Netherlands Food and Consumer Product Safety Authority
Ministry of Agriculture, Nature and Food Quality

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To the Minister of Medical Care and Sports

from the Director of the Office for Risk Assessment & Research

Advice on PFOA and GenX in food

Office for Risk Assessment & Research

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Our reference
NVWA/BuRO/2019/4294

Date
July 16, 2019

Background

Perfluoroalkylated substances (PFASs) have been used, and are being used, in a range of industrial and chemical applications, e.g. as processing aids in impregnation agents for a wide range of products. The best known PFASs are perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA).
PFASs are thermally and chemically stable and can be taken up and accumulate in organisms. Their chemical properties and the widespread use led to worldwide distribution in the environment and thus to human exposure. In many countries there is pressure on industry to reduce, or even ban, the use of PFASs and to find and apply alternatives.

GenX is such an alternative to the use of PFOA. It is a polymerisation aid that is used for the production of fluoropolymers, such as Teflon® and denotes two substances:

- ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902) and
- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903).

Under environmental and physical conditions (e.g. in water or blood) FRD-902 and FRD-903 dissociate into the ion HFPO-DA (hexafluoropropyleneoxide dimer acid). The HFPO-DA ion is relevant for toxicological effects. In this advice the HFPO-DA ion is called GenX.

In the past, the companies DuPont/Chemours in Dordrecht and Custom Powders in Helmond emitted PFOA and GenX to the air. As a consequence, the areas around the sites of these companies have been polluted. In 2017 and 2018 the 'Expertisecentrum PFAS' has investigated the deposition of PFOA and GenX via air in the surroundings of DuPont Chemours in Dordrecht[1] and Custom Powders in Helmond[2]. PFOA and GenX were found to be present in soil and water due to air

[1]Report is available via https://www.ozhz.nl/fileadmin/uploads/bodeminformatie/PFOA_in_bodem/Onderzoek_Expertisecentrum_-_Maart_2018
[2]First report is available via https://www.helmond.nl/Media%20Helmond.nl/Documenten%20Helmond/Actueel/Nieuws/Nieuws%202018/2018-10-23%20VO%20GenX%20en%20PFOA%20Helmond%20definitief%20incl%20bijlagen.pdf

deposition and may thus enter the food chain. Livestock might be exposed if polluted soil, grass or water is consumed[3]. Subsequently, consumers might be exposed via the consumption of products of animal origin (e.g. dairy products or meat), leading to a elevated risk for human health.

The Office for Risk Assessment & Research (BuRO) of the Netherlands Food and Consumer Product Safety Authority (NVWA), therefore, investigated in a pilot study in 2018 the presence of PFOA and GenX in feed and food. The results of this study were used for a preliminary assessment to answer the question:

> Is there a possible risk for human health due to exposure to PFOA and GenX in food?

Approach

The sites of the companies DuPont/Chemours in Dordrecht and Custom Powders in Helmond are two 'hot spots' related to PFOA and GenX emissions. BuRO requested the directorate Enforcement of the NVWA to collect egg, milk, cheese, yoghurt and silage samples at farms in the vicinity of DuPont/Chemours in Dordrecht and Custom Powders in Helmond. As a starting point, farms were selected based on locations where soil samples were taken for air deposition studies as performed by the 'Expertisecentrum PFAS'. Subsequently, the directorate Enforcement was requested to collect fish samples at a fishing pond in the close vicinity of the site of Custom Powders in Helmond.

The collected samples were sent to Wageningen Food Safety Research (WFSR, formerly known as RIKILT) for analysis. Next, BuRO sent the results of the analysis to the Front Office Food and Product Safety (FO) of the National Institute for Public Health and the Environment (RIVM) addressing the following questions:

1. Describe the toxicology of PFOA and GenX.
2. Estimate the intake of PFOA and GenX by consumers based on the measured concentrations of PFOA and GenX in dairy products, eggs and fish.
3. Perform a risk assessment of PFOA and GenX in contaminated food of animal origin.
4. Model the transfer of PFOA and GenX from ditch water to edible products from lactating cows and sheep (milk and meat).
5. Estimate the intake of PFOA and GenX by consumers based on the theoretical (modelled) concentrations in cow's milk and meat and sheep's milk and meat.
6. Calculate the possible concentrations of PFOA and GenX in ditch water when concentrations of PFOA and GenX occur at the analytical limit of quantification (LOQ) of 0.01 ng/g in milk (based on reversed dosimetry modelling).
7. Estimate the transfer of PFOA and GenX in silage to milk and meat from lactating cows and sheep.

FO divided their report in two parts. Part one addresses questions 1 – 3 and part two addresses questions 4 – 7. The FO risk assessments are added to this advice as appendices 1 and 2. BuRO used the FO risk assessments as a starting point for this advice. However, BuRO did not stick to the exposure assessment performed

Second report is available via https://www.helmond.nl/Media%20Helmond.nl/Documenten%20Helmond/Actueel/Nieuws/Nieuws%202018/2019-03-14%20Definitief%20onderzoeksrapport%20fase%202%20inclusief%20bijlagen%20SECURED.pdf

[3] The NVWA received questions from farmers who wanted to know if they could let their livestock drink with PFOA or GenX contaminated ditch water.

by the FO. BuRO compared the actual PFOA and GenX exposure via the consumption of products of animal origin to tolerable daily intakes (TDI)[4] of both PFOA and the GenX.

Regarding PFOA and GenX, FO used the TDI as derived by RIVM in its risk assessment. FO did not use the provisional TDI for PFOA as derived by the European Food Safety Authority (EFSA) in 2018. According to FO the risk assessments based on the TDI derived by RIVM should be considered provisional until EFSA has finalized their evaluation on PFOA.

In this advice BuRO uses the provisional TDI for PFOA provided by EFSA and the TDI's for PFOA and GenX provided by RIVM for the risk assessment. EFSA did not derive a health based guidance value for GenX.

Findings

Toxicology PFOA

- After oral administration PFOA is readily absorbed in the gastrointestinal tract in mammals, including humans, and distributed to plasma and liver. PFOA is not metabolized and is excreted unchanged in urine and faeces. PFOA crosses the placenta leading to prenatal exposure of the foetus. PFOA is also present in breastmilk. The estimated half-life for PFOA in humans is between 2 – 4 years.
- Short-term, subchronic and chronic oral PFOA toxicity studies using experimental animals report developmental effects, liver and kidney toxicity, immune effects and cancer (liver, testicular and pancreatic). Developmental effects observed in animals include decreased survival, delayed eye opening and reduced ossification, skeletal defects, altered puberty and altered mammary gland developments.

Toxicology GenX

- The biokinetics of GenX were studied in rats, mice and monkeys. The results indicate that GenX has lower potential for bioaccumulation compared to PFOA in these species (half-lives in experimental animals between hours and days for GenX and between hours and weeks for PFOA). Data on the half-life of GenX in humans are lacking. Toxicokinetic data indicate that GenX is mainly distributed to liver and blood.
- Apart from the tumorigenic response in rats, the main affected organs in rodents resulting from repeated exposure to GenX are liver, kidneys, haematological system and immune system.

Health based guidance values

- In 2016, RIVM derived a tolerable daily intake (TDI) for PFOA of 12.5 ng/kg body weight per day. Hepatotoxicity was considered by RIVM to be the critical effect. In 2018, the EFSA Panel on Contaminants in the Food Chain (CONTAM) derived a provisional TDI for PFOA of 0.8 ng/kg body weight per day. The increase of serum cholesterol was considered by EFSA to be the critical effect.
- For GenX, RIVM derived a provisional TDI of 21 ng/kg body weight per day. An increase in albumin and albumin/globulin ratio in male rats was considered the critical effect, possibly indicating immunotoxic effects.

Exposure assessment

- Table 1 provides an overview of the worst-case exposure of children (1-18 years old; average body weight 38.5 kg) and adults (19-79 years old; average body weight 81.9 kg) to PFOA and GenX via the consumption of contaminated milk (cow/sheep), meat (cow/sheep), cheese, yoghurt, egg,

[4] A TDI estimates the amount of a potentially harmful substance or contaminant in food or water that can be ingested per day over a lifetime without risk of adverse health effects.

eel and carp. BuRO assumed a high intake (P95) of these foods based on the Dutch Food Consumption Survey 2012-2016. A further assumption was that the PFOA or GenX concentration was equal to the quantification limit if a PFOA or GenX concentration was reported to be below the quantification limit.

Table 1. The exposure of children (1-18 years old) and adults (19-79 years old) to PFOA and GenX via the consumption of contaminated milk (cow/sheep), meat (cow/sheep), cheese, yoghurt, egg, eel and carp.

		Concentration (ng/g)		P95 consumption rate of food or beverage (g/day)	Exposure (ng/kg body weight per day)	
	Product	**PFOA**	**GenX**		**PFOA**	**GenX**
Children (1-18 years)	Milk (cow)[1]	0.06[2]	0.01[3]	446.1[7]	0.70	0.12
	Milk (cow)	0.01[5]	0.10[5]	446.1[7]	0.12	1.16
	Milk (sheep)[1]	0.2 – 0.7[4]	0.04 - 0.14[4]	446.1[7]	2.32 – 8.11	0.46 – 1.62
	Meat (cow)[1]	0.28[2]	0.06[3]	15.5[7]	0.11	0.02
	Meat (sheep)[1]	0.2[4]	0.04[4]	15.5[7]	0.08	0.02
	Cheese	0.10[5]	0.10[5]	44.1[7]	0.11	0.11
	Yoghurt	0.10[5]	0.10[5]	138.2[7]	0.36	0.36
	Egg	0.14[6]	0.25[5]	20.3[7]	0.07	0.13
	Eel	0.05[5]	0.01[5]	0[8]	0	0
	Carp	1.3[6]	4.7[6]	37[9]	0.87	3.15
Adults (19-79 years)	Milk (cow)[1]	0.06[2]	0.01[3]	365.5[10]	0.27	0.04
	Milk (cow)	0.01[5]	0.10[5]	365.5[10]	0.04	0.45
	Milk (sheep)[1]	0.2 – 0.7[4]	0.04 - 0.14[4]	365.5[10]	0.89 – 3.12	0.18 – 0.62
	Meat (cow)[1]	0.28[2]	0.06[3]	29.6[10]	0.10	0.02
	Meat (sheep)[1]	0.2[4]	0.04[4]	29.6[10]	0.07	0.01
	Cheese	0.10[5]	0.10[5]	68.7[10]	0.08	0.08
	Yoghurt	0.10[5]	0.10[5]	189.5[10]	0.23	0.23
	Egg	0.14[6]	0.25[5]	30.1[10]	0.05	0.09
	Eel	0.05[5]	0.01[5]	300[11]	0.18	0.37
	Carp	1.3[6]	4.7[6]	101[12]	1.48	5.35

[1]Based on exposure through contaminated ditch water;
[2]Concentration calculated by FO via an adjusted transfer model for PFOS in dairy cows;
[3]Concentration reasoned by FO;
[4]Concentration estimated by FO based on experimental data from literature;
[5]Concentration < LOQ;
[6]Positive concentration (>LOQ);
[7]Data on usual intake based on high consumption from the Dutch Food Consumption Survey 2012-2016 by children (1-18 years old; average body weight 38.5 kg). Assumption that the same amount of milk or meat is consumed regardless if it is from cow or sheep;
[8]Data on actual intake based on high consumption from the Dutch Food Consumption Survey 2012-2016 by children (1-18 years old; average body weight 38.5 kg);
[9]High consumption based on the Dutch Food Consumption Survey 2012-2016. Female consumers (9-18 years old; average body weight 55.2 kg);
[10]Data on usual intake based on high consumption from the Dutch Food Consumption Survey 2012-2016 by adults (19-79 years old; average body weight 81.9 kg). Assumption that the same amount of milk or meat is consumed regardless if it is from cow or sheep;

[11]Data on actual intake based on high consumption from the Dutch Food Consumption Survey 2012-2016 by adults (19-79 years old; average body weight 81.9 kg);
[12]High consumption based on the Dutch Food Consumption Survey 2012-2016. Male consumers (51-79 years old; average body weight 88.8 kg).

Risk assessment

- The exposure to PFOA via the consumption of sheep's milk and carp by children and adults exceeds the provisional EFSA-TDI (0.8 ng/kg body weight per day) for PFOA, indicating a possible risk for human health (see also table 5 in substantiation).
- Both the TDI's for PFOA and the TDI for GenX are not exceeded after the consumption of cow's milk, meat (cow/sheep), cheese, yoghurt, egg and eel by children and adults. The consumption of these products does not pose a risk for human health (see also table 5 in substantiation).
- A calculated PFOA concentration of 810 – 1100 ng/L in ditch water could lead to a PFOA concentration at the analytical limit of quantification (LOQ; being 0.01 ng/g) in milk of dairy cows after the consumption of contaminated ditch water (80 L or 110 L).

Answers to the questions

1. *Is there a possible risk for human health due to exposure to PFOA and GenX in food?*

Despite the fact that the exposure of children and adults to PFOA via the consumption of carp exceeds the provisional EFSA-TDI of 0.8 ng/kg body weight per day, the risk for human health is expected to be low. A TDI is a health based guidance value based on chronic (long term) exposure. The carp was caught in a fishing pond in the close vicinity of the factory of Custom Powders in Helmond. Fish from this pond will probably only, on occasion, be eaten by specific consumers (sport fishermen) leading to acute (short term) exposure. Furthermore, the risk assessment of carp was based on one fish and this fish does not provide an overview of the PFOA distribution in fish from the fishing pond.

Based on a comparison with the provisional EFSA-TDI of 0.8 ng/kg body weight per day, the exposure of children and adults to PFOA via the consumption of sheep's milk might pose a risk to human health. The risk assessment for sheep's milk is based on experimental transfer data from two sheep that do not show the same kinetics. Compared to dairy cows, the transfer of PFOA to milk in sheep is higher than one might expect. Therefore, no firm conclusion about the human health risk can be drawn.

The exposure of children and adults to PFOA and GenX via the consumption of cow's milk, meat (cow/sheep), cheese, yoghurt, egg and eel does not pose a risk for human health.

A calculated PFOA concentration of 810 – 1100 ng/L in ditch water would lead to a PFOA concentration at the analytical limit of quantification (LOQ; being 0.01 ng/g) in milk of dairy cows after the consumption of contaminated ditch water (intake of 80 L or 110 L). If the PFOA concentration is higher than 1100 ng/L contamination of milk with PFOA may occur.

As no transfer model for GenX was available, no maximum GenX concentration in ditch water that would lead to a GenX concentration in milk at the present analytical limit of quantification (LOQ; 0.1 ng/g) could be calculated.

Office for Risk Assesment & Research

Date

July 16, 2019

Our reference
NVWA/BuRO/2019/4294

Advice

To the minister of Medical Care and Sports
Initiate additional toxicological research to investigate the risk caused by exposure to (mixtures of) PFAS substances; this because many PFAS substances are already on the market, new PFAS substances are being developed, while currently the main focus of regulatory and scientific authorities is on PFOS, PFAS and GenX.

To the Head of Agency

- Monitor the presence of PFAS in food of animal origin to allow assessment of the potential exposure of humans; this in spite of the fact that this preliminary assessment does not indicate increased risks for human health due to the current exposure to PFOA and GenX by food consumption in general
- However, inform the municipality of Helmond that increased health risk should not be excluded after regular consumption of fish from the specific fishing pond in the close vicinity of the factory of Custom Powders.

Yours sincerely,

Prof. dr. Antoon Opperhuizen
Director of the Office for Risk Assessment & Research

SUBSTANTIATION

Background

Perfluoroalkylated substances (PFASs) are compounds consisting of a hydrophobic alkyl chain of varying length and a hydrophilic end group (EFSA CONTAM Panel, 2018). PFASs are thermally and chemically stable. They have, therefore, been used since decades in a range of industrial and chemical applications as processing aids for impregnation of textiles, carpets, paper, packaging materials, furniture, shoes, cleaning agents, paints and varnish, wax, floor polishing agents, fire-extinguishing liquids, photo paper and insecticide formulations (EFSA, 2012; EFSA CONTAM Panel, 2018). This widespread use led to their global distribution in the environment including humans. The best known PFASs are perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). PFOA has an anionic head group and belongs to the perfluoroalkyl carboxylic acids (PFCAs) (EFSA CONTAM Panel, 2018).

GenX is a polymerisation aid that is used for the production of fluoropolymers, such as Teflon®, without the use of PFOA (Beekman et al., 2016; Bokkers et al., 2018; FO, 2019a). GenX is used to denote two substances:

- ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902) and
- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903).

Under environmental and physical conditions (e.g. in water or blood) FRD-902 and FRD-903 dissociate into the ion HFPO-DA (hexafluoropropyleneoxide dimer acid). The HFPO-DA ion is responsible for the observed toxicological effects (Bokkers et al., 2018; FO, 2019a). In this advice the HFPO-DA ion is called GenX.

In the Netherlands, the companies DuPont/Chemours in Dordrecht and Custom Powders in Helmond emitted PFOA and GenX in to the air. The emission of GenX by DuPont/Chemours is ongoing. Consequently, the area around the sites of these companies (soil, water and vegetation) is polluted (FO, 2019a).

Legislation

PFOA

Based on the REACH Regulation (Registration, Evaluation, Authorisation and Restriction of Chemicals; Regulation (EC) No 1907/2006[5]) PFOA is a persistent, bioaccumulative and toxic (PBT) substance. In 2013 PFOA was included in the Candidate List of Substances of Very High Concern (SVHC) for possible inclusion into Annex XVI of the REACH Regulation. Annex XVI describes a list of substances subject to authorisation.

Via Commission Regulation (EU) 2017/1000[6], PFOA was included in Annex XVII of the REACH Regulation. Annex XVII describes restrictions on the manufacture, placing on the market and use of certain dangerous substances, mixtures and articles. From July 4th 2020 PFOA substances shall not

- be manufactured or placed on the market as substances on their own;

[5] REGULATION (EC) No 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

[6] COMMISSION REGULATION (EU) 2017/1000 of 13 June 2017 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards perfluorooctanoic acid (PFOA), its salts and PFOA-related substances.

- be used in the production of, or placed on the market in (a) another substance, as a constituent (b) a mixture (c) an article in a concentration equal to or above 225 ppb of PFOA including its salts or 1000 ppb of one or a combination of PFOA-related substances.

In the annex PFOA is specified as:

- PFOA (CAS No 335-67-1) and it salts
- Any related substance (including its salts and polymers) having a linear or branched perfluoroheptyl group with the formula C_7F_{15}- directly attached to another carbon atom, as one of the structural elements.
- Any related substance (including its salts and polymers) having a linear or branched perfluorooctyl group with the formula C_8F_{17}- as one of the structural elements, excluding:
    - C_8F_{17}-X, where X = F, Cl, Br.
    - C_8F_{17}-C(=O)OH, C_8F_{17}-C(=O)O-X′ or C_8F_{17}-CF_2-X′ (where X′ = any group, including salts).

There are a few exceptions where the restrictions will enter into force at a later point in time:

- equipment used to manufacture semi-conductors and latex printing inks (July 4th 2022).
- textiles for the protection of workers from risk to their health and safety, membranes intended for use in medical textiles, filtration in water treatment, production processes and effluent treatment and plasma nano-coatings (July 4th 2023).

There are also some exceptions that are not restricted:

- PFOS and its derivatives, which are listed in Part A of Annex I to Regulation (EC) No 850/2004[7].
- The manufacture of a substance where this occurs as an unavoidable by-product of the manufacture of fluorochemicals with a carbon chain equal to or shorter than six atoms.
- A substance that is to be used, or is used as a transported isolated intermediate, proved that the conditions in points (a) to (f) of Article 18(4) of the REACH Regulation are met.
- A substance, constituent of another substance or mixture that is to be used, or is used:
    - In the production of implantable medical devices within the scope of Directive 93/42/EEC[8].
    - In photographic coatings applied to films, papers or printing plates.
    - In photo-lithography processes for semiconductors or in etching processes for compound semiconductors
- Concentrated fire-fighting foam mixtures that were placed on the market before 4 July 2020 and are to be used, or are used in the production of other fire-fighting foam mixtures.

Commission Regulation (EC) No 10/2011[9] states that PFOA can be used as a polymer production aid only to be used in repeated use plastic articles that come into contact with food, sintered at high temperatures.

PFOA is not listed in Regulation (EC) No 1881/2006[10] setting maximum levels for certain contaminants in food.

[7] REGULATION (EC) No 850/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004 on persistent organic pollutants and amending Directive 79/117/EEC.
[8] COUNCIL DIRECTIVE 93/42/EEC of 14 June 1993 concerning medical devices.
[9] COMMISSION REGULATION (EU) No 10/2011 on plastic materials and articles intended to come into contact with food.
[10] COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

GenX
The Netherlands (represented by the Dutch Ministry of Infrastructure and Water Management) proposed "2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, its salts and its acyl halides (covering any of their individual isomers and combinations thereof)" to be identified as a SVHC. A dossier in accordance with the requirements set out in Annex XV to REACH was prepared. Comments on this dossier can be submitted by all interested parties before April 29th 2019[11]. When the public consultation is finalised and GenX is identified as a SVHC, it will be added to the Candidate List for eventual inclusion in the Authorisation List.

According to Commission Regulation (EC) No 10/2011[12] perfluoro[2-(n-propoxy)propanoic acid] or 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoic acid are only to be used in the polymerisation of fluoropolymers that are processed at temperatures at or above 265 °C and are intended for use in repeated use articles.

GenX is not listed in Regulation (EC) No 1881/2006[13] setting maximum levels for certain contaminants in food.

Toxicology
Below a short summary of toxicology of PFOA and GenX is presented, extracted from the report by the RIVM/RIKILT Front Office Food and Product Safety (FO). More detailed information on PFOA and GenX can be found in the FO report (Appendix 1)(FO, 2019a). Comprehensive reviews on the toxicity of PFOA are available (US EPA, 2016; DWQI, 2017; ATSDR, 2018; EFSA CONTAM Panel, 2018). The description of the GenX toxicology is mainly based on data available in the REACH registration dossier (Beekman et al., 2016; FO, 2019a).

PFOA
After oral administration PFOA is readily absorbed in the gastrointestinal tract in mammals, including humans, and distributed to plasma and liver. PFOA is not metabolized and is excreted unchanged in urine and faeces. PFOA crosses the placenta leading to prenatal exposure of a fetus. PFOA is also present in breastmilk. The estimated half-life for PFOA in humans is between 2 – 4 years (EFSA CONTAM Panel, 2018). This half-life is rather long compared to the period of several weeks which was reported for experimental animals (Zeilmaker et al., 2016). In contrast to classic lipophilic organic pollutants (e.g. dioxins) PFOA primarily binds to proteins instead of lipids (FO, 2019a).

Short-term, subchronic and chronic oral PFOA toxicity studies using experimental animals report developmental effects, liver and kidney toxicity, immune effects and cancer (liver, testicular and pancreatic). Developmental effects observed in animals include decreased survival, delayed eye opening and reduced ossification, skeletal defects, altered puberty and altered mammary gland developments (FO, 2019a).

Regarding PFOA toxicity, the liver is a target organ in rodents. PFOA is a ligand of the nuclear receptor peroxisome proliferator activated receptor-alpha (PPARα) and induces liver growth, proliferation of peroxisomes and inductions of peroxisomal β-oxidation in rodents. Elevated peroxisomal β-oxidation in rodents may lead to

[11] At https://echa.europa.eu/substances-of-very-high-concern-identification/-/substance-rev/22907/term
[12] COMMISSION REGULATION (EU) No 10/2011 on plastic materials and articles intended to come into contact with food.
[13] COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

hepatic lipid peroxidation and subsequently to cell death and enhanced release of liver transaminases (EFSA CONTAM Panel, 2018).

PFOA has developmental neurotoxicity potential and widespread effects on the expression of genes relevant for signal transmission in the brain. Exposure of rodents to PFOA during pregnancy led to increased liver weight in pups and mothers (EFSA CONTAM Panel, 2018).

The International Agency for Research on Cancer (IARC) stated that there is limited evidence for carcinogenicity in experimental animals and moderate evidence for mechanisms of PFOA-associated carcinogenesis, including some evidence for these mechanisms being operative in humans. PFOA was assigned to group 2B as being possibly carcinogenic to humans (IARC, 2016; EFSA CONTAM Panel, 2018). From *in vitro* and *in vivo* genotoxicity studies, there is no evidence for a direct genotoxic mode of action of PFOA (FO, 2019a).

Human epidemiological studies report associations between PFOA exposure and a number of disorders and diseases. The National Institute for Public Health and the Environment (RIVM) reviewed these associations and concluded that the weight of evidence was variable and that uncertainty remains about the causality of the observed associations (Rijs & Bogers, 2017). In contrast, EFSA concluded that an association between PFOA exposure and adverse affected serum antibody response following vaccination in children is likely to be causal. For metabolic outcomes, human epidemiological studies provide strong support for causal associations between exposure to PFOA and increased serum levels of cholesterol and support for a causal association between exposure to PFOA and increased serum levels of the liver enzyme alanine transferase (ALT) (EFSA CONTAM Panel, 2018).

GenX

The majority of the toxicity studies using experimental animals are performed with FRD-902. Read-across of the toxicological properties of FRD-902 to FRD-903 is justified (Beekman et al., 2016; FO, 2019a). Under environmental and physical conditions (e.g. in water or blood) FRD-902 and FRD-903 dissociate into the ion HFPO-DA (hexafluoropropyleneoxide dimer acid). The HFPO-DA ion is responsible for the observed toxicological effects (Bokkers et al., 2018; FO, 2019a). In this advice the HFPO-DA ion is called GenX.

The biokinetics of GenX were studied in rats, mice and monkeys (Gannon et al., 2016). The results indicate that GenX has lower potential for bioaccumulation compared to PFOA in these species (half-lives between hours and days for GenX and between hours and weeks for PFOA) (FO, 2019a). Data on half-life on GenX in humans are lacking. The limited data available suggests that GenX binds to fatty acid-binding proteins in the liver (Sheng et al., 2018) and to serum proteins (albumin) in blood. Although no data are available on a direct interaction of GenX with albumin, toxicokinetic data illustrates that GenX mainly distributes to the liver and the blood. Overall, tissue and serum concentrations are higher in males compared to females, suggesting that females are able to eliminate GenX more effectively (FO, 2019a).

Apart from the tumorigenic response in rats, the main affected organs in rodents resulting from repeated exposure to GenX are the liver, the kidneys, the haematological system and the immune system (FO, 2019a). With regard to developmental toxicity, GenX crosses the placenta and distributes into the foetus and causes early deliveries and decreased birth weight in pups without causing severe parental toxicity at 100 mg/kg body weight per day. Information is inconclusive with respect to potential effects to the reproductive system (FO,

2019a). The observed liver effects are suggested to be (at least partly) explained (directly or indirectly) by activation of the peroxisome proliferator-activated receptor alpha (PPARα), a biological pathway mainly responsible for lipid metabolism (FO, 2019a). A recent study suggests that activation of PPAR signalling pathways is not solely responsible for the observed toxicity effects in pregnant rats and their offspring exposed to GenX (Conley et al., 2019).

Health based guidance values

PFOA

In 2016, RIVM derived a tolerable daily intake (TDI) for PFOA, at 12.5 ng/kg body weight per day (Zeilmaker et al., 2016). A TDI estimates the amount of a potentially harmful substance or contaminant in food or water that can be ingested per day over a lifetime without risk of adverse health effects. Hepatotoxicity was considered to be the critical effect. Male CrL:CD®BR rats were orally exposed to ammonium perfluorooctanoate concentrations of 0, 1, 10, 30 or 100 ppm (equivalent to 0, 0.06, 0.64, 1.94 and 6.5 mg/kg body weight per day) for 13 weeks (Perkins et al., 2004). After exposure for 4, 7 and 13 weeks increased liver weights (absolute and relative) and increased hepatocyte hypertrophy were observed at a dose of 10 ppm. When exposure was ceased the effects were reversible. From this rat study a lowest observed adverse effect level (LOAEL) and a no observed adverse effect level (NOAEL) can be derived, being 10 ppm (0.64 mg/kg body weight per day) and 1 ppm (0.06 mg/kg body weight per day). The PFOA serum concentration in rats related to the derived NOAEL was 7.1 µg/ml. RIVM translated the rat NOAEL to a Human Equivalent Dose for semi-chronic intake, being 0.001 mg/kg body weight per day. RIVM applied an assessment factor of 1 for interspecies differences, because rats are more sensitive to hepatotoxicity compared humans. RIVM also applied an assessment factor of 10 for intraspecies differences, resulting in a semi-chronic health based guidance value of 0.0001 mg/kg body weight per day (100 ng/kg body weight per day, corresponding to a human serum concentration of 710 ng/ml). An additional assessment factor of 8 was applied to translate the semi-chronic to a chronic health based guidance value of $12.5 * 10^{-6}$ mg/kg body weight per day (12.5 ng/kg body weight per day, corresponding to a human serum concentration of 89 ng/ml).

In 2018, the EFSA Panel on Contaminants in the Food Chain (CONTAM) derived a provisional tolerable weekly intake (TWI) for PFOA, being 6 ng/kg body weight per week (EFSA CONTAM Panel, 2018). A TWI estimates the amount of a potentially harmful substance or contaminant in food or water that can be ingested per week over a lifetime without risk of adverse health effects. A TWI is usually calculated for substances that are persistent (i.e. having a long half-life).
The increase of serum cholesterol is considered to be the critical effect. EFSA used the data of two studies (Steenland et al., 2009; Eriksen et al., 2013) on serum cholesterol to perform benchmark dose (BMD) modelling. The BMD modelling resulted in an estimated chronic daily intake of about 0.8 ng/kg body weight per day. This was considered to be an appropriate reference point for the establishment of the TWI (6 ng/kg body weight per week = 0.8 * 7). EFSA decided not to apply any additional uncertainty factor because the BMD modelling was based on large epidemiological studies from the general population, including potentially sensitive subgroups. EFSA also took into account that the BMD modelling was performed on risk factors for disease rather than disease (EFSA CONTAM Panel, 2018). How this was done is not further substantiated in the opinion.

Instead of the EFSA approach RIVM, ECHA[14] (European Chemicals Agency) and Danish EPA[15] have used a different approach for deriving a health based guidance value for PFOA (Danish EPA, 2015; ECHA, 2015; Zeilmaker et al., 2016). These different approaches were discussed during an expert meeting (EFSA, 2018). With regards the derived TWI by EFSA, RIVM identified three main issues:

- The suitability of the information in the epidemiological studies available for deriving a Point of Departure (PoD).
- The assumptions made in the derivation of the PoD.
- The inconsistency of the applied BMD analysis with the existing EFSA guidance.

In general, RIVM follows the health based guidance values set by EFSA. However, due to the above mentioned difference and the ongoing evaluation by EFSA, RIVM maintains its own TDI for PFOA presently. Risk assessments based on this value should be considered provisional until the EFSA evaluation is finalised (FO, 2019a).

GenX

RIVM derived a provisional TDI of 0,000021 mg/kg body weight per day (i.e. 21 ng/kg body weight per day). A NOAEL of 0,1 mg/kg body weight per day was considered as the point of departure (POD). The NOAEL, for a chronic oral gavage study in rats, is based on an increase in albumin and the albumin/globulin ratio in male rats. This effect indicates possible immunotoxic effects (Beekman et al., 2016). In agreement with the REACH guidance RIVM applied the following assessment factors to the oral NOAEL (Janssen et al., 2017):

- Standard interspecies for differences in kinetics 4
- Additional factor for potential kinetic differences 66
- Interspecies remaining toxicodynamic differences 1,8
- Intraspecies factor human 10

Livestock exposure

PFOA and GenX in ditch water and silage

Single samples of ditch water were taken at five different sites within a distance (radius) of four kilometres from the Dupont/Chemours factory in Dordrecht (van Poll, 2018). The average concentrations PFOA and GenX at these sites are given in table 1.

[14] In 2015, the committee for risk assessment (RAC), established a 'Derived No Effect Level' (DNEL) of 800 ng/mL serum for PFOA for the general population (ECHA, 2015). The DNEL was based on a study with mice where a decreased pup growth rate in the order of 25-30% during post-natal days 13-23 was observed at doses of 3 mg/kg/day and higher, leading to a NOAEL of 1 mg/kg/day. The corresponding NOAEL in serum was approximately 20,000 ng/mL. RAC used a total assessment factor of 12.5 (2.5 x 5), resulting in a worker DNEL of 1600 ng/mL serum. The corresponding DNEL for the general population was 800 ng/mL serum, using an intraspecies assessment factor of 10 (total assessment factor 2.5 x 10).

[15] The Danish EPA referred to the 2014 assessments by the US EPA (US EPA 2014a,b) to establish TDIs for PFOS and PFOA. The endpoint of liver toxicity in rats was used to derive TDIs of 0.03 µg/kg bw per day and 0.1 µg/kg bw per day for PFOS and PFOA, respectively. Human studies were considered in the Danish EPA 2015 assessment, however they were not considered to be adequate.

Table 1. Average PFOA- and GenX-concentrations in ditch water (ng/L) at five different locations around the factory in Dordrecht.

Location number	Distance (km)	PFOA (ng/L)	GenX (ng/L)
8	< 1	4670	956.5
6	1-2	660.5	133.5
4	1-2	556	97.5
3	2-3	172.5	24.5
10	3-4	40.5	9.7

The Netherlands Food and Consumer Product Safety Authority (NVWA) took ten samples of silage at farms in the vicinity of Dordrecht and Helmond. No GenX could be detected in these samples (<250 ng/kg). PFOA could only be detected in two samples in the vicinity of Dordrecht, concentrations were 540 and 600 ng/kg (measurements on basis of whole product).

Exposure of lactating cows

FO calculated the average intake of PFOA and GenX via ditch water by lactating cows (600 kg) assuming:

1. Maximum exposure – highest PFOA (4670 ng/L) and GenX (956.5 ng/L) concentration.
2. A maximum drinking water consumption of 110 L per day for mature lactating cows (weight 600 kg; milk yield 35 kg per day)
3. Cows solely consume contaminated ditch water.

The average intake of PFOA by lactating cows is approximately 510,000 ng PFOA per day (≈110 * 4670) and the average intake of GenX is approximately 110,000 ng GenX per day (≈110 * 956.5).

FO also calculated the average intake of PFOA via silage by lactating cows assuming:

1. Silage intake during winter time (worst case scenario). An average of 25 to 38.5 kg (grass) silage per day wet weight is consumed.
2. Cows solely consume contaminated silage.

The average intake of PFOA by lactating cows based on a worst case scenario is approximately 23,000 ng PFOA per day (≈38.5 * 600).
FO did not calculate the average intake of GenX via silage by lactating cows as GenX was not detected (<250 ng/kg).

Exposure of lactating sheep

FO calculated the average intake of PFOA and GenX via ditch water by lactating sheep (60 kg) assuming:

1. Maximum exposure – highest PFOA (4670 ng/L) and GenX (956.5 ng/L) concentration.
2. A daily drinking water consumption of 6 L per day.
3. Sheep solely consume contaminated ditch water.

The average intake of PFOA by lactating sheep is approximately 28,000 ng PFOA per day (≈6 * 4670) and the average intake of GenX is approximately 5700 ng GenX per day (≈6 * 956.5).

FO also calculated the average intake of PFOA via silage by lactating sheep assuming:

1. Daily silage intake of 2.7 kg wet weight grass silage daily.
2. Sheep solely consume contaminated silage.

The average intake of PFOA by lactating sheep based on the scenario above is approximately 1600 ng PFOA per day (≈2.7 * 600).

FO did not calculate the average intake of GenX via silage by lactating sheep as GenX was not detected is silage (<250 ng/kg).

Human exposure via food of animal origin

Dairy products, meat, egg and eel

FO modelled the PFOA concentration in milk and meat of cows exposed to ditch water and silage by using the adjusted transfer model for PFOS in dairy cows (van Asselt et al., 2013; FO, 2019b). The GenX concentrations (due to the absence of a transfer model) were reasoned by FO. FO did not scale the PFOA/PFOS transfer model from dairy cows to sheep. Allometric scaling does not apply, because renal clearance of PFOS/PFOA differs between animal species. Consequently one does not know if the PFOS/PFOA concentration in tissues of different animals is the same. Instead the experimental transfer of PFOA from contaminated feed into milk and meat of two sheep was used to estimate the PFOA concentration in milk of sheep exposed to contaminated ditch water (Kowalczyk et al., 2012; FO, 2019b). Table 2 provides an overview of the PFOA and GenX concentration in milk and meat of cows and sheep exposed to contaminated ditch water and silage.

Table 2. The PFOA and GenX concentration (ng/g) in milk and meat of cows and sheep exposed to contaminated ditch water or silage.

Animal	Product	PFOA (ng/g)		GenX (ng/g)	
		Ditch water	Silage	Ditch water	Silage
Cow	Milk	0.06[1]	0.003[1]	<0.01[2]	X[3]
	Meat	0.28[1]	0.01[1]	<0.06[2]	X[3]
Sheep	Milk	0.2 - 0.7[4]	0.01 – 0.04[4]	0.04 - 0.14[2]	X[3]
	Meat	0.2[4]	0.01[4]	0.04[2]	X[3]

[1]Modelled; [2]Reasoned assumption; i.e. assuming less efficient transfer of GenX relative to PFOA at comparable exposure; [3]X: negligible; [4]Estimated based on a pilot experiment (N=2)(Kowalczyk et al., 2012).

Samples of dairy products (milk, cheese and yoghurt), eggs and fish were taken by the NVWA from farms in the vicinity of Dordrecht and Helmond. One fish sample was taken from a fishing pond closely to Custom Powders in Helmond. Table 3 provides an overview of the PFOA- and GenX-concentrations in these samples.

Table 3. Analyzed PFOA- and GenX-concentrations in dairy products, egg and fish sampled near the companies DuPont/Chemours in Dordrecht and Custom Powders in Helmond.

Location	Product	Concentration (ng/g)		
		N	PFOA	GenX
Dordrecht	Dairy products			
	Milk[1]	15	<0.01[4]	<0.10
	Cheese[2]	1	<0.10	<0.10
	Yoghurt[2]	1	<0.10	<0.10
	Egg[3]	1	0.14	<0.25
Helmond	Dairy products			
	Milk[2]	2	<0.01	<0.10
	Egg[3]	1	<0.025	<0.25
	Fish			
	Eel (farmed)	1	<0.05	<0.10
	Carp	1	1.3	4.7

[1]Cow (N=14) and goat (N=1); [2]Cow; [3]Chicken; [4]< means <LOQ

Subsequently, BuRO took the PFOA and GenX concentrations in the products mentioned in tables 2 and 3 and calculated the worst-case exposure of children (1-18 years old) and adults (19-79 years old) (Table 4) by assuming:

1. That the PFOA or GenX concentration was equal to the quantification limit if a PFOA or GenX concentration was reported to be below the quantification limit.
2. High consumption (P95) based on the Dutch Food Consumption Survey 2012-2016.
    a. One consumes the same amount of milk or meat regardless if it is from a cow or sheep, as no consumption data for sheep's milk or meat are available in the Dutch Food Consumption Survey 2012-2016.
    b. Data on usual intake of milk, cheese, yoghurt, egg and beef.
    c. Data on the acute intake of eel, as no usual intake could be calculated in the Dutch Food Consumption Survey.
    d. No consumption data for carp were available in the Dutch Food Consumption Survey, therefore fish consumption was used as an alternative. See also the FO rapport (FO, 2019a).
3. Average body weight of 38.5 kg (children) and 81.9 kg (adults).

BuRO did not follow the exposure assessment performed by the FO. BuRO compared the actual PFOA and GenX exposure via the consumption of products of animal origin to both PFOA TDI's and the GenX TDI.

Table 4. The exposure of children (1-18 years old) and adults (19-79 years old) to PFOA and GenX via the consumption of contaminated milk (cow/sheep), meat (cow/sheep), cheese, yoghurt, egg, eel and carp.

		Concentration (ng/g)		P95 consumption rate of food or beverage (g/day)	Exposure (ng/kg body weight per day)	
	Product	**PFOA**	**GenX**		**PFOA**	**GenX**
Children (1-18 years)	Milk (cow)[1]	0.06[2]	0.01[3]	446.1[7]	0.70	0.12
	Milk (cow)	0.01[5]	0.10[5]	446.1[7]	0.12	1.16
	Milk (sheep)[1]	0.2 – 0.7[4]	0.04 - 0.14[4]	446.1[7]	2.32 – 8.11	0.46 – 1.62
	Meat (cow) [1]	0.28[2]	0.06[3]	15.5[7]	0.11	0.02
	Meat (sheep) [1]	0.2[4]	0.04[4]	15.5[7]	0.08	0.02
	Cheese	0.10[5]	0.10[5]	44.1[7]	0.11	0.11
	Yoghurt	0.10[5]	0.10[5]	138.2[7]	0.36	0.36
	Egg	0.14[6]	0.25[5]	20.3[7]	0.07	0.13
	Eel	0.05[5]	0.01[5]	0[8]	0	0
	Carp	1.3[6]	4.7[6]	37[9]	0.87	3.15
Adults (19-79 years)	Milk (cow)[1]	0.06[2]	0.01[3]	365.5[10]	0.27	0.04
	Milk (cow)	0.01[5]	0.10[5]	365.5[10]	0.04	0.45
	Milk (sheep)[1]	0.2 – 0.7[4]	0.04 - 0.14[4]	365.5[10]	0.89 – 3.12	0.18 – 0.62
	Meat (cow) [1]	0.28[2]	0.06[3]	29.6[10]	0.10	0.02
	Meat (sheep) [1]	0.2[4]	0.04[4]	29.6[10]	0.07	0.01
	Cheese	0.10[5]	0.10[5]	68.7[10]	0.08	0.08
	Yoghurt	0.10[5]	0.10[5]	189.5[10]	0.23	0.23
	Egg	0.14[6]	0.25[5]	30.1[10]	0.05	0.09
	Eel	0.05[5]	0.01[5]	300[11]	0.18	0.37
	Carp	1.3[6]	4.7[6]	101[12]	1.48	5.35

[1]Based on exposure through contaminated ditch water;
[2]Concentration calculated by FO via an adjusted transfer model for PFOS in dairy cows;
[3]Concentration reasoned by FO;
[4]Concentration estimated by FO based on experimental data from literature;
[5]Concentration < LOQ;
[6]Positive concentration (>LOQ);
[7]Data on usual intake based on high consumption from the Dutch Food Consumption Survey 2012-2016 by children (1-18 years old; average body weight 38.5 kg). Assumption that the same amount of milk or meat is consumed regardless if it is from cow or sheep;
[8]Data on actual intake based on high consumption from the Dutch Food Consumption Survey 2012-2016 by children (1-18 years old; average body weight 38.5 kg);
[9]High consumption based on the Dutch Food Consumption Survey 2012-2016. Female consumers (9-18 years old; average body weight 55.2 kg);
[10]Data on usual intake based on high consumption from the Dutch Food Consumption Survey 2012-2016 by adults (19-79 years old; average body weight 81.9 kg). Assumption that the same amount of milk or meat is consumed regardless if it is from cow or sheep;
[11]Data on actual intake based on high consumption from the Dutch Food Consumption Survey 2012-2016 by adults (19-79 years old; average body weight 81.9 kg);
[12]High consumption based on the Dutch Food Consumption Survey 2012-2016. Male consumers (51-79 years old; average body weight 88.8 kg).

Risk assessment

Table 5 provides an overview of the percentages PFOA and GenX covering the TDI's of both substances, being 0.8 ng/kg body weight per day (provisional by EFSA) or 12.5 ng/kg body weight per day (RIVM) for PFOA and 21 ng/kg body weight per day for GenX. If the percentage is higher than 100%, the TDI is exceeded and consumption of the related products might pose a risk for human health.

Table 5. Overview of the percentages PFOA and GenX covering the TDI's of both substances, being 0.8 ng/kg body weight per day (provisional by EFSA) or 12.5 ng/kg body weight per day (RIVM) for PFOA and 21 ng/kg body weight per day for GenX.

		Exposure (ng/kg body weight per day)		%TDI		
	Product	**PFOA**	**GenX**	**PFOA[2]**	**PFOA[3]**	**GenX**
Children (1-18 years)	Milk (cow)[1]	0.70	0.12	87	6	1
	Milk (cow)	0.12	1.16	14	1	6
	Milk (sheep)[1]	2.32 – 8.11	0.46 – 1.62	290 – 1014[4]	19 – 65	2 – 8
	Meat (cow) [1]	0.11	0.02	14	1	0
	Meat (sheep) [1]	0.08	0.02	10	1	0
	Cheese	0.11	0.11	14	1	1
	Yoghurt	0.36	0.36	45	3	2
	Egg	0.07	0.13	9	1	1
	Eel	0	0			
	Carp	0.87	3.15	109	7	15
Adults (19-79 years)	Milk (cow)[1]	0.27	0.04	33	2	0
	Milk (cow)	0.04	0.45	6	0	2
	Milk (sheep)[1]	0.89 – 3.12	0.18 – 0.62	111 – 390	7 – 25	1 – 3
	Meat (cow) [1]	0.10	0.02	13	1	0
	Meat (sheep) [1]	0.07	0.01	9	1	0
	Cheese	0.08	0.08	10	1	0
	Yoghurt	0.23	0.23	29	2	1
	Egg	0.05	0.09	6	0	0
	Eel	0.18	0.37	23	1	2
	Carp	1.48	5.35	185	12	25

[1]Based on exposure through contaminated ditch water; [2]Based on a provisional EFSA-TDI of 0.8 ng/kg body weight per day; [3]Based on a RIVM-TDI of 12.5 ng/kg body weight per day; [4]Red numbers indicate an exceedance of the TDI.

Table 5 shows that the consumption of sheep's milk and carp by children and adults exceeds the provisional EFSA-TDI (0.8 ng/kg body weight per day) for PFOA, indicating a possible risk for human health. Both TDI's for PFOA and the TDI for GenX are not exceeded after the consumption of cow's milk, meat (cow/sheep), cheese, yoghurt, egg and eel by children and adults. The consumption of these products does not pose a risk for human health.

Maximum PFOA and GenX concentration in ditch water

FO calculated the maximum PFOA concentration in ditch water that would lead to a PFOA concentration in milk at the present analytical limit of quantification (LOQ; being 0.01 ng/g = 0.01 ng/mL). In literature a transfer model for PFOS in dairy cows is available (van Asselt et al., 2013), which was adjusted to PFOA by FO (FO, 2019b). For their calculation FO assumed that no additional exposure occurs from

other sources than ditch water. Using a PFOA concentration of 0.01 ng/g in milk as input, a theoretical intake of 89,000 ng per day was calculated. This results in a calculated PFOA concentration in ditch water of approximately 1100 ng/L (≈89,000/80) or 810 ng/L (≈89,000/110) depending on the ditch water intake (80 L or 110 L).

As no transfer model for GenX was available, FO could not calculate the maximum GenX concentration in ditch water that would lead to a GenX concentration in milk at the present analytical limit of quantification (LOQ; 0.1 ng/g).

Discussion

The exposure assessment and subsequently the risk assessment performed by BuRO are based on a very limited number of samples of possible contaminated foods, such as milk, cheese and eggs. This explorative sample strategy was chosen by BuRO to obtain an indication of the possible risk for human health due to exposure to PFOA and GenX in foods.

In interpreting the sheep transfer calculations it should be noted that the transfer to milk was observed in only two sheep showing quite different PFOA kinetics. The available transfer data in dairy cattle and lactating sheep indicate that PFOA transfer to organs and tissues is comparable in both species, but transfer to milk is not. Regarding the latter, the limited available data suggest a much higher transfer (i.e. up to 6 – 20 fold) of PFOA from the blood to milk in lactating sheep than from the blood to milk in dairy cattle. Therefore, FO concluded that the observed transfer of PFOA in lactating sheep to milk needs to be confirmed beyond the pilot experiment in which it was assessed in order to draw a more definitive conclusion on the relevance of such transfer for human risk assessment (FO, 2019b).

Due to the absence of consumption amounts of fish by persons fishing in the fish pond in Helmond, the consumption rates of fish by the general Dutch population were used in the risk assessment. Persons fishing in this pond possibly consume fish more frequently than the general population. They may also consume fish in larger amounts when eating fish. By using the consumed amount at the 95th percentile of the consumption distribution, this was partly addressed (FO, 2019a).

People living in the vicinity of either of the two sites are not only exposed to PFOA and GenX through the consumption of dairy products, egg and fish. As a result of emissions from the DuPont/Chemours site in Dordrecht and Custom Powders in Helmond, PFOA and GenX have been emitted into the environment via the air. As a consequence, these substances may have been deposited at a vegetable garden in the vicinity of the sites and local authorities were concerned whether it is safe to eat their home-grown vegetables. Therefore, RIVM performed a risk assessment of PFOA and GenX in vegetable garden crops in Dordrecht (including Papendrecht and Sliedrecht) and Helmond. In both assessments RIVM assumed that the persons in question would eat exclusively home-grown vegetables every day throughout their life. As a worst-case scenario, the calculated exposure is therefore probably higher than the actual exposure to PFOA and GenX of vegetable garden owners in the vicinity of the factories. Information about the amount and frequency in which the vegetables and potatoes are consumed was obtained from the Dutch food consumption survey (Mengelers et al., 2018; Boon et al., 2019).

<u>Dordrecht, Papendrecht and Sliedrecht</u>

RIVM concludes that the TDI for PFOA and the TDI for GenX are not exceeded via food. However, residents are also exposed to these substances via air and drinking water. Therefore, RIVM advises that vegetable garden crops grown within

a radius of one kilometer from the company should be consumed in moderation (not too often or too much). Outside this area, the concentrations were so low that the crops can be safely consumed even if one takes into account the two other sources of exposure (Mengelers et al., 2018).

Helmond
RIVM concludes that persons with a vegetable garden near the company Custom Powders in Helmond can safely eat their home-grown vegetables. In the past, this company emitted the substances PFOA and GenX into the air. However, RIVM-TDI's of PFOA and Genx for exposure were not exceeded by oral intake (Boon et al., 2019).

Other relevant sources of exposure in the vicinity of both companies are drinking water and air (Mengelers et al., 2018; Boon et al., 2019) and possibly sheep meat and milk (FO, 2019b). In Helmond, also swimming water was identified as a potential source of exposure (Beekman, 2018; Muller & te Biesebeek, 2018). These sources need also to be considered to determine whether there is a health risk related to the exposure to PFOA and GenX.

In 2011, Noorlander and colleagues calculated the high level intake (99th percentile) of PFOA via food (flour, fatty fish, lean fish, pork, eggs, crustaceans, bakery products, vegetables/fruit, cheese, beef, chicken/poultry, butter, milk, vegetable oil and industrial oil) and drinking water for the Dutch population, being 0.6 ng/kg body weight per day (Noorlander et al., 2011). This concentration is lower than the EFSA-TDI of 0.8 ng/kg body weight per day.

Conclusion
Despite the fact that the exposure of children and adults to PFOA via the consumption of carp exceeds the provisional EFSA-TDI of 0.8 ng/kg body weight per day, the risk for human health is expected to be low. A TDI is a health based guidance value based on chronic (long term) exposure. This carp was caught in a fishing pond in the close vicinity of the factory of Custom Powders in Helmond. Fish from this pond will probably only, on occasion, be eaten by specific consumers (sport fishermen) leading to acute (short term) exposure. Furthermore, the risk assessment of carp was based on one fish and this fish does not provide an overview of the PFOA distribution in fish from this fishing pond in general.

Based on a comparison with the provisional EFSA-TDI of 0.8 ng/kg body weight per day, the exposure of children and adults to PFOA via the consumption of sheep's milk might pose a risk to human health. The risk assessment for sheep's milk is based on experimental transfer data from two sheep that do not show the same kinetics. Compared to dairy cows, the transfer of PFOA to sheep's milk is higher than one might expect. Therefore, no firm conclusion about the human health risk regarding sheep's milk can be drawn.

The exposure of children and adults to PFOA and GenX via the consumption of cow's milk, meat (cow/sheep), cheese, yoghurt, egg and eel does not pose a risk for human health.

References

ATSDR, 2018. Toxicological Profile for Perfluoroalkyls Draft for Public Comment June 2018. Agency for Toxic Substances and Disease Registry, Division of Toxicology and Human Health Sciences. Available online: https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf

Beekman M, 2018. Voorlopige risicoschatting PFOA in recreatieplas Berkendonk in Helmond. RIVM Memo d.d. 28 mei 2018. Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven.

Beekman M, Zweers P, Muller A, de Vries W, Janssen P & Zeilmaker MJ, 2016. Evaluation of substances used in the GenX technology by Chemours, Dordrecht. RIVM, Bilthoven. Available online: https://www.rivm.nl/bibliotheek/rapporten/2016-0174.pdf

Bokkers B, van de Ven B, Janssen P, Bil W, van Broekhuizen F, Zeilmaker MJ & Oomen A, 2018. Per- and polyfluoralkyl substances (PFASs) in food contact material. RIMV, Bilthoven. Available online: https://www.rivm.nl/bibliotheek/rapporten/2018-0181.pdf

Boon P, Zeilmaker M & Mengelers M, 2019. Risicobeoordeling van GenX en PFOA in moestuingewassen in Helmond. Risk assessment of GenX and PFOA in home-grown garden crops in Helmond. Rijksinstituut voor Volksgezondheid en Milieu. Available online: https://doi.org/10.21945/rivm-2019-0024

Conley JM, Lambright CS, Evans N, Strynar MJ, McCord J, McIntyre BS, Travlos GS, Cardon MC, Medlock-Kakaley E, Hartig PC, Wilson VS & Gray LE, 2019. Adverse Maternal, Fetal, and Postnatal Effects of Hexafluoropropylene Oxide Dimer Acid (GenX) from Oral Gestational Exposure in Sprague-Dawley Rats. Environmental Health Perspectives, 127 (3), 037008. Available online: https://doi.org/doi:10.1289/EHP4372

Danish EPA, 2015. Perfluoroalkylated substances: PFOA, PFOS and PFOSA Evaluation of health hazards and proposal of a health based quality criterion for drinking water, soil and ground water Environmental project No. 1665, 2015. The Danish Environmental Protection Agency, Copenhagen. Available online: https://www2.mst.dk/Udgiv/publications/2015/04/978-87-93283-01-5.pdf

DWQI, 2017. Health-based maximum contaminant level support document: Perfluorooctanoic acid (PFOA). New Jersey Drinking Water Quality Institute - Health Effects Subcommittee. Available online: https://www.nj.gov/dep/watersupply/pdf/pfoa-appendixa.pdf

ECHA, 2015. Committee for Risk Assessment (RAC) Opinion on an Annex XV dossier proposing restrictions on Perfluorooctanoic acid (PFOA), its salts and PFOA-related substances ECHA/RAC/RES-O-0000006229-70-02/F. European Chemicals Agency. Available online: https://echa.europa.eu/documents/10162/3d13de3a-de0d-49ae-bfbd-749aea884966

EFSA, 2012. Perfluoroalkylated substances in food: occurrence and dietary exposure. EFSA Journal, 10 (6), 2743. Available online: https://doi.org/10.2903/j.efsa.2012.2743

EFSA CONTAM Panel, 2018. Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA Journal, 16 (12), e05194. Available online: https://doi.org/doi:10.2903/j.efsa.2018.5194

Eriksen KT, Raaschou-Nielsen O, McLaughlin JK, Lipworth L, Tjønneland A, Overvad K & Sørensen M, 2013. Association between Plasma PFOA and PFOS Levels and Total Cholesterol in a Middle-Aged Danish Population. PLoS One, 8 (2), e56969. Available online: https://doi.org/10.1371/journal.pone.0056969

FO, 2019a. Risk Assessment of GenX and PFOA in food - Part 1: Toxicity of GenX and PFOA and intake through contaminated food of animal origin. Front Office Food and Product Safety, National Institute for Public Health and the Environment and Wageningen University & Research, Bilthoven.

FO, 2019b. Risk Assessment of GenX and PFOA in food - Part 2: Transfer of GenX and PFOA in ditchwater and silage to edible products of food producing animals. Front Office Food and Product Safety, National Institute for Public Health and the Environment, Wageningen University & Research, Bilthoven.

Gannon SA, Fasano WJ, Mawn MP, Nabb DL, Buck RC, Buxton LW, Jepson GW & Frame SR, 2016. Absorption, distribution, metabolism, excretion, and kinetics of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid ammonium salt following a single dose in rat, mouse, and cynomolgus monkey. Toxicology, 340, 1-9. Available online: https://doi.org/10.1016/j.tox.2015.12.006

IARC, 2016. Monograph Perfluorooctanoic acid. International Agency for Research on Cancer,. Available online: https://monographs.iarc.fr/wp-content/uploads/2018/06/mono110-01.pdf

Janssen P, Muller A & Zeilmaker MJ, 2017. Derivation of a lifetime drinking-water guideline for 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903) – Revised version January 2017. RIVM, Bilthoven.

Kowalczyk J, Ehlers S, Furst P, Schafft H & Lahrssen-Wiederholt M, 2012. Transfer of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) from contaminated feed into milk and meat of sheep: pilot study. Arch Environ Contam Toxicol, 63 (2), 288-298. Available online: https://doi.org/10.1007/s00244-012-9759-2

Mengelers M, te Biesebeek J, Schipper M, Slob W & Boon P, 2018. Risicobeoordeling van GenX en PFOA in moestuingewassen in Dordrecht, Papendrecht en Sliedrecht. Risk assessment of GenX and PFOA in vegetable garden crops in Dordrecht, Papendrecht and Sliedrecht. Rijksinstituut voor Volksgezondheid en Milieu RIVM. Available online: https://www.rivm.nl/bibliotheek/rapporten/2018-0017.pdf

Muller A & te Biesebeek JD, 2018. Voorlopige risicoschatting GenX in oppervlaktewater rondom het bedrijf Custom Powders in Helmond. Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven.

Noorlander CW, van Leeuwen SP, Te Biesebeek JD, Mengelers MJ & Zeilmaker MJ, 2011. Levels of perfluorinated compounds in food and dietary intake of PFOS and PFOA in the Netherlands. J Agric Food Chem, 59 (13), 7496-7505. Available online: https://doi.org/10.1021/jf104943p

Perkins RG, Butenhoff JL, Kennedy GL & Palazzolo MJ, 2004. 13-Week Dietary Toxicity Study of Ammonium Perfluorooctanoate (APFO) in Male Rats. Drug and Chemical Toxicology, 27 (4), 361-378. Available online: https://doi.org/10.1081/DCT-200039773

Rijs KJ & Bogers RP, 2017. PFOA exposure and health - A review of scientific literature. National Institute for Public Health and the Environment (RIVM), Bilthoven. Available online: https://www.rivm.nl/bibliotheek/rapporten/2017-0086.pdf

Sheng N, Cui R, Wang J, Guo Y, Wang J & Dai J, 2018. Cytotoxicity of novel fluorinated alternatives to long-chain perfluoroalkyl substances to human liver cell line and their binding capacity to human liver fatty acid binding protein. Arch Toxicol, 92 (1), 359-369. Available online: https://doi.org/10.1007/s00204-017-2055-1

Steenland K, Tinker S, Frisbee S, Ducatman A & Vaccarino V, 2009. Association of Perfluorooctanoic Acid and Perfluorooctane Sulfonate With Serum Lipids Among Adults Living Near a Chemical Plant. American Journal of Epidemiology, 170 (10), 1268-1278. Available online: https://doi.org/10.1093/aje/kwp279

US EPA, 2016. Health Effects Support Document for Perfluorooctanoic Acid (PFOA). U.S. Environmental Protection Agency, Washington, DC. Available online: https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_hesd_final-plain.pdf

van Asselt ED, Kowalczyk J, van Eijkeren JC, Zeilmaker MJ, Ehlers S, Furst P, Lahrssen-Wiederholt M & van der Fels-Klerx HJ, 2013. Transfer of perfluorooctane sulfonic acid (PFOS) from contaminated feed to dairy milk. Food Chem, 141 (2), 1489-1495. Available online: https://doi.org/10.1016/j.foodchem.2013.04.035

van Poll R, 2018. GenX en PFOA in grond en irrigatiewater in moestuinen rondom DuPont Chemours Fase twee van het 'Moestuinonderzoek'. Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven. Available online: https://www.rivm.nl/sites/default/files/2018-11/GenX%20en%20PFOA%20in%20grond%20en%20irrigatiewater%20in%20moestuinen%20rondom%20DuPont%20Chemours.pdf

Zeilmaker MJ, Janssen P, Versteegh A, van Pul A, de Vries W, Bokkers B, Wuijts S, Oomen A & Herremans J, 2016. **Risicoschatting emissie PFOA voor omwonenden** Locatie: DuPont/Chemours, Dordrecht, Nederland. Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven.

Appendix 1: Risk assessment of GenX and PFOA in food
Part 1: Toxicity of GenX and PFOA and intake through contaminated food of animal origin

Office for Risk Assesment & Research

Date

July 16, 2019

Our reference
NVWA/BuRO/2019/4294

Appendix 2: Risk assessment of GenX and PFOA in food
Part 2: Transfer of GenX and PFOA in ditch water and silage to edible products of food producing animals

Office for Risk Assesment & Research

Date

July 16, 2019

Our reference
NVWA/BuRO/2019/4294

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

WAGENINGEN UNIVERSITY & RESEARCH

FRONT OFFICE FOOD AND PRODUCT SAFETY

RISK ASSESSMENT OF GenX AND PFOA IN FOOD
PART 1: TOXICITY OF GenX AND PFOA AND INTAKE THROUGH CONTAMINATED FOOD OF ANIMAL ORIGIN

Risk assessment requested by:	Office for Risk Assessment and Research
Risk assessment performed by:	RIVM and RIKILT[1]
Date of request:	06-06-2018
Date of risk assessment:	17-04-2019
Project number:	V/093130

Subject

In the past, the companies DuPont/Chemours in Dordrecht and Custom Powders in Helmond emitted GenX[1] and perfluoro-octanoic acid (PFOA) into the air. The emission of GenX by DuPont/Chemours is ongoing. Consequently, the area around these companies (soil, water and vegetation) has been polluted. In May 2018, the Netherlands Food and Consumer Product Safety Authority (NVWA) took samples (dairy products, egg, fish and silage) in these areas. At that moment, the detection and quantification limits of the analytical method to analyse these compounds were not low enough for performing a risk assessment. In other words, if all concentrations would be below these limit values, the calculated exposure using concentrations at these limit values (worst case) would exceed the health-based guidance value. In that case, a conclusion about a possible health risk cannot be drawn. RIKILT-WUR resolved this analytical issue, and on 10 January 2019 sent the analysed concentrations of GenX and PFOA in dairy products, egg, fish and silage to the Front Office Food and Product Safety (FO).

Questions

Given the GenX and PFOA concentrations in dairy products, egg and fish, the Office for Risk Assessment and Research (BuRO) has asked the FO several questions, which are answered in this FO assessment (Part 1). BuRO has also asked questions related to the GenX and PFOA concentrations in silage. These questions are answered in a separate FO assessment (Part 2). The questions addressed in this Part 1 FO assessment are:

1. Describe the toxicology of GenX and PFOA.
2. Estimate the intake of GenX and PFOA for consumers based on the measured concentrations of GenX and PFOA in dairy products, egg and fish.
3. Perform a risk assessment of GenX and PFOA in contaminated food of animal origin.

[1] GenX refers to hexafluoropropyleneoxide dimer acid (HPFO-DA), or to its ammonium salt, as used in the GenX technology.

Conclusions

1) In 2017, RIVM derived a tentative tolerable daily intake (t-TDI) of 21 ng/kg body weight (bw) per day for GenX, based on an increased albumin/globulin ratio in serum of rats (Janssen, 2017). For PFOA, RIVM derived a TDI of 12.5 ng/kg bw per day based on liver toxicity in rats in 2016 (Zeilmaker et al., 2016).
2) The exposure to GenX and PFOA through the consumption of dairy products (milk, cheese and yoghurt), egg and eel was negligible. A rough, maximum exposure to GenX and PFOA through the consumption of carp was estimated at 5.3 and 1.3 ng/kg bw per day, respectively, based on
   - concentrations in one carp caught in a fish pond in Helmond;
   - a high consumption level of fish from the Dutch National Food Consumption Survey of 2012-2016.
3) GenX and PFOA concentrations in dairy products (milk, cheese and yoghurt), egg, and fish (eel and carp) do not pose a health risk for people living in the environment of both companies.

Question 1: Toxicology of GenX and PFOA

Below the toxicity data underlying the derivation of the tolerable daily intakes (TDI) of GenX and PFOA used to perform a risk assessment (question 3) are described, as well as the recent EFSA evaluation of PFOA (EFSA, 2018b). For a more extended description of the toxicity of both compounds, see Appendix A for GenX and Appendix B for PFOA.

GenX

The chemicals FRD-902 and FRD-903, also known as "GenX chemicals", are the main substances associated with the GenX processing aid technology that enables the production of fluoropolymers. FRD-902 is the dimer ammonium salt (ammonium-2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate; CAS no. 62037-80-3) and FRD-903 is the dimer acid (2,3,3,3-tetrafluoro-2 (heptafluoropropoxy)propanoic acid; CAS no. 13252-13-6) (Figure 1). Under environmental and physical conditions, such as in water or in blood, FRD-902 and FRD-903 dissociate into the ion HFPO-DA (hexafluoropropyleneoxide dimer acid), which is responsible for the observed toxicological effects. In this assessment, the ion HFPO-DA is called GenX.

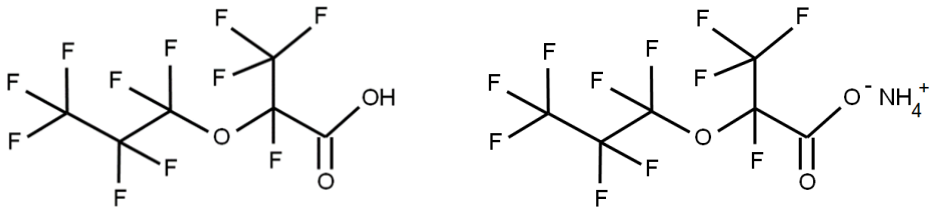

Figure 1. The chemical structure of the acid FRD-903 (left) and the ammonium salt FRD-902 (right)

In 2017, RIVM derived a tentative TDI (t-TDI) of 21 ng/kg bw per day for GenX (Janssen, 2017). This t-TDI was based on an overall no-observed adverse level (NOAEL)[2] of 0.1 mg/kg body weight (bw) per day from a chronic oral study in rats with increased albumin/globulin ratio in serum as the critical effect (Beekman et al., 2016), and the following assessment factors (Janssen, 2017):

- interspecies (for toxicokinetic differences related to metabolic rate): 4
- interspecies (for toxicodynamic differences): 1.8
- intraspecies (standard factor): 10
- extra factor for possible bioaccumulation: 66

[2] The highest dose administered in an animal study at which no adverse effects are observed

PFOA

Perfluoro-octanoic acid (CAS no. 335-67-1; PFOA) and its salts are used as processing aids in the production of fluoro-elastomers and fluoropolymers, with polytetrafluoretheen (PTFE; brand name is 'Teflon') being an important fluoropolymer. In addition, PFOA-related compounds are used as surfactants in non-food applications (in fire-fighting foams, wetting agents and cleaners) and for the manufacture of side-chain fluorinated polymers (used as surface finishes for textiles and apparel, leather, paper and cardboard, paints, lacquers etc.). The chemical structure of PFOA is given in Figure 2.

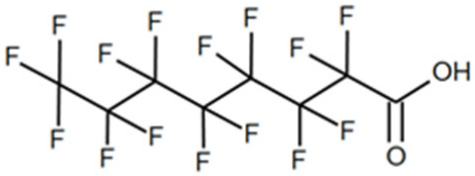

Figure 2. The chemical structure of perfluoro-octanoic acid (PFOA)

In its 2016 risk assessment of PFOA, RIVM concluded that liver effects represented the most sensitive endpoint for PFOA-toxicity (Zeilmaker et al., 2016). According to the approach previously developed for polychlorinated dioxins, which is a group of substances with a high potential for bioaccumulation in humans, RIVM used a quantitative approach to derive a TDI for PFOA based on a critical PFOA blood serum concentration (Zeilmaker et al., 2016). The reason for this is that PFOA belongs to the group of per- and polyfluoroalkyl substances, which has, as dioxins, a high potential to accumulate in humans (EFSA, 2018b).

The TDI was derived from the (mean) NOAEL PFOA concentration of 7.1 µg PFOA/mL in rat from a semi-chronic study by Perkins et al. (2004) using one compartmental modelling to calculate the corresponding chronic human oral dose (Human Equivalent Dose ((HED) of 1000 ng/kg bw per day. The TDI of 12.5 ng/kg bw per day was subsequently derived by dividing this HED by an "overall" assessment factor of 80. This "overall" factor was composed of the following sub-factors:

- Interspecies extrapolation:
  A correction for interspecies differences in kinetics and toxicodynamics was not needed, and therefore the assessment factor was set at 1. Interspecies differences in kinetics were explicitly considered in the derivation of the TDI, and based on mechanistic considerations it was assumed that rats are more sensitive for liver toxicity than humans.
- Intraspecies extrapolation
  In order to correct for intraspecies differences in toxicokinetics and toxicodynamics the default assessment factor of 10 was used.
- Semi-chronic → chronic extrapolation
  The NOAEL was based on a semi-chronic study. To extrapolate the TDI based on semi-chronic exposure to chronic exposure, an assessment factor of 8 was used. This factor was based on an empirically derived distribution for this assessment factor as proposed by the International Programme on Chemical Safety (IPCS) and has a coverage of 95%; there is a 95% confidence that this factor is sufficiently large to account for possible semi-chronic versus chronic differences in toxicity (for technical details, see Zeilmaker et al., 2016, Annex TA-3).

This approach of deriving a TDI is in agreement with the approach used by the US EPA (2016), DWQI (2017) and ATSDR (2018).

EFSA (2018b) has recently re-evaluated PFOA (and perfluoroctane sulfonic acid (PFOS)) and derived a health-based guidance value (HBGV) for both compounds based on health effects from epidemiological studies. For PFOA, the critical effect was an increase in serum total cholesterol. Based on benchmark modelling, EFSA established a tolerable weekly intake (TWI) of 6 ng/kg bw per week for PFOA (equivalent to 0.8 ng/kg bw per

day). PFOA (and PFOS) belong to the group of per- and polyfluoroakyl substances (PFAS). In 2019, EFSA will finalise a scientific opinion on "The risk to human health related to the presence Perfluoroalkylated substances, other than Perfluorooctane sulfonate and Perfluorooctanoic acid, in food" (EFSA-Q-2017-00549, scheduled December 2019)[3], with the possible application of the forthcoming Scientific Committee guidance on combined exposure to multiple chemicals[4]. Until then, EFSA's derived tolerable weekly intake for PFOA (as well as for PFOS) has to be considered provisional.

In general, RIVM follows the HBGVs derived by EFSA. However, in the case of PFOA, RIVM has questioned EFSA's HBGV derivation (EFSA, 2018a)[5]. Given EFSA's ongoing evaluation, RIVM maintains presently its own TDI for PFOA. However, also risk assessments based on this HBGV should be considered provisional until the EFSA evaluation is finalised.

Question 2 and 3: Exposure and risk assessment

Concentration GenX and PFOA in dairy products, egg and fish
GenX and PFOA were analysed in dairy products (milk, cheese and yoghurt), egg and fish sampled near the companies Chemours/DuPont in Dordrecht and Custom Powders in Helmond. Table 1 lists the product concentrations per location as provided by RIKILT-WUR. The majority of the concentrations were below the limit of quantification (LOQ). Only the PFOA concentration in one egg sampled in Dordrecht and that of both compounds in one carp caught in a fish pond in Helmond were above the LOQ (Table 1).

Table 1. Analysed concentrations of GenX and PFOA in dairy products, egg and fish sampled near the companies Chemours/DuPont in Dordrecht and Custom Powders in Helmond

Product and location	Concentration (ng/g)[1]		
	n	GenX	PFOA
Dordrecht			
Dairy products			
Milk[2]	15	<0.10	<0.01
Cheese[2]	1	<0.10	<0.10
Yoghurt[2]	1	<0.10	<0.10
Egg[3]	1	<0.25	0.14
Helmond			
Dairy products			
Milk[2]	2	<0.10	<0.01
Egg[3]	1	<0.25	<0.025
Fish			
Eel (farmed)	1	<0.10	<0.05
Carp	1	4.7	1.3

PFOA: perfluoro-octanoic acid
[1] Samples with concentrations reported as '<' may contain GenX and PFOA, but the concentrations did not exceed the limit of quantification of the analytical method
[2] Cow and one sample of goat in Dordrecht
[3] Chicken

20% TDI concentrations
In September 2018, the FO calculated how low the LOQ for the analysis of GenX and PFOA in animal products should be for performing a risk assessment. For this, GenX and PFOA concentrations for egg, meat (beef) and cow's milk were calculated at which a high consumption of each product would result in an exposure equal to 20% of the TDI of

[3] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2017-00549
[4] https://www.efsa.europa.eu/en/topics/topic/chemical-mixtures
[5] https://www.rivm.nl/en/news/discussion-regarding-health-based-guidance-value-of-pfoa

GenX or PFOA (FO, 2018). The percentage of 20% accounted for the exposure to GenX and PFOA through the consumption of other foods than the product itself. In this present assessment, these concentrations are referred to as '20% TDI concentrations'. No such concentrations were derived for cheese and yoghurt.

These 20% TDI concentrations were calculated using high consumptions of egg, meat (beef) and milk on an arbitrary day among children and adults and corresponding body weights (Table 2) combined with the RIVM TDIs of GenX and PFOA (see question 1) with the following equation:

$$20\%\ TDI\ concentration = \left(\frac{TDI}{(High\ consumption \div Body\ weight)}\right) \div 5 \qquad \text{Equation 1}$$

20% TDI concentration	= Concentration of GenX and PFOA at which a high consumption of a product results in an exposure equal to 20% of the TDI in ng/g
TDI	= Tolerable daily intake of GenX and PFOA in ng/kg bw per day
High consumption	= High consumption of a product in gram per day
Body weight	= Body weight in kg

The 20% TDI concentrations per product that were calculated in this way are listed in Table 3. If the measured concentrations of GenX and PFOA are below these 20% TDI concentrations, a health risk can be excluded. If the measured concentrations are higher, a risk assessment is required to assess possible health risks.

Table 2. High consumptions on an arbitrary day per product used to derive the 20% TDI concentrations for GenX and PFOA

Product	Age (years)	Body weight (kg)	High consumption (gram)
Egg	2 - 6	18.8[1]	65[3]
Meat (beef)	19 - 69	66[2]	210[4]
Milk	2 - 6	18.8	750[5]

bw: body weight; DNFCS: Dutch Food Consumption Survey; TDI: tolerable daily intake
[1] The average body weight of children aged 2 to 6 years in DNFCS 2005-2006 among children within this age range (Ocké et al., 2008).
[2] This body weight is the weight of boys aged 14 to 18 in DNFCS 2007-2010 (van Rossum et al., 2011). The average body weight in this DNFCS for the adult population from which the estimated meat (beef) consumption was obtained (footnote 4 of this table) was about 80 kg. The high consumption per kg bw (210/66 = 3.2 g/kg bw) used to derive the 20% TDI concentrations for meat (beef) was therefore higher than when the average body weight for adults had been used (210/80 = 2.6 g/kg bw). This has resulted in lower (more conservative) 20% TDI concentrations.
[3] The consumed amount of egg was comparable to the 95th percentile consumption of boiled egg in DNFCS 2005-2006 among children aged 2 to 6 and equals about one large egg.
[4] This consumed amount of meat (beef) was equal to the 95th percentile of consumption of meat (beef) by persons aged 19-69 in DNFCS 2007-2010.
[5] This consumed amount of milk was higher than the maximally reported consumption of 520 gram of semi-skimmed milk reported in DNFCS 2005-2006 among children aged 2 to 6.

Table 3. The 20% TDI concentrations for GenX and PFOA per product[1]

Product	20% TDI concentration (ng/g)	
	GenX	PFOA
Egg	1.2	0.7
Meat (beef)	1.3	0.8
Milk	0.1	0.06

PFOA: perfluoro-octanoic acid; TDI: tolerable daily intake
[1] See equation 1 and Table 2
[2] TDI for GenX is 21 ng/kg bw per day and for PFOA 12.5 ng/kg bw per day

Exposure and risk assessment of dairy products, egg and eel
The LOQs of the analytical method were either equal (GenX in milk) or lower (GenX in egg and PFOA in milk and egg) than the 20% TDI concentrations (Table 1 and 3). Assuming that eel is not consumed at higher amounts than meat (beef), also the analytical LOQ of eel was below the corresponding 20% TDI concentration. As the concentrations are all at or below the 20% TDI concentration, these products (milk, egg and eel) are not likely to pose a health risk, even if consumed in combination. The reasons for this are:

1. GenX and PFOA may cause adverse effects if ingested at doses higher than the TDI over a lifelong period. However, the consumed amounts on which the 20% TDI concentrations were based reflect large consumed amounts on an arbitrary day. These amounts overestimate the consumed amounts over a lifelong period, when large consumed amounts of a food will be alternated with lower amounts or days on which these products are not consumed;
2. The 20% TDI concentrations were set in such a way that the exposure to GenX and PFOA would equal 20% of the TDI if analysed concentrations were at these concentrations;
3. Except for GenX in milk, the LOQs used in the analyses were even lower than the 20% TDI concentrations (Table 1 and 3).

To substantiate this further, we performed a rough, worst case exposure assessment. For this, we selected the maximum consumption of milk, eel and eggs as such per kg body weight on one of the two recording days in the Dutch National Food Consumption Survey (DNFCS) of 2012-2016 among persons aged 1 to 79[6] (Table 4). The highest consumed amounts of milk and egg were reported for a 1-year old child and that of eel by an adult aged 59. The adult with the maximum consumption of eel also consumed milk and egg on that specific day. Using the analysed concentration of PFOA in egg (0.14 ng/g) and assuming that the concentrations below LOQ equalled a concentration at the LOQ, the GenX and PFOA intake for these three persons would maximally equal 14% of both TDIs (Table 4). These intakes reflect a possible intake on one day and overestimate the intake expected over a lifelong period, the relevant time period for GenX and PFOA exposure (see above).

For cheese and yoghurt, the analysed concentrations were also below the LOQ (Table 1). For these products, no 20% TDI concentrations were derived for risk assessment (FO, 2018). As the analytical LOQs were only slightly higher than the 20% TDI concentrations' for milk, it is not likely that the analysed levels would have resulted in an intake of GenX and PFOA that would pose a health risk. The consumption of yoghurt and cheese is not likely to exceed 750 gram per day in children aged 2 to 6 (Table 2).

Risk assessment of GenX and PFOA through the consumption of carp
Using the same assumption for carp as for eel, i.e. the 20% TDI concentration for meat is also applicable to carp, the analysed concentrations of GenX and PFOA in carp were above the 20% TDI concentration for meat (beef). We therefore performed a risk assessment for carp to establish if the consumption of this fish could pose a health risk. The carp was caught in a fish pond in the vicinity of Custom Powders in Helmond. The concentrations refer to the concentrations in fish meat.

To assess the risk, we first calculated the exposure to GenX and PFOA using the food consumption data among 4313 individuals aged 1 to 79 of DNFCS 2012-2016. In this survey, individuals, or their caretakers in case of young children, recorded what and how much they consumed on two arbitrary days. As there are no consumption levels of carp in this survey, the consumption of fish was used as a proxy. Fish included all types of

[6] Wateetnederland.nl

Table 4. Maximal consumed amounts per kg body weight for milk, eel and eggs on one of the two recording days in DNFCS 2012-2016

Age (years)	Body weight (kg)	Product	Consumed amount (gram/kg bw)	Concentration (ng/g)		Exposure (ng/kg bw)				Exposure as % of the TDI[1]	
						Per product		On one day			
				GenX	PFOA	GenX	PFOA	GenX	PFOA	GenX	PFOA
1	8.6	Milk	29.9	0.1	0.01	3	0.3	3	0.3	14	2
		Eel	-	0.1	0.05	-	-				
		Egg		0.25	0.14						
59	60	Milk	7.1	0.1	0.01	0.7	0.07	1.4	0.4	7	3
		Eel	5	0.1	0.05	0.5	0.25				
		Egg	0.8	0.25	0.14	0.2	0.1				
1	11.2	Milk	-	0.1	0.01	-	-	2.2	1.3	10	10
		Eel		0.1	0.05						
		Egg	8.9	0.25	0.14	2.2	1.3				

bw: body weight; DNFCS: Dutch National Food Consumption survey; PFOA: perfluoro-octanoic acid; TDI: tolerable daily intake
[1] TDI for GenX is 21 ng/kg bw per day and for PFOA 12.5 ng/kg bw per day

fish, such as salmon, tuna and pangasius. The consumption of crustaceans and fish products, such as fish fingers, was not included as being considered not representative of the amounts in which carp may be consumed. Table 5 lists the mean and high (95th percentile) consumption of fish for different age groups and sex for all days in the survey and for only those days on which the consumption of fish was reported ("consumption days").

Table 5. Mean and high (95th percentile) consumed amounts of fish1 per age group and sex based on DNFCS 2012-2016

Age group (year) + sex	Consumed amount (gram per day)				Percentage consumption days[4]
	All days[2]		Consumption days only[3]		
	Mean	High	Mean	High	
1-3	1.5	14.5	46.5	122	4
4-8	3	24.5	71.5	178.5	5
9-18 man	3	15	73.5	219	5
9-18 female	3.5	37	63.5	141	6
19-50 man	12	88	119	286	10
19-50 female	10	71	91	208	11
51-79 man	18	101	126	301.5	15
51-79 female	15.5	80	97.5	239	16

DNFCS: Dutch National Food Consumption Survey
[1] Based on consumed amounts of all types of fish reported in DNFCS 2012-2016. Consumed amounts of crustaceans and fish products were not included.
[2] Based on all days, irrespective of whether fish was consumed or not. Mean and high (95th percentile) consumed amounts were calculated based on mean consumed amounts across the two consumption days per individual
[3] Based on only the days on which the consumption of fish was reported. Mean and high (95th percentile) consumed amounts were calculated based on consumed amounts per consumption day per individual
[4] Percentage of the days on which consumption of fish was reported.

GenX and PFOA may be harmful when ingested at amounts above the TDI over a lifelong period. Therefore, we should use the consumed amounts of fish that best reflect lifelong consumed amounts of fish for the risk assessment. Based on the information in Table 5, the best estimate for this is the consumed amounts based on 'all consumption days', assuming that individuals are not likely to consume daily fish obtained from the fish pond. We used the high (95th percentile) consumed amounts to estimate the intake of GenX and PFOA to also protect possible high consumers of fish (Table 5).

Based on the analysed concentrations of GenX and PFOA in carp and high consumed amounts of fish per age group and sex, the intake was estimated per age – sex group using the following equation:

$$Intake = \frac{Consumption \times Concentration}{Body\ weight}$$ Equation 2

Intake	= Intake of GenX and PFOA in ng/kg bw per day
Consumption	= Consumption of carp in gram (Table 5)
Concentration	= Concentration of GenX and PFOA in carp in ng/g (Table 1)
Body weight	= Body weight in kg (Table 5)

Using this equation, the maximum intake of GenX and PFOA was estimated at 5.3 ng/kg bw per day for GenX and 1.5 ng/kg bw per day for PFOA, both in men aged 51 to 79 (Table 6). The maximum exposure to GenX and PFOA through the consumption of carp was equal to 25% and 12% of the TDI, respectively. Therefore, the consumption of this specific fish at the analysed concentrations does not pose a health risk for GenX and PFOA.

Table 6. Intake of GenX and PFOA through a high (95th percentile) consumption of fish contaminated with GenX and PFOA at 4.7 and 1.3 ng/g[1], respectively

Age group (year) + sex	High consumption of fish (gram per day)[2]	Body weight (kg)	Intake			
			ng/kg bw per day		% of the TDI[3]	
			GenX	PFOA	GenX	PFOA
1-3	14.5	14.5	4.9	1.4	23	11
4-8	24.5	24.5	4.8	1.3	23	10
9-18; man	15	56.6	1.3	0.4	6	3
9-18; female	37	55.2	3.3	0.9	16	7
19-50; man	88	84.6	4.9	1.4	23	11
19-50; female	71	75.7	4.4	1.2	21	10
51-79; man	101	88.8	5.3	1.5	25	12
51-79; female	80	76.9	4.9	1.4	23	11

bw: body weight; TDI: tolerable daily intake
[1] Concentration analysed in fish meat of carp
[2] High consumption based on all consumption days within the food consumption survey, irrespective of whether fish was consumed (Table 5)
[3] TDI for GenX is 21 ng/kg bw per day and for PFOA 12.5 ng/kg bw per day

Discussion points

- The risk assessment of GenX and PFOA in carp was based on only one concentration per compound. There is no information available about the distribution of concentrations of GenX and PFOA in carp present in the same fish pond, as of other fish present that may be caught for consumption. It is therefore unclear if the analysed concentrations are representative of those in carp and other fish that may be caught in the future or have been caught in the past. Because of this, the intake of GenX and PFOA may be under- or overestimated. Also the number of concentrations of GenX and PFOA analysed in dairy products, egg and eel was limited, except possibly for milk sampled in Dordrecht.
  Due to the absence of consumed amounts of fish by persons fishing in the fish pond, consumed amounts of fish by the general Dutch population were used in the risk assessment. Persons fishing in this fish pond possibly consume fish more frequently than the general population. They may also consume fish in larger amounts when eating fish. By using the consumed amount at the 95th percentile of the consumption distribution, this was partly addressed.
- People living in the vicinity of both companies are not only exposed to GenX and PFOA through the consumption of dairy products, egg and fish. Other relevant sources of exposure in the vicinity of both companies are home grown vegetables, fruits and potatoes, drinking water and air (Mengelers et al., 2018; Boon et al., 2019), and possibly meat (Part 2). In Helmond, also swimming water was identified as a potential source of exposure (Beekman, 2018; Muller & te Biesebeek, 2018). These sources need to be considered to determine whether there is a health risk related to the exposure to these compounds.

Overall conclusion

1) In 2017, RIVM derived a tentative tolerable daily intake (t-TDI) of 21 ng/kg body weight (bw) per day for GenX, based on an increased albumin/globulin ratio in serum of rats (Janssen, 2017). For PFOA, RIVM derived a TDI of 12.5 ng/kg bw per day based on liver toxicity in rats in 2016 (Zeilmaker et al., 2016).
2) The exposure to GenX and PFOA through the consumption of dairy products (milk, cheese and yoghurt), egg and eel was negligible. A rough, maximum exposure to GenX and PFOA through the consumption of carp was estimated at 5.3 and 1.3 ng/kg bw per day, respectively, based on
   - concentrations in one carp caught in a fish pond in Helmond;
   - a high consumption level of fish from DNFCS 2012-2016.

3) GenX and PFOA concentrations in dairy products (milk, cheese and yoghurt), egg, and fish (eel and carp) do not pose a health risk for people living in the environment of both companies.

References

ATSDR (2018). Toxicological profile for perfluoroalkyls. Draft for public comment June 2018. Agency for Toxic Substances and Disease Registry, Division of Toxicology and Human Health Sciences. Available online: www.atsdr.cdc.gov.

Beekman M (2018). Voorlopige risicoschatting PFOA in recreatieplas Berkendonk in Helmond. RIVM Memo d.d. 28 mei 2018. Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven.

Beekman MPZ, Muller A, de Vries W, Janssen P, Zeilmaker M (2016). Evaluation of substances used in the GenX technology by Chemours, Dordrecht. RIVM Letter report 2016-0174. National Institute for Public Health and the Environment (RIVM), Bilthoven. Available online: www.rivm.nl.

Boon PE, Zeilmaker MJ, Mengelers MJ (2019). Risicobeoordeling van GenX en PFOA in moestuingewassen in Helmond. RIVM Briefrapport 2019-0024. Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven. Available online: www.rivm.nl.

Brendel, S., Fetter, E., Staude, C., Vierke, L., and Biegel-Engler, A. (2018). Short-chain perfluoroalkyl acids: environmental concerns and a regulatory strategy under REACH. Environ Sci Eur, 30:9, 1-11.

Caverly Rae JM, Craig L, Slone TW, Frame SR, Buxton LW, Kennedy GL (2015). Evaluation of chronic toxicity and carcinogenicity of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate in Sprague-Dawley rats. Toxicol Rep, 2, 939-949.

DWQI (2017). Health-based maximum contaminant level support document: Perfluorooctanoic acid (PFOA) New Jersey Drinking Water Quality Institute - Health Effects Subcommittee February 15, 2017.

EFSA (2018a). Minutes of the expert meeting on perfluooroctane sulfonic acid and perfluorooctanoic acid in food assessment. EFSA/CONTAM/3503. European Food Safety Authority, Parma. Beschikbaar online: www.efsa.europa.eu.

EFSA (2018b). Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA Journal 2018;16(12):5194, 284 pp., doi: 10.2903/j.efsa.2018.5194. Beschikbaar online: www.efsa.europa.eu.

FO (2018). Discussiestuk benodigde kwantificeringslimiet (LOQ) voor de risicoschatting van PFOA en GenX in verschillende voedingsmiddelen. Front Office Voedsel- en Productveiligheid, Rijksinstituut voor Volksgezondheid en Milieu RIVM, RIKILT-WUR, Bilthoven, Wageningen.

Gannon SA, Fasano WJ, Mawn MP, Nabb DL, Buck RC, Buxton LW, Jepson GW, Frame SR (2016). Absorption, distribution, metabolism, excretion, and kinetics of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid ammonium salt following a single dose in rat, mouse, and cynomolgus monkey. Toxicol, 340, 1-9.

Hall AP, Elcombe CR, Foster JR, Harada T, Kaufmann W, Knippel A. Kuttler K, Malarkey DE, Maronpot RR, Nishikawa A, Nolte T, Schulte A, Strauss V, York MJ (2012). Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop. Toxicol Pathol, 40, 971-94.

Janssen P (2017). Bijlage bij brief 0148/2016/M&V/EvS/AV. Derivation of a lifetime drinking-water guideline for 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903) – Revised version January 2017.

Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM, Deluca JG, Lai DY, Mckee RH, Peters JM, Robert RA,Fenner-Crisp PA (2003). PPARalpha agonist-induced rodent tumors: modes of action and human relevance. Crit Rev Toxicol, 33, 655-780.

Li CH, Ren XM, GUO LH (2019). Adipogenic Activity of Oligomeric Hexafluoropropylene Oxide (Perfluorooctanoic Acid Alternative) through Peroxisome Proliferator-Activated Receptor gamma Pathway. Environ Sci Technol.

Mengelers MJB, te Biesebeek JD, Schipper M, Slob W, Boon PE (2018). Risicobeoordeling van GenX en PFOA aanwezig in moestuingewassen in Dordrecht, Papendrecht en Sliedrecht. RIVM Briefrapport 2018-0017. Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven. Available online: www.rivm.nl.

Muller A, te Biesebeek JD (2018). Voorlopige risicoschatting GenX in oppervlaktewater rondom het bedrijf Custom Powders in Helmond. Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven.

Ocké MC, van Rossum CTM, Fransen HP, Buurma EJM, de Boer EJ, Brants HAM, Niekerk EM, van der Laan JD, Drijvers JJMM, Ghameshlou Z (2008). Dutch National Food Consumption Survey - Young children 2005/2006. RIVM Report 350070001/2008. National Institute for Public Health and the Environment (RIVM), Bilthoven. Available online: www.rivm.nl.

OECD (2013). OECD/UNEP Global PFC Group, Synthesis paper on per- and polyfluorinated chemicals (PFCs), Environment, Health and Safety, Environment Directorate, OECD.

OECD (2015). Working towards a global emission inventory of PFASs: Focus on PFCAs - Status quo and the way forward, OECD Environment, Health and Safety Publications Series on Risk Management No. 30.

OECD (2018). Toward a new comprehensive global database of per- and polyfluoroalkyl substances (PFASs): Summary report on updating the OECD 2007 list of per- and polyfluoroalkyl substances (PFASs) Series on Risk Management No. 39, ENV/JM/MONO(2018)7.

Perkins, R, Butenhoff J, Kennedy G, Palazzolo M. (2004) 13-Week dietary study of ammonium perfluoroctanoate (APFO) in male rats. Drug Chem Tox, 27, 361-378 (as cited in Zeilmaker et al., 2016).

Rijs K, Bogers R (2017). PFOA exposure and health – A review of scientific literature. RIVM Report 2017-0086. National Institute for Public Health and the Environment (RIVM), Bilthoven. Available online: www.rivm.nl.

Rushing BR, Hu Q, Franklin JN, McMahen R, Dagnino S, Higgins CP, Strynar MJ, DeWitt JC (2017). Evaluation of the immunomodulatory effects of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate in C57BL/6 mice. Toxicol Sci.

Sheng N, Cui R, Wang J, Guo Y, Wang J, Dai J (2018). Cytotoxicity of novel fluorinated alternatives to long-chain perfluoroalkyl substances to human liver cell line and their binding capacity to human liver fatty acid binding protein. Arch Toxicol, 92, 359-369.

US EPA (2016). Health Effects Support Document for Perfluorooctanoic Acid (PFOA). EPA 822-R-16-003 May 2016. U.S. Environmental Protection Agency, Washington DC. Available online: www.epa.gov.

US EPA (2018). Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3): Also Known As "GenX Chemicals". U.S. Environmental Protection Agency, Washington DC. Available online: www.epa.gov.

van Rossum CTM, Fransen HP, Verkaik-Kloosterman J, Buurma-Rethans EJM, Ocké MC (2011). Dutch National Food Consumption Survey 2007-2010. Diet of children and adults aged 7 to 69 years. RIVM Report 350050006/2011. National Institute for Public Health and the Environment (RIVM), Bilthoven. Beschikbaar online: www.rivm.nl.

Wang J, Wang X, Sheng N, Zhou X, Cui R, Zhang H, Dai J (2017). RNA-sequencing analysis reveals the hepatotoxic mechanism of perfluoroalkyl alternatives, HFPO2 and HFPO4, following exposure in mice. J Appl Toxicol, 37, 436-444.

Yang CH, Glover KP, Han X (2010). Characterization of cellular uptake of perfluorooctanoate via organic anion-transporting polypeptide 1A2, organic anion transporter 4, and urate transporter 1 for their potential roles in mediating human renal reabsorption of perfluorocarboxylates. Toxicol Sci, 117, 294-302.

Zeilmaker MJ, Janssen P, Versteegh A, van Pul A, de Vries W, Bokkers B, Wuijts S, Oomen A, Herremans J (2016). Risicoschatting emissie PFOA voor omwonenden. Locatie: DuPont/Chemours, Dordrecht, Nederland. RIVM Letter report 2016-0049. National Institute for Public Health and the Environment (RIVM), Bilthoven. Available online: www.rivm.nl.

Appendix A Toxicity of GenX

The main body of information regarding mammalian toxicity are studies performed with FRD-902. Read-across of the toxicological properties of FRD-902 to FRD-903 is considered justified, because under environmental conditions, such as in water or in blood, both FRD-902 and FRD-903 will dissociate into the ion HFPO-DA (hexafluoropropyleneoxide dimer acid ion), which is responsible for the observed toxicological effects. In this assessment, the ion HFPO-DA is called GenX.

The large majority of the toxicity data is obtained from the REACH registration dossier of FRD-902. Additionally, the original study reports for FRD-902 (which largely comprise the same information as in the REACH registration dossier) and the original study reports for FRD-903, published via the Health & Environmental Research Online (HERO) database of the US EPA, are the main sources of information. Other than that, six scientific publications and two scientific reports are available in the public literature (Beekman et al., 2016; Caverly Rae et al., 2015; Gannon et al., 2016; Li et al., 2019; Rushing et al., 2017; Sheng et al., 2018; US EPA, 2018; Wang et al., 2017).

The REACH registration dossier and the HERO database contain:

- *in vitro* and *in vivo* studies on toxicokinetics,
- *in vitro* and *in vivo* studies on skin irritation/corrosion,
- an *in vivo* study on eye irritation,
- subacute and subchronic oral toxicity studies in rats and mice,
- a chronic oral toxicity/carcinogenicity study in rats,
- *in vitro* and *in vivo* genotoxicity studies,
- an oral prenatal developmental toxicity study in rats,
- an oral reproduction/developmental screening toxicity study in mice.

All studies were performed according to OECD test guidelines, except for the studies covering toxicokinetics. Additional information for GenX is available for:

- immunotoxicity (Rushing et al., 2017),
- half-lives in experimental animals (Gannon et al., 2016),
- carcinogenicity (Caverly Rae et al., 2015),
- *in vitro* liver protein binding (Sheng et al., 2018),
- mode of action (Li et al., 2019; Wang et al., 2017).

No epidemiological studies are available for GenX.

Both GenX and PFOA are part of the subgroup of perfluoroalkyl acids (PFAAs), which belong to a larger group of per- and polyfluoroalkyl substances (PFASs) (OECD 2013; 2015; 2018). Many PFASs can degrade to PFAAs that are their final degradation products under relevant environmental conditions. These PFAAs, both with short- and long chain length, show high persistency due to the bond between carbon and fluorine. This bond is stable and requires high energy input to break (Brendel et al., 2018). Besides that GenX and PFOA show high persistency, the substances show also similarities in toxicological profiles in experimental animals at comparable doses (e.g. carcinogenicity, liver toxicity) as illustrated in this Appendix and Appendix B.

The biokinetics of GenX were studied in rats, mice and monkeys (Gannon et al., 2016). The results indicate that GenX has a lower potential for bioaccumulation compared to PFOA in these species (half-lives between hours and days for GenX and between hours and weeks for PFOA). For other PFASs, such as PFOA, it is known that the human half-life is significantly higher compared to other species, which cannot solely be explained by allometric differences. Whether interspecies differences in terms of bioaccumulation also apply to GenX is uncertain, because half-life data for GenX in humans are lacking. This issue is currently under investigation in a Substance Evaluation under REACH, where more information is requested on the half-life of GenX in humans by performing a human biomonitoring study in volunteering workers at a manufacturing site.

In contrast to the classic lipophilic organic pollutants that primarily bind to fatty tissues, PFASs primarily bind to proteins. The limited data available suggests that GenX binds to fatty acid-binding proteins in the liver (Sheng et al., 2018) and to serum proteins (i.e. albumin) in blood. Although no data are available on a direct interaction of GenX with albumin, toxicokinetic data illustrates that GenX mainly distributes to the liver and the blood. Overall, tissue and serum concentrations are higher in males compared to females, suggesting that females are able to eliminate GenX more effectively. Protein binding may be one of the aspects leading to slower elimination, resulting in a longer half-life in humans. Additionally, it is argued that the half-life of PFOA is longer in humans compared to other species, because of stronger reabsorption from the lumen of the kidney back into the blood by organic anion transporters (OATs) in humans (Yang et al., 2010). No data is available on OAT efficacy for GenX in humans. It is therefore not known what effect GenX has on the functioning of OATs and if resorption of GenX in the lumen of the kidney will occur in humans or not.

Chronic exposure to GenX results in a statistically significant induction of pancreatic acinar cell adenomas/carcinomas and Leydig cell tumours in male rats at 50 mg/kg bw per day, and a statistically significant induction of hepatocellular adenomas and carcinomas in female rats at 500 mg/kg bw per day (Caverly Rae et al., 2015). All genotoxicity tests carried out with GenX (Ames test, *in vitro* mammalian cell gene mutation assay, *in vitro* mammalian cell chromosome aberration test, and *in vivo* micronucleus-test in mice) were negative. In 2016, RIVM (Beekman et al., 2016) concluded that the available mutagenicity studies and mechanistic information indicate a non-genotoxic mode of action for the observed tumours in the 2-year study. There is concern that GenX may be a human carcinogen as well, but data are currently insufficient to conclude on the substance's full carcinogenic potential. Therefore, this issue is currently under investigation in a Substance Evaluation under REACH, where more information is requested on the carcinogenic potential of GenX in mice.

Apart from the tumorigenic response in rats, the main affected organs in rodents resulting from repeated exposure to GenX are the liver, the kidneys, the haematological system, and the immune system. In rodents, GenX consistently caused increases in liver weight, changes in clinical chemistry parameters related to liver toxicity (AST, ALT, ALP), decreased serum cholesterol, liver hypertrophy, and liver microscopical changes (i.e. single cell necrosis and (multi)focal necrosis). Single cell necrosis was observed in male mice at doses as low as 0.5 mg/kg bw per day. Overall, males showed higher sensitivity to the substance compared to females, with liver effects being more severe and occurring at lower doses.

In the kidneys, exposure to GenX resulted in increased kidney weight, kidney hypertrophy, microscopically observed kidney damage, and increased blood urea nitrogen in rodents. These kidney effects generally occur at higher concentrations than the observed liver effects (5 mg/kg bw per day and above), and are more apparent in females compared to males.

Haematological effects include changes in red blood cell parameters (e.g. decreased number of red blood cells, decreased haemoglobin). These changes are overall relatively mild, with parameters not exceeding 10% change from control up to dosages of 50 mg/kg bw per day in a chronic study in rats. However, data from female rats dosed at 1000 mg/kg bw per day under a subchronic exposure regimen illustrate that FRD-902 may promote severe anaemic conditions.

Increased albumin (A) and/or decreased globulin (G), and associated increases in A/G ratio occurred in rodents administered with 1 mg FRD-902/ kg bw per day and above. Decreased globulin and corresponding increases in A/G ratio are considered early signs of potential reduced immune function. Furthermore, Rushing et al. (2017) observed suppression of the T cell-dependent antibody response (TDAR) in females and increased

T lymphocyte numbers (but no suppression of TDAR) in males exposed to the substance at a dose of 100 mg/kg bw per day for 28 days. Additionally, decreased spleen weight was detected in some studies. This suggests that the immune system might be affected upon treatment with GenX, but there is little information available, which hampers full assessment of the immunomodulatory effects of GenX.

With regard to developmental toxicity, GenX crosses the placenta and distributes into the foetus, and causes early deliveries and decreased birth weight in pups without causing severe parental toxicity at 100 mg/kg bw per day. Maternal toxicity includes mortality, lower mean body weight gain and decreased food consumption, decreased gravid uterine weight, higher mean kidney weight, liver hypertrophy, and microscopic changes in the liver at 1000 mg/kg bw per day. Also, at 100 mg/kg bw per day, GenX caused a mean decreased gravid uterine weight and focal necrosis in the liver. RIVM (Beekman et al., 2016) set the NOAEL for developmental toxicity at 10 mg/kg bw per day, based on early deliveries and decreased birth weight in pups.

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

Based on the currently available data, FRD-902 does cause serious eye damage, but does not result in skin irritation. Furthermore, FRD-903 was graded as corrosive to the skin in an *in vitro* Corrositex assay. FRD-902 is not considered to be a skin sensitizer. No information is available on respiratory sensitisation. Lastly, no studies are available providing insight into potential endocrine (disrupting) mode of action for GenX, such as *in vivo* modulation of thyroid hormone (T3, T4 and TSH), androgenic/estrogenic effects, or *in vitro* receptor binding studies.

The observed liver effects are suggested to be (at least partly) explained (directly or indirectly) by activation of the peroxisome proliferator-activated receptor alpha (PPARα), a biological pathway mainly responsible for lipid metabolism. This is, amongst others, indicated by treatment-related increases in fatty acid beta oxidation in rodents upon exposure to GenX, and a mechanistic study which showed that many lipid metabolism associated genes were upregulated in mice treated with GenX for 28 days (Wang et al., 2017). Rodents are known to be more susceptible to PPARα mediated liver effects (i.e. hepatocellular hypertrophy and increased liver weight) (Hall et al., 2012) and PPARα mediated formation of liver tumours (Klaunig et al., 2003) than are primates. For PFOA the interaction with PPARα in rodents has been studied extensively (especially many studies using *pparα* knockout or *pparα* humanised mice) and the effects seen in rodents with GenX closely match those seen with PFOA.

It should however be noted in this context that the liver effects for PFOA in rodents have been demonstrated to be in part non PPARα mediated, and whether this also is the case for GenX still needs further inquiry. At least it cannot be excluded that the liver effects as well as the tumorigenic responses are induced by a non-PPARα related mode of action. A recent study has indicated that GenX is able to bind to- and to activate PPARγ (Li et al., 2019), just as PFOA. Moreover, it should also be noted that although rodents are more susceptible than humans, PPARα mediated effects do occur in humans: this is clearly demonstrated by the fact that certain drugs (fibrates) that act via PPARα activation are applied in human medicine as a treatment for cholesterolemia. Based on the above, the hepatic effects as seen in rodents after treatment with GenX and PFOA are considered

relevant for the risk assessment for humans. This is in agreement with RIVM (Beekman et al., 2016; Zeilmaker et al., 2016a), US EPA (2016), DWQI (2017) and ATSDR (2018).

Appendix B. Toxicity of PFOA

Production and use of PFOA have been reduced significantly following the discovery of its wide-spread occurrence in the environment and in humans due to its persistence and its bioaccumulative potential in addition to its toxic potential.

The toxicity and toxicokinetics of PFOA have been studied extensively in experimental animals and in human biomonitoring and epidemiology studies. Comprehensive reviews of these data are provided by US EPA (2016), DWQI (2017), ATSDR (2018) and EFSA (2018b). RIVM has evaluated the toxicity and toxicokinetics of PFOA as part of its risk assessment of the emission of PFOA by the DuPont/Chemours chemical plant in Dordrecht, The Netherlands (Zeilmaker et al., 2016). As a supplement to this assessment, RIVM provided a review specifically of the available epidemiological studies for PFOA (Rijs & Bogers, 2017).

The biokinetics of PFOA and its salts has been studied in rats, mice, monkeys and humans. A remarkably high potential for bioaccumulation in humans has been determined, with an estimated half-life for clearance from human serum as long as 3-4 years. This contrasts with the half-life for PFOA in experimental animals (monkeys, rats, mice), which is only several weeks at the most (11-21 days) (Zeilmaker et al., 2016). As explained in the Appendix on GenX toxicity, in contrast to classic lipophilic organic pollutants (e.g. DDT, dioxins) which accumulate via linking into fat metabolism leading to accumulation in fatty tissues, PFOA primarily bind to proteins. The long half-life of PFOA in humans compared to other species has been hypothesized to be due to stronger reabsorption from the lumen of the kidney back into the blood by organic anion transporters (OATs) (Yang et al., 2010).

Animal toxicity experiments have been carried out using both PFOA and its ammonium salt ammonium pentadecafluorooctanoate (APFO). Since in aqueous environments both PFOA and APFO lead to the presence of perfluorooctanoate as the dominant chemical species the read across from the salt to the acid is considered valid. Animal toxicity studies with orally dosed PFOA of short-term, subchronic and chronic duration are available in multiple species including monkeys, rats, and mice. In addition, developmental toxicity and reproductive toxicity and carcinogenicity were studied in mice and rats. These studies report developmental effects, liver and kidney toxicity, immune effects, and cancer (liver, testicular, and pancreatic). Developmental effects observed in animals include decreased survival, delayed eye opening and reduced ossification, skeletal defects, altered puberty (delayed vaginal opening in females and accelerated puberty in males) and altered mammary gland development.

Human epidemiology data report associations between PFOA exposure and a number of disorders and diseases. The examined populations were workers at PFOA production plants, a high-exposure community population near a production plant in the United States (the C8 cohort) and members of the general population in the United States, Europe, and Asia. In its review of the epidemiological evidence for PFOA, RIVM (Rijs & Bogers, 2017) selected the following effects reported in the available epidemiology studies for further evaluation: increased liver enzymes and liver disease, testicular and kidney cancer, pregnancy-induced hypertension and pre-eclampsia, decreased birth weight, increased serum uric acid concentration, ulcerative colitis, changes in blood lipid concentrations, decreased vaccination response and thyroid disorders. The weight of evidence for an association between PFOA and these effects was concluded to be variable. For some effects inconsistencies were noted, for others the influence of possible confounders could not be ruled and for others the biological significance was doubtful. The evidence for decreased birth weight and increased cholesterol is strongest but even for these effects uncertainty remains about the causality of the observed association (Rijs & Bogers, 2017). Because of the limitations in the available epidemiological evidence, most international organizations do not use these data for quantitative dose response analysis and risk assessment.

In animal studies with PFOA, changes in liver weight and hepatocellular hypertrophy were the most common effects observed with or without other hepatic indicators of adversity. Liver contains the highest levels of PFOA when analysed after test animal sacrifice. The increases in liver weight and hypertrophy as seen in rodent studies may be associated with activation of the cellular peroxisome proliferator-activated receptor α (PPARα). The PPARα response in the liver is known to be greater in rodents than in humans. But increased liver weight and hypertrophy were also observed in monkeys. In its 2016 risk assessment for PFOA, RIVM concluded liver effects to represent the most sensitive endpoint for PFOA-toxicity (Zeilmaker et al., 2016). In the derivation of a health-based guidance value (HBGV) for PFOA, RIVM (Zeilmaker et al., 2016) initially selected a subchronic study in monkeys in which increased liver weight and hypertrophy were seen, but rejected this study upon evaluation due to inconsistencies in the serum PFOA-levels that were reported for the different dose groups. As a replacement a subchronic study in rats (Perkins et al., 2004) was used, which included measurement of the serum PFOA levels. In deriving the HBGV for PFOA, the NOAEL from this rat study was divided by a reduced interspecies factor because of the known higher susceptibility to liver effects by PFOA in rodents compared to humans (for the complete derivation see below).

In reproduction toxicity studies in mice reduced fertility and reduced sperm counts were observed (NOAEL of 2.5 mg/kg bw per day). Developmental toxicity in mice and rats showed decreases in pup weight as the most sensitive effect (NOAEL of 1 mg/kg bw per day). In addition, several developmental toxicity studies in mice showed delayed mammary gland development in female offspring at very low maternal dose levels. The biological significance of this effect is unknown. RIVM noted that other hormone-related parameters in these studies showed no effect and concluded that further research on this possible effect is needed. This effect was therefore not used for deriving an NOAEL.

Rat bioassays showed increased incidences of tumours in liver, testes and pancreas. Epidemiological studies in a population living in the vicinity of a PFOA production plant in the USA and in workers of this plant showed an association between PFOA exposure and testicular cancer and kidney cancer. As stated above, IARC concluded the rat bioassay results to represent limited evidence in experimental animals and the positive associations as seen in the epidemiological studies to represent limited evidence for a carcinogenic effect by PFOA in humans. Available information on PFOA genotoxicity and mechanistic information for the induction of the observed tumours indicates a non-genotoxic mode of action (DWQI, 2017; US EPA, 2016; Zeilmaker et al., 2016). For the derivation of a HBGV for PFOA this means that a threshold in its toxic action is assumed and that a HBGV can be derived via application of the appropriate assessment factors to a selected point of departure in the form of an adequate NOAEL or BMDL.

National Institute for Public Health
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Ministry of Health, Welfare and Sport

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FRONT OFFICE FOOD AND PRODUCT SAFETY

RISK ASSESSMENT OF GenX AND PFOA IN FOOD
PART 2: TRANSFER OF GenX AND PFOA IN DITCHWATER AND SILAGE TO EDIBLE PRODUCTS OF FOOD PRODUCING ANIMALS

Risk assessment requested by:	Office for Risk Assessment and Research
Risk assessment performed by:	RIVM and RIKILT
Date of request:	19-12-2018
Date of risk assessment:	18-04-2019
Project number:	V/093130

Subject

In the past, the companies DuPont/Chemours in Dordrecht and Custom Powders in Helmond emitted the substances GenX[1] and PFOA into the air and surface water. The emission of GenX by DuPont/Chemours is ongoing. Consequently, the area in the vicinity of these companies (soil, water and vegetation) became polluted, as communicated by the National Institute for Public Health and the Environment (RIVM) with the the city council of Dordrecht in July and September 2018. One of the conclusions was that ditchwater showed high levels of GenX and PFOA. This might be a potential concern for livestock drinking from that ditchwater. At that moment no firm conclusions were drawn regarding the transfer of GenX and PFOA to milk and edible tissue of livestock consuming this ditchwater.
The Office for Risk Assessment and Research (BURO), has requested the Front Office Food and Product Safety to address three questions related to the transfer of GenX and PFOA in ditchwater to lactating cows and sheep. A fourth question related to the transfer of GenX and PFOA in silage to lactating cows was added at a later stage to this request.

Questions

BuRO has asked the following questions with respect to the transfer of GenX and PFOA in ditchwater and silage to lactating cows and/or sheep.

1. Model the transfer of GenX and PFOA from ditchwater to edible products from lactating cows and sheep (milk and meat);
2. Estimate the intake of GenX and PFOA for consumers based on the theoretical (modelled) concentrations in milk and meat of dairy cattle and lactating sheep;
3. Calculate the possible concentrations of GenX and PFOA in ditchwater when concentrations of GenX and PFOA occur at the analytical LOQ of 0.01 ng/g in cow's milk (based on reversed dosimetry modelling).
4. Estimate the transfer of GenX and PFOA from silage to milk and meat from lactating cows and sheep.

[1] GenX refers to hexafluoropropyleneoxide dimer acid (HFPO-DA), or to its ammonium salt, as used in the GenX technology.

Conclusions

1) A transfer model for PFOS in dairy cows was adapted for the transfer of PFOA from ditchwater to cow's milk and muscle meat. The highest intake of PFOA through the consumption of contaminated ditchwater (510 µg/day) resulted in modelled concentrations in milk and meat of 0.06 ng PFOA/g and 0.28 ng PFOA/g, respectively.
In species which show extensive renal PFOA clearance such as dairy cattle (and lactating sheep) it was assumed that comparable exposure of GenX and PFOA leads to lower concentrations of GenX in tissues and milk than PFOA.
Therefore, given the fact that the exposure to GenX from ditchwater is lower (approximately a factor 5), the expected concentration in milk and meat of dairy cattle is equal to or lower than 0.01 ng GenX/g resp. 0.06 ng GenX/g.
Only one pilot study described the kinetics of PFOA in sheep (n=2) after exposure from silage and the information does not allow us to develop a transfer model for this matrix or ditchwater in sheep. Reported transfer of PFOA to the carcass was (more or less) similar for sheep and dairy cattle, whereas transfer of PFOA to milk was possibly higher in sheep. These data need experimental confirmation before the relevance of the transfer of PFOA (and GenX) to milk and meat of sheep for human exposure can be evaluated.

2) For milk and meat of dairy cattle, the human exposure to PFOA and GenX, based on the calculated transferred concentrations of PFOA and GenX in milk and meat of dairy cattle, will be negligible and therefore do not pose a health risk. For milk and meat of sheep, more data on transfer are needed before a conclusion on human health risk can be drawn.

3) Reverse dosimetry could only be performed for PFOA. A PFOA concentration in milk at the analytical LOQ level (0.01 ng/g) leads to a modelled intake of 89 µg PFOA per dairy cow per day. This intake corresponds to a calculated PFOA concentration in ditchwater of (approximately) 810-1100 ng/L.

4) Due to the fact that (excluding other sources) the intake of PFOA through silage is 22 times lower than the intake through ditchwater it is concluded that in dairy cattle the concentrations in meat and milk will be 0.01 ng PFOA/g or 0.003 ng/g. As levels of GenX in silage were below the LOQ the transfer of GenX from silage to milk and meat of dairy cattle is considered negligible.
As mentioned under 1) calculations for the transfer of GenX and PFOA from silage to milk and meat from lactating sheep cannot yet be assessed.

Introduction

As a result of long-lasting emissions from the DuPont/Chemours chemicals company in Dordrecht, the substances GenX and PFOA have been emitted into the environment via the air and surface water. Consequently, the area in the vicinity of the factory (soil, water and vegetation) became polluted. In July 2018 the National Institute for Public Health and Environment (RIVM) has informed the city council of Dordrecht in a letter on the provisional results of research carried out in soil and irrigation water (RIVM 2018a). One of the conclusions was that ditchwater showed high levels of GenX and PFOA. This might be of concern for livestock consuming that ditchwater. In this letter it was mentioned that watering livestock at the observed high levels of GenX and PFOA in ditchwater should be avoided. In September a final report and an accompanying letter were sent to the city council of Dordrecht (RIVM 2018b). In the report, amongst others, concentrations of GenX and PFOA in ditchwater at five different locations in the vicinity of the DuPont/Chemours factory were given. At that moment no conclusions were drawn regarding the transfer of GenX and PFOA to milk and meat of livestock consuming that ditchwater.

In May 2018, the Netherlands Food and Consumer Product Safety Authority (NVWA) took samples of edible products of food producing animals (dairy products and fish) and silage in the vicinity of DuPont/Chemours company in Dordrecht and Custom Powders in Helmond. At that moment, the detection and quantification limits of the analytical method to analyse these compounds were not low enough for performing a risk assessment. In other words, if all concentrations would be below these limit values, the calculated exposure using concentrations at these limit values (worst case) would exceed the health-based guidance value. In that case, a realistic conclusion about a possible health risk cannot be drawn.

In September 2018, the FO calculated how low the LOQ for analysis of GenX and PFOA in animal products should be to be able to perform a quantitative risk assessment. Therefore, GenX and PFOA concentrations for animal products including egg, meat (beef) and cow's milk at which a high consumption of each product would result in an exposure equal to 20% of the TDI of GenX or PFOA were calculated (FO, 2018). These concentrations are referred to as '20% TDI concentrations'. No such concentrations were derived for cheese and yoghurt. The 20% TDI concentrations of GenX and PFOA in milk were respectively 0.1 and 0.06 ng/g (or ng/mL). For meat they are 1.3 ng GenX/g and 0.8 ng PFOA/g. The way these concentrations were derived is described in more detail in "Risk assessment of GenX and PFOA in food; Part 1: Toxicity of GenX and PFOA and intake of contaminated food of animal origin" (FO, 2019). If all concentrations (whether measured, modelled or reasoned) of GenX and PFOA are below these 20% TDI concentrations, a health risk can be excluded. If the concentrations are higher, an exposure assessment is required to assess possible health risks.

On 10 January 2019 the concentrations of GenX and PFOA in dairy products, fish and silage were sent to the Office for Risk Assessment and Research (BuRO). BuRO has requested the FO Food and Product Safety to address the above-mentioned questions related to the transfer of GenX and PFOA in ditchwater to lactating cows and sheep and the transfer of GenX and PFOA in silage to lactating cows and sheep. In Part 1, the risk related to the consumption of contaminated milk and meat was addressed (FO, 2019).

Toxicology

PFOA

Perfluoro-octanoic acid (CAS no. 335-67-1) (pentadeca-fluoro-octanoic acid, PFOA) and its salts are used as processing aid in the production of fluoro-elastomers and fluoropolymers, with PTFE being an important fluoropolymer. In addition, PFOA-related compounds are used as surfactant (in fire-fighting foams, wetting agents and cleaners) and for the manufacture of side-chain fluorinated polymers (used as surface finishes for textiles and apparel, leather, paper and cardboard, paints, lacquers etc.). In 2016, RIVM derived a tentative TDI (t-TDI) of 12.5 ng/kg bw per day for PFOA (Janssen, 2017). This t-TDI was based on an overall no-observed adverse level (NOAEL) of 0.06 mg/kg body weight (bw) per day for liver toxicity in a semi-chronic oral study in rats (Perkins et al. (2004).
For information on the toxicity of PFOA we refer to part 1 of the risk assessment of GenX and PFOA in food (FO, 2019).

GenX

The chemicals FRD-902 and FRD-903, referred to as "GenX chemicals", are the main substances associated with the GenX processing aid technology that enables the production of fluoropolymers. FRD-902 is the dimer ammonium salt (ammonium-2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate; CAS no. 62037-80-3) and FRD-903 is the dimer acid (2,3,3,3-tetrafluoro-2 (heptafluoropropoxy)propanoic acid; CAS no. 13252-13-6). Under environmental and physiological conditions, such as in water or in blood, FRD-902 and FRD-903 will dissociate into the ion HFPO-DA (hexafluoropropylene-oxide dimer acid ion), which is responsible for the observed toxicological effects. In this assessment, the ion HFPO-DA is referred to as GenX. In 2017, RIVM derived a tentative TDI (t-TDI) of 21 ng/kg bw per day for GenX (Janssen, 2017). This t-TDI was based on an overall no-observed adverse level (NOAEL)[2] of 0.1 mg/kg body weight (bw) per day from a chronic oral study in rats with increased albumin/globulin ratio in serum as the critical effect (Beekman et al., 2016).
For information on the toxicity of GenX we refer to part 1 of the risk assessment of GenX and PFOA in food (FO, 2019).

Rationale of transfer assessment

This assessment focuses on the transfer of PFOA and GenX from contaminated ditch water or silage to milk and meat from dairy cattle and lactating sheep. Quantifying such transfer needs experimentally observed transfer, ideally in conjunction with a computer model describing the experimental observations (the latter enabling extrapolation of the experimental settings across dosage, matrix and exposure time duration, etc.).

PFOA: Dairy cattle (modelling approach)

As shown below an experimental study on the transfer of PFOA and its structure analogue PFOS from contaminated (grass) silage and hay to milk and meat of dairy cattle (N=6) is available. Only a PFOS transfer model based on this study is available. However, the PFOA transfer data of this study enabled the scaling of the PFOS model to PFOA (this assessment, for details, see Annex 1). This scaled PFOA model was used to quantify the transfer of this compound from ditch water or silage to milk and meat of dairy cattle (not taking into account other sources of exposure).

PFOA: Lactating sheep (experimental approach)

With regard to lactating sheep (N=2), only a pilot study on the transfer of PFOS and PFOA from contaminated (corn) silage and hay to milk and meat is available (see below). The results of this study therefore do no warrant the development of a transfer model for either of these PFASs. Furthermore, in livestock allometric scaling of PFAS kinetics does

[2] The highest dose administered in an animal study at which no adverse effects are observed

not apply (compare for example dairy cattle (Kowalczyk *et al*., (2013) with pigs (Numata *et al.* 2014). Therefore, the PFOA/PFOS transfer model in dairy cattle was not allometrically scaled from this species to sheep. In this assessment the transfer of PFOA from ditch water *or* silage to milk and meat of lactating sheep was based on the available experimental data (not taking into account other sources of exposure). The available study being a pilot, however, urges to consider the assessed transfer only as indicative.

GenX (reasoning approach)
Dairy cattle and lactating sheep show comparable, extensive renal clearance of PFOA. GenX and PFOA show comparable renal clearance in rodents and monkeys (see below). Due to lack of information on the transfer of GenX to farming animals it is assumed that dairy cattle and lactating sheep also show extensive renal clearance of GenX (for reasoning, see below).

Literature search: Transfer models
Different PubMed search strings were used to obtain information on the transfer of GenX or PFOA in dairy cows, cattle, sheep or lamb. The search initially addressed GenX and PFOA. However, as information on GenX and PFOA appeared limited the search was extended to PFOS. For dairy cattle only a transfer model for PFOS was found. In the case of lactating sheep no transfer model was found for PFOS, PFOA or GenX.

Ditch water and silage sampling of PFOA and GenX
Single samples of ditchwater were taken at five different sites within a distance (radius) of four kilometres from the DuPont/Chemours factory (Van Poll, 2018). The samples were analysed in duplicate. The average concentrations of GenX and PFOA at these sites (including their distance from the factory) are given in table 1. These results are also depicted in figure 1, which is taken from the report of Van Poll (Van Poll, 2018).

Table 1. Average concentrations of GenX and PFOA in ditchwater (in ng/L) at five different locations (codes and distances of locations are given) around Dordrecht.

Location code	Sub code	Location number	Distance (km)	GenX (ng/L)	PFOA (ng/L)
G3LOC4	WA2	8	< 1	956.5	4670
G2LOC3	WA1	6	1-2	133.5	660.5
G2LOC1	WA2	4	1-2	97.5	566
G1LOC3	WA2	3	2-3	24.5	172.5
G3LOC3	WA2	10	3-4	9.7	40.5

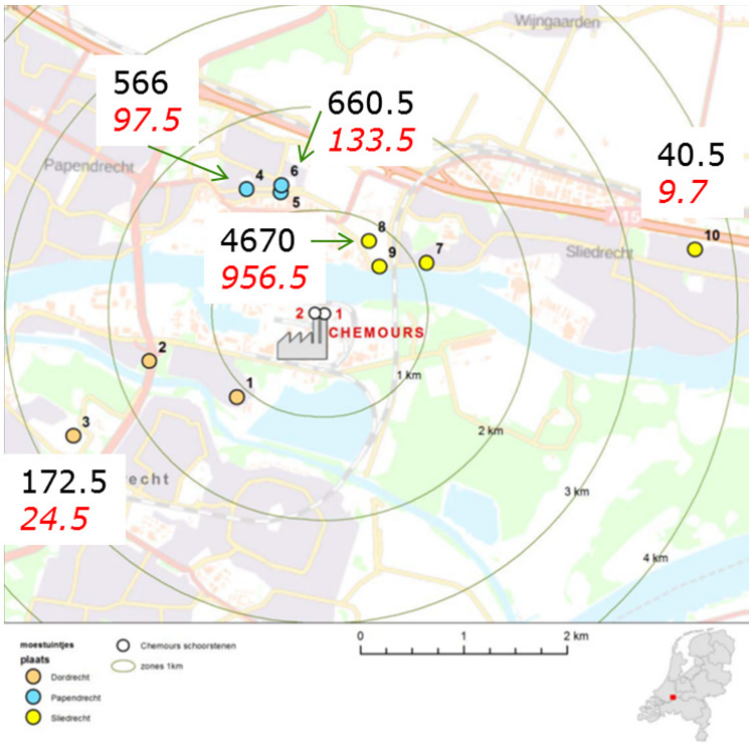


Figure 1. Average concentrations (in ng/L) of GenX (in red and italic) and PFOA (in black) in ditchwater at five different locations around Dordrecht. Ditchwater samples were taken at locations, numbered 3, 4, 6, 8 and 10.

Ten samples of silage were taken in the vicinity of Dordrecht and Helmond. No GenX could be detected in these samples (< 250 ng/kg). Only in two samples could PFOA be detected, concentrations were 540 and 600 ng/kg (measurement on basis of whole product).

Exposure scenarios

Lactating cow: PFOA and GenX exposure from ditchwater or silage

As shown in Table 1 the measured concentration of PFOA in ditchwater ranged from 40.5 to 4670 ng/L. Similarly, the GenX concentration ranged from 9.7 to 956.5 ng/L. Regarding the drinking water intake, lactating cows consume different volumes of drinking water per day, with (total) water intake depending on factors like ambient temperature, body weight, dry matter intake, milk production, etc. (Kume *et al.*, 2010; Meyer *et al.*, 2004; Murphy MR, 1992; National Research Council, 2001). Average drinking water consumption was used in combination with the conservative assumption that cows solely consume contaminated ditchwater. As model input for PFOA transfer model calculations, an average drinking water intake of 80 L/day for (mature) lactating cows (weighing 600 kg; milk yield: 29.5 kg day^{-1}) ranging to a maximum drinking water intake of 110 L/day (weighing 600 kg; milk yield: 35 kg day^{-1}) was used (Kume *et al.*, 2010; Meyer *et al.*, 2004; National Research Council, 2001). Then, given the maximal measured GenX and PFOA concentrations, the ditchwater intake for lactating cows is calculated at 110*956,5 ≈ 110,000 ng GenX/day respectively 110*4670 ≈ 510,000 ng PFOA/day.
PFOA, but not GenX, was detected in two samples of silage at comparable concentrations of 540 and 600 ng/kg. On average dairy cows consume 25 to 38.5 kg (grass) silage per day wet weight (Berende, 1998; Vestergren et al. 2013). As worst case (winter) scenario, the average intake of PFOA through silage for lactating cows is approximately 38.5*600 ≈ 23,000 ng PFOA/day.

Lactating cow: PFOA transfer (modelling)

Van Asselt *et al.* (2013) described a transfer model for PFOS in dairy cows. This model represents the body to be composed of blood and carcass, with PFOS being eliminated by milk clearance from the blood compartment. The model was calibrated on experimental results of a transfer experiment in which dairy cows were exposed to contaminated hay/grass silage for a 28 day period, followed by a 22 day wash-out period (Kowalczyk *et al.,* 2013; intake: 7.6 $\pm$ 3.2 µg PFOS kg bw^{-1} day^{-1}). Note that the modal calibration resulted in complete absorption of PFSA from the silage matrix. Model output consists of the PFOS concentration in the blood, carcass, milk and urine.

Next to PFOS, Kowalczyk *et al.* (2013) also provide PFOA transfer data in dairy cows. These data allowed the scaling of the PFOS model to PFOA. In concordance with the differences of PFOS and PFOA kinetics in dairy cows this scaling consisted of introducing renal clearance as the major route of excretion (while maintaining milk clearance as a minor PFOA route of excretion) in conjunction with minimizing PFOA transport from the blood to the carcass. Carcass is assumed to consist (mainly) of muscle, liver and kidney. Concentrations in muscle are expected to be half of the carcass concentrations. Furthermore, as for found for PFOS, the model applies complete absorption of PFOA from silage and, hence, ditchwater. As shown in Annex 1, Figure 4 this scaling resulted in an acceptable description of PFOA transfer data in dairy cows.

Lactating cow: PFOA reverse dosimetry (modelling back calculation from concentration in cow's milk to corresponding ditchwater concentration)

In order to calculate the possible concentrations of PFOA in ditchwater based on reversed dosimetry modelling (question 3) the above mentioned RIVM PFOA transfer model was used. The present analytical LOQ of PFOA in cow's milk (0.01 ng/g), was used as model input for the reverse dosimetry calculation. In this calculation the cow's model was used to calculate the PFOA intake through ditchwater which would lead to a milk concentration corresponding with the current analytical LOQ. Applying the above-mentioned water consumption of lactating cows, the corresponding concentration of PFOA in ditchwater can be calculated (assuming no additional exposure from other sources than ditchwater).

Lactating cow: GenX transfer (assumption)

In rodents and monkeys, GenX and PFOA preferentially partition into the blood (Butenhoff *et al.*, 2004; Gannon *et al.*, 2016). Though renal clearance is the major excretion pathway for both compounds, GenX is removed from the blood faster than PFOA (Gannon *et al.*, 2013 and references therein). From this it concluded that, due to a more efficient renal clearance of GenX, its partitioning from blood to milk and tissues is less efficient than that of PFOA. Therefore, in species which show extensive renal PFOA clearance such as dairy cattle (and lactating sheep) it was assumed that comparable exposure of GenX and PFOA leads to lower concentrations of GenX in tissues and milk than PFOA.

Lactating sheep: PFOA and GenX exposure from ditchwater and silage

Given a body weight of around 60 kg for lactating sheep (Kowalczyk *et al.*, 2012) and a daily drinking water consumption of 6 L/day, i.e. 10% of body weight, containing a (maximal) concentration of 4670 ng PFOA/L corresponds with a maximum intake of 6*4670 ≈ 28,000 ng PFOA/day, i.e. 28,000/60 ≈ 500 ng PFOA/kg bw/day (thereby excluding all other exposure sources). Similarly, taking a (maximum) concentration of 956,5 ng GenX/L corresponds to a maximum intake of 6*956,5 ≈ 5700 ng GenX/day, i.e. 5700/60 ≈ 95 ng GenX/kg bw/day.

For lactating sheep, Kemme *et al.* (2005) mention for dairy sheep a yearly meadow grass consumption of 364 kg dry matter plus 58 kg of wet weight grass silage. Assuming meadow grass to contain 40% dry matter (http://eurofins-agro.com/nl-nl/wiki/drogestof) this yearly corresponds to 364/0,40 + 58 ≈ 970 kg wet weight grass silage, i.e. around 2.7 kg wet weight grass silage daily. Given a PFOA concentration of 600 ng PFOA/kg (see above) this results in a daily intake of 2.7 * 600 ≈ 1600 ng PFOA, i.e. 1600/60 ≈ 27 ng PFOA/kg bw/day.

Lactating sheep: PFOA transfer (experimental)
As mentioned above, no transfer model was found for PFOS, PFOA or GenX transfer in dairy cattle or lactating sheep. However, Kowalczyk *et al.* (2012) describe a pilot experiment in two sheep on the transfer of PFOA from contaminated corn silage and hay to milk (N=2) and meat (N=1). One sheep was exposed for a period of 21 day to a dose of 0.53 µg/kg bw/day, after which PFOA was measured in milk, liver, kidneys and muscle tissue. The other sheep was exposed for 21 days to 0.43 µg PFOA/kg bw/day, followed by a wash-out period of 21 days. In both sheep PFOA was mainly eliminated by renal clearance. In this sheep PFOA was completely cleared from the tissues within 10 days after the PFOA feeding period had stopped. The results of this experiment were used to estimate the transfer of PFOA and GenX from ditchwater to milk and meat, i.e. muscle tissue, and PFOA from silage.

Lactating sheep: GenX transfer (assumption)
As in dairy cattle it was assumed that comparable exposure of GenX and PFOA to lactating sheep leads to lower concentrations of GenX in tissues and milk than PFOA.

Results

Dairy cattle: PFOA

PFOA transfer from ditch water to cow's milk and meat
In the PFOA transfer model a (maximum) intake of 510 µg/day from ditchwater leads to PFOA concentrations in milk and muscle meat of 0.06 ng/g and 0.28 ng/g (carcass: 0.54 ng/g), respectively (see Annex 1). This low transfer to milk and meat is mainly due to a high renal excretion of PFOA in dairy cows. The modelled concentrations are at or lower than the 20% TDI concentration of PFOA for milk (0.06 ng/g) and meat (0.8 ng/g).

PFOA reverse dosimetry (back extrapolation from cow's milk to ditch water)
When the (analytical) LOQ of PFOA in milk (0.01 ng/g) was used in the model to back extrapolate the (theoretical) intake of dairy cows, a dose of 89 µg/day was calculated. This results in a calculated PFOA concentration in ditch water of (approximately) 1100 ng/L (for a ditch water intake of 80 L/day) and 810 ng/L (for a ditch water intake of 110 L/day). This means that whenever the concentration of PFOA in ditch water is below (approximately) 810 ng/L, it is likely that concentrations in milk will not exceed the (analytical) LOQ of PFOA in milk.

PFOA transfer from silage to cow's milk and meat
The intake of PFOA through consumption of contaminated silage of approximately 23 µg/day is much lower than the intake of PFOA through ditch water, i.e. 510 µg/day. The modelled transferred PFOA concentration to milk and meat from silage is 23/510* 0.06 ≈ 0.003 ng/g milk and 23/510*0.28 ≈ 0.01 ng/g meat.

Dairy cattle: GenX
The (maximal) exposure to GenX via ditchwater is almost five-fold lower than the (maximal) PFOA intake, i.e. 110 vs. 510 µg/day. The *assumed* GenX concentrations in milk and muscle meat then are *lower* than 0.06/5 ≈ 0.01 ng/g resp. 0.28/5 ≈ 0.06 ng/g. Note that the calculated GenX concentrations in milk and muscle meat also are lower than the GenX 20% TDI concentration of 0.1 ng/g for milk and 1.3 ng/g for meat and the the analytical LOQ of 0.1 ng/g for GenX in milk.

Sheep: PFOA and GenX
As mentioned above, one publication addressed the transfer of PFOA (and PFOS) from contaminated feed to milk and meat of two sheep (Kowalczyk, 2012).

In one sheep (sheep 2) the distribution of PFOA was experimentally determined over a 21 day exposure period at a dose of 0.53 µg/kg bw/day. In plasma PFOA increased dur-

ing the first 9 days of the 21 day exposure period to a peak concentration. Milk was collected across the exposure period. After slaughter at the end of the exposure period, PFOA was measured in the liver, the kidneys and the muscle tissue, i.e. meat. As in dairy cows, the concentration of PFOA in milk was around or just above the LOD (see Table 2).

The other sheep (sheep 1) was exposed for 21 days to 0.43 µg PFOA/kg bw/day, followed by a wash-out period of 21 days. In this sheep plasma levels hardly peaked during the exposure period and were found substantially lower than in sheep 2 across the 21 day exposure period. Milk was collected across the exposure period. In this sheep PFOA was completely cleared from the plasma within 10 days after the PFOA feeding period had stopped.

Given a body weight of around 60 kg for lactating sheep (Kowalczyk *et al.*, 2012) and a daily drinking water consumption of 6 L/day, i.e. 10% of body weight, containing a (maximal) concentration of 4670 ng PFOA/L corresponds with an exposure of 6*4670/60 ≈ 500 ng PFOA/kg bw/day (thereby excluding all other exposure sources). At this exposure level the PFOA concentration in milk is expected to be approximately 0.2 - 0.7 ng/g resp. and in meat 0.2 ng/g (see Table 2).

The exposure to PFOA from silage was calculated at 27 ng PFOA/kg bw/day. The corresponding PFOA concentration range in milk is estimated to be ≈ 0.01 ng/g (27/500*0.2) – 0.04 ng/g (27/500*0.7). In meat this is approximately 0.01 ng/g (27/500*0.2).

The exposure to GenX from ditch water was calculated at 95 ng GenX/kg bw/day (approximately one fifth of the PFOA exposure). The GenX concentration in sheep milk is expected to be lower than 0.2/5 - 0.7/5 ≈ 0.04 - 0.14 ng GenX/g and in meat lower than 0.2/5 ≈ 0.04 ng/g. These concentrations are at or below the analytical LOQ of 0.1 ng/g for GenX in milk.

Table 2. Comparison of the concentration (ng/g wet weight, $\pm$ SD) of PFOA in dairy cows (N=3) exposed for 28 days to 2.0 $\pm$ 1.1 µg/kg bw/day from contaminated hay/grass silage and two lactating sheep exposed for 21 days to 0.43 µg/kg bw/day (sheep 1, S1) resp. 0.53 µg/kg bw/day (sheep 2, S2) from contaminated hay/corn silage (data taken from Kowalczyk *et al.* 2012; 2013).

Organ	**Cow**	**Sheep**
Liver	10.1 $\pm$ 1.9[3]	S2: 2.6[3]
Kidney	8.7 $\pm$ 3.9[3]	S2: 4.8[3]
Muscle	0.6 $\pm$ 0.3[3]	S2: 0.2[3]
Milk	0.14 $\pm$ 0.05[1]	S1: 0.2 $\pm$ 0.1[2] S2: 0.7 $\pm$ 0.5[2]

[1] Figure 3B, obtained from LOD: 0.1 ng/g; [2] LOD: 0.2 ng/g, average of 15 samples during the 21 day exposure period (Ranges: Sheep 1: < LOD – 0.5 ng/g; Sheep 2: < LOD – 1.3 ng/g); [3] LOD: 0.2 ng/g.

Consequences for human exposure

Table 3 provides an overview of the calculated PFOA transfer in dairy cattle. As shown the calculated transfer from ditch water or silage to milk remains below the 20% TDI concentration for PFOA in milk, i.e. 0.06 ng PFOA/g or meat, i.e. 0.8 ng PFOA/g.
In the case of milk in sheep the transfer calculations suggest otherwise (see above). However, in interpreting the sheep transfer calculations it should be noted that the transfer to milk was observed in only two sheep showing quite different (and partly aberrant) PFOA (and PFOS) kinetics. Secondly, taking Table 2 as a reference, the available transfer data in dairy cattle and lactating sheep indicate that PFOA transfer to organs and tissues is comparable in both species, but transfer to milk not. Regarding the latter, the limited

available data suggest a much higher transfer, i.e. up to 6 - 20 fold, of PFOA from the blood to milk in lactating sheep than in dairy cattle. For this reason it is concluded that the observed transfer of PFOA in lactating sheep to milk needs to be confirmed beyond the pilot experiment in which it was assessed in order to draw a more definitive conclusion on the relevance of such transfer for human exposure assessment. Furthermore, consumption data on sheep milk (by different population groups) in the Netherlands is not available in the Dutch Food Consumption Survey.

Table 3. Overview of calculated PFOA or GenX concentrations (ng/g) in milk and meat of dairy cattle after exposure to these chemicals via contaminated ditch water or grass silage.

Dairy cattle	PFOA		GenX	
	Milk	Meat	Milk	Meat
Ditch water	0.06[1]	0.28[1]	< 0.01[2]	< 0.06[2]
Silage	0.003[1]	0.01[1]	X[3]	X[3]

[1] Modelled; [2] Reasoned assumption, i.e. assuming less efficient transfer of GenX relative to PFOA at comparable exposure; [3] X: negligible, in other words: below LOD.

Answers

Based on the results described above the answers to the four questions asked by the Office for Risk Assessment and Research are given underneath.

Question 1

Model the transfer of GenX and PFOA from ditch water to edible products from lactating cows and sheep (milk and meat).

Answer 1

A transfer model for PFOS in dairy cows was adapted for the transfer of PFOA from ditchwater to cow's milk and muscle meat. The highest intake of PFOA through the consumption of contaminated ditchwater (510 µg/day) resulted in modelled concentrations in milk and meat of 0.06 ng PFOA/g and 0.28 ng PFOA/g, respectively.

In species which show extensive renal PFOA clearance such as dairy cattle (and lactating sheep) it was assumed that comparable exposure of GenX and PFOA leads to lower concentrations of GenX in tissues and milk than PFOA.

Therefore, given the fact that the exposure to GenX from ditchwater is lower (approximately a factor 5), the expected concentration in milk and meat of dairy cattle is equal to or lower than 0.01 ng GenX/g resp. 0.06 ng GenX/g.

Only one pilot study described the kinetics of PFOA in sheep (n=2) after exposure from silage and the information does not allow us to develop a transfer model for this matrix or ditchwater in sheep. Reported transfer of PFOA to the carcass was (more or less) similar for sheep and dairy cattle, whereas transfer of PFOA to milk was possibly higher in sheep. These data need experimental confirmation before the relevance of the transfer of PFOA (and GenX) to milk and meat of sheep for human exposure can be evaluated.

Question 2

Estimate the intake of GenX and PFOA for consumers based on the theoretical (modelled) concentrations in cow's milk and meat.

Answer 2

For milk and meat of dairy cattle, the human exposure to PFOA and GenX based on the calculated transferred concentrations of PFOA and GenX in milk and meat of dairy cattle will be negligible and therefore do not pose a health risk. For milk and meat of sheep, more data on transfer are needed before a conclusion on human health risk can be drawn.

Question 3
Calculate the possible concentrations of GenX and PFOA in ditchwater when concentrations of GenX and PFOA occur at the current limit of quantification of GenX and PFOA in cow's milk (based on reversed dosimetry modelling).
Answer 3
Reverse dosimetry could only be performed for PFOA. A PFOA concentration in milk at the analytical LOQ level (0.01 ng/g) leads to a modelled intake of 89 µg PFOA per dairy cow per day. This intake corresponds to a calculated PFOA concentration in ditchwater of (approximately) 810-1100 ng/L.

Question 4
Estimate the transfer of GenX and PFOA in silage to milk and meat from lactating cows and/or sheep.
Answer 4
Due to the fact that (excluding other sources) the intake of PFOA through silage is 22 times lower than the intake through ditchwater it is concluded that in dairy cattle the concentrations in meat and milk will be 0.01 ng PFOA/g or 0.003 ng/g. As levels of GenX in silage were below the LOQ the transfer of GenX from silage to milk and meat of dairy cattle is considered negligible.
As mentioned under 1) calculations for the transfer of GenX and PFOA from silage to milk and meat from lactating sheep cannot yet be assessed.

References

ATSDR (2018). Toxicological profile for perfluoroalkyls. Draft for public comment June 2018. Agency for Toxic Substances and Disease Registry, Division of Toxicology and Human Health Sciences. Available online: www.atsdr.cdc.gov.

Beekman MPZ, Muller A, de Vries W, Janssen P, Zeilmaker M (2016). Evaluation of substances used in the GenX technology by Chemours, Dordrecht. RIVM Letter report 2016-0174. National Institute for Public Health and the Environment (RIVM), Bilthoven. Available online: www.rivm.nl

Butenhoff JL, Kennedy GL, Hinderliter PM, Lieder PH, Jung R, Hansen KJ, Gorman GS, Noker PE, Thomford PJ (2004) Pharmacokinetics of perfluorooctanoate in Cynomolgus monkeys, Toxicological Sciences, 82: 394-406.

DWQI (2017). Health-based maximum contaminant level support document: Perfluorooctanoic acid (PFOA) New Jersey Drinking Water Quality Institute - Health Effects Subcommittee February 15, 2017.

FO, 2018. Front Office Voedsel- en Productveiligheid: Discussiestuk benodigde kwantificeringslimiet (LOQ) voor de risicoschatting van PFOA en GenX in verschillende voedingsmiddelen. September 2018.

FO, 2019. Front Office Food and Product Safety: Risk assessment of GenX and PFOA in food; Part 1: Toxicity of GenX and PFOA and intake through contaminated food of animal origin.

Gannon SA, Fasano WJ, Mawn MP, Nabb DL, Buck RC, Buxton LW, Jepson GW, Frame SR (2016) Absorption, distribution, metabolism, excretion and kinetics of 2,3,3,3-tertafluoro-2-(heptafluoropropoxy)propanoic acid ammonium salt following a single dose in rat, mouse, and cynomolgus monkey, Toxicology, 340: 1-9.

Janssen P (2017) Bijlage bij brief 0148/2016/M&V/EvS/AV. Derivation of a lifetime drinking-water guideline for 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903) – Revised version January 2017.

Kemme P, Heeres-van der Tol J, Smolders G, Valk H, Van der Klis JD (2005) Schatting van de uitscheiding van stikstof en fosfor door diverse categorieën graasdieren, WUR Animal Science Group, Report 05/I00653 Nutrition and Food.

Kowalczyk J, Ehlers S, Fürst P, Schafft H, Larssen-Wiederholt M (2012) Transfer of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) from contaminated feed into milk and meat of sheep: Pilot study. Archives of Environmental Contaminants and Toxicology, 63: 288-298.

Kowalczyk J, Ehlers S, Oberhausen A, Tisher M, Fürst P, Schafft H, Lahrssen-Wiederholt M (2013) Absorption, distribution, and milk secretion of the perfluoroalkyl acids PFBS, PFHxS, PFOS, and PFOA by dairy cows fed naturally contaminated feed. Journal of Agricultural and Food Chemistry, 61: 2903-2912.

Kume S, Nonaka K, Oshita T, Kozakai T (2010) Evaluation of drinking water intake, feed water intake and total water intake in dry and lactating cows fed silages, Livestock Science, 128: 46-51.

Lupton SJ, Huwe JK, Smith DJ, Dearfield KL, Johnson JJ (2013) Absorption and excretion of ^{14}C-perfluorooctanoic acid (PFOA) in Angus cattle (*Bos Taurus),* Journal of Agricultural and Food Chemistry, 60: 1128-1134.

Meyer U, Everinghoff M, Gädeken D, Flachowsky G (2004). Investigations on the water intake of lactating dairy cows, Livestock Production Science, 90: 117– 121.

Murphy MR (1992). Water metabolism of dairy cattle. Journal of Dairy Science, 75: 326-333.

National Research Council (2001). Nutrient requirements of dairy cattle, Seventh Revised Edition, Chapter 8: Water Requirements. National Academy Press, Washington, D.C., U.S.A.

Numata J, Kowalczyk J, Adolphs J, Ehlers S, Schafft H, Fürst P, Müller-Graf C, Larssen-Wiederholt M, Greiner M (2014) Toxicokinetics of seven Perfluoroalkyl sulfonic and carboxylic acids in pigs fed a contaminated diet, The Journal of Agricultural and Food Chemistry, 62: 6861-6870.

Perkins, R, Butenhoff J, Kennedy G, Palazzolo M (2004) 13-Week dietary study of ammonium perfluoroctanoate (APFO) in male rats. Drug Chem Tox, 27, 361-378 (as cited in Zeilmaker et al., 2016).

RIVM (2018a). Letter to the city council of Dordrecht, July 2018. Moestuinonderzoek GenX en PFOA: resultaten van de analyse van GenX en PFOA in grond- en irrigatiewatermonsters. RIVM kenmerk 114/2018 DMG BL/TT, 9 juli 2018.

RIVM (2018b). Letter to the city council of Dordrecht, September 2018. Aanbieding rapportage Moestuin, fase 2 'Grond en irrigatiewatermonsters'. RIVM kenmerk 132/2018 M&V/EvS/RvP, 17 september 2018.

US EPA (2016). Health Effects Support Document for Perfluorooctanoic Acid (PFOA). EPA 822-R-16-003 May 2016. U.S. Environmental Protection Agency, Washington DC. Available online: www.epa.gov.

Van Asselt, ED, Kowalczyk J, Eijkeren JCH van, Zeilmaker MJ, Ehlers S, Fürst P, Lahrssen-Wiederholt M, Fels-Klerx HJ van der (2013) Transfer of perfluorooctane sulfonic acid (PFOS) from contaminated feed to dairy milk, Food Chemistry, 141: 1489-1495.

Van Poll R, 2018. Report 132. Annex to the letter to the city council of Dordrecht, September 2018.

Vestergren R, Orata F, Berger U, Cousins IT (2013) Bioaccumulation of perfluoroalkyl acids in dairy cows in a naturally contaminated environment, Environmental Science and Pollution Research, 20: 7959-7869.

Zeilmaker MJ, Janssen P, Versteegh A, van Pul A, de Vries W, Bokkers B, Wuijts S, Oomen A, Herremans J (2016) Risicoschatting emissie PFOA voor omwonenden. Locatie: DuPont/Chemours, Dordrecht, Nederland. RIVM Letter report 2016-0049. National Institute for Public Health and the Environment (RIVM), Bilthoven. Available online: www.rivm.nl.

Annex 1. Transfer models for contaminants in dairy cows: PFOS/PFOA

1. Introduction

In dairy cows PFOS and PFOA show quite different kinetics. For example, in the case of PFOS, milk is found the major route of excretion, with urinary excretion being negligible. Milk clearance however does not prevent PFOS accumulation in the blood and the carcass (as represented by muscle, liver and kidney), with the concentration in blood ≈ liver > kidneys >> muscle>> milk. In contrast, PFOA is only marginally detected in milk, i.e. levels just up to twofold above the LOD (0.1 µg L^{-1}). Levels in tissues were negligible when compared to an equal PFOS dosing. PFOA excretion occurs mainly via the urine with concentrations in urine >> plasma >> milk (Kowalczyk *et al.*, 2013).

The modeling of PFOS has been addressed before (Van Asselt *et al.*, 2013 and specifications herein). Here the basics of the PFOS model are summarized and its scaling to PFOA is described.

2. PFOS transfer model

The PFOS transfer model for dairy cows describes the uptake of PFOA from a feed matrix into the (free) PFOS fraction of blood serum. From circulating blood PFOS may be distributed into the cow's carcass or cleared towards bound serum PFOS, which in turn is cleared into the milk or urine (Van Asselt *et al.*, 2013). The model contains six unknown parameters, i.e. the fraction PFOS absorbed from hay/grass silage feed matrix, the free → bound clearance in the serum, the bound serum → milk clearance, the bound serum → urinary clearance, the serum flow-rate to the carcass and the serum-carcass partition coefficient. Analogous to Derks *et al.* (1993) the modeled cow's net body weight was set at 600 kg, the liver percentage of net body weight at 1.9%, the kidney fraction of net body weight at 0.3%, the blood volume fraction of net body weight at (9.3%) and the muscle fraction of net body weight at 35%. The carcass PFOS concentration was calculated as the weighted mean of the muscle, liver and kidney concentrations.

The PFOS transfer model was calibrated/verified on the basis of experimental results of Kowalczyk *et al.*(2013). In this study dairy cows (Holstein Friesian, body weight: 583 kg; N=6) were continuously exposed to hay-grass silage obtained from contaminated farmland for 28 days (upload phase, for intake data see Figure 1, N=3) or for 28 days followed by a wash-out period of 22 days (N=3). During the upload phase the overall average was 7.6 $\pm$ 3.2 µg kg bw^{-1} day^{-1}. As shown in Figure 1 for PFOS and Figure 3 for PFOA the intake during the 28 day upload phase showed a relative high intake between day 8 to 14, probably reflecting a quite high variability in the contamination level of different farmland batches. The experimental results of the exposure + wash out period for 3 cows were used to estimate unknown model parameters, whereas the results of the upload phase for the other 3 cows were used for validation purposes.

Of the six unknown parameters three, i.e. the fraction PFOS absorbed from hay/grass silage feed matrix and the milk and urinary clearances could unconditionally be identified. The remaining three parameters, i.e. the free → bound clearance in the blood, the blood flow-rate to the carcass and the blood-carcass partition coefficient appeared conditionally identifiable (for details, see Van Asselt *et al.*, 2013). As shown in Van Asselt *et al.* (2013, Figures 2 and 3, corresponding model specifications: see Table 1) the model clearly indicated PFOS to accumulate in blood serum, milk and carcass, with urinary excretion being negligible, eventually leading to a "steady state" situation (see Figure 2). Note that, as expected for bioaccumulating compounds, the time course of the accumulation does not visually reflect the time course of the daily intake.

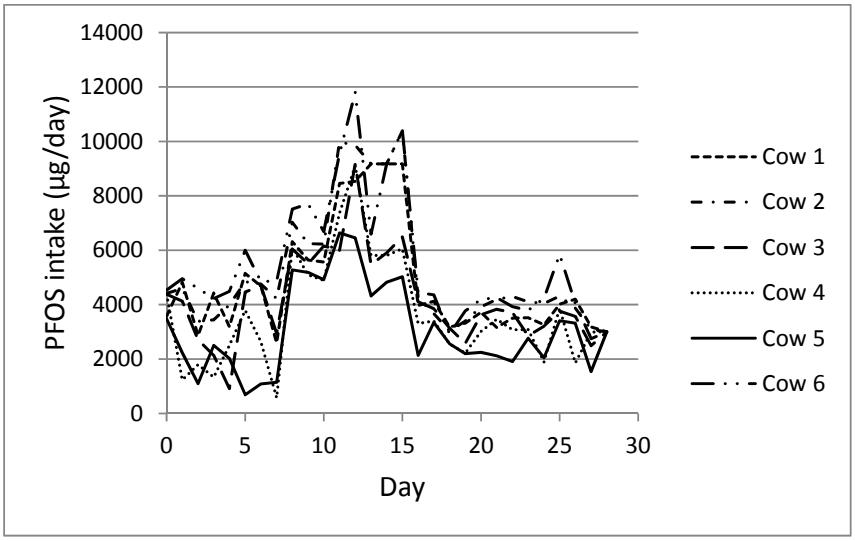


Figure 1. The daily intake (µg/day) of PFOS from contaminated hay-grass silage for a period of 28 days in dairy cows (individual data corresponding with Kowalczyk et al. (2013) supplied by WUR/RIKILT).

Table 1. PFOS model specifications (Van Asselt *et al.*, 2013).

Daily intake:	Individual intake as specified in Kowalczyk *et al*. (2013)(µg day^{-1})
Milk yield:	Individual milk yield as specified in Kowalczyk *et al*. (2013)(L day^{-1})
Fraction PFOS absorbed	1
$Serum_{free} \rightarrow Serum_{bound}$ clearance (CL_a)[1]	3.6 L day^{-1}
$Serum_{bound} \rightarrow$ Milk clearance (CL_m)	0.017 L day^{-1}
$Serum_{bound} \rightarrow$ Urine clearance (CL_u)	0 L day^{-1}
Carcass $\leftrightarrow Serum_{free}$ blood flow (Q_c)	13.4 L day^{-1}
$Serum_{free}$ -carcass partition coefficient (P_c)	28

[1] nomenclature as in Van Asselt *et al.* (2013)

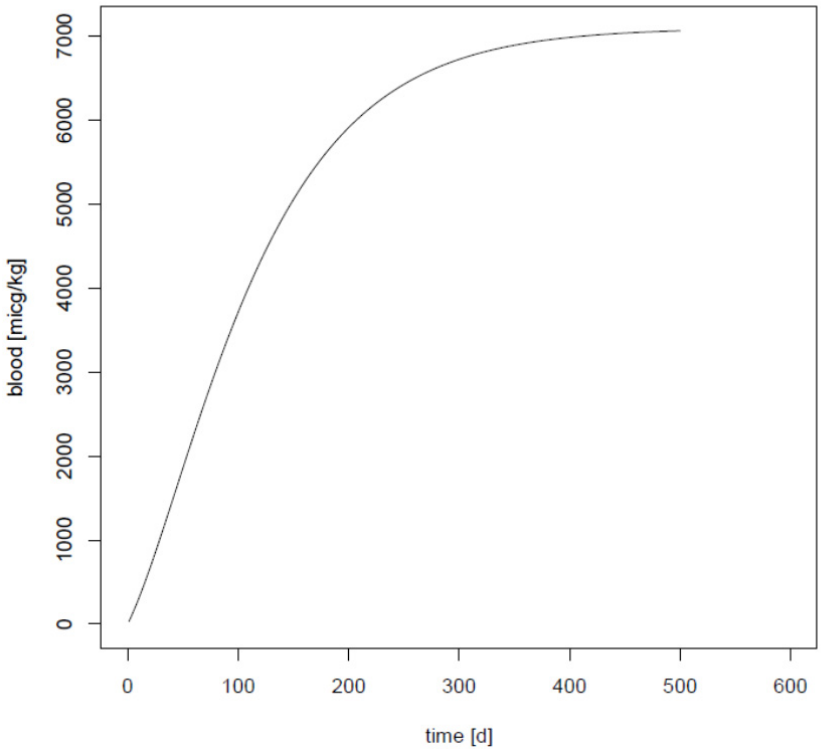


Figure 2. The time-course of the accumulation of PFOS in blood serum after continuous intake of 3000 µg day^{-1} from contaminated hay-grass silage in dairy cows. PFOS model specifications as described in Table 1.

3. PFOA transfer model

Next to PFOS the cows in Kowalczyk *et al.* (2013) were concomitantly exposed to (on average) 2.0 $\pm$ 1.2 µg PFOA kg bw^{-1} day^{-1} (see Figure 3).

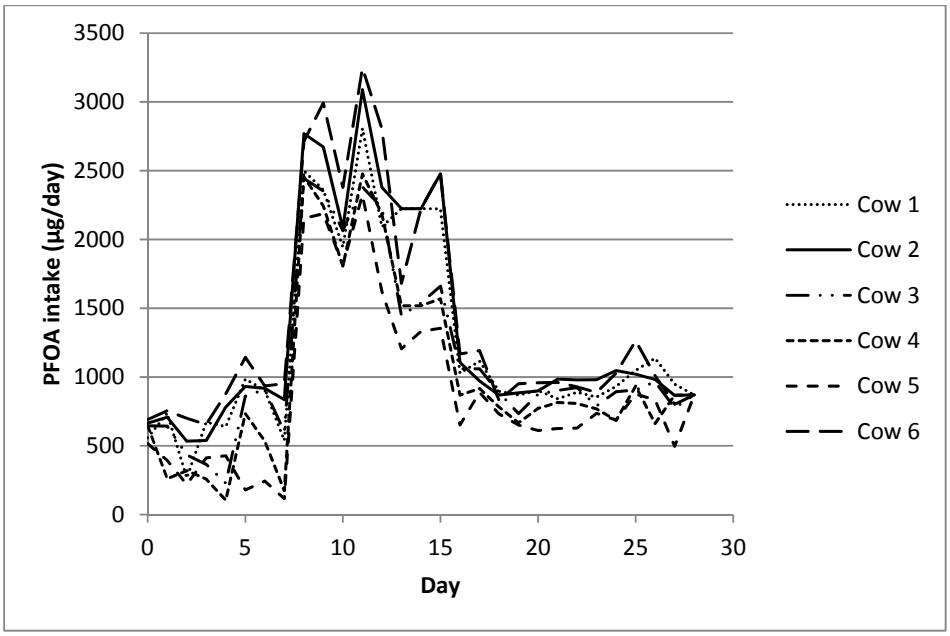


Figure 3. The daily intake (µg/day) of PFOA from contaminated hay-grass silage for a period of 28 days in dairy cows (individual data corresponding with Kowalczyk et al. (2013) supplied by WUR/RIKILT).

PFOA was excreted in the urine, with urinary concentrations ranging from 20 to 80 µg L^{-1} (to be compared with negligible PFOS urine levels). Observed levels in milk were at or just above the Limit of Detection of 0.1 µg L^{-1}. Moreover, in contrast to PFOS, the simulation characteristics closely follow PFOA intake characteristics, thereby reflecting instantaneous absorption and elimination kinetics of PFOA in dairy cows. At the end of the 28 day exposure period, levels in the liver, kidneys and muscle amounted 10.1, 8.7 and 0.6 µg kg^{-1}, corresponding with a carcass concentration around 1 µg kg^{-1} (to be compared with 295 µg PFOS kg^{-1}!). Corresponding levels in blood ranged from 9 to 16 µg L^{-1} (to be compared with around 2000 µg L^{-1} in the case of the PFOS exposure!).

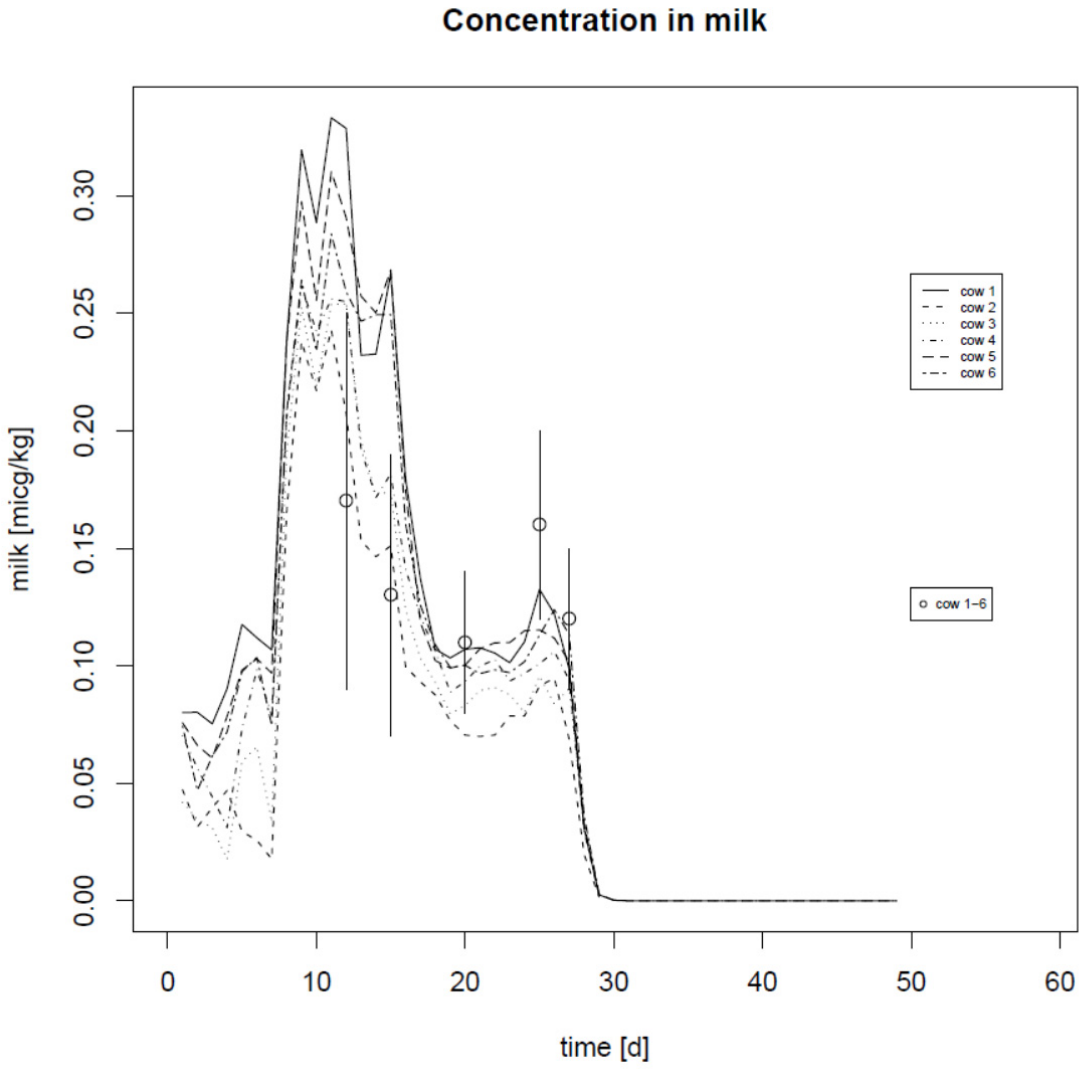
Clearly, describing PFOA kinetics within the same model concept as PFOS needs re-calibration of the latter, i.e. a decrease of the transfer of PFOA from the serum to the carcass, and an increase of the mass-flow towards urinary clearance (while maintaining clearance via milk).

The PFOA re-calibration was performed as follows. The decrease of the transfer of PFOA to the carcass was simulated by lowering the serum carcass partition coefficient from 28 (PFOS) to 0.25 (PFOA). To increase the mass-flow towards urinary clearance the free → bound clearance in blood serum was increased from 3.6 L day^{-1} (PFOS) to 10 L day^{-1} (PFOA) and the bound blood serum → urine clearance from 0 L day^{-1} (PFOS) to 15 L day^{-1} (PFOA), thereby enabling a relative high PFOA mass flow to the urine. The corresponding bound blood serum → milk clearance was found to be 0.040 L day^{-1} (PFOA, to be compared with 0.017 L day^{-1} for PFOS)(See Table 2). As shown in Figure 4A and 4B, this re-calibration a vu led to a satisfactory description of the observed transfer of PFOA from feed to milk.

Table 2. PFOA model specifications.

PFOA intake: Individual intake as specified in Kowalczyk *et al*. (2013)(µg day^{-1})	
Milk yield: Individual milk yield as specified in Kowalczyk *et al*. (2013)(L day^{-1})	
$Serum_{free}$ → $Serum_{bound}$ clearance (CL_a)	10 L day^{-1}
$Serum_{bound}$ → Milk clearance (CL_m)	0.040 L day^{-1}
$Serum_{bound}$ → Urine clearance (CL_u)	15 L day^{-1}
$Serum_{free}$ ↔ carcass partition coefficient (P_c)	0.25

A.



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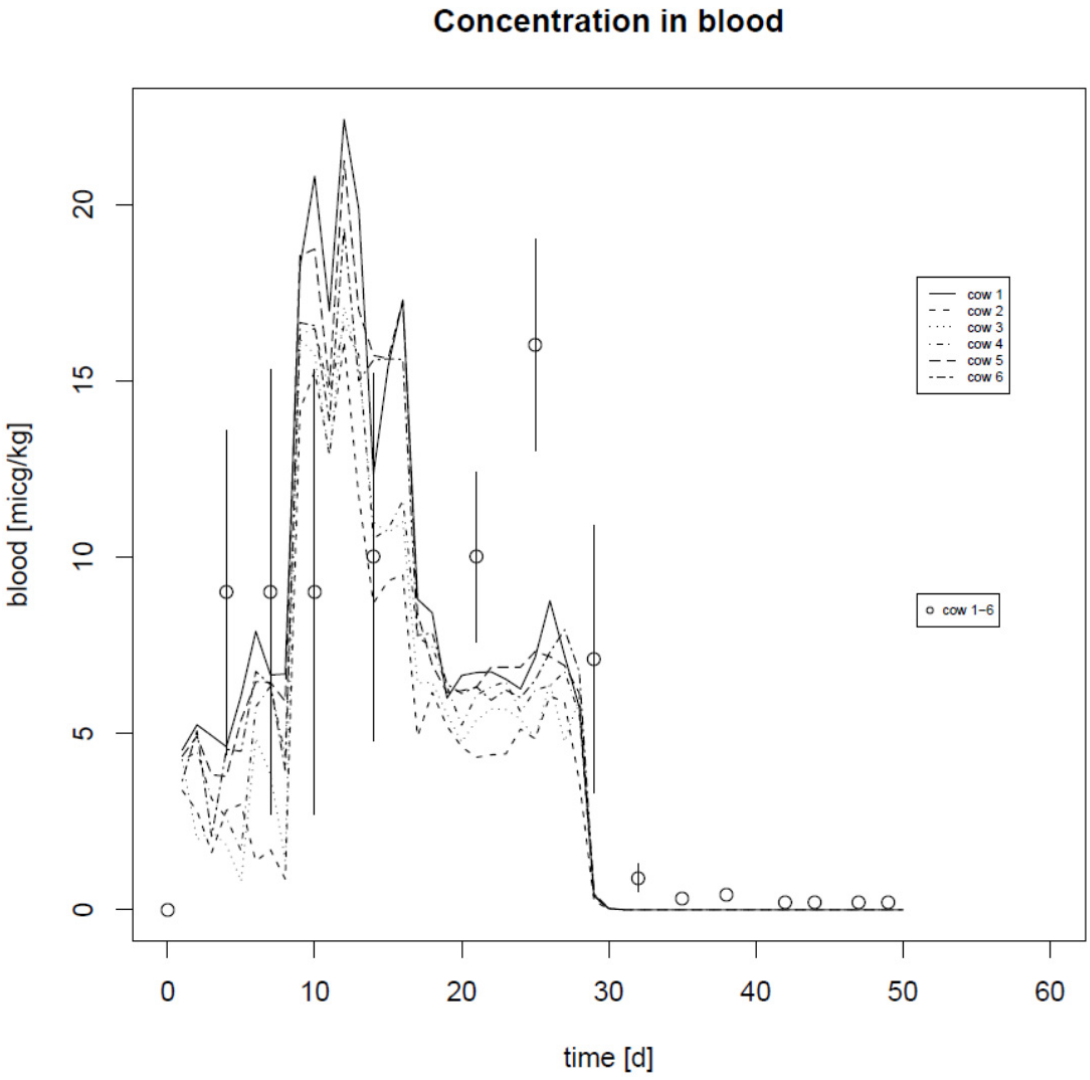
Concentration in blood
blood [micg/kg]
time [d]
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5
10
15
20
0
10
20
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C.

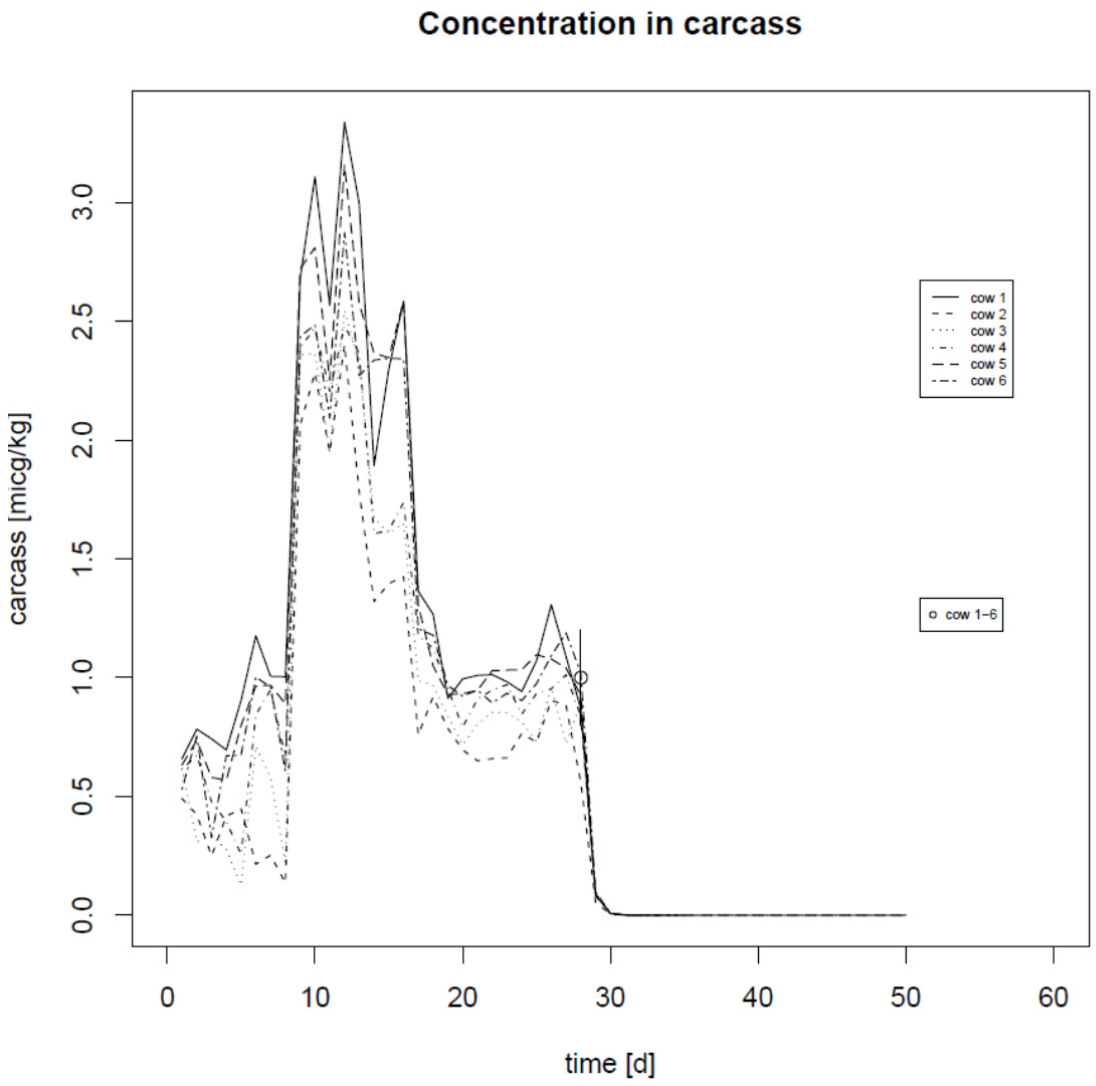

Figure 4. Model simulation of the transfer of PFOA from contaminated hay-grass silage to milk (A), blood serum (B) and carcass (C). PFOS transfer model recalibrated as specified in Table 2. Lines: cow specific individual kinetics. Symbols: experimental data (milk: upload phase, mean ± SD, to be compared with Kowalczyk et al., 2013, Figure 3 ; blood: upload + wash out phase, mean ± SD, to be compared with Kowalczyk et al., 2013, Figure 1).

4. Application of PFOA transfer model: FO question

In 2018 Dutch milk cows on average produced 28.1 kg milk day^{-1} (https://www.cooperatie-crv.nl, dd. 21-03-2019). The corresponding drinking water consumption was obtained from the study of Kume *et al.* (2010). In this study the drinking water consumption and corresponding milk yield were experimentally determined in lactating Holstein cows (body weight: 609 kg, N=16, water consumption and milk yield determined in a metabolic chamber during a 4-day time period). The average milk yield was determined at 29.5 kg day^{-1} (minimum: 21.9 kg day^{-1}; maximum: 35.3 kg day^{-1}. Similarly, the average drinking water intake was determined at 77.6 L day^{-1} (minimum: 57.0 L day $^{-1}$; maximum: 110.3 L day^{-1}).

Taking the drinking water consumption of Kume as representative for Dutch dairy cows the transfer of PFOA in drinking water in such cows to milk and meat was calculated given a daily intake of 80 liter water (corresponding with a daily milk yield of 29.5 kg day^{-1}) resp. 110 liter water (corresponding to a daily milk yield of 35 kg day^{-1}) containing 4.67 µg PFOA L^{-1}, i.e. resulting in a total daily intake of 374 µg resp. 514 µg PFOA. Given 600 kg for a cow's net body weight this corresponds with an intake of 0.62 µg resp. 0.86 PFOA/kg bw/day. Note that such intake exceeds the PFOA intake of dairy cows under uncontaminated pasture conditions, i.e. around 0.6 µg per day (Vestergren *et al.*, 2013) and is somewhat lower than in Kowalczyk *et al.* (2013).
For the exposure of 0.62 µg PFOA/kg bw/day the PFOA transfer model calculates a concentration of 0.04 µg kg^{-1} for milk and 0.20 µg kg^{-1} for muscle, i.e. meat, after repeated exposure. For the 0.86 µg PFOA/kg bw/day exposure corresponding concentrations are 0.06 µg kg^{-1} for milk and 0.28 µg kg^{-1} for muscle.

Given a level of 0.01 µg L^{-1} in milk the model back-calculates a daily PFOA intake of 89.2 µg, corresponding with a ditchwater concentration ranging from 89200/110 ≈ 810 ng L^{-1} to 89200/80 ≈ 1100 ng L^{-1}, at a milk yield of 25 kg day^{-1}.

References

Asselt, ED van, Kowalczyk J, Eijkeren JCH van, Zeilmaker MJ, Ehlers S, Fürst P, Lahrssen-Wiederholt M, Fels-Klerx HJ van der (2013) Transfer of perfluorooctane sulfonic acid (PFOS) from contaminated feed to dairy milk. Food Chemistry, 141: 1489-1495.

Derks HJGM, Berende PLM, Olling M, Everts H, Liem AKD, De Jong AJPM (1994) Pharmacokinetic modeling of polychlorinated dibenzo-p-dioxins (PCDDs) and furans (PCDFs) in cows. Chemosphere, 28(4): 711-715.

Kowalczyk J, Ehlers S, Oberhausen A, Tisher M, Fürst P, Schafft H, Lahrssen-Wiederholt M (2013) Absorption, distribution, and milk secretion of the perfluoroalkyl acids PFBS, PFHxS, PFOS, and PFOA by dairy cows fed naturally contaminated feed. Journal of Agricultural and Food Chemistry, 61: 2903-2912.

Kume S, Nonaka K, Oshita T, Kozakai T (2010) Evaluation of drinking water intake, feed water intake and total water intake in dry and lactating cows fed silages. Livestock Science, 128: 46-51.

Vestergren R, Orata F, Berger U, Cousins IA (2013) Bioaccumulation of perfluoralkyl acids in dairy cows in a naturally contaminated environment. Environmental Science and Pollution Research, 20: 7959-7969.