hepatocellular hypertrophy, and changes in serum lipids and proteins, increased body weight, decreases in red cell mass (<10%), increased adrenal weight and adrenal cortical hypertrophy, hepatocellular single cell necrosis | Effects which may require classification (almost no information on effect size) as STOT RE including single cell necrosis and changes in serum lipids and proteins were observed at dose levels clearly below the guidance value for STOT RE 2. | Exp supporting repeated dose toxicity: oral.003 |
| Oral, 7-day rat (screening study)<br>30, 300 and 1000 mg/kg bw/day | 1000 mg/kg bw/day | 1000 mg/kg bw/day: reduced body weight (males), reduced red cell mass parameters, increase reticulocytes and neutrophils (females), decreases in serum lipids, increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BUN), and Glucose; and decreased sorbitol dehydrogenase (SDH), creatinine, and calcium, increased liver weights, hepatocellular hypertrophy. | As there is almost no information on the effect size, it is difficult to assess the adversity of the observed effects. | Exp supporting repeated dose toxicity: oral.004 |
| Oral, chronic rat<br>Males: 0.1, 1 and 50 mg/kg bw/day<br>Females: 1, 50 and 500 mg/kg bw/day | 12.5 mg/kg bw/day | 50 mg/kg bw/day: liver: focal cystic degeneration, focal necrosis, centrilobular necrosis, increase liver enzymes, increase in albumin (16%), increase A/G ratio, reduced red cell mass (males) (<10%), reduced red cell mass (females) (<6%), A/G ratio (females)<br>50 mg/kg bw/day: Mild focal necrosis and minimal focal cystic degeneration was also observed in some animals at the one-year interim section (guidance value 25 mg/kg bw/day). | Difficult to assess as the effects at 50 mg/kg bw/day warrant STOT RE classification but the dose is too high whereas at 1 mg/kg bw/day the effects do not warrant classification. | Exp Key repeated dose toxicity: oral.005 |
| Oral, 7-day male mouse | 1000 mg/kg | 30 mg/kg bw/day: increased liver and body weight, minimal single | The observed effects do not warrant classification | Exp supporting |

| Study | STOT RE 2 guidance value | Effects observed (dose in mg/kg bw/day) | RIVM remark | Reference [17] |
|---|---|---|---|---|
| (screening study) 30 mg/kg bw/day | bw/day | cell necrosis, moderate hypertrophy and increase in mitotic figures | but the tested dose level is clearly below the guidance value for STOT RE 2. | repeated dose toxicity: oral.006 |
| Oral, 90-day mouse 0.1, 0.5 and 5 mg/kg bw/day | 100 mg/kg bw/day | 5 mg/kg bw/day: liver single cell necrosis (minimal) and other minimal to mild effects | Effects which not require classification as STOT RE were observed at dose levels clearly below the guidance value for STOT RE 2. | Exp supporting repeated dose toxicity: oral.007 |

Overall, the requirement of STOT RE 2 is difficult to assess because the dose levels tested in mice, with effects that may or may not warrant classification, are clearly below the guidance values and this may be taken as an indication that STOT RE 2 is needed. The effects in the rat are borderline and sometimes difficult to assess due to the large steps in the dose levels.

The registrant does not classify FRD-902 as carcinogenic because the observed increase in liver tumours in females and increases in pancreas and Leydig cell tumours in male rats are not considered relevant to humans. RIVM agrees that there are some species differences with regard to the relevance of these typical tumours for peroxisome proliferators for humans. In line with RAC and IARC, we consider the level of evidence sufficient to show that these tumours are relevant for humans. However, as tumours were only observed in one species, classification as a category 2 carcinogen is justified (suspected human carcinogen).

The available in vitro (OECD TG 471, 476 and 473) and in vivo (OECD TG 474, 475 and 486) genetic toxicity and mutagenicity studies show that FRD-902 is not mutagenic. EFSA (2008) concluded that FRD-902 is non-genotoxic based on the same dataset.
The registrant proposes no classification for reproductive toxicity. In the developmental toxicity study in rats, the only effect on reproduction was early delivery of the offspring at 100 and 1000 mg/kg bw/day. However, the adversity of this effect is uncertain as the offspring was alive and there was no increase in resorptions. In addition, these reproductive effects were observed at dose levels also inducing maternal toxicity. Therefore, classification based on the early delivery is doubtful and in category 2 at most. Other effects include decreased foetal weights at 100 and 1000 mg/kg bw/day and increases in variations at 1000 mg/kg bw/day. These effects in the presence of maternal toxicity do not normally warrant classification.

In the modified one-generation study in mice, postnatal reduced body weight and body weight gain was observed at the highest dose level in the presence of maternal toxicity (liver effects). Secondary delays in development were observed based on time after birth but not based on body weight.

These effects, observed in presence of maternal toxicity, do not normally result in classification.

In Annex I an elaborated overview of the available human health data for FRD-902 is given.

## 4.2 Conclusion on CMR and STOT RE properties FRD-902

- Carcinogenic: As tumours were only observed in one species, classification as a category 2 carcinogen is justified.
- Mutagenic: The available in vitro and in vivo genetic toxicity and mutagenicity studies show that FRD-902 is not mutagenic.
- Reproductive toxicity: The limited effects observed in presence of maternal toxicity do not normally result in classification.
- STOT RE: The requirement of STOT RE 2 is difficult to assess due to dose levels tested in mice clearly below the guidance values, which may be taken as an indication that STOT RE 2 is needed. The effects in the rat are borderline and difficult to assess due to the large steps in the dose levels.

## 4.3 Comparison FRD-902 and APFO

As FRD-902 is used as a replacement of PFOA and its ammonium salt (APFO) for the production of Teflon, a comparison of the toxicological properties of both ammonium salts (FRD-902 and APFO) is considered relevant. An exact comparison is not possible due to differences in applied dose levels. The data in table 5 show that excretion of FRD-902 is much faster in all tested animals compared to APFO. However, comparable PPAR-α effects and tumour types were observed in the available sub-chronic and chronic studies at roughly comparable exposure levels. As comparable effects occurred at comparable external dose levels, but at lower FRD-902 internal concentrations, the interaction of FRD-902 with its toxicological target is probably stronger. Differences are observed in the type of developmental effect between both substances.

*Table 5. Comparison of the toxicological properties of FRD-902 and APFO.*

| | | **FRD-902** | **APFO** | **References APFO** |
|---|---|---|---|---|
| **Study type** | **Parameter** | **Result** | **Result** | |
| Kinetics | Half-life mouse | 5.2 hours | 17-19 days | Lau et al, 2007 |
| | Half-life rat (male) | 3.2 hours | 4-6 days | Lau et al, 2007 |
| | Half-life monkey (male) | 2.3 hours | 20.9 days | Butenhof et al, 2004 |
| | Half-life human | unknown | 1378 days | Olsen et al, 2007 |
| Acute oral toxicity | LD50 rat | 1750 mg/kg bw | 250 – 500 mg/kg bw | RAC, 2011 |
| Skin irritation | CLP classification | No classification | Inconclusive | RAC, 2011 |
| Eye irritation | CLP classification | Category 1 | Category 1 | RAC, 2011 |



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| | | FRD-902 | APFO | References APFO |
|---|---|---|---|---|
| **Study type** | **Parameter** | **Result** | **Result** | |
| 90-day study rat | Effects LOAEL | PPAR- α related effects | Liver hypertrophy | Zeilmaker, 2016 |
| | NOAEL/LOAEL | 0.1 / 10 mg/kg bw/day | 0.06 / 0.64 mg/kg bw/day | Zeilmaker, 2016 |
| Chronic study rat | Effects LOAEL | Increased A/G ratio<br>PPAR-a related effects at higher dose levels | Body weight, liver changes | US-EPA, 2016 |
| | NOAEL/LOAEL | 0.1 / 1.0 mg/kg bw/day | 1.3 / 14.2 – 16.1 mg/kg bw/day | US-EPA, 2016 |
| Carcinogenicity | Type of tumours | Liver cell adenomas<br>Leydig cell adenomas<br>Pancreas acinar cell tumours | Liver cell adenomas<br>Leydig cell adenomas<br>Pancreas acinar cell adenomas | RAC, 2011 |
| | LOAEL/NOAEL | 50 / 1 mg/kg bw/day | 15 / 1 mg/kg bw/day | RAC, 2011 |
| Developmental toxicity rat | Type of effects | Early delivery | No developmental effects | RAC, 2011 |
| | LOAEL/NOAEL | 100 / 10 mg/kg bw/day | - / 150 | RAC, 2011 |
| Generation study mice | Type of effects | No reproductive or developmental effects | Resorptions, stillbirth, postnatal mortality, early preputial separation | RAC, 2011 |
| | LOAEL/NOAEL | - / 5 mg/kg bw/day | 1 / - mg/kg bw/day | RAC, 2011 |

In comparing the toxicity of both substances it is useful to view toxicity as being the result of toxicokinetics and toxicodynamics. As to toxicodynamics, as already stated, the data (in particular the chronic and semichronic studies) indicate that FRD-902 interacts more strongly with its toxicological target than does APFO. As to toxicokinetics, however, the available non-human data for FRD-902 indicate a more favourable profile compared to APFO. As concluded in the present report, human data on the bioaccumulation of FRD-902 are lacking. If human data would confirm that FRD-902 indeed is considerably less bioaccumulative than APFO, overall its long term toxicity for humans can be judged as being lower. It should be noted that for the developmental toxicity endpoint these considerations do not apply. For this endpoint the mouse studies show a clearly lower potency for FRD-902 than for APFO whereas in rats FRD-902 was somewhat more potent (induced early delivery in combination with maternal toxicity at a dose level where APFO induced no effect). Overall with a view to reproductive

toxicity the information on FRD-902 do not normally warrant classification (see sections 4.1 and 4.2), whereas APFO is classified as toxic for the reproduction (category 1B).

## 4.4 Human health hazards FRD-903

All available toxicological studies were performed with the ammonium salt (FRD-902). Read-across of the toxicological properties of the ammonium salt to the acid is considered justified for systemic effects as after dissolution and dissociation of the acid and the salt the absorption in the intestinal tract and the lungs and distribution over the body of the anion (2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate) will be the same. However, local effects to the lung may differ between the two substances as acids normally have a higher irritating effect than neutral salts.

## 4.5 Human health hazards E1

Only limited toxicological information is available on E1, consisting of a number of study reports provided by Chemours and a summary of the EFSA evaluation of the mutagenicity. Chemours could provide not all studies as some studies contained information on several substances. These are available upon request after redaction to remove all other data. Study summaries of the provided study reports on E1 and further details on the read-across are included in Annex 2.

The available oral kinetic studies indicate low oral absorption of E1. The observed effects after inhalation exposure indicate effects on the central nervous system. This indicates that some absorption can occur via this route. The absence of mortality after high dermal exposure indicates low dermal uptake.

The available acute toxicity studies via the oral (>17000 mg/kg bw), dermal (> 37500 mg/kg bw) and inhalation route (>576000 ppm) show no mortality at high dose levels indicating low overall toxicity and no requirement for classification.

The only repeated dose study is limited to a 10-day inhalation exposure over a period of 12 days and was performed using only male animals. The results show low toxicity limited to CNS depression during exposure. A NOAEC of 25000 ppm was derived.
The available in vitro and in vivo studies show no evidence of a mutagenic potential of E1, as also concluded by EFSA. The Ames test was negative. However, due to the likely evaporation of E1 in the in vitro chromosomal aberration study and the possibly limited amount of E1 in the in vivo inhalation micronucleus test that reached the bone marrow as no change in the PCE/NCE ratio was observed, no conclusion on the mutagenic properties concerning chromosome aberrations can be drawn from these studies.

Read-across
Read-across from FRD-902 to E1 is not justified because of the differences in chemical-physical properties (solid versus liquid with high vapour pressure, acid or salt versus neutral, more lipophilic substance).



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In addition, the available toxicological data indicate that E1 is less toxic than FRD-902.
Expert systems including 'Oncologic', 'OECD toolbox' and 'ISS' do not indicate a strong concern for mutagenicity or carcinogenicity.
The two closest analogues identified using the OECD QSAR toolbox, enflurane and isoflurane are used as inhalation anaesthetic used for narcosis at high concentrations and show low toxicity. A range of fluorinated compounds collected from the RepDose database (Frauenhofer) showed limited toxicity with NOECs always above 50 ppm.

Exposure limits
Acceptable Exposure Limit (DuPont): 500 ppm 8 and 12 hour TWA (MSDS, 2007).

## 4.6 Conclusion on CMR and STOT RE properties E1

Information on the toxicity of E1 is limited but the available information indicates that E1 has a low to very low toxicity. This is supported by the repeated dose toxicity information on some structural analogues. However, all available studies were performed in male animals and were of limited duration. Overall, the available in vitro and in vivo data on mutagenicity combined with the read-across data show that E1 is unlikely to be mutagenic. In addition, the available data indicate no requirement for classification for acute toxicity and probably STOT RE via inhalation but the requirement for classification for other hazard classes including carcinogenicity, reproductive toxicity and STOT RE via oral exposure is unknown.

## 4.7 Derivation of a general population exposure limit for FRD-902

### *4.7.1 Approach*

For the derivation of an exposure limit for FRD-902 for the general population the REACH method as described in the "Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health" is used (version 2.1 November 2012). Although FRD-902 induces carcinogenicity in experimental animals, the available mutagenicity studies and mechanistic information indicate a non-genotoxic mode of action and therefore a threshold approach can be applied.

The use of an internal dose per ml of serum as dose metric has recently been applied for PFOA by RIVM (Zeilmaker et al, 2016). However, applying this principle to FRD-902 is considered not feasible. The reason for this is that in contrast to the critical studies with PFOA, no information on the serum levels of FRD-902 is available from the critical animal toxicity study. Furthermore, no kinetic model is available for FRD-902 in humans. Moreover, the available data in test animals show quick elimination of FRD-902 (T1/2 for elimination from serum in rats 2.8 h in males and 0.2 h in females), which leads to the serum values in the toxicity studies being strongly dependent on the time after the last exposure. Crucially, no information is available regarding the half-life of FRD-902 in humans or regarding serum concentrations in humans. Therefore, the derivation of a limit value on the basis of serum levels as was done for PFOA is unfeasible. Instead, for FRD-902 a method for



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deriving a limit value based on the external concentration in air is applied.

Application of the GenX technology leads to emission of FRD-902 via air. As a result, the general population may be exposed to FRD-902 via air, food and/or drinking water. However, no information is currently available regarding levels of FRD-902 in drinking water or food. Therefore, only inhalation exposure is assumed in the present assessment and only a limit value for air is derived. As it cannot be excluded that this exposure will continue for years, a chronic inhalation limit value is determined.

### 4.7.2 *Toxicity studies*

The NOAELs derived from the oral repeated dose toxicity studies are summarised in table 6 below.

*Table 6. Derived NOAELs for repeated dose toxicity.*

| Species | Duration | NOAEL mg/kg bw/day | LOAEL mg/kg bw/day | Effects | Reference |
|---|---|---|---|---|---|
| Rat | 28 days | 0.3 | 30 | Reduction in cholesterol | Exp supporting repeated dose toxicity: oral.001 |
| | 90 days | 0.1 | 10 | A/G ratio increased<br>Reduction in cholesterol<br>Increased liver weight<br>Increased kidney weight | Exp supporting repeated dose toxicity: oral.002 |
| | Chronic | 0.1 | 1 | A/G ratio increased | Rae et al, 2015 |
| Mouse | 28 days | 0.1 | 3 | A/G ratio increased<br>Reduced Hb<br>Liver single cell necrosis | Exp supporting repeated dose toxicity: oral.003 |
| | 90 days | 0.1 | 1 | Increased liver weight<br>Liver hypertrophy | Exp supporting repeated dose toxicity: oral.007 |
| Rat | Carcinogenicity | 1 | 50 | Increase in testis and pancreatic tumours | Rae et al, 2015 |
| | Developmental study | 10 | 100 | Early delivery<br>Reduced fetal weights | Developmental toxicity/teratogenicity |
| Mouse | 1-generation study | 0.1 | 0.5 | Single cell necrosis in the liver | Exp Supporting Toxicity to reproduction.002 |

### 4.7.3 *Selection of the most appropriate point of departure*

Exposure of the general population due to emissions to ambient air is normally limited to low level of exposures over a long period. The exposure can be intermittent depending on the applied process, release and distribution in the environment. However, based on the available



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emission data, only an average concentration per year can be estimated for the general population depending on the distance from the source. Therefore, acute effects after a single high exposure and local effects except local effects on the airways are considered not relevant for the general population in the present case. Accordingly, the assessment is based on effects observed after prolonged low level exposure. For FRD-902 this includes the NOAELs/LOAELs from repeated dose studies, carcinogenicity studies and reproductive toxicity studies.

As no inhalation studies are available with FRD-902 but only oral (gavage) studies, an oral study is used and route-to-route extrapolation is applied for deriving the exposure limit for air. Overall, the NOAEL of 0.1 mg/kg bw/day in the oral chronic study in rats is considered the best available point of departure (POD) for derivation of an exposure limit. This NOAEL is based on an increase in albumin and the albumin/globulin ratio in male rats, an effect that indicates possible immunotoxic effects. This effect was also observed with other PPAR-α inducers and secondary to binding to the PPAR-α receptor (Gervois et al, 2004). As changes in albumin and albumin/globulin ratio also occur in humans after exposure to PPAR-α inducers (Gervois et al, 2004), this effect is considered relevant to humans.

### 4.7.4 *Inhalation exposure limit*

The NOAEL of 0.1 mg/kg bw/day from the oral (gavage) chronic study is used as POD. Via route-to-route extrapolation the corresponding POD-concentration in air is derived. The chronic study used gavage exposure, meaning that the whole daily dose was applied at once. The possible inhalation of FRD-902 is expected to be evenly distributed over the whole day. Because of the half-life in male rats of only 3 hours, this difference may result in a different internal exposure pattern. The peaks of internal exposure (Cmax) are expected to be higher under the conditions of the chronic rat study. However, the effect of this difference on the toxicity of FRD-902 in humans is unknown as it is not known whether the peak exposure (Cmax) or the integrated dose (AUC) determines the critical toxicological effect of FRD-902. No additional safety factor is applied for this difference. In the route-to-route extrapolation, an additional factor for difference in absorption between the oral and the inhalation route is required as the oral absorption has been shown to be 100% but the inhalation absorption is unknown. Available information from comparable substance like PFOA show absorption after inhalation exposure in animals. However, no absorption percentage is provided in the available summaries. Therefore, a default value of 100% is applied in line with the REACH guidance. This is further justified by the absence of metabolism showing that first pass effects are not relevant. Route-to-route extrapolation was performed by dividing the point of departure of 0.1 mg/kg bw/day by 1.15 $m^3$/kg bw/day[18] resulting in a POD for the air concentration of 0.087 mg/$m^3$.

Besides this allometric scaling factor of 4, normally an interspecies factor of 2.5 for remaining toxicokinetic and toxicodynamic differences

[18] Inhalation volume per kg bw per day in rats which is compatible with a factor 0.25 for allometric scaling from rats to humans, 70 kg bw and 20 $m^3$/day as the daily ventilation volume for humans: (20 $m^3$/day * 4) / 70 kg bw = 1.15 $m^3$/kg bw/day



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and an intraspecies factor of 10 are used in agreement with the REACH guidance.

***Additional factor for potential kinetic difference***
However, there is concern regarding the potential difference in half-lives between the tested animal species and humans. FRD-902 is used as a replacement of PFOA and is also a fully fluorinated carboxylic acid. The half-life of PFOA in humans is much longer than those in all tested animal species (mouse, rat, monkey) (Zeilmaker et al, 2016) probably due to stronger reabsorption from the lumen of the kidney back into the blood by organic anion transporters (OATs) (Yang et al, 2010). There are genetic differences in OATs between humans and the tested animal species (Yang et al, 2010). As FRD-902 is also an anion, this mechanism cannot be excluded. The elimination of FRD-902 was tested in three animal species (Gannon et al, 2016) and the results show that in these species the half-life of FRD-902 was clearly shorter than those of PFOA, suggesting that for FRD-902 reabsorption by OATs is lower or absent entirely, at least in these species. However, because of the genetic differences of the OATs between the tested animal species and humans (Yang et al, 2010) this cannot be directly extrapolated to humans. Thus, for humans the involvement of OATs in the elimination of FRD-902 in cannot be excluded. Moreover, contrary to other perfluorinated compounds, no data are available for FRD-902 to confirm whether the fast elimination and absence of accumulation as seen in several animal species also applies to humans.

In view of the above, an additional toxicokinetic assessment factor is applied to take into account the uncertainty in the human elimination rate of FRD-902. This additional toxicokinetic factor is based on the difference in half-lives between cynomolgus monkeys and humans as determined for PFOA. Using a half-life of 1378 days in humans (mainly males)(Olsen et al, 2007) and of 20.9 days in male cynomolgus monkeys (Butenhoff et al, 2004), leads to an additional toxicokinetic factor of 66 (1378 / 20.9).

The PFOA half-life in male cynomolgus monkey is used in deriving this additional factor instead of the half-life for PFOA in male rats (the species used in the pivotal chronic study) because for FRD-902 the half-lives in male rats and cynomolgus monkeys were similar in size whereas for PFOA the half-life in cynomolgus monkeys is much longer than that in male rats (20.9 days in male cynomolgus monkeys versus 6-7 days in male rats). This indicates that for FRD-902 the use of the factor between male rats and humans for PFOA is not appropriate.

***Interspecies remaining difference***
Interspecies extrapolation corrects for the differences in the sensitivity between experimental animals and humans. This covers differences in toxicodynamics and toxicokinetics. Some of the toxicokinetic differences can be explained by body size in relation to the basal metabolic rate. The latter is linked to the inhalation volume per kg bw. By default, in the extrapolation from animals to humans an interspecies correction for metabolic rate is applied (a factor of 4 in case of rats), as described above. An additional factor of 2.5 for remaining differences, i.e. toxicokinetic differences not related to metabolic rate (small part) and



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toxicodynamic differences (larger part). As the REACH guidance points out, in case substance-specific information shows specific susceptibility differences between species, which are not related to differences in basal metabolic rate (not covered by allometric scaling), the additional factor of 2.5 for 'remaining differences' should be modified accordingly. The potential difference in half-life of FRD-902 between the tested animal species and humans is a potential difference in toxicokinetics which is probably not related to the metabolic rate.

Therefore, the calculated potential difference in half-life is used to replace the toxicokinetic part of the additional factor of 2.5. As the toxicodynamic part is the larger part of the remaining difference, a factor of 1.8 is applied as the remaining factor for toxicodynamic interspecies extrapolation. The factor of 1.8 was selected as being the larger part of the 2.5 factor which is not quantified in the ECHA guidance R.8.

No assessment factor for duration of exposure is applied as the point of departure is a chronic study. In addition, no factor is applied for the dose-response relationship as the point of departure is a NOAEL. Also, no factor is applied for quality of the database as repeated dose toxicity studies in two species, a carcinogenicity study and reproductive toxicity studies are available.

Overall, the following assessment factors are applied:

- Additional factor for potential kinetic difference 66
- Interspecies remaining toxicodynamic difference 1.8
- Intraspecies 10

Therefore, the overall assessment factor is 1188. Combining this assessment factor with the point of departure of 0.087 mg/m$^3$, results in an chronic inhalation exposure limit of 73 ng/m$^3$.

Although local effects on the lung due to the irritating properties of FRD-902 at the inhalation point of departure of 0.087 mg/m$^3$ cannot be excluded, it is considered unlikely that such effects could be critical for the limit value as the derivation of limit values based on local irritating effects does not require additional assessment factors for possible differences in accumulation. Therefore, the limit value of 73 ng/m$^3$ is considered to be also protective for local irritating effects.

Using the additional toxicokinetic factor as above represents a pragmatic worst-case approach based on the data as currently available. Additional information on the bioaccumulation of FRD-902 in humans and on the inhalatory absorption rate would allow derivation of an improved exposure limit.

## 4.8 Derivation of a general population exposure limit for FRD-903

All available toxicological studies were performed with the ammonium salt (FRD-902). Read-across of the toxicological properties of the ammonium salt to the acid is considered justified for systemic effects as after dissolution and dissociation of the acid and the salt the absorption in the intestinal tract and the lungs and distribution over the body of the



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anion (2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate) will be the same.

Local effects to the upper airways and lungs may differ between FRD-902 and FRD-903 as acids normally have a higher irritating effect than neutral salts. Thus the derived limit value in air for FRD-902 might underestimate the local toxicity of FRD-903. However, it is considered unlikely that such effects are determinative for the limit value as the derivation of limit values based on local irritating effects does not require additional assessment factors for possible differences in accumulation. Therefore, the limit value of 73 ng/m$^3$ is considered to adequately cover the local irritating effects of FRD-903 and FRD-902.

## 4.9 Derivation of a general population exposure limit for E1

As indicated before, the information on the toxicity of E1 is limited and no studies were performed using female animals. However, the available information indicates that E1 has a low to very low toxicity. This is supported by the repeated dose toxicity information on some structural analogues. However, the data are insufficient for deriving an inhalation exposure limit.



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# 5 Indicative concentrations around the Chemours plant due to FRD-903 and E1 emission

As indicated in section 2.2, FRD-903 and E1 are emitted to air from the Teflon PTFE and from the Teflon FEP plants (see tables 7 to 10). The presented data are based on the maximum emission indicated in the permits, the emission recorded in the Electronic Environmental Year report of Chemours and additional data provided by Chemours.

*Table 7. Emissions to air of FRD-903 for both plants in kg/year.*

| **Year** | **Permitted PTFE** | **Recorded PTFE** | **Permitted FEP** | **Recorded FEP** |
|---|---|---|---|---|
| 2012 | 600 | 197 | 40 | 10 |
| 2013 | 600 | 292 | 40 | 27 |
| 2014 | 600 | 386 | 40 | 31 |
| 2015 | 600 | 288 | 40 | 27 |

*Table 8. Emissions to air of FRD-903 per stack for both plants in kg/year.*

| | | Emissions to air FRD 903 | | |
|---|---|---|---|---|
| | | 2013 | 2014 | 2015 |
| FEP | TL20 scrubber | 5 | 6 | 6 |
| | TL33 membrane unit | 12 | 14 | 12 |
| | TL34 vacuum filter 1 | 1 | 2 | 1 |
| | TL35 vacuum filter 2 and 3 | 8 | 9 | 8 |
| | Total FEP | 27 | 31 | 27 |
| | | | | |
| PTFE | TL05 dryer granular | 24 | 19 | 20 |
| | TL10a waxtrap | 13 | 16 | 12 |
| | TL10b wash separators | 13 | 17 | 13 |
| | TL12 scrubber | 242 | 334 | 243 |
| | Total PTFE | 292 | 386 | 288 |

*Table 9. Emissions to air of E1 for both plants in kg/year.*

| **Year** | **Permitted PTFE** | **Recorded PTFE** | **Permitted FEP** | **Recorded FEP** |
|---|---|---|---|---|
| 2012 | 750 | 205 | 450 | 11 |
| 2013 | 750 | 293 | 450 | 42 |
| 2014 | 750 | 390 | 450 | 48 |
| 2015 | 750 | 288 | 450 | 46 |

*Table 10. Emissions to air of E1 per stack for both plants in kg/year.*

| | | Emissions to air E1 | | |
|---|---|---|---|---|
| | | 2013 | 2014 | 2015 |
| FEP | TL20 scrubber | 7 | 8 | 7 |
| | TL31 flotation tanks | 25 | 29 | 29 |
| | TL36 vacuum system | 10 | 11 | 11 |
| | Total FEP | 42 | 48 | 46 |
| | | | | |
| PTFE | TL01 vacuum granular claaf | 11 | 9 | 9 |



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| | | Emissions to air E1 | | |
|---|---|---|---|---|
| | | 2013 | 2014 | 2015 |
| | TL05 dryer granular | 1 | 1 | 1 |
| | TL10a waxtrap | 17 | 22 | 17 |
| | TL10b wash separators | 18 | 23 | 17 |
| | TL12 scrubber | 227 | 313 | 228 |
| | TL13a dryer east | 10 | 12 | 9 |
| | TL13b dryer west | 9 | 10 | 8 |
| | Total PTFE | 293 | 390 | 288 |

The characteristics of the stacks are copied from the request for revision of the permit of 2013. These characteristics are presented in table 11. At the moment the calculations were carried out no information about the exact locations of all stacks was available. It was assumed all stacks in the FEP-plant have the same location as TL20 and all stacks in the FTPE plant as TL12. For FEP TL36 characteristics were invalid, instead TL20 characteristics were used. Same for FTPE TL01, here TL12 characteristics were used. Since not all information was available these calculations should be considered as indicative.

*Table 11. Reported emission characteristics of the stacks (request for revision of the permit of 2013).*

| Plant | FEP | | | | | | |
|---|---|---|---|---|---|---|---|
| Stackname | TL20 | TL31 | TL33 | TL34 | TL35 | TL36 | TL37 |
| x-coordinate RDM (m) | 109817 | | | | | | |
| y-coordinate RDM (m) | 425858 | | | | | | |
| Stack height (m) | 28 | 19 | 11.7 | 0 | 0 | 40 | 5.9 |
| diameter (m) | 0.4 | 0.08 | 0.434 | 0.08 | 0.21 | 0.08 | 0.125 |
| temperature (K) | 353 | 323 | 293 | 293 | 293 | 373 | 293 |
| flow rate ($Nm^3/u$) | 700 | 200 | 250 | 150 | 850 | | 50 |
| heat content (Mw) | 0.016 | 0.003 | 0.000 | 0.000 | 0.002 | | 0.000 |
| Plant | PTFE | | | | | | |
| Stackname | TL01 | TL5 | TL10a | TL10b | TL12 | TL13a | TL13b |
| x-coordinate RDM (m) | | | | | 109726 | | |
| y-coordinate RDM (m) | | | | | 425865 | | |
| Stack height (m) | | 12 | 12 | 12 | 20 | 14.7 | 5.6 |
| diameter (m) | | 0.36 | 0.25 | 0.5 | 0.8 | 0.68 | 0.4 |
| temperature (K) | | 333 | 293 | 293 | 323 | 318 | 333 |
| flow rate ($Nm^3/u$) | | 6500 | 2000 | 2500 | 20000 | 1900 | 1750 |
| heat content (Mw) | | 0.106 | 0.004 | 0.005 | 0.253 | 0.021 | 0.028 |

Based on the permitted and recorded (2014) emissions to air the air concentrations around the Chemours plant are calculated using the RIVM model OPS-PRO version 4.5.0[19]. As no information on particle-size,

[19] http://www.rivm.nl/media/ops/v4.5.0/OPS-model-v4.5.0.pdf

coagulation or deposition is available, in the calculations it is assumed all substance maintains in the air without any (wet or dry) deposition. This can be seen as a worst case. The distribution is calculated in a 50 x 50 km grid. Within this grid, the concentration is calculated in cells of 100 x 100 m. The calculated result does not give the exact concentration at a certain point, but is the average concentration of the cell.

The calculated air concentrations in the nearest populated areas (along the dike at the other side of the river) are around 20 ng/m$^3$ (permitted emissions) versus 15 ng/m$^3$ (recorded emissions) for FRD-903 and around 40 ng/m$^3$ (permitted emissions) versus 20 ng/m$^3$ (recorded emissions) for E1.



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# 6 Possible health effects in residents in the vicinity of the Chemours plant

In chapter 4, the available human health effects information on FRD-902/903 is evaluated, based on which a chronic inhalation exposure limit of 73 ng/m$^3$ is derived (paragraph 4.6.4). In chapter 5, the year-average air concentrations were calculated, in first instance based on the permitted emissions and, secondly also for the recorded emission for 2014. This led to estimated concentrations in air of about 20 ng/m$^3$ for the nearest populated areas (along the dike at the opposite side of the river) and lower concentrations at greater distances from the plant based on the permitted concentrations. Based on the recorded emissions for 2014 the estimated concentrations for the nearest populated areas are about 15 ng/m$^3$.

Comparing these concentrations with the limit value of 73 ng/m$^3$ leads to the conclusion that based on the available data, no health risk is expected for people living in the vicinity of the Chemours Dordrecht plant due to exposure to FRD-903.

To illustrate the results, figure 1 shows the calculated concentration FRD-903 in air based on the recorded emission in 2014 compared to the exposure limit derived in this report. The data from 2014 have been used, because in this year the highest emission was recorded. The figure shows that only directly next to the stack, air concentrations above 73 ng/m$^3$ are calculated. Annex 3 presents the calculated concentration FRD-903 in air based on the permitted emissions.

In chapter 4 also the available human health effects information on E1 is evaluated. The conclusion was that limited toxicological information and structure-activity relations indicate that this chemical has low toxic potential only. However, the data are insufficient for deriving an inhalation exposure limit. In chapter 5, the year-average air concentrations for E1 were calculated based on the permitted emissions. This led to estimated concentrations in air of about 40 ng /m$^3$ for the nearest populated areas (along the dike at the opposite side of the river) and lower concentrations at greater distances from the plant. Based on recorded emissions for 2014 the estimated concentrations for the nearest populated areas are about 20 ng/m$^3$.

Due to the insufficient health effects information available for E1, these concentrations cannot be evaluated as to the possible health risk they might pose for people living in the vicinity of the Chemours plant in Dordrecht.



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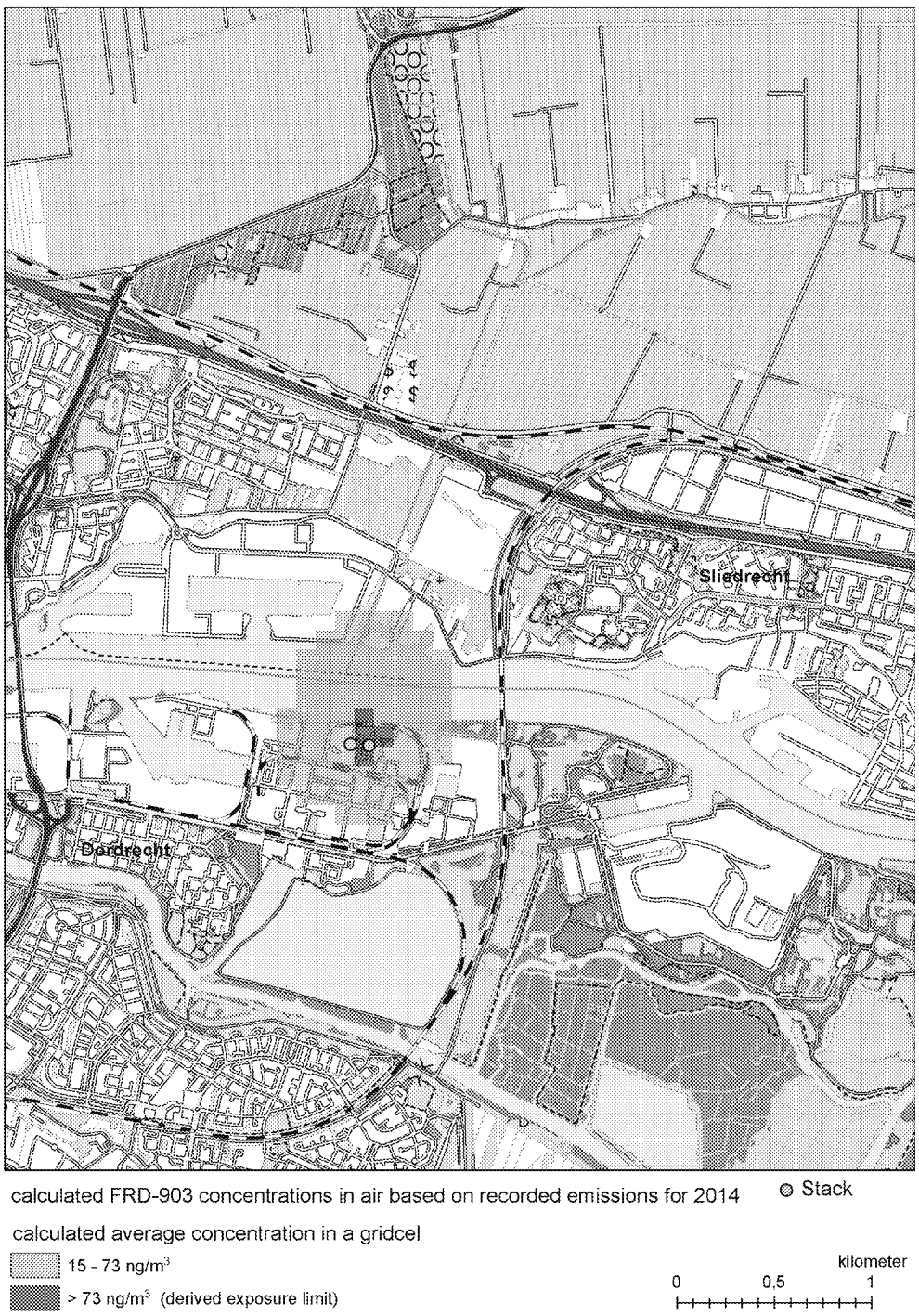


*Figure 1. Calculated air concentrations FRD-903 based on the recorded emissions (in ng/m$^3$).*

# 7 Conclusions

In chapter 3 of this report, on PBT-properties, the conclusion is that it cannot be excluded that the GenX related substances meet the PBT/vPvB criteria. All evaluated substances (FRD-903, FRD-902 and E1) are perfluorinated compounds and should therefore be regarded as certainly P/vP. Since FRD-903 and FRD-902 in animals are more rapidly eliminated than PFOA, it is expected that both substances bioaccumulate less than PFOA. However, it is not possible to reach a conclusion on the human bioaccumulation potential in absence of data on the human clearance time. For the substance E1, insufficient information is available to draw a conclusion about the bioaccumulation potential for FRD-902 and FRD-903. Since E1 contains no hydrophilic group, the human clearance time of the substance and the bioaccumulation potential are expected to be higher than for PFOA, although E1 has the potential to be excreted via exhalation. Finally, FRD-903 and FRD-902 are considered less toxic compared to PFOA. However, no definitive conclusion on the T criteria can be reached. E1 will most likely not meet the T criteria.

Chapter 4 evaluates the CMR and STOT RE properties of the three substances. It is concluded that classification as carcinogenic category 2 (suspected human carcinogen) is justified for FRD-902. The available studies show that both substances are not mutagenic. On reproductive toxicity the limited effects observed in presence of maternal toxicity do not normally result in classification, whereas PFOA is classified as toxic for the reproduction (category 1B). The requirement of STOT RE 2 (like liver and kidney) is difficult to assess due to dose levels tested in mice clearly below the guidance values, which may be taken as an indication that STOT RE 2 is needed. The effects in the rat are borderline and difficult to assess due to the large steps in the dose levels. Effects on the liver are observed at the similar dose levels for FRD-902 and PFOA.

All available toxicological studies were performed with the ammonium salt (FRD-902). Read-across of the toxicological properties of the ammonium salt to the acid is considered justified for systemic effects as after dissolution and dissociation of the acid and the salt the absorption in the intestinal tract and the lungs and distribution over the body of the anion (2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate) will be the same. However, local effects to the lung may differ between the two substances as acids normally have a higher irritating effect than neutral salts.

Information on the toxicity of E1 is limited but the available information indicates that E1 has a low to very low toxicity. This is supported by the repeated dose toxicity information on some structural analogues. Contrary to what is usual in toxicology, all available studies with E1 were performed in male animals only. In addition only studies of limited duration are available. Overall, the available in vitro and in vivo data on mutagenicity combined with the read-across data show that E1 is unlikely to be mutagenic. In addition, the available data indicate no requirement for classification for acute toxicity and probably STOT RE



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via inhalation but the requirement for classification for other hazard classes including carcinogenicity, reproductive toxicity and STOT RE via oral exposure is unknown.

Chapter 4 completes with the derivation of an chronic inhalation exposure limit of 73 ng/m$^3$ for FRD-903 and FRD-902 which includes an additional toxicokinetic factor in a pragmatic worst-case approach. The available information on the toxicity of E1 is limited. The data are insufficient for deriving an inhalation exposure limit.

Based on the permitted and recorded emission to air, the air concentrations around the Chemours plant are calculated in chapter 5. Comparing the calculated concentrations in air with the limit value of 73 ng/m$^3$ in chapter 6 leads to the conclusion that based on the available data, no health risks are expected for people living in the vicinity of the Chemours Dordrecht plant due to exposure to FRD-903. Due to the insufficient health effects information available for E1, these concentrations cannot be evaluated as to the possible health risk they might pose for people living in the vicinity of the Chemours plant in Dordrecht.



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# Annex 1. Human health toxicity FRD-902

**Introduction**
Most study summaries were copied from the robust study summaries in the registration dossier. The summaries were checked and conclusions by the registrant to which RIVM did not agree or were questioned were removed or it was added that this was a conclusion of the registrant. No attempt was made to have an RIVM conclusion on a parameter if the parameter was not determinative for the derivation of the NOAEL. The RIVM conclusion on the NOAEL is included at the end of each study summary. In addition, the study reports of the key studies (90-day mouse, 90-day rat and chronic rat) were provided by Chemours. A comparison of the summaries in the registration dossier with the study report showed that the summaries were copied from the study report. In addition, the results were compared with the detailed study results and additional details were added were necessary.

**A1.1 Kinetics FRD-902**
Gannon et al. (2016) tested the absorption, distribution, metabolism and excretion (ADME) and kinetics of FRD-902 (the ammonium salt) in rats, mice and cynomolgus monkeys. Pharmacokinetics was determined by measuring in blood samples from rats and mice at multiple time points after a single oral dosing at 10 or 30 mg/kg. In addition, pharmacokinetics after single intravenous exposure (10 mg/kg) were measured at multiple time points up to 7 days in rats and up to 21 days in cynomolgus monkeys. ADME parameters were measured in tissue and excreta up to 168 hours after oral dosing in mice (dose: 3 mg/kg) and in rats (dose: 30 mg/kg). A hepatocyte metabolism test indicated that FRD-902 is not metabolized by rat hepatocytes, which was supported by the absence of metabolites and the complete recovery of the dosed FRD-902 in rat and mouse urine. As shown in table A1, FRD-902 is rapidly absorbed after oral exposure and shows biphasic elimination with a very rapid alpha-phase and a slower beta-phase elimination. The alpha-phase elimination half-life was faster in female rats compared to male rats. Furthermore, it is mentioned in the publication that because the urinary elimination rate is very rapid (nearly the entire dose was eliminated within 12-24 hours), the sex difference observed in the plasma kinetics was not readily apparent in the urine kinetics. No test substance was detected in the blood of monkeys 16 days after dosing probably due to the much slower elimination in the beta-phase. For both rats and monkeys the alpha phase was very rapid and the contribution of the beta-phase was considered negligible. Therefore, the authors concluded that the beta phase elimination did not contribute to potential accumulation after multiple dosing in rats or monkeys. Nearly the whole administered dose was eliminated in the urine (table A2) in rats and mice. A small amount of the test substance was recovered in the faeces, but this was likely due to contamination of the faeces with urine. Tissues were not analyzed, because at the conclusion of the study (at 168 h) the entire dose was recovered in the urine, faeces, and cage wash. No metabolites were found.



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Pharmacokinetics differed between rats and mice, with a slower elimination rate in mice compared to rats. The elimination rate in monkeys was more similar to male rats.

*Table A1. Pharmacokinetic parameters of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate (FRD-902) as presented in Gannon et al 2016.*

| **Constant** | **Units** | **Rat, intravenous** | | **Rat, oral** | | **Mouse, oral** | | **Cynomolgus monkey, intravenous** | |
|---|---|---|---|---|---|---|---|---|---|
| | | **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 |

*Table A2. Material balance of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate (FRD-902) dosing in rats and mice, as presented in Gannon et al 2016.*

| | **Rat (30 mg/kg)** | | | | **Mouse (3 mg/kg)** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Male** | | **Female** | | **Male** | | **Female** | |
| | **Mean (%)** | **SD (%)** | **Mean (%)** | **SD (%)** | **Mean (%)** | **SD (%)** | **Mean (%)** | **SD (%)** |
| Urine | 103 | 2.7 | 100 | 6.4 | 90 | 6.9 | 92 | 6.0 |
| Feces | 1 | 1.0 | 1 | 0.6 | 2 | 1.0 | 2 | 0.6 |
| Cage wash | 1 | 0.5 | 5 | 5.1 | 10 | 4.0 | 6 | 3.2 |
| Total | 105 | 2.2 | 106 | 1.4 | 101 | 3.2 | 99 | 3.2 |

In the REACH registration dossier, 10 studies in experimental animals and two *in vitro* studies were available for basic toxicokinetics. The *in vitro* studies were carried out with rat hepatocytes (Exp Supporting Basic Toxicokinetics.012) and trout hepatocytes (Exp Supporting Basic Toxicokinetics.011) and showed no indication of metabolism. Several



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studies included in the registration were published by Gannon et al (2016) and are described above. Other studies in orally exposed rats (doses ranging from 10 – 30 mg/kg) showed that the test compound was almost completely eliminated in urine and reported half-lives of between 13.2 and 18.8 hours, clearance times (98.4%) at doses of 10 and 30 mg/kg of 12 h and 22 h for male rats, and of 4 h and 8 h for female rats, respectively. No test compound was recovered in the fat of male and female rats and no test compound was recovered in the liver of female rats. In male rats, the tissue:plasma ratio in the liver was 2.2 at a dose of 10 mg/kg, and 0.8 at a dose of 30 mg/kg, respectively (Exp Supporting Basic Toxicokinetics.008).

In another study (Exp Supporting Basic Toxicokinetics.007), rats were exposed by intravenous injection with 10 or 50 mg/kg. Reported clearance times were 22 h and 3 h for male and female rats, respectively, at a dose of 10 mg/kg. At a dose of 50 mg/kg, reported clearance times were 17 hours and 4 hours for male and female rats, respectively.

One additional study in mice was available (Exp Supporting Basic Toxicokinetics.010), in which mice were given a single oral dose of 10 or 30 mg/kg. At 10 mg/kg, the plasma clearance time was 143 h and 57 h for male and female mice, respectively. At 30 mg/kg, the plasma clearance time was 139 h and 62 h for male and female mice, respectively. The tissue:plasma ratio for fat was >0.1 in male rats exposed to 30 mg/kg. In male rats in the 10 mg/kg dose group, no test substance was detected in fat. The tissue:plasma ratio for the liver was 0.5 for male rats in both the 10 mg/kg and 30 mg/kg dose groups. In female rats, no test substance was detected in fat or liver.

One study in cynomolgus monkeys is reported in the registration dossier (Exp Supporting Basic Toxicokinetics.003). From the results presented it could not be determined whether these data also refer to the Gannon et al (2016) study. Therefore, the study report in cynomolgus monkeys is presented here as well. Monkeys were exposed to 10 mg/kg by a single intravenous injection. Blood was collected at multiple time points (approximately 0.083 (5 min), 0.167 (10 min), 0.25 (15 min), 0.5 (30 min), 1, 2, 4, 8, 12, and 24 hours post dose). Additional blood samples were collected once daily on day 3 – 21. Half-lives at a time interval of 0-12 h were 1.8 h and 1.6 h for male and female monkeys, respectively (Table 5). Clearance times were reported to be 11 hours for males and 10 hours for females.



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*Table A3. Half-life in cynomolgus monkeys as reported in the REACH registration dossier for ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate; study report Exp. Supporting Basic toxicokinetics.003.*

| **Half-life of Test Substance in primate plasma over the time interval corresponding to clearance time** | | | |
|---|---|---|---|
| | Time Interval (hr) | Lambda (1/hr) | Half-life (hr) |
| Male | 0-12 | 0.3845 | 1.8 |
| | 4-12 | 0.2666 | 2.6 |
| Female | 0-12 | 0.4288 | 1.6 |
| | 4-12 | 0.3047 | 2.3 |

One study on toxicokinetic data in rats after prenatal exposure was available in the registration dossier (Exp Supporting Basic Toxicokinetics.006). Pregnant rats were exposed to a daily dose of 5, 10, 100 or 1000 mg/kg/day by oral gavage during gestational days (GD) 6 – 20. Plasma concentrations were measured in the fetuses on GD20, in dams on GD20, and additionally on GD6 in dams in the highest exposure group (1000 mg/kg/day). A linear dose-plasma concentration relation was observed between 5 and 100 mg/kg/day, levelling off at 1000 mg/kg/day. The mean plasma concentration on GD20 was less than that on GD6, indicating that a steady state was achieved by GD6 and no accumulation occurred in the dams between GD6 and GD20. The plasma concentration in fetuses (pooled concentration) was approximately one-third of the plasma concentration in the dam at GD20.

One report on dermal absorption of FRD-902 (purity 86%) was available in the registration dossier performed according to OECD TG 428 in 2008. Dermal absorption was studied in a static diffusion cell setup with rat and human skin at a concentration of 124 mg/ml. In rat skin, a lag time of 0.82 ± 0.77 hours was observed and in human skin the observed lag time was 1.73 ± 1.01 hours. Steady state penetration was 70 ± 5.3 ug/cm2/h and Kp was 5.7E-4 ± 4.3E-5 cm/h in rat skin. In human skin, steady state penetration was 6.2 ± 5.3 ug/cm2/h and Kp was 5.0E-5 ± 4.3E-5 cm/h.

*Conclusions on ADME.*
The available data indicate that FRD-902 is quickly absorbed after oral and absorbed after dermal exposure, not metabolized, and eliminated almost completely within approximately 24 hours via urine in rats, mice and monkeys. The substance distributes into the fetus. The elimination was significantly higher in female rats compared to male rats but no such difference was observed in mice and cynomolgus monkeys.

### A1.2 Acute toxicity

***Oral***

Two studies in rats and one study in mice were available from the registration dossier on acute toxicity by the oral exposure route. The studies were performed according to OECD Guideline 425 and EPA OPPTS 870.1100. Test substance (86% purity) was applied by oral

gavage at doses of 175, 550, 1750, and 5000 mg/kg for rats and 175, 550 and 1750 mg/kg for mice. Animals were observed during 14 days and then necropsied.

All female rats in the highest dose group (5000 mg/kg) 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 (Exp Support Acute Tox: oral.001).

All male rats in the highest dose group (5000 mg/kg) and one male rat in the 1750 mg/kg dose group died. These rats showed lethargy, skin stain, expanded lungs, eye discoloration and stomach discoloration. One rat in the 175 mg/kg dose group also showed lethargy. Other clinical findings in the 550 and 1750 mg/kg 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, with 95% profile likelihood confidence interval 1239 – 4450 mg/kg. This study was selected by the registrant as the key study for acute toxicity after oral exposure (Exp Key Acute Tox: oral.003).

In mice, all mice in the highest dose group (1750 mg/kg) died. These mice exhibited lethargy and low posture. One mouse in the 550 mg/kg 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 registrant. The oral LD50 for mice was 1030 mg/kg (Exp Support Acute Tox: oral.002).

Three additional studies in male rats were available in the registration dossier, but have been marked as 'not reliable' by the registrant because the test substance composition was insufficiently defined.

Based on the key study in male rats with an LD50 of 1750 mg/kg bw, classification as Acute Tox 4; H302 is warranted.

***Inhalation***

One study on acute inhalation toxicity was available in the registration dossier (Acute Toxicity: inhalation). The study was performed according to OECD Guideline 403. 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 (however, microscopic analysis was not performed in the 5200

mg/m$^3$ dose group). Body weight loss between 2.5% - 6.8% as compared to controls was observed in rats in the highest dose group. 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$^3$. Based on this study classification is not warranted.

***Dermal***
Two acute toxicity studies for the dermal exposure route were available in the registration dossier; one in rabbits and one in rats.

Rabbits were exposed by occlusive patch for 24 hours to a dose of 5000 mg/kg. No mortality was observed (2 rabbits were used in the study). There was no mortality. 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 ALD of > 5000 mg/kg was reported (Exp Supporting Acute Toxicity: dermal.002).

The study in rats was performed according to international guidelines (OECD Guideline 402 / EPA OPPTS 870.1200 / EEC, Method B.3 Directive 92/69/EEC) 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 (Exp Key Acute Toxicity: dermal.001). No mortality was observed. Reversible local effects were observed on the treated skin.

Based on these studies classification is not warranted.

**A1.3 Irritation and corrosion**
The available skin irritation study according to OECD TG 404 (Skin irritation/corrosion) showed limited and reversible erythema (score1 or 2) at 1 hour after removal of FRD-902 (86% purity). Based on this study classification for skin irritation is not warranted.

The available eye irritation study according to OECD TG 405 (Eye irritation) showed irreversible effects in the tested rabbit including cornea opacity, iritis and conjunctival chemosis and discharge. The rabbit was euthanized the day after treatment for humane reasons. Based on this study classification as Eye Damage 1; H318 is warranted.

**A1.4 Sensitisation**
In a LLNA test according to OECD TG 429 (Exp Key Skin sensitisatopn.002), FRD-902 dissolved in dimethylformamide at 0, 5, 25, 50 and 100% induced no increase in the stimulation index above 3. 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 and undefined mixture was tested and positive with an EC3 of 37%. However, the relation of the tested substance with the marketed



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substance was questioned. Therefore, based on the first test the marketed substance need not to be classified for skin sensitisation.

**A1.5 Mutagenicity**
In an Ames test according to OECD TG 471 (Exp Key genetic toxicity in vitro.001) up to 5000 ug/plate using plate incorporation, FRD-902 was negative with and without metabolic activation. A comparable study (Exp supporting in genetic toxicity in vitro.003) with a test substance which was not sufficiently characterised according to the registrant, was also negative. In a mammalian cell gene mutation assay according to OECD TG 476 (Exp Key genetic toxicity in vitro.002) in which the pH was adjusted to neutral, FRD-902 was negative with and without metabolic activation. In an in vitro mammalian chromosome aberration test according to OECD TG 473 (Exp Key genetic toxicity in vitro.005), 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. In the first test, there was no statistically significant increase at this concentration but the control value for numerical aberrations was outside the historical control range. In a confirmatory trial, the structural and numerical aberrations were increased compared to the concurrent control at the highest dose level. A comparable study (Exp supporting in genetic toxicity in vitro.004) with a test substance which was not sufficiently characterised according to the registrant, was negative.

In a mouse micronucleus test according to OECD TG 474 (Exp Key genetic toxicity in vivo.001) 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 reaches 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 (Exp Key genetic toxicity in vivo.003) 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.

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.

**A1.6 Carcinogenicity**
In a combined chronic and carcinogenicity study performed according to OECD Guideline 453, 80 rats per dose and sex were exposed to FRD-902 (purity 84%) by gavage (water). The dose levels were males: 0.1, 1, 50 mg/kg bw/day and females: 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)(Rae et al, 2015).



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In high dose females, a significant increased incidence of hepatocellular adenoma and hepatocellular carcinoma was observed. In high dose males, a statistically significant increase was observed in the incidence of pancreatic acinar cell adenoma/carcinoma combined, but not adenoma or carcinoma alone. The incidence of interstitial cell adenoma of the testes was increased in males at 50 mg/kg/day, and one interstitial cell adenoma was also present in one male in the 50 mg/kg/day group at the interim necropsy. Also the incidence of interstitial cell hyperplasia was increased in this group and outside the historical control range. These findings were not statistically significant, amongst others due to a relatively high incidence of these lesions in the controls. The increase in uterus stromal polyps was within the range of the historical controls. Therefore, it is uncertain whether this statistical significant increase in polyps is substance related.

*Table A4. Tumor incidences and related histological changes in the OECD 453 study in rats.*

| Tumor type | Sex | Hist control | Control | Low | Mid | High |
|---|---|---|---|---|---|---|
| Hepatocellular adenoma (%) | Females | 0-5% | 0 | 0 | 0 | 11 (15.71%)* |
| | Males | | 1 | 2 | 1 | 1 |
| Hepatocellular carcinoma | Females | 0-1.7% | 0 | 0 | 0 | 4 (5.71%)* |
| | Males | | 1 | 0 | 0 | 2 |
| Pancreatic acinar cell adenoma | Males | 0-5% | 0 | 1 | 0 | 3 (4.29%) |
| Pancreatic acinar cell carcinoma | Males | 0-1.7% | 0 | 0 | 0 | 2 (2.86%) |
| Combined acinar cell tumors | 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 in at least 2/3 statistical tests | | | | | | |

It was suggested by the study authors that the observed increase in tumors was induced by non-genotoxic peroxisome proliferation, which is specific for rodents. We agree that the available data do indicate a non-genotoxic mechanism. However, we do not currently agree, as further substantiated in the chapter on the mode of action, that it is sufficiently shown that these types of tumours via this mechanism are not relevant for humans. The NOAEL for carcinogenicity is 1 mg/kg bw/day in males based on an increase in combined adenoma and carcinoma of the



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pancreas and 50 mg/kg bw/day in females based on an increase in liver tumours at 500 mg/kg bw/day.

**A1.7 Reproductive toxicity**

A study on developmental toxicity (Developmental toxicity/teratogenicity) was conducted in rats, according to OECD Guideline 414. Pregnant rats were exposed to FRD-902 (84% purity) at 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 fetuses 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/day groups, respectively, delivered early on gestation day 21. The mortality in the 1000 mg/kg/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 occurred only in the highest dose group. Decreased gravid uterine weights were found in the 100 and 1000 mg/kg/day groups. Increased liver weight was found in the 100 and 1000 mg/kg/day groups and was considered in the report to be related to PPARa activation. In addition, focal necrosis was observed in the liver of some animals at these dose levels and hepatocellular hypertrophy at the highest dose level. Mean fetal weight was reduced by 8,8% in the 100 mg/kg/day group and by 28.1% in the 1000 mg/kg/day group. No effects were found on fetal survival, on malformations or on variations, except a higher incidence of 14th rudimentary ribs in the highest dose group which was not considered adverse by the registrant.

The increase in early delivery was confirmed in a second study at 1000 mg/kg bw/day in which 3 early deliveries were observed in an unknown number of dams versus none in the controls. The fetal weight was decreased. In addition, comparable maternal effects were observed as in the main study.

The no-observed-adverse-effect level (NOAEL) for maternal toxicity was considered to be 10 mg/kg/day, based on mortality and lower mean body weight gains and food consumption at 1000 mg/kg/day and early deliveries, and microscopic findings in the liver (focal necrosis) at 100 and 1000 mg/kg/day.

The no-observed-adverse-effect level (NOAEL) for developmental toxicity was considered to be 10 mg/kg/day, based on early deliveries and lower mean fetal weights at 100 and 1000 mg/kg/day.

In a one-generation study (OECD TG 421) in mice (n=25) with exposure by oral gavage at dose levels of 0.1, 0.5 and 5 mg FRD-902/kg bw/day (purity 84%), 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



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were selected for toxicokinetic evaluation were dosed through the day of euthanasia (lactation day 21) for a total of 54 to 65 doses.

Parental animals: no effect on mortality. In the higher dose groups, an increase in body weight was found with a corresponding increase in food consumption. Increased liver weight in the 0.5 and 5 mg/kg/day dose groups. In the highest dose group, an increase in kidney weight was found (only significant in females). Hypertrophy was found in the liver of males and females in the middle and high dose groups, and in the kidneys of males in the middle and high dose groups. Liver necrosis (focal or single cell) and an increase in the presence of mitotic figures was observed at the highest dose level. An increase in single cell necrosis of the liver was also observed in males at 0.5 mg/kg bw/day.

There was no effect on reproductive performance. Mean numbers of F1 pups born, live litter size, percentage of males at birth, postnatal survival, and the general physical condition of the F1 pups were unaffected by test substance administration at all dosage levels. F1 survival was unaffected by test substance administration at all dosage levels following weaning. At the highest dose level, a reduction in body weight gain was observed in male pups, and in female pups only during the pre-weaning period. Minimal delays in sexual maturation were observed, but were related to the reduction in body weight. For both maternal animals and their offspring, male and female mice behaved in a kinetically similar manner, with an approximately linear relationship between dose and blood levels of the test substance. The plasma level in pups on post-natal day (PND) 4 were 2-4 fold below the maternal levels and on PND 21, 40-60 fold lower. On PND 40, after direct gavage exposure of the F1, the plasma levels were comparable between the dams and offspring. Based on these results, the no-observed-adverse-effect level (NOAEL) for reproductive toxicity was 5 mg/kg/day, as no effects on reproduction were observed at any of the doses levels tested. The results indicate limited transfer of FRD-902 via lactation. The NOAEL for systemic toxicity in parental animals was 0.1 mg/kg/day based on the low incidences of single cell necrosis observed in the liver of males at 0.5 mg/kg/day. The NOAEL for systemic toxicity in the offspring was 0.5 mg/kg/day based on body weight decrements in the F1 males and females in the 5 mg/kg/day group during the pre-weaning period.

### A1.8 Specific target organ toxicity – repeated exposure

***Mice***

In a range-finding study not performed according to OECD and GLP, male mice (n=5) were exposed to FRD-902 (86.6% purity) by gavage (water) at 30 mg/kg bw/day. Body (105%) and liver weight (200%) were increased. Histopathology showed an increase in liver hypotrophy, single cell necrosis and mitotic figures (Exp Supporting Repeated dose toxicity:oral.006).

In a 28-day study 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 0.1, 3 and 30 mg/kg bw/day. The reversibility of the effects in the high dose mice was determined after a 4-week recovery period (Exp Supporting Repeated dose toxicity:oral.003).



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Body weights were significantly higher compared to controls in the 30 mg/kg/day group males and females at the end of the dosing period. Body weight gain slowed during the recovery period and body weights in the 30 mg/kg/day animals were comparable to the control group by the end of the study. Statistically significant, test substance-related decreases in red cell mass parameters (red blood cells, hemoglobin and/or hematocrit) were present in the 3 and 30 mg/kg/day group males. The changes in red cell mass parameters were minimal (decreased less than 10% compared to controls). Test substance-related serum chemistry findings included changes in liver enzymes and serum proteins in males and females administered 3 or 30 mg/kg/day. Liver enzyme levels (alanine aminotransferase, alkaline phosphatase and sorbitol dehydrogenase) were higher in the 3 and 30 mg/kg/day group males and 30 mg/kg/day group females at study week 4. Aspartate aminotransferase levels were also higher in the 3 and 30 mg/kg/day group males at study week 4. These liver enzyme level changes were consistent with hepatocellular injury, and single cell necrosis was noted microscopically in some animals in these groups. Liver enzyme changes were reversible in both males and females, as levels for all liver enzymes were similar to controls following 4 weeks of recovery. Minimal changes of higher albumin, lower globulin, and associated changes of increased total protein and increased albumin/globulin ratio were present in the 30 mg/kg/day group males. Decreased globulin and increased albumin/globulin ratio were also present in the 3 mg/kg/day group males. A similar pattern of change in serum proteins was present in females administered 3 or 30 mg/kg/day. These serum protein changes were reversible, as there were no statistically significant changes in these parameters in males or females by study week 8. Blood urea nitrogen was slightly increased in the 30 mg/kg/day group males at the end of exposure. Blood urea nitrogen was similar to control values following the 4-week recovery period. The slight increase in urea nitrogen in 30 mg/kg/day group males was not associated with changes in related clinical chemistry parameters or with test substance-related microscopic findings in the kidney. A statistically significant decrease in cholesterol was present in the 3 mg/kg/day group males. This decrease was not dose-related, as mean cholesterol in the 30 mg/kg/day group males was not statistically different from controls and was higher than that of the 3 mg/kg/day group males. However, several individual cholesterol values in treated male groups were below the study control range, and thus, a test substance-related effect of decreased cholesterol cannot be ruled out. However, individual cholesterol values in treated groups were within the testing laboratory historical control values (with the exception of one male in the 3 mg/kg/day group).

Test substance-related gross necropsy findings included enlarged liver in the 30 mg/kg/day group males at the primary necropsy. There were no test substance-related gross necropsy findings at the recovery necropsy. Liver weights were increased in the 3 and 30 mg/kg/day group males and females at the end of the exposure period. These changes correlated with hepatocellular hypertrophy microscopically and with increases in beta-oxidation. Liver weights were mostly, but not completely, reversible in the 30 mg/kg/day males and females. At this dose, liver weight relative to body weight in the 30 mg/kg/day group males was increased by 163.1% above controls at the end of exposure,

and was reduced to 21.5% of control after the 4-week recovery period. Similarly, in 30 mg/kg/day group females, liver weight relative to body weight was increased by 102.7% above controls at the end of exposure and was reduced to 14.3% of control after the 4-week recovery period. Adrenal gland weights (absolute and relative to body and brain weights) were increased in the 3 and 30 mg/kg/day group males at the end of the exposure period. In the 30 mg/kg/day group males, these adrenal weight changes correlated with minimal adrenal cortical hypertrophy microscopically. Adrenal weight changes were reversible following the 4-week recovery period. Decreased uterus weights (absolute and relative to body and brain weights) were present in the 30 mg/kg/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. Minimal adrenal cortical hypertrophy was observed microscopically in the 30 mg/kg/day group males at the primary necropsy. This change correlated with increased adrenal weights in this group. Adrenal cortical hypertrophy was not observed in the 30 mg/kg/day group males at the recovery necropsy.

Hepatocellular hypertrophy was observed in the 3 and 30 mg/kg/day group males and females at the primary necropsy. This change was consistent with increased liver weights noted in these groups. The hepatocellular hypertrophy was characterized by expansion of the hepatocellular cytoplasm by numerous fine eosinophilic granules. Other findings in the liver included multifocal single cell hepatocellular necrosis in the 3 and 30 mg/kg/day group males and 30 mg/kg/day group females at the primary necropsy, and increased mitoses distributed multifocally throughout the liver section in the 30 mg/kg/day group males and females at the primary necropsy. Incidences of these changes were higher in the males compared to the females. Hepatocellular hypertrophy, single cell hepatocellular necrosis and increased mitoses in the liver were not observed in the 30 mg/kg/day group males and females at the recovery necropsy.

There was an increased number of animals in the diestrus stage of the estrous 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 estrous cycling. The significance of the differences in estrous stage distribution between the 30 mg/kg/day group females and control group females is uncertain. The number of animals in the diestrus stage of the estrous cycle was equal in the control and 30 mg/kg/day group females at the recovery necropsy.

The test substance was an inducer of hepatic peroxisomal beta-oxidation activity, a measure of peroxisome proliferation, in male mice after administration of 0.1, 3 and 30 mg/kg/day and in female mice after administration of 3 and 30 mg/kg/day for 28 days. Total hepatic microsomal cytochrome P-450 enzyme content was decreased at a dosage of 3 and 30 mg/kg/day in male mice but not in females. Beta-oxidation activity in both male and female mice had returned to control levels after approximately 28 days of recovery, while total cytochrome P-450 content remained below control levels in the males.



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The NOAEL in this study was 0.1 mg/kg bw/day based on several effects mainly in males including liver single cell necrosis, reduction in red blood cell parameters, increased liver weights, hepatocellular hypertrophy, and changes in albumin/globulin ratio at 3 mg/kg bw/day.

In a 90-day study 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 were exposed for evaluation of the plasma concentration of the substance at 2 hours after exposure on day 0, 28 and 95 (Exp Supporting Repeated dose toxicity:oral.007).

There were no test substance-related clinical observations or deaths. No adverse, test substance-related effects on mean body weight or mean body weight gain were observed in any female group. 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/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. No adverse, test substance-related effects on mean food consumption or food efficiency were observed in any female group. Statistically significant increases in mean overall (test days 0-91) food consumption and food efficiency were observed in the male 5 mg/kg/day group, relative to control. Mean overall food consumption and food efficiency were 111% and 127% of control, respectively. The higher food efficiency is likely due to increased body weight due to enlarged livers in this group. No ophthalmological signs were observed in any mouse in any group. There were no adverse or treatment-related changes in group mean hematology parameters at test day 96 (males) or 97 (females). Test substance-related increases in a number of liver-related clinical chemistry parameters were present in male and female mice administered 5 mg/kg/day (see Table A5). Increases were mild to severe, were consistently more severe in males compared to females, and included increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), alkaline phosphatase (ALKP) and total bile acids (TBA). Changes in these parameters were consistent with hepatocellular damage and/or cholestasis, correlated microscopically with hepatocellular single cell necrosis in male (10/10) and female (1/10) mice at this dose, and thus were considered to be adverse effects. Total protein (TP) and albumin (ALB) were minimally increased in male mice dosed with 5 mg/kg (110% and 114% of control, respectively). ALB was also minimally increased in female mice at the same dose (104% of control). These changes were considered to be treatment-related due to the consistency of change among individual animals. However, minimally increased total protein and albumin have no toxicological significance; therefore, these changes were considered to be non-adverse by the registrant. Cholesterol (CHOL) was mildly decreased in male mice dosed with 5 mg/kg (74% of control). There are no known adverse effects associated with minimal decreases in cholesterol. Therefore, these changes were considered test substance related but non-adverse by the registrant. Potassium (K) was decreased in male and female mice dosed with 5 mg/kg (87% and 91% of control,

respectively). Decreased K typically occurs when there is a shift of K from extra cellular fluid to intracellular fluid (e.g., in metabolic alkalosis), a decreased dietary intake of K, or an increased loss of K via kidneys (e.g., polyuria), alimentary tract (e.g., diarrhea) or skin (e.g., sweating). In the present study, the relationship of this finding to test substance administration is uncertain. However, there were no clinical signs suggestive of hypokalemia and no test substance-related alterations in sodium (Na). Therefore, the minimal change in K was not considered to be adverse. Chloride (CL) was slightly higher (102% of control) in male mice dosed with 5 mg/kg. Based on the minimal nature of the change and lack of any correlative findings, this change was considered to be unrelated to treatment and non-adverse by the registrant. Under the conditions of the study, the test substance had no effect on neurobehavioral parameters in either males or females.

*Organ weights*
A test substance related increase in mean liver weight parameters was observed in mice exposed to ≥ 0.5 mg/kg/day in males and 5 mg/kg/day in females (see Table A6). In the 5 mg/kg/day males, mean absolute and mean relative (% brain weight and % body weight) liver weights were increased to 263%, 242%, and 230% of control, respectively. These increases were statistically significant. In the 0.5 mg/kg/day males, mean absolute and mean relative (% brain weight and % body weight) liver weights were also increased (not statistically significant) to 112%, 113%, and 111% of control, respectively. In 5 mg/kg/day females, mean absolute and mean relative (% brain weight and % body weight) liver weights were increased (statistically significant) to 169% , 167% and 169% of control, respectively. Increased liver weight parameters were considered test substance related in males given ≥ 0.5 mg/kg/day and in females given 5 mg/kg/day. The increase in liver weight parameters in both sexes correlated with a treatment-related increase in enlarged liver and microscopic hepatic changes.

Mean relative (to brain) weight of kidneys was increased (statistically significant) in male mice given 5 mg/kg/day of test substance as compared to controls. Although minimal renal tubular hypertrophy was present in this group, the change in kidney weight relative to brain weight was not associated with changes in mean absolute or relative (% body weight) kidney weights. Mean weights of brain and epididymides relative to body weight were lower, and mean weight of heart relative to brain weight was higher in male mice given 5 mg/kg/day of test substance as compared to controls (all statistically significant). These changes occurred without correlative changes in other weight parameters for these organs or with microscopic findings.

*Gross pathology*
At the terminal sacrifice, enlarged and/ or discolored livers were observed in 4/10 and 9/10 male mice exposed to 0.5 mg/kg/day and 5 mg/kg/day of test substance respectively. In the 5 mg/kg/day group 3/10 female mice had enlarged livers (see Table A7). These gross changes were considered test substance related. Liver enlargement and discoloration correlated with test substance related increases in liver

weights and microscopic hepatocellular hypertrophy.

*Histopathology: non-neoplastic*
Test substance related and adverse microscopic findings were present in the liver of male and female mice administered 5 mg/kg/day of the test substance (see Table A8). At 0.5 mg/kg/day, test substance related microscopic changes were limited to male mice which had minimal hepatocellular hypertrophy, without evidence of liver cell injury. In the 5 mg/kg/day male and female groups, test substance-related hepatocellular hypertrophy was present in all animals. Hypertrophy was graded as mild (grade 2 out of 4) in males and minimal (grade 1 out of 4) or mild in females. The distribution of the hepatocellular hypertrophy was centrilobular when of minimal severity and diffuse when of mild severity. Hypertrophy was morphologically consistent with peroxisome proliferation and was characterized by increase in the size of hepatocytes due to increased amount of finely granular eosinophilic cytoplasm and enlarged nuclei with occasional binucleated cells. Additional liver changes in the 5 mg/kg/day group occurred most consistently in males and included increased numbers of mitotic figures (males only), increased pigment (likely lipofuchsin) in Kupffer cells, and single cell hepatocellular necrosis. The latter change was characterized by isolated eosinophilic bodies with occasional pyknotic nuclear fragments and unaccompanied by inflammation, and thus was consistent with apoptosis. Hepatic lesions correlated with increased absolute and relative liver weight and increased total bile acid and liver enzyme levels (AST, ALT, SDH, ALP). Minimal bile duct hyperplasia was present in the liver of one male mouse in the 5 mg/kg/day group. Since similar changes were not seen in any other treated mice, the relationship of this finding to test substance administration is uncertain. In the 0.5 mg/kg/day groups, liver changes were limited to minimal hepatocellular hypertrophy in males only. In females, focal necrosis was present in both treated and control mice with slightly increased incidence in the 5 mg/kg/day females (1/10, 0/10, 2/10, 3/10 in control, 0.1, 0.5, and 5 mg/kg/day groups, respectively). Focal hepatic necrosis is a common background lesion in mice, and there was no difference in morphology or severity of this lesion in treated female mice as compared to controls. In addition, test substance-related focal necrosis did not occur in males, the more sensitive gender for liver effects. Therefore, the minimal increase in the incidence of this lesion in high dose females was considered spurious and unrelated to treatment.

Test substance related changes in the kidney were limited to minimal tubular epithelial hypertrophy in 9/10 male mice given 5 mg/kg/day of the test substance. Hypertrophy was characterized by slightly enlarged epithelial cells containing increased amounts of fine granular eosinophilic cytoplasm. Tubular epithelial hypertrophy was not associated with renal tubular cell degeneration/necrosis. Also there was no change in clinical pathology parameters indicative of renal injury.

*Plasma Concentration Evaluation*
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. This is consistent with a test substance that was cleared rapidly from the blood within one dosing

interval. The test substance concentration in blood from male mice was lower on day 0 than on day 28, and for the high dose the concentrations on day 28 were slightly lower than day 95 concentrations, indicating steady state may not have been achieved by day 28. Compared to female mice, male mice 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. Test substance was not present in plasma from control animals.

*Table A5. Clinical chemistry.*

| | **Male Mice – Test Day 96** | | | | **Female Mice – Test Day 97** | | | |
|---|---|---|---|---|---|---|---|---|
| Dosage (mg/kg) | 0 | 0.1 | 0.5 | 5 | 0 | 0.1 | 0.5 | 5 |
| AST (U/L) | 62 | 67[a] | 84 | **128** | 68 | 71 | 69 | 74 |
| | | 108%[b] | 135% | **206%** | | 104% | 101% | 109% |
| ALT (U/L) | 49 | 62 | 66 | **255** | 36 | 36 | 32 | **51** |
| | | 127% | 135% | **520%** | | 100% | 89% | **142%** |
| SDH (U/L) | 26.6 | 26.0 | 25.8 | **108.5** | 25.3 | 22.9 | 23.6 | **33.5** |
| | | 98% | 97% | **408%** | | 118% | 111% | **243%** |
| ALKP (U/L) | 50 | 55 | 70 | **617** | 65 | 77 | 72 | **158** |
| | | 110% | 140% | **1234%** | | 118% | 111% | **243%** |
| TBA (µmol/L) | 1.2 | 1.2 | 1.4 | **11.1** | 4.3 | 2.3 | 2.7 | 13.2 |
| | | 100% | 117% | **925%** | | 53% | 63% | 307% |
| [a] – mean, [b] - % of control, **bold** = statistically significant | | | | | | | | |

*Table A6.Test Substance-Related Effects on Mean Absolute and Relative Liver Weights.*

| **Dose (mg/kg/day)** | **0** | **0.1** | **0.5** | **5** |
|---|---|---|---|---|
| Male | | | | |
| Number of mice | 10 | 10 | 10 | 10 |
| Mean final body weight (grams) | 38.7 | 40.3 | 38.9 | 44.3* |
| Liver | | | | |
| absolute weight (grams) | 1.955 | 2.024 | **2.186** | **5.144*** |
| Liver weight/body weight x 100 | 5.06 | 5.028 | **5.618** | **11.637*** |
| Female | | | | |
| Number of mice | 10 | 10 | 9 | 9 |
| Mean final body weight (grams) | 32.2 | 32.1 | 32.6 | 32.4 |
| Liver | | | | |
| absolute weight (grams) | 1.693 | 1.697 | 1.745 | **2.867*** |
| Liver weight/body weight x 100 | 5.225 | 5.309 | 5.337 | **8.811*** |
| * Statistically significant as compared to control value.<br>**Bold** values were interpreted to be test-substance related increases, as compared to control values. | | | | |

*Table A7. Test Substance-Related Gross Observations in Mice.*

| | **Male** | | | | **Female** | | | |
|---|---|---|---|---|---|---|---|---|
| Dose (mg/kg/day) | 0 | 0.1 | 0.5 | 5 | 0 | 0.1 | 0.5 | 5 |
| mice/group: | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Liver | (10) | (10) | (10) | (10) | (10) | (10) | (10) | (10) |
| Large | 0 | 0 | **1** | **9** | 0 | 0 | 0 | **3** |
| Discoloration | 0 | 0 | **4** | **5** | 1 | 0 | 0 | **3** |
| Numbers in parentheses are the number of tissues examined within each group.<br>**Bold** values were interpreted to be test substance-related gross findings. | | | | | | | | |

*Table A8. Incidences of Test Substance-Related Microscopic Findings in the Liver of Male and Female Mice.*

| | **Male** | | | | **Female** | | | |
|---|---|---|---|---|---|---|---|---|
| Dose (mg/kg/day) | 0 | 0.1 | 0.5 | 5 | 0 | 0.1 | 0.5 | 5 |
| mice/group | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Liver | (10) | (10) | (10) | (10) | (10) | (10) | (10) | (10) |
| Hepatocellular hypertrophy | 0 | 0 | **8** | **10** | 0 | 0 | 0 | **10** |
| Hepatocellular single cell necrosis | 0 | 0 | 0 | **10** | 0 | 0 | 0 | **1** |
| Mitotic figures | 0 | 0 | 0 | **9** | 0 | 0 | 0 | 0 |
| Pigment increased, Kupffer cells | 0 | 0 | 0 | **10** | 0 | 0 | 0 | **2** |
| Numbers in parentheses are the number of tissues examined within each group.<br>**Bold** values were interpreted to be test substance-related gross findings. | | | | | | | | |

RIVM opinion: Several statistically significant effects observed in this study were considered substance related but not adverse by the registrant because of the small effect size. We agree that not all statistically significant effects are also biologically significant. In addition, a minimal effect size is applied in the Bench Mark Dose approach to derive limit values. However, for some of the effects in the current study, the justification for the absence of adversity based on the effect size is too limited and not accepted. The NOAEL in this study is 0.1 mg/kg bw/day based on an increase in liver weight and hypertrophy observed at 0.5 mg/kg bw/day. According to the EFSA opinion on PFOA (EFSA, 2008) "These changes [liver] are often classified as adaptive and reversible. However, as these represent biological changes possibly related to effects such as tumour promotion and/or changes in drug-metabolizing enzyme activities, […] the findings should be critically evaluated.". In addition, the reversibility is of less relevance for substances with potential continues and lifelong exposure.

***Rats***
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 30, 300 and 1000 mg/kg bw/day (Exp Supporting Repeated dose toxicity:oral.004). Additional animals at 30 mg/kg bw/day were used to collect toxicokinetic information which was not reported in the robust study summary.

Effects on body weight was significant at the high dose level in male rats, 92.4% of control ± 2.0%. Statistically significant decreases in red cell mass parameters (red blood cell, hemoglobin and hematocrit) were observed in male rats at 300 and 1000 mg/kg/day and in females at 1000 mg/kg/day. Statistically significant increases in red cell distribution width, reticulocytes and neutrophils were also present in 1000 mg/kg/day females. Decreases in serum lipids (triglycerides and/or cholesterol) and globulins were present in all dosed male groups and in females at 300 and/or 1000 mg/kg/day. Other changes in clinical chemistry parameters occurred at 300 and/or 1000 mg/kg/day and included increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BUN), and Glucose; and decreased sorbitol dehydrogenase (SDH), creatinine, and calcium.

Increased liver weight parameters were present in males at all dose levels and in females in the 1000 mg/kg/day group. These liver weight changes were correlative to microscopic hepatocellular hypertrophy in the liver. Other organ weight changes included decreases in heart weight parameters (1000 mg/kg/day males) and increases in some kidney weight parameters (1000 mg/kg/day females). There were no correlative 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 1000 mg/kg/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.

A statistically significant increase in peroxisomal beta-oxidation activity was present in the 30, 300, and 1000 mg/kg/day male groups and in the 1000 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 300 and 1000 mg/kg/day male groups and in the 1000 mg/kg/day female group at the 7-day sacrifice.

In a 28-day repeated dose toxicity study according to OECD 407, groups of 10 Crl:CD(SD) rats per dose and sex were exposed to FRD-902 (purity 88%) by gavage (water). Males were exposed to 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 (Exp Supporting Repeated dose toxicity:oral.001).

All animals survived to the scheduled necropsies. Yellow material around the urogenital area was noted occasionally for 9 females in the 300 mg/kg/day group at 1 to 2 hours post-dosing from study day 3 to 25. This finding was not noted during the recovery period. There were no test substance-related effects on body weight. Minimal, statistically

significant decreases in red cell mass parameters (RBC, hemoglobin and hematocrit) were present in the 3 and 30 mg/kg/day male groups. These decreases were associated with minimal increases in absolute reticulocyte counts. The decreases in red cell mass parameters were minimal (≤7.9% below the control mean for all parameters), and values for red cell mass parameters and reticulocyte counts in individual animals in the 3 and 30 mg/kg/day male groups were within the testing laboratory historical control ranges for the respective parameters. There were no statistically significant changes in red cell mass parameters or reticulocytes following the 4-week recovery period. Test substance-related and statistically significant decreases in cholesterol were present in all treated male groups. Decreases were minimal (not reported), as values for most animals were within or only slightly below the testing laboratory historical control range. Based on the minimal nature of the changes, as well as the direction of change (decreased rather than increased), these changes in cholesterol were not considered to be adverse by the registrant. However, as the effect size is unknown this conclusion is not agreed by the RIVM. Comparable reductions were observed in other studies and can be related to the increased beta-oxidation. Effects on cholesterol were reversible as cholesterol values were actually increased compared to controls following the approximately 4-week recovery period, although cholesterol values for all animals in the 30 mg/kg/day recovery group were within the testing laboratory historical control range. Higher albumin and lower globulin levels, as well as associated increases in albumin/globulin ratio, were present in the 3 and 30 mg/kg/day male groups. Increased albumin and albumin/globulin ratio were also present in the 300 mg/kg/day female group. Changes in globulin were minimal (not reported), as individual values for all animals in the 3 and 30 mg/kg/day male groups were within the testing laboratory historical control range, with the exception of one rat in the 30 mg/kg/day group whose value was just below the testing laboratory historical control range. Similarly, increases in albumin in the affected male and female groups were within the testing laboratory historical control range, or, for some animals in the 30 mg/kg/day male group, were only slightly above the testing laboratory historical control range. The changes in serum proteins were considered to be test substance related. However, these changes were not considered to be adverse based on their minimal nature at all dose levels by the registrant. In addition, all serum protein changes were reversible, as mean values were similar to controls following the 4-week recovery period. Urea nitrogen was minimally increased in the 30 mg/kg/day group males. This increase was not associated with changes in creatinine or with treatment-related microscopic changes in the kidney. The minimal increase in urea nitrogen is likely of non-renal origin. The pattern of changes in urea nitrogen, as well as those noted above for serum proteins, is consistent with those reported for other peroxisome proliferators. Changes in urea nitrogen were reversible in males, as there were no statistically significant changes in these parameters following the recovery period. Glucose levels were minimally increased (15.2% higher than the control group mean) in the 30 mg/kg/day group males at study week 4, but were lower than the control group at study week 8. These increases were within the testing laboratory historical control ranges and were not considered adverse. Mean triglyceride values in treated male groups were lower than

controls. These decreases did not occur in a dose-related manner and were statistically significant only in the 3 mg/kg/day group. The group means for the treated groups were actually similar to the historical control mean, while the concurrent study control group mean of 72 mg/dL was higher than the mean of the historical control data, which was 48 mg/dL. Individual triglyceride values in animals from all treated male groups were within the testing laboratory historical control range. While some peroxisome proliferators have been shown to lower triglycerides in rodents, it is unclear if the triglyceride effects in the current study are test substance-related. The effects are not considered to be adverse by the registrant, as changes were minimal and individual triglyceride values in treated groups were similar to those seen normally in this species and strain. There were no significant elevations in group mean liver enzyme values in test substance-treated males and females.

Significantly higher liver weights occurred in a dose-related manner in males administered 3 or 30 mg/kg/day group and in females in the 300 mg/kg/day group. These findings correlated with histologic evidence of centrilobular hypertrophy. Following the recovery period, the absolute liver weight and organ-to-body-weight ratios of males from the 30 mg/kg/day group and females from the 300 mg/kg/day group did not significantly differ from the control group values. There were no other test substance-related effects on organ weights. However, some statistically significant differences were observed when the control and test substance-treated groups were compared. The absolute kidney weight was higher for the 3 and 30 mg/kg/day group males relative to the control group and kidney weights relative to body or brain weight were higher for the 30 mg/kg/day group males relative to the control group. These differences were small in magnitude and lacked a morphologic or clinical pathology correlate. Therefore, the kidney weight effects were not considered to be adverse according to the registrant.

Test substance-related changes of multifocal centrilobular hypertrophy were observed in the liver of 3 and 30 mg/kg/day group males and the 300 mg/kg/day group females. The tissue alteration was characterized by enlargement of hepatocytes surrounding central veins. Changes, graded minimal and mild, were diagnosed as a relative change when compared to periportal hepatocytes. Although females were administered higher doses of test substance, changes were more subtle than in males. Histologic examination of the liver from recovery animals revealed no evidence of centrilobular hypertrophy.

In male rats, beta-oxidation activity was statistically significantly increased at the 28-day time point at all dosage levels. At 0.3 mg/kg/day the increase was minimal (about 1.4-fold higher than control), with more moderate increases of about 3.7- and 8.7-fold above control in the 3 and 30 mg/kg/day male groups, respectively. In female rats dosed with 30 and 300 mg/kg/day test substance, beta-oxidation activity was statistically significantly increased (about 1.5- and 3.0-fold higher than controls, respectively) at the 28-day time point. Beta-oxidation activity had returned to control levels after approximately 28 days of recovery in both male and female rats. A minimal, statistically significant increase in total cytochrome P-450 was present in the 30 mg/kg/day male group at the 28-day time point, but had returned to



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control levels after approximately 28-days recovery. There were no effects on total cytochrome P-450 content in female rats.

No NOAEL could be derived from this study because at the lowest dose of 0.3 mg/kg bw/day in males, a decrease in cholesterol levels was observed. The level of decrease is unknown. However, reductions in cholesterol were also observed in other studies and could be related to the increase in beta-oxidation. Therefore, currently this effect cannot be discounted as non-adverse. In addition, an increase in beta-oxidation of 1.4 times the control level was determined. This effect at such low level of increase is not considered adverse. If the additional details of the study report provide sufficient justification to conclude that the decrease in cholesterol is not adverse, a NOAEL of 0.3 mg/kg bw/day can be derived from this study.

In a 90-day repeated dose toxicity study according to OECD 408, groups of 10 Crl:CD(SD) rats per dose and sex were exposed to FRD-902 (purity 84%) by gavage (water). Males were exposed to 0.1, 10 and 100 mg/kg bw/day whereas females were exposed to 10, 100 and 1000 mg/kg bw/day. Additional animals were used to determine the recovery within 4-weeks (Exp Supporting Repeated dose toxicity:oral.002).

There were 2 test-substance-related deaths and 1 death of uncertain relationship to test substance administration in the 1000 mg/kg/day group females. Female no. 7323 was euthanized in extremis on study day 8 with clinical observations of impaired use of the hindlimbs and forelimbs. Female nos. 7315 and 7318 were found dead on study days 21 and 37, respectively. All 3 females were noted with clear material around the mouth and/or yellow material on various body surfaces at approximately 1-2 hours post-dosing on study days 7 and 8 (no. 7323) or intermittently from study days 0 to 20 (no. 7315) and 27 to 36 (no. 7318). At necropsy, the female euthanized in extremis (no. 7323) had gross lesions of red areas in the stomach, urinary bladder, and thymus and microscopically observed necrosis, hemorrhage, and thrombus of the spinal cord, thrombosis, myocardial fiber degeneration, and necrosis in the heart, necrosis of the glandular stomach, and hemorrhage in the lung, thymus, and urinary bladder. The first female found dead (no. 7315) had renal tubular and papillary necrosis, hepatocellular hypertrophy, and lymphoid depletion in multiple tissues. The second female found dead (no. 7318) had renal papillary necrosis and necrosis of portions of an adrenal gland, hyperplasia of the transitional epithelium of the urinary bladder, hepatocellular hypertrophy, and lymphoid depletion in multiple tissues. The early death of female nos. 7315 and 7318 was considered to be test substance-related because both females shared similar microscopic findings (renal papillary necrosis, hepatocellular hypertrophy, and lymphoid depletion in multiple tissues). The other early death female (no. 7323) in this group died earlier (study day 8) than the unscheduled death female nos. 7315 and 7315 (study days 21 and 37, respectively) and had microscopic findings that were not observed in the other animals in this group. Thus, the relationship of this early death of female no. 7323 to treatment was uncertain.
There were no adverse clinical observations or effects on survival for any test substance-treated male groups and for the 10 and 100

mg/kg/day group females. Clinical observations in the 1000 mg/kg/day group females were noted in at least half of the surviving females and included clear material around the mouth, neck, and/or forelimb(s), yellow material on various body surfaces (at time of dosing and approximately 1-2 hours post-dosing) and red material on various body surfaces (1-2 hours post-dosing) beginning on the third day of dosing for some females.

There were no test substance-related effects on body weight at any dosage level. Mean body weights for the high-dose group males (100 mg/kg/day) and females (1000 mg/kg/day) were approximately 97% and 102% of the control group mean value, respectively (neither statistically significant). Mean overall food consumption during the dosing period for the high-dose group males (100 mg/kg/day) and females (1000 mg/kg/day) were 100% and 111% of the control group value, respectively (statistically significant in females). In males, significantly lower mean food consumption was recorded for study week 0 to 1 for the 10 and 100 mg/kg/day group, and from study week 1 to 2 for the 100 mg/kg/day group.

No ophthalmic lesions indicative of toxicity were observed in any of the test substance-treated groups.

Test substance-related hematology changes in red cell mass parameters (red blood cell counts, hemoglobin, and hematocrit) were present in the high-dose group males (100 mg/kg/day) and females (1000 mg/kg/day) at the end of the dosing period. These parameters were approximately 11%-13% lower in males and 18%-28% lower in females when compared to the respective control group. In addition, individual values for these erythrocyte parameters in several animals at these dose levels were below historical control reference ranges. The lower red cell mass parameters were associated with higher absolute reticulocyte counts in both sexes, and in females, were associated with changes in red cell parameters, including higher mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), and lower mean corpuscular hemoglobin concentration (MCHC). The changes in reticulocyte counts and red cell parameters indicated a regenerative response to the lower red cell mass. Consistent with their regenerative nature, the red cell changes in the high-dose group males and females showed recovery following the approximate 4-week recovery period. In females, recovery was complete as values for some red cell mass parameters were statistically higher (along with lower reticulocytes and an equivocal higher MCV) when compared to the control group. In males, recovery was present but was not complete as slightly lower (about 5% below the control group) red cell mass parameters were still present at the end of the recovery period when compared to the control group. In addition, absolute reticulocyte counts remained minimally elevated in this group. Based on the regenerative response noted in the high-dose group males at the recovery evaluation, complete recovery would be expected with increased recovery time. Statistically significant lower erythrocyte parameters were also present in the 10 mg/kg/day group males when compared to the control group. At this dose level, the magnitude of changes were minimal (approximately 7% below the control group), and values for individual animals were within historical control reference

ranges (except for the hematocrit values in 2 males which were 0.2 percentage points below the reference range). Consistent with the minimal nature of the erythrocyte changes at this dose, there were no statistically significant changes in absolute reticulocyte counts. Based on the minimal nature of the effects on red cell parameters, the lack of an increase in reticulocyte counts suggesting a lack of an erythropoietic stimulus, and the absence of anemia in individual animals, the erythrocyte effects in the 10 mg/kg/day male group were not considered to be adverse by the registrant. Some other statistically significant differences in hematology parameters were noted when the control and test substance-treated groups were compared. These findings included lower activated partial thromboplastin time (APTT) at the recovery (study week 17) evaluation for the 100 mg/kg/day group males and lower absolute basophil counts at study week 13 for the 100 mg/kg/day group males. These group mean differences were not considered test substance-related because according to the registrant they did not occur in a time-related manner or they were not of a magnitude that would be considered toxicologically important.

Test substance-related and statistically significant changes in several serum chemistry parameters were present in the 10 and 100 mg/kg/day group males and the 100 and 1000 mg/kg/day group females when compared to the control group. Most changes were consistent with PPARα activation. Test substance-related lower (variable statistical significance) cholesterol values were present in the 10 and 100 mg/kg/day group males (-31%) and the 100 (-20%) and 1000 mg/kg/day group (-31%) females. The differences from the control group were minimal, as values for most animals in the affected groups were within historical control reference ranges. There are no known adverse effects associated with minimally lower levels in cholesterol according to the registrant. As such, these changes were considered by the registrant to be test substance-related but non-adverse. However, this is questioned by the RIVM. Test substance-related effects on cholesterol values were reversible in both males and females as there were no statistically significant changes in cholesterol values in the high-dose group when compared to the control group following the 4-week recovery period. In addition, there were no test substance-related changes in triglycerides in male or female rats at any of the dosage levels tested. Higher albumin (males only +10% and 12%) and lower globulin levels (both sexes -12% and -15% and -33%), as well as associated higher albumin/globulin ratios (+26% and +35% and +58%), were present in the 10 and 100 mg/kg/day group males and the 1000 mg/kg/day group females when compared to the control group. A lower total protein level (due to lower globulin) was also present in the 1000 mg/kg/day group females (-10%). Individual values for these protein parameters were outside the historical control reference ranges in 2 high-dose group females. All serum protein changes were reversible, as mean values were similar to the control group following the 4-week recovery period. The biological significance of the changes (lower) in total protein levels is uncertain. The pattern of change in serum proteins (lower globulin and higher albumin levels) was consistent with the known anti-inflammatory properties of a PPARα agonist. The anti-inflammatory response to PPARα activation is characterized by lower levels of acute phase proteins (which contribute

to the globulin fraction), and higher levels of negative acute phase protein (albumin).

The urea nitrogen level was minimally higher (+38%) in the 100 mg/kg/day group males when compared to the control group. This higher level was likely of non-renal origin, as it was not associated with changes in creatinine, urinalysis parameters, or renal histopathology. As with the serum protein changes, the pattern of changes in urea nitrogen was consistent with those reported for other peroxisome proliferators. Changes in urea nitrogen levels were reversible in males, as there were no statistically significant changes in these parameters when compared to the control group following the approximate 4-week recovery period. Alkaline phosphatase levels were minimally higher in the 10 (+48%) and 100 mg/kg/day group (+106%) males and in the 1000 mg/kg/day group females (+66%) when compared to the control group. Alkaline phosphatase levels may be higher in association with cholestatic liver disease; however, in this study, other markers of cholestatic liver injury were not increased (bilirubin and gamma glutamyltransferase levels were actually lower in the 1000 mg/kg/day group females), and there were no effects on other enzymes indicative of hepatocellular injury (alanine aminotransferase, aspartate aminotransferase, and sorbitol dehydrogenase levels). Additionally, there was no histopathological evidence of liver cytotoxicity. Therefore, these minimally higher alkaline phosphatase levels were the result of extra-hepatic factors and were likely due to induction of liver microsomal enzymes. Total bilirubin and gamma glutamyltransferase values were lower in the 1000 mg/kg/day group females when compared to the control group. Total bilirubin was also lower in the 100 mg/kg/day group females. These changes were considered to be test substance-related but non-adverse based on the direction of change (lower rather than higher). The changes in both parameters were reversible following the approximate 4-week recovery period. At study week 17 (recovery evaluation), lower creatinine and higher potassium levels were noted for the 100 mg/kg/day group males when compared to the control group. These group mean differences were not considered to be test substance-related because the values did not show a time-related response, were of a magnitude that would be considered to be toxicologically unimportant, or involved a change in a direction of no known biological importance.

There were no test substance-related effects on urinalysis parameters in the 0.1, 10, and 100 mg/kg/day group males and the 10 and 100 mg/kg/day group females. Test substance-related higher urine volume (statistically significant) and a lower urine osmolality (not statistically significant) suggestive of diuresis were present in the 1000 mg/kg/day group females at study week 13 as compared to the control group. Lower urine pH (statistically significant) was also present in the 1000 mg/kg/day group females at study week 13 as compared to the control group.

There were no significant changes in the functional observation battery.

Test substance-related organ weight changes consisted of higher kidney and liver weights. Higher liver weights in the 10 and 100 mg/kg/day group males and the 1000 mg/kg/day group females correlated with



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microscopic hepatocellular hypertrophy, but they were not associated with degeneration or necrosis in the liver or with changes in serum chemistries suggestive of liver toxicity. Therefore, higher liver weights were not considered to be adverse by the registrant. However, this is questioned by the RIVM. In the 100 mg/kg/day group males, liver weight changes were reversible except for liver weight relative to body weight, which was mostly, but not completely reversible. In the 1000 mg/kg/day group females, liver weight changes showed partial recovery, but were not completely reversible following the 4-week recovery period.

All kidney weight parameters (absolute, relative to body and brain weight) were minimally higher in the high-dose group males (100 mg/kg/day) and females (1000 mg/kg/day) when compared to the control group. In the 1000 mg/kg/day group females, these changes were associated with evidence of diuresis (increased urine volume and decreased urine osmolality) and microscopic changes in the kidneys, most commonly in the early death animals. In the 100 mg/kg/day group males, there were no clinical pathology or microscopic changes suggestive of kidney injury. Minimally higher kidney weights were also present in males at the recovery evaluation but not in females.
Kidney weight relative to body weight was also higher and statistically significant in the 10 mg/kg/day group males and females and the 100 mg/kg/day group females when compared to the control group. However, at these dosage levels, there were no changes in other kidney weight parameters (absolute and relative to brain weight), and no correlative changes in serum chemistry, urinalysis, or histopathology suggestive of renal toxicity. Thus, higher kidney weights relative to body weight in the 10 mg/kg/day group males and females and the 100 mg/kg/day group females were not considered to be adverse by the registrant.

There were no test substance-related macroscopic findings noted at the scheduled necropsies. Macroscopic findings of uncertain pathogenesis were noted for the 1000 mg/kg/day group female (no. 7323) euthanized in extremis on study day 8 and consisted of red areas in the stomach, urinary bladder, and thymus.

In addition to the microscopic changes observed in the 1000 mg/kg/day group females found dead or euthanized in extremis, one 1000 mg/kg/day group female (animal no. 7279) had minimal renal tubular necrosis and regeneration at the study week 13 primary necropsy.

Minimal hepatocellular hypertrophy was observed in the liver of some males in the 10 and 100 mg/kg/day groups and some females in the 1000 mg/kg/day group at the primary necropsy (see Table A10). Hepatocellular hypertrophy was associated with increased eosinophilic granularity of the hepatocyte cytoplasm consistent with peroxisome proliferation. 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.



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*Table A9. Test Substance-Related Organ Weight Changes.*

| Parameter | Direction and magnitude of change | Dosage level (mg/kg/day) | Sex | Necropsy |
|---|---|---|---|---|
| **Kidney** | | | | |
| Absolute | ↑10.6%* | 100 | M | Primary |
| Relative to body weight | ↑12.9%*, 16.2%** | 10, 100 | M | Primary |
| Relative to brain weight | ↑11.6%* | 100 | M | Primary |
| Absolute | ↑10.8%** | 100 | M | Recovery |
| Relative to body weight | ↑11.5%** | 100 | M | Recovery |
| Relative to brain weight | ↑9.4%** | 100 | M | Recovery |
| **Kidney** | | | | |
| Absolute | ↑18.3%** | 1000 | F | Primary |
| Relative to body weight | ↑9.3%*,9.5%*, 23%** | 10, 100, 1000 | F | Primary |
| Relative to brain weight | ↑17.9%** | 1000 | F | Primary |
| **Liver** | | | | |
| Absolute | ↑22.8%*, 59.4 %** | 10, 100 | M | Primary |
| Relative to body weight | ↑30.9%**, 67.0%** | 10, 100 | M | Primary |
| Relative to brain weight | ↑25.5%**, 61.2%** | 10, 100 | M | Primary |
| Relative to body weight | ↑10.6%** | 100 | M | Recovery |
| **Liver** | | | | |
| Absolute | ↑77.3%** | 1000 | F | Primary |
| Relative to body weight | ↑84.6%** | 1000 | F | Primary |
| Relative to brain weight | ↑76.7%** | 1000 | F | Primary |
| Absolute | ↑18.5%* | 1000 | F | Recovery |
| Relative to body weight | ↑14.6%** | 1000 | F | Recovery |
| Relative to brain weight | ↑17.6%* | 1000 | F | Recovery |
| * = Significantly different from the control group at 0.05 using Dunnett's test<br>** = Significantly different from the control group at 0.01 using Dunnett's test | | | | |



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*Table A10. Incidence of Hepatocellular Hypertrophy at the Primary Necropsy.*

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dosage Level (mg/kg/day) | 0 | 0.1 | 10 | 100 | 0 | 10 | 100 | 1000 |
| Liver[a] | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Hepatocellular hypertrophy Minimal | 0 | 0 | 3 | 10 | 0 | 0 | 0 | 0 |
| [a] = Number of tissues examined from each group. | | | | | | | | |

A NOAEL of 0.1 mg/kg bw/day was derived from this study based on decreased red blood cell parameters, cholesterol and increased A/G ratio, liver weight and hypertrophy and kidney weight at the next higher dose level of 10 mg/kg bw/day.

In a 2-year oral exposure study according to OECD TG 453, rats (n=80) were dosed by gavage with 0.1, 1, or 50 mg/kg bw/day for up to 104 weeks (male rats) or with 1, 50, or 500 mg/kg bw/day for up to 101 weeks (female rats). Interim section was performed on 10 animals per dose and sex after one year (Rae et al, 2015).

Exposure to FRD-902 (purity 84%) did not affect survival. A single test article-related cause of death/moribundity was inflammation/necrosis of the kidneys which occurred in seven of the 500 mg/kg/day females and was characterized by papillary necrosis. In males the most common causes of death/moribundity were pituitary tumours and undetermined. In females the most common causes of death/moribundity were mammary tumour and pituitary tumour. 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/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. There were no test article-related clinical observations.

Mean body weight in 50 mg/kg/day males was statistically significantly below control over most of the first year, although mean body weight was only 4% below control in males at Week 52 (not statistically significant), and exceeded the control value at termination. Mean body weight gain in this group was 6% below control in males over Weeks 1 to 52 and exceeded the control value over the two year period. Based on the small magnitude of the changes, the effect among males at 50 mg/kg/day was not considered adverse. Exposure to 500 mg/kg/day of the test substance produced adverse reductions in body weight and body weight gain in females. In this group, statistically significantly lower mean body weight was observed from weeks 30 through 86. Mean body weight was 13% below control at Week 52, and mean body weight gain was 20% below controls over Weeks 1 to 52 (both statistically significant). Mean final body weight (week 100) and overall body weight gain (Weeks 1-100) were comparable to the control value. However, these body weight changes were considered adverse at this dose based on the difference during the first year on study.

There were no adverse test article-related effects on food consumption in either sex or in food efficiency in males at any dose. Adverse effects on food efficiency were observed in 500 mg/kg/day females. In this group, food efficiency was 23% below control (statistically significant) over the first year and 11% below control (statistically significant) overall (Weeks 1-100). Lower mean food efficiency was noted over the first year in males at 50 mg/kg/day. However, overall (Weeks 1-104) food efficiency was comparable to controls. No effects were noted in any other dose group. No test article-related findings were noted in the interim or terminal ophthalmoscopic examination.

At the 3, 6, and 12 month intervals there were mild decreases in red cell mass (erythrocytes, haemoglobin, and hematocrit in females receiving 500 mg/kg/day. Effects were mild in females (up to 28% less than control) and were not associated with any test article-related effects on erythrocyte morphology. Appropriate increases in reticulocytes (106% above respective) occurred in response to the decreases in red cell mass. The increases in reticulocytes were associated with expected decreases in MCHC and increases MCV. This collection of findings is suggestive of red cell loss or haemolysis although the exact mechanisms involved are unknown. Statistically significant decreases in red cell mass were also present in males receiving 50 mg/kg/day at the 3- and 6-month interval. However, the decreases were small (-9 to -7%) and did induce a non-statistically significant increase in reticulocytes. In addition, red mass changes were transient— at the 12 month interval there were no statistically significant changes in any red cell mass parameter, and values in individual animals in the 50 mg/kg/day group were similar to controls. Therefore, the red cell mass changes in 50 mg/kg/day males were considered to be test article-related but non-adverse by the registrant. Bilirubin levels were statistically significant reduced in females at the mid (-21 to -31%) and high dose groups (-33 to -47%) at almost all intervals.

At the 12-month interval in males receiving 50 mg/kg/day, there were mild increases relative to controls in enzymes indicative of liver injury including alkaline phosphatase, ALT, AST and sorbitol dehydrogenase (sorbitol dehydrogenase and AST not statistically significant). These enzyme changes correlated with microscopic findings of minimal cystic degeneration and minimal to mild focal necrosis in the liver of males at 50 mg/kg/day. Therefore, these enzymes changes were considered test article-related and adverse. Minimal but statistically significant increases in alkaline phosphatase were also present at the 3- and 6-month intervals in the 50 mg/kg/day male group. At these intervals, increases in alkaline phosphatase were less than those present at 12 months and were not associated with statistically significant changes in other enzymes indicative of hepatic or hepatobiliary injury. Therefore, the changes in alkaline phosphatase in the 50 mg/kg/day male group at the 3 and 6 month intervals may be due in part or in whole to test article-related enzyme induction, as the test article was previously shown to produce an increase in total P450 enzyme activity in male rats at 30 mg/kg/day. There were no test article-related changes in liver enzymes in males receiving 1 or 0.1 mg/kg/day or in females at any of the dose levels tested (up to 500 mg/kg/day).

Serum Proteins: Minimal, statistically significant increases in albumin were present in males receiving 50 mg/kg/day at all intervals (up to 16% above controls) and in females receiving 500 mg/kg/day at the 3-month interval (10% above controls). In addition, statistically significant decreases (of up to 17% below control) in globulin were present in females at 500 mg/kg/day at all intervals (an associated decrease in total protein was also present in this group at the 6-month interval). No statistically significant decreases in globulin were present in males at any dose or interval except for males at 50 mg/kg bw/day after 3-months, small decreases in individual values for these parameters in individual animals in the 50 mg/kg/day male group may have been test article-related. The changes in albumin and globulin in the high-dose male (+18 to +28%) and female groups (+20 to +23%) also resulted in statistically significant increases in albumin/globulin ratio in these groups at all intervals. The test article is a peroxisome proliferator, and the pattern of change in serum proteins observed in high dose males and females-lower globulin and higher albumin-is a well-established response to PPARα activation. Peroxisome proliferators are anti-inflammatory, producing decreases in acute phase proteins (which contribute to the globulin fraction), and increases in negative acute phase protein (albumin). However, no adverse biological outcomes have been associated with such changes in these serum proteins according to the registrant. Therefore, these changes in serum proteins in high dose males and females were considered test article-related although they were not considered biologically relevant by the registrant based on their small magnitude and lack of association with known adverse outcomes. In addition to the serum protein changes noted above, minimal, statistically significant increases in albumin/globulin ratio were present in the 1 mg/kg/day males (+16%) and 50 mg/kg/day females (+9%) at all intervals. Also, in some individual animals in these groups, albumin tended to be higher and globulin lower than controls. However, group mean albumin and globulin in these groups were not statistically different from controls (with the exception of elevated albumin in the 1 mg/kg/day male group at 12 months and decreased globulin in the 50 mg/kg/day female group at 6 months), and differences from control group means for both albumin and globulin were ≤8% at all intervals. Therefore, the statistically significant changes in albumin/globulin ratio in these groups were also considered by the registrant to be test article-related but non-adverse based on the minimal nature of the changes. However, the changes in albumin and albumin/globulin ratio are indicative of effects on the acute phase response of the immune system. These effects were also observed with other PPAR-α inducers, occurs in humans and is secondary to binding to the PPAR-α receptor (Gervois et al, 2004). Therefore, these effects are considered adverse by the RIVM.

Phosphorus was statistically higher than control in the 500 mg/kg/day female group at the 12-month interval. The relationship to treatment for this difference is uncertain; however values in individual rats in this group were similar to controls except for one animal, and there were no statistically significant changes in phosphorus in any treated group at any other time point. Therefore, based on the minimal nature of these changes, they were not considered to be adverse. Phosphorus was statistically higher in the 0.1 at the 3-month interval and 50 mg/kg male groups at the 3 and 6-months interval and non-significantly after 12-

months. These differences at 0.1 mg/kg bw/day were considered to be unrelated to test article administration since they did not occur in a dose-related manner and there were no statistically significant differences in phosphorus in any treated group relative to control at the 6- and 12- month intervals. Calcium was statistically higher in the 50 mg/kg/day males at the 12-month interval. One fraction of serum calcium exists as "bound" to albumin, and increases in albumin are necessarily associated with physiologically appropriate increases in calcium. Changes in bound calcium have no effect on unbound ("ionized") calcium, which is the physiologically active form of calcium. Therefore, the increase in calcium in the 50 mg/kg/day male group at 12 months was considered to be secondary to albumin changes, physiologically irrelevant, and thus non-adverse. Urea nitrogen was statistically higher than the respective control in the 50 mg/kg/day male group at the 3 and 6-month interval. These differences were were not consistent across time, and there were no correlative changes in related clinical chemistry parameters or with microscopic changes in the kidneys. Chloride was statistically higher than control in females at 1 and 500 mg/kg/day (but not at 50 mg/kg/day) at the 6-month interval. These differences were not considered to be test article-related as they very slight (only 2% above control), did not occur in a dose-related manner, and were not associated with changes in chloride at any other interval.

In females receiving 500 mg/kg/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 and not associated with changes in kidney-related chemistry parameters (e.g., urea nitrogen, creatinine), 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.
Urine pH was increased and urine volume after 12-months decreased in males at all dose levels. These changes are of uncertain relationship to administration of the test article based on the lack of a clear dose response across the affected groups. Based on the lack of any correlative findings suggestive of an effect on the urogenital system, the changes were considered nonadverse. In addition, no such effects were observed in males in the 90-day study up to 100 mg/kg bw/day.

Interim: Test article-related organ weight changes were limited to the high dose groups. Increased liver weights occurred in males at 50 mg/kg/day and in female rats at 500 mg/kg/day. In males, the increase was small and only the mean liver relative to body weight was statistically significantly increased (14.53% above control). In females, the liver weight increase was larger (mean liver relative to body weight was 66.75% above control) and all parameters (absolute and relative to both brain and body weight) were statistically significantly increased. The liver weight changes in the affected male and female groups were associated with microscopic changes in the liver (discussed below). Mean final body weight at the interim necropsy was 19.51% less than control in the 500 mg/kg/day females. As a result of this decrement in mean final body weight, the brain, kidney, and thyroid/parathyroid relative to body weight were statistically significantly increased. Aside

from a slight increase in severity of chronic progressive nephropathy in the kidneys, there were no microscopic changes in these organs associated with the increased weights, and mean absolute weights were not increased. Thus, these changes were considered secondary to the body weight decrement at 500 mg/kg/day. Additionally, mean absolute and relative to brain weights of the spleen in the 500 mg/kg/day females were statistically significantly lower than controls. These differences were not considered test article-related by the registrant, as there were no microscopic changes in the spleen in either sex.

Terminal: No test article-related or statistically significant organ weight changes occurred in males. In females, the only test article-related effect on organ weights was an increase in liver and kidney weights at 500 mg/kg/day. Mean absolute and relative to both body and brain weights were increased compared to control, with mean liver relative to body weight 41.61% greater than control. There were several test article-related microscopic changes to account for the increased weights, as described below. Absolute and relative to bodyweight kidney weights were increased and related to microscopic changes.

Interim: A test article-related macroscopic observation, "irregular surface" of the kidneys, was noted in the kidneys of one 500 mg/kg/day (high dose) female. This observation correlated with mild chronic progressive nephropathy in this animal and was indicative of a slight increase in severity of chronic progressive nephropathy in the 500 mg/kg/day female group at one year.

Terminal: No test article-related macroscopic observations were noted in males. In females, test article-related macroscopic observations were noted in the kidneys and liver. In the kidneys, "irregular surface" was noted in 16 of 70 animals at 500 mg/kg/day (not present in controls or any of the lower dose groups), while in the liver, "tan focus/foci" was noted in 1, 1, 1, and 8 of 70 animals each at 0, 1, 50, and 500 mg/kg/day, respectively, and "mass/nodule" was noted in 14 of 70 animals at 500 mg/kg/day (not present in controls or any of the lower dose groups). These macroscopic observations were correlative to test article-related microscopic findings described below.

Interim: Test article-related microscopic findings were noted in the liver of both male and female rats, and in the kidneys of females, in the high-dose groups (50 and 500 mg/kg/day for males and females, respectively). In males, there was a slight increase in minimal focal cystic degeneration of the liver (0, 0, 0, and 3 at 0, 0.1, 1, and 50 mg/kg/day, respectively). This finding was more pronounced in the terminal portion of the study. Also in males, there was a slight increase in minimal to mild focal necrosis of the liver (1, 1, 0, and 5 at 0, 0.1, 1, and 50 mg/kg/day, respectively). In females, the only microscopic finding in the liver was centrilobular hypertrophy, which occurred in all 10 of the 500 mg/kg/day females. This change was of minimal or mild severity and was characterized primarily by a slight increase in size of centrilobular hepatocytes with increased red granularity to the cytoplasm and is consistent with peroxisome proliferation. Also in females, there was a very slight increase in incidence and severity of chronic progressive nephropathy in the kidneys at 500 mg/kg/day. This

change was characterized by foci of basophilic tubules, some with thickening of basement membranes. In the 500 mg/kg/day group, most incidences were of mild severity, while in the other groups, including controls, the incidences were primarily of minimal severity, although in a single control female the incidence was of moderate severit. In males, there was a single interstitial cell adenoma of the testes at 50 mg/kg/day; incidences of interstitial cell hyperplasia were 1, 0, 0, and 3 at 0, 0.1, 1, and 50 mg/kg/day. The incidences of these changes in treated groups were not statistically different from controls (historical data for rats of this age were not available). Proliferative interstitial cell lesions are discussed in more detail under microscopic findings for the terminal sacrifice. All other microscopic findings were considered incidental, and typical of those seen in rats of this strain and age.

Terminal: Test article-related non-neoplastic microscopic changes were observed in the liver and adrenal of males and in the liver, kidneys, nonglandular stomach (limiting ridge), and tongue of females at the highest doses tested, 50 mg/kg/day in males and 500 mg/kg/day in females. Focal vacuolation of the adrenal in males was reduced at all dose levels compared to controls but showed no dose response relation. Therefore, this effect was not considered substance related. In the liver of males at 50 mg/kg/day there were statistically significantly increased incidences of focal cystic degeneration, centrilobular hepatocellular hypertrophy, and centrilobular hepatocellular necrosis. Periportal liver vacuolation was reduced. Cystic degeneration was characterized by the presence of multilocular cystic spaces containing finely granular or flocculent material without endothelial or epithelial cells lining the spaces. Centrilobular hypertrophy, morphologically consistent with peroxisome proliferation, was characterized by hepatocytes with red granular cytoplasm sometimes containing small amounts of pigment morphologically compatible with lipofuscin. Centrilobular hepatocellular necrosis was typically of the coagulative type with strongly eosinophilic-staining cytoplasm and pyknotic nuclei. Test article-related findings in the liver of females at 500 mg/kg/day were similar to those noted in males at 50 mg/kg/day, and also included low incidences of panlobular hepatocellular hypertrophy and individual cell hepatocellular necrosis. Panlobular hepatocellular hypertrophy was characterized by enlargement of hepatocytes (as described above for centrilobular hypertrophy) throughout the entire liver. Individual cell necrosis was characterized by the presence of scattered single hepatocytes with features characteristic of apoptosis. Liver periportal vacuolation was reduced at the highest dose in females.

Statistically significantly increased microscopic findings in the kidneys of females at 500 mg/kg/day included tubular dilatation, oedema of the renal papilla, transitional cell hyperplasia in the renal pelvis, tubular mineralization, renal papillary necrosis, and chronic progressive nephropathy. Tubular dilatation frequently occurred in an ascending pattern extending from the papilla to the outer cortex, while at other times it was more prominent in the papilla. Oedema of the papilla was characterized by increased rarefaction or myxomatous change in the papillary interstitium, sometimes with polypoid protrusions from the lateral surface of the papilla. The oedema and tubular dilatation were often associated with hyperplasia of the transitional cell epithelium lining

the papilla and pelvis. In some animals, necrosis of the tip of the papilla was present. In some 500 mg/kg/day females with the renal papillary changes, lesions diagnosed as chronic progressive nephropathy (CPN) were comprised of dilated tubules (often in an ascending pattern as described above), mononuclear cell infiltrates, and basophilic tubules, but with less thickening of tubular basement membranes than typically seen in CPN. In these animals, the constellation of lesions diagnosed as CPN may be more representative of retrograde nephropathy, rather than typical CPN.

The nonglandular stomach (limiting ridge only) and the tongue had statistically significantly increased incidences of hyperplasia of squamous epithelium at 500 mg/kg/day. In the tongue, subacute/chronic inflammation occurred in association with squamous epithelial cell hyperplasia. There is no data describing incidence of epithelial hyperplasia of the limiting ridge of the nonglandular stomach in the historical control database for 2 year studies. The incidence of squamous cell hyperplasia of the tongue at 500 mg/kg/day (18.6%) exceeds the historical control range of 0-3.3%. There was also a single incidence of squamous cell carcinoma (1.4%) in the tongue of females at 500 mg/kg/day. This is well within the historical control range of 0-1.7% and the finding of a single such tumour was not considered a direct result of test article administration.

A statistically significant increase in the incidence of alveolar histiocytosis was present in females at 500 mg/kg/day. The incidences were 22, 20, 21, 42 (61%) at 0, 1, 50, and 500 mg/kg/day, respectively. The incidence at 500 mg/kg/day was statistically significant by both the Fisher Exact test and the Cochran-Armitage trend test and is at the upper end of the historical control range of 9.2-61.7%. The increased incidence of this common background finding may be secondary to aspiration of dosing formulation at this high concentration; however, a definitive mechanism for this increase could not be determined. A slight but statistically higher (by the Cochran-Armitage Trend test) incidence of pancreatic acinar cell hyperplasia occurred in females at 50 and 500 mg/kg/day; incidences were 0, 2, 5, 5 (7.1%) at 0, 1, 50, and 500 mg/kg/day, respectively. The incidences of acinar cell hyperplasia at the two highest doses slightly exceeded the historical control range of 0-4.6%, but were not significant by the Fisher Exact test and were not associated with pancreatic acinar cell tumours. In addition, acinar cell hyperplasia did not occur in a clear dose response manner, as incidences in the 50 and 500 mg/kg/day groups were the same despite the order of magnitude difference in dose. In contrast, all other test article-related changes observed at 500 mg/kg/day occurred with a clear dose response. Therefore, the slight increase in acinar cell hyperplasia in the 50 and 500 mg/kg/day females was considered by the registrant most likely spurious and not test article-related. A statistically significant increase (by both the Fisher Exact test and the Cochran-Armitage trend test) in the incidence of alopecia/hypotrichosis was present in females at 500 mg/kg/day. The incidences were 1/70, 2/48, 5/55, and 9/70 (12.9%). However, the relevance of alopecia/hypotrichosis is more appropriately made by interpretation of the incidence of this finding in the clinical observations of the study

rather than the microscopic observations. Therefore, for microscopic purposes, this was not considered at potential target organ.

Finally, incidences of cataract of the lens of the eye, pelvic mineralization of the kidney, and angiectasis of the liver were statistically significantly increased. Cataract of the eye and angiectasis of the liver were statistically significantly increased by the Cochran-Armitage trend test at 500 mg/kg/day while pelvic mineralization of the kidney was statistically significantly increased by the Cochran-Armitage trend test and Fisher's exact test at 500 mg/kg/day, and Fisher's exact test at 1 mg/kg/day. Incidences of cataract of the eye were 0/69, 0/48, 0/55, and 3/70 (4.29%) at 0, 1, 50, and 500 mg/kg/day, respectively. The historical control range for cataract is 0 to 10.8%. Incidences of pelvic mineralization of the kidney were 52/70, 63/70, 58/70, and 63/70 (90.0%) at 0, 1, 50, and 500 mg/kg/day, respectively. The historical control range is 45.0 to 87.7% (note: two studies in the historical control database with an incidence of 0/60 reflect that this change was simply not tracked as pelvic mineralization in the studies). Incidences of angiectasis of the liver were 1/70, 0/70, 3/70, and 5/70 (7.14%) at 0, 1, 50, and 500 mg/kg/day, respectively. The historical control range is 0 to 10.0%. For each of the changes, the incidence was well within the historical control range, except pelvic mineralization, which is a very common background finding, only slightly exceeded the historical control range. Thus, these changes were not considered test article-related.

This study was used as a key study in the REACH registration dossier for repeated dose toxicity and carcinogenicity. A NOAEL of 1 mg/kg bw/day for male rats was established by the registrant based on liver effects and equivocal increases in pancreatic and Leydig cell tumours. For female rats, a NOAEL of 50 mg/kg bw/day was established by the registrant, based on reductions in body weight and body weight gain, mild decrease in red cell mass, effects on the liver, kidneys, nonglandular stomach and tongue, and increase in liver tumours. The RIVM derives a NOAEL of 0.1 mg/kg bw/day based on an increase in A/G ratio in males at the next higher dose level of 1 mg/kg bw/day.

### A1.9 Mode of action

In view of the RIVM, the observed effects with FRD-902 including increased beta-oxidation, liver hypertrophy, reduction in serum cholesterol, increased albumin / globulin ratio and observed tumour types are typical for peroxisome proliferators. Peroxisome proliferators act mainly by binding to the peroxisome proliferator-activated receptor alpha (PPAR-a). However, no direct information on the interaction of FRD-902 with PPAR-a is available. A large volume of information on this interaction is available for the structural analogue PFOA which induces comparable effects in repeated dose toxicity studies and carcinogenicity studies. The results indicate that substances like PFOA and therefore possible also FRD-902 can interact also with other nuclear receptors. According to published reviews, the human relevance of the hepatic and carcinogenic effects of PFOA cannot be excluded (EFSA, 2008) (US-EPA, 2016) (RAC, 2011) (IARC, 2016). Therefore, the observed effects with FRD-902 are also considered relevant for humans.



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# Annex 2. Human health toxicity E1

**Kinetics**
Three male rats (CrL:CD(SD), 6-8 weeks old) were orally exposed (gavage in water) once to 20 mg E1/kg bw. The concentration of E1 in urine collected at several time points after exposure was determined using GC/MS. The E1 concentration in urine was below the LOD of 0.04 ug/mL or below the LOQ of 0.02 ug/mL in all animals at all time points (Anonymous, 2007). This is a very limited report. It is unclear how the rats were dosed as the stated concentration of 5 mg E1/ml water is above the water solubility of 7 mg/L.

Three male and three female rats (CrL:CD(SD), 7-12 weeks old) were orally exposed (gavage in water) once to 10 or 30 mg E1/kg bw. E1 was determined in blood samples at 14 time points after administration and once before administration and in liver and fat samples after necropsy. E1 plasma levels were below the LOQ (LOQ level not stated) at all time points. Also all liver samples were below the unstated LOQ. Some fat samples appeared to contain low concentration of E1. The results (not stated) were not proportional with dose or consistent within or across the sexes. The spectrum of the analyte could not be confirmed (Anonymous, 2008). This is a very limited report lacking details on the LOQs and the measured concentrations.

In an in vitro test on metabolism using male rat S9 no metabolism and difference between active and heat treated S9 was observed indicating absence of metabolism. This study was not provided but is available upon request.

**Acute Toxicity**
In an acute oral toxicity study a single male rat was exposed to 7500, 11000, 17000 or 25000 mg/kg bw E1 by gavage. The rats were observed for 14 days after which liver weight was determined and liver histopathology performed. No mortality occurred but some limited toxic signs were observed. No effects upon final body weight, liver weight or liver pathology was observed (Anonymous, 1967a). This is a very limited report of a study using only a single animal per dose whereas normally several rats per dose levels are required to estimate the $LD_{50}$.

Rabbits dose dermally with 25000 or 37500 mg/kg bw E1 did not display mortality or systemic histopathological effects. Slight CNS effects occurred during exposure. Local irritation in the form of reversible erythema was observed. This study was not provided but is available upon request.

In an acute inhalation toxicity study groups of 4 male rats (Chr-CD) were exposed whole body for 4 hours to E1 at nominal concentrations of 5000, 15000 or 30000 ppm as a vapour. The rats were observed for 14 days after which the relative liver weight and lung pathology was determined. No mortality was observed. Clinical signs of toxicity exhibited during exposure were mild lacrimation, red ears, inactivity and deep respiration at the highest concentration with lesser effects at lower



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concentrations. There was no effect on relative liver weight. Mild irregular lung congestion was observed at the two highest concentrations at 14 days after the exposure (Anonymous, 1965). The study report was very limited.

In an acute inhalation toxicity study groups of 6 male rats (Chr-CD) were exposed whole body for 4 hours to E1 at nominal concentrations of 5870, 13130 or 23540, 62226 or 195114 ppm as a vapour. Some of the tested batches of E1 contained a mixture of n-propyl and isopropyl isomers. At E1 concentrations above 100000 ppm, additional oxygen was supplied to enrich the air. The post exposure period was not stated. No mortality occurred. Several clinical effects were observed during exposure including tremor and convulsions but not after exposure. The clinical effects indicate respiratory irritation and possible effects on the central nervous system (Anonymous, 1967b).

In an acute inhalation toxicity study groups of 6 male rats (Chr-CD) were exposed whole body for 4 hours to E1 at nominal concentrations of 396800 or 576000 ppm as a vapour. Additional oxygen was supplied to enrich the air up to 20% oxygen. Gross and histopathological examination was performed on day 1,2 and 7 of exposure on 2 rats per dose. No mortality occurred. Several clinical effects were observed during exposure including tremor but not after exposure. The clinical effects indicate respiratory irritation and possible effects on the central nervous system. No histopathological changes were observed in a range of tissues including liver and lungs (Anonymous, 1968).

An additional acute inhalation study in dogs challenged with epinephrine resulted in a NOAEC of 100000 ppm and a LOAEC of 200000 ppm. This study was not provided but is available upon request.

**Repeated dose toxicity**
In a two-week repeated dose inhalation study, groups of 10 male rats (CrL:CD®BR) were exposed whole body during 10 days for 6 hours a day to nominal concentrations of 0, 5000, 25000 or 175000 ppm E1 as a vapour. Additional oxygen was supplied at the highest dose level to ensure an oxygen content of at least 19%. The test atmosphere was produced by evaporation of E1 and resulted in mean analytical concentrations within 1% of the target concentration. Necropsy was performed on 5 rats per group directly after the last exposure whereas the other 5 rats were sacrificed 2 weeks after the last exposure. The determined parameters included body weights, clinical effects, hematology, clinical chemistry, urine analyses and macroscopic and microscopic pathology. In addition, micronuclei were determined as described below.
The mean analytical concentration was within 1% of the nominal concentration. No mortality or effects on body weight were observed. There were no changes in hematology, clinical chemistry, organ weights and urine analyses. A compound-related increase in the hyaline droplets within kidney tubules of 3 out of 5 rats exposed to 175000 ppm was observed microscopically. This observation in the kidney was minimal in severity, unaccompanied by cell necrosis, and judged not to be biologically or toxicologically significant by the authors. A lack of response to an alerting stimulus and occasional tremors was observed

during exposure at the highest concentration. The NOAEC is determined at 25000 ppm. The absence of an increase in fluorine in urine compared to controls indicates the absence of metabolism resulting in the release of fluorine from E1 (Anonymous, 1995).

**Mutagenicity**
In an in vitro study on the mutagenicity of E1 in *Salmonella typhimurium* (Ames test, OECD 471) using strains TA100, TA1535, TA97 and TA98 with and without exogenous metabolic activation, no increase in reverse mutations was observed at dose levels up to 5000 ug/plate (Anonymous, 1994). However, considering the low water solubility of the substance and the high vapour pressure of the substance it is deemd likely that most E1 evaporated from the plates during incubation at 37°C, as taping of the plates to reduce evaporation is not mentioned. Therefore, this study cannot be used to demonstrate the absence of a mutagenic potential.

In an additional bacterial reverse mutation test of E1 in *Salmonella typhimurium* (Ames test, OECD 471) using strains TA100, TA1535, TA97 and TA98 and *Escherichia coli* using strain WP2 uvrA$^-$ with and without exogenous metabolic activation, no increase in reverse mutations was observed at dose levels up to 5000 ug/plate (Anonymous, 2009). Pre-incubation was performed using ice cold E1 in acetone in sealed test tubes and plates were sealed with a vinyl sack per concentration and experimental condition to minimise evaporation. The study was negative.

In an in vitro test for chromosome aberrations in human lymphocytes, E1 was stated to be negative with and without metabolic activation. However, considering the low water solubility and the high vapour pressure it is deemed likely that E1 evaporated from the culture vessel during incubation. Therefore, this study cannot be used to demonstrate the absence of a mutagenic potential. This study was not provided but is available upon request.

An in vivo micronucleus test was performed as part of a two week inhalation study as described above. An additional group containing 5 male rats exposed by IP injection to cyclophosphamide 24 hours before necropsy was included as positive control. Directly after the last exposure, bone marrow smears were prepared. Two thousand PCEs per animal were evaluated for micronuclei after staining with acridine orange. In addition, the PCE/NCE ratio was determined. No increase in micronuclei or change in the PCE/NCE ratio was observed except for the positive control (Anonymous, 1995).

***Read-across***
Read-across from FRD-902 to E1 is not justified because of the differences in chemical-physical properties (solid versus liquid with high vapour pressure, acid or salt versus neutral, more lipophilic substance). In addition, the available toxicological data indicate that E1 is less toxic than FRD-902. For example the acute oral LD50 of FRD-902 was 1750 mg/kg bw in male rats. This is at least 10-times below the LD50 of E1 in male rats.



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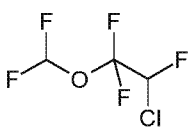
Several expert systems including 'Oncologic', 'OECD toolbox' and 'ISS' indicate that E1 is an alpha, beta-haloether or alpha haloether which could be direct-acting alkylating agents leading to mutagenicity and carcinogenicity. However, the alert is based on chloro-ethers, limited to monohalo methyl or ethyl ethers and probably less relevant for fluoro-ethers as the C-F bond is more stable and less relevant for di halo ethyl or methyl ethers. In addition, no in vivo mutageniticy was observed for the FRD-902, which also fulfils the alpha haloether alert, and the carcinogenicity was limited to an increased number of tumours typical for peroxisome proliferators in rats. This confirms the limited relevance of the alert for fluoro-ethers. Overall, the expert systems do not indicate a strong concern for mutagenicity or carcinogenicity. However, these systems are only designed to detect (sub) structures that could result in a specified effect but not for identifying the absence of effects. Therefore, mutagenic and carcinogenic properties cannot be excluded based on read-across.

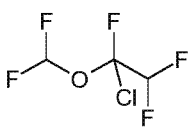
Read-across based on structural analogues as identified using the OECD QSAR toolbox indicates limited toxicity after repeated dose inhalation exposure. Two fluorinated ethers were identified as the closest analogues and are shown below. However, both contain one chlorine atom instead of only fluor atoms. As the bond between fluor and carbon is stronger than between chlorine and carbon, the reactivity and toxicity is expected to be lower for the fluorinated compound. Therefore, these analogues have some benefit for the assessment of E1.

***1. Enflurane***




CAS: 13838-16-9 EINECS: 237-553-4
Name: Enflurane; ethane, 2-chloro-1-(difluoromethoxy)-1,1,2-trifluoro-.
Enflurane is an inhalation anaesthetic used for narcosis in concentrations of 5000 to 15000 ppm. A MAK value of 20 ppm (150 mg/m$^3$) is available (http://onlinelibrary.wiley.com/doi/10.1002/3527600418.mb1383816e0009/full). The kinetic data show limited metabolisation.

***2. Isoflurane***




CAS: 26675-46-7 EINECS: 247-897-7
Name: Isoflurane; 2-chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane.
Isoflurane is an inhalation anaesthetic used for narcosis in concentrations of 12000 or 23000 ppm. No MAK was derived because the available database was inadequate (http://onlinelibrary.wiley.com/doi/10.1002/3527600418.mb2667546e0007/full). The NOEL from chronic studies in mice was 1000 ppm (7470 mg/m$^3$) and nervous system effects were observed in humans after brief exposures to 1150 ppm.

In addition, information on fluorinated compounds was collected from the RepDose database (Frauenhofer). However, the available fluorinated alkanes did not contain ethers and no general conclusion on the repeated dose toxicity via inhalation could be determined as the NOEC values from these studies varied more than 1000 fold but all NOECs were above 50 ppm. Overall, the information on analogues indicates limited toxicity for E1.



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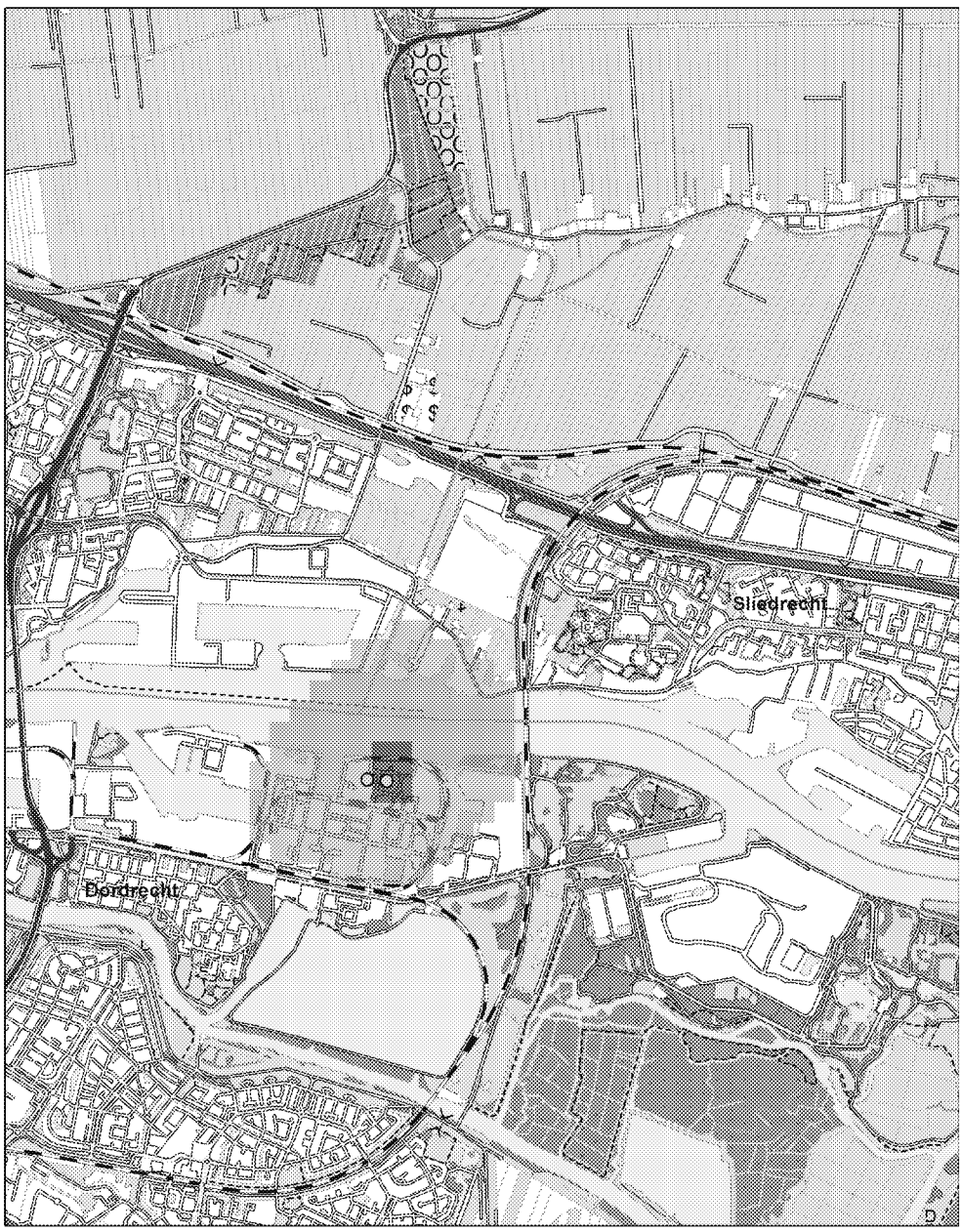
# Annex 3. Calculated air concentrations FRD-903 based on the permitted emissions (in ng/m$^3$)




calculated FRD-903 concentrations in air based on permitted emissions for 2014

Stack

calculated average concentration in a gridcel

15 - 73 ng/m$^3$

> 73 ng/m$^3$ (derived exposure limit)

kilometer

0 0,5 1

**RIVM**
Committed to *health and sustainability*

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