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

FRONT OFFICE FOOD AND PRODUCT SAFETY

REVISED RISK ASSESSMENT OF GENX AND PFOA IN FOOD PART 1: TOXICITY OF GENX AND PFOA AND INTAKE THROUGH CONTAMINATED DAIRY PRODUCTS, EGGS AND FISH

Risk assessment requested by:	Office for Risk Assessment and Research (BuRO)
Risk assessment performed by:	RIVM
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Subject

In the past, the companies Chemours in Dordrecht and Custom Powders in Helmond emitted the perfluoroalkyl substances (PFAS) GenX[1] and perfluorooctanoic acid (PFOA) into the air and surface water. The emission of GenX by Chemours is ongoing, although the local permit for emission was reduced (De Kort et al., 2019). 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, eggs, fish and silage) in these areas. In January 2019, Wageningen Food Safety Research (WFSR) analysed GenX and PFOA in these samples. The results were sent to the Front Office Food and Product Safety (FO) for risk assessment. The 2019 FO risk assessment was based on health-based guidance values (HBGVs) applicable at that time. In 2020, the European Food Safety Authority (EFSA) established an HBGV for the sum of four PFAS (hereinafter called the EFSA-4) based on new scientific information. This new HBGV is lower and, thus, stricter than the HBGVs applicable in 2019. This means that the FO assessment of 2019 is no longer valid.

Questions

Given the new HBGV derived by EFSA, the Office for Risk Assessment and Research (BuRO) asked the FO to update the risk assessment of GenX and PFOA. In 2019, BuRO asked the FO several questions related to GenX and PFOA concentrations in dairy products, eggs and fish. These questions were answered in the 2019 FO risk assessment (Part 1), which is updated in the current assessment. BuRO also asked questions related to GenX and PFOA concentrations in silage. Those questions are answered in a separate FO assessment (Part 2), which will also be updated. 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, eggs 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.

1) Toxicological starting point

In 2020, the European Food Safety Authority (EFSA) established a tolerable weekly intake (TWI) for the sum of four perfluoroalkyl substances (PFAS; hereinafter called EFSA-4), including perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS)), of 4.4 ng/kg body weight (bw) per week based on a reduced antibody response after vaccination in infants (EFSA, 2020a).

Although EFSA recognized that there were potency differences for PFASs on other toxicological endpoints, EFSA was not able to establish Relative Potency Factors (RPFs) for immune effects due to a lack of suitable studies. Therefore, EFSA assumed equipotency. However, knowing that PFAS are not equipotent for other effects (for example liver effects), RIVM considers it plausible that various PFAS are also not equipotent for their immune effects.

In the Netherlands, PFAS other than the EFSA-4, such as GenX, are detected in environmental media. Therefore, RIVM deemed it necessary to incorporate an additional step prior to being able to apply the EFSA TWI when performing risk assessments. RIVM will use the TWI derived for the EFSA-4 in combination with RPFs derived for liver effects for risk assessment of the combined exposure to PFAS. The RPF for GenX is 0.06, meaning this substance is 17 times less potent compared to PFOA based on hepatotoxicity.

The proposed RPF method is subject to further refinement. Currently research is ongoing at RIVM to further validate the RPFs.

2) Conclusions intake assessment

A simple chronic dietary risk assessment was performed for the summed concentrations of GenX and PFOA, expressed as PFOA-equivalents using RPFs, in products using a lower bound scenario (in which analytical results in samples below the level of quantification (LOQ) equalled 0) and an upper bound scenario (in which concentrations in samples below the LOQ equalled the value of the LOQ). Samples were taken in the areas around the companies Chemours in Dordrecht and Custom Powders in Helmond.

Because EFSA derived a TWI, the weekly chronic exposure to the sum of GenX and PFOA was estimated per product. To investigate whether frequent consumption of large portions of dairy products, eggs or fish locally produced or caught in the Dordrecht or Helmond area would pose a health risk, a point estimate was used. For this, high consumption statistics (95th percentile of consumption) of dairy products, eggs and fish were obtained from the Dutch National Food Consumption Survey of 2012-2016 and multiplied with the summed concentration of GenX and PFOA in the particular products. The maximum weekly intakes for the different age and sex-groups varied between:

- 0-5.8 ng PFOA-equivalents per kg bw for the consumption of dairy products sampled in the Dordrecht and Helmond area. If the exposure was calculated for a 35-year old lactating woman, taking into account her 35 exposure years and hence reflecting the situation on which the TWI was based, the exposure via dairy products varied between 0 and 2.0 ng PFOA-equivalents per kg bw;
- 0.7-2.2 ng PFOA-equivalents per kg bw for the eggs sample collected from the Dordrecht area;
- 0-0.68 PFOA-equivalents per kg bw for the eggs sample collected from the Helmond area;
- 0-0.45 ng PFOA-equivalents per kg bw for one eel obtained from an eel farm in the Helmond area;

- 13 ng PFOA-equivalents per kg bw for one carp caught in a fish pond in Helmond.

It should be noted that the upper bound exposure via dairy products, the eggs sample collected in Helmond and the eel sample was completely driven by the value of the LOQ.

3) Conclusions risk assessment

The conclusions regarding risk assessment for the products of animal origin sampled from the Dordrecht and Helmond area are uncertain because of the small number of samples of which the majority had analytical results below the LOQ, and the lack of background dietary exposure estimates based on recent concentration data.

The following conclusions can be drawn:

- Consumption of dairy products and eggs samples collected in the Dordrecht area and in the Helmond area, and that of the eel sample in the Helmond area for a prolonged period of time would not pose a health risk on the condition that:
  - Background exposure from other (non) dietary sources is sufficiently low;
  - The sampled eggs and eel are representative for all eggs and eel produced or caught in the particular area.
- Consumption of carp from the Helmond area for a prolonged period of time may pose a health risk. Given that only one carp was sampled, it is not clear whether this carp is representative for all carps or other freshwater fish in the Helmond area.

It is noted that the population is also exposed to GenX and PFOA via other dietary and non-dietary sources. When assessing the risk of GenX and PFOA intake from locally produced or caught foods from animal origin, this background exposure should be taken into account. RIVM recently performed an indicative dietary exposure assessment according to the RPF approach, based on data from 2009, which showed that exposure to the EFSA-4 via drinking water and food exceeded the TWI. It was concluded that more recent concentration data in food were needed for an up to date exposure assessment. For drinking water, concentration data were up-to-date.

Question 1: Toxicology of GenX and PFOA

Below, the toxicity data underlying the RIVM risk assessment strategy for combined exposure to GenX and perfluorooctanoic acid (PFOA) (Question 3) are described, as well as the critical study identified in the recent EFSA evaluation on perfluoroalkyl substances (PFAS), which formed the basis for the derivation of the tolerable weekly intake (TWI) for PFAS (EFSA, 2020a). An overview of the toxicity data may be found in ECHA (2019) for GenX and EFSA (2020a) for PFOA. Both compounds belong to the group of PFAS.

In summary, RIVM uses the TWI established by EFSA together with relative potency factors (RPFs) for PFAS for the risk assessment of this group of compounds (Annex B; RIVM, 2021a). Consequently, the RIVM tolerable daily intake (TDI) for PFOA (Zeilmaker et al., 2016) and the tentative TDI (t-TDI) for GenX (Janssen, 2017) have become obsolete.

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 (with polytetrafluorethylene (PTFE; brand name 'Teflon') being an important fluoropolymer). These substances are used as substitutes for PFOA

and its salts, that were formerly used as processing aids in the production of fluoro-elastomers and 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.

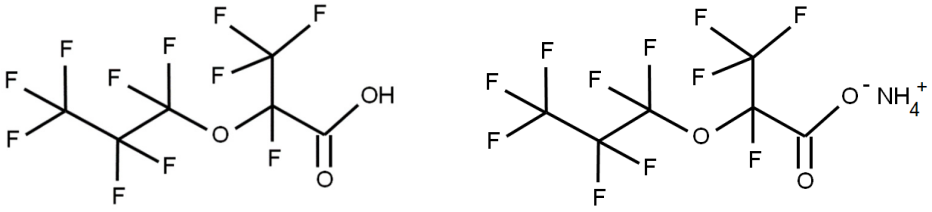

Figure 1. The chemical structure of the acid FRD-903 (left) and the ammonium salt FRD-902 (right)

PFOA
PFOA (CAS no. 335-67-1) and its salts are as of July 2020 only allowed for derogated uses defined under the amendment of Annex I (EU 2020/784) of the persistent organic pollutants regulation (EU 2019/1021) such as in fire-fighting foams, pharmaceutical products, and certain textiles. The chemical structure of PFOA is given in Figure 2.

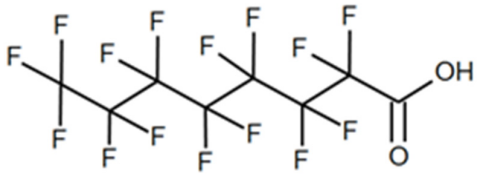

Figure 2. The chemical structure of perfluorooctanoic acid (PFOA)

TWI for the sum of four PFAS (EFSA, 2020a)
EFSA established a 10% benchmark dose lower confidence limit ($BMDL_{10}$) of 17.5 ng/mL for the sum of PFOA, perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS) ('the EFSA-4') in blood serum of 1-year-old children (fed mothers milk or infant formulae), based on the reverse association between serum levels and antibody titers against diphtheria observed in an epidemiological study (Abraham et al., 2020; Annex A). This $BMDL_{10}$ was used to estimate the serum concentration in the mother, i.e. 6.9 ng/mL for the sum of the EFSA-4, that would correspond with a concentration in breastmilk, which in turn would lead to the 17.5 ng/mL in blood serum of 1-year old children. It was estimated that a daily intake of 0.63 ng/kg bw per day for the sum of the EFSA-4, of the mother, would lead to the serum level of 6.9 ng/mL at the age of 35 years. Subsequently, this leads to a TWI of 7 x 0.63 = 4.4 ng/kg bw per week. This TWI should prevent that mothers reach a body burden that results in levels in breast milk that would lead to serum levels in the infant, associated with a decrease in vaccination response. The TWI derivation takes a relative high PFAS exposure of breastfed infants into account. EFSA's TWI derivation therefore explicitly states that the intake of PFAS by infants should not be compared with this TWI (EFSA 2020a). The assumptions and steps to derive the TWI are summarized and discussed in EFSA (2020a) and the RIVM memorandum (RIVM, 2021a).

Combined use of TWI with RPFs (RIVM, 2021a)
RIVM uses the TWI together with relative potency factors (RPFs) for PFAS (RIVM, 2021a), after discussion of scientific arguments and weighing several options for implementation of the TWI (RIVM, 2020a; RIVM, 2020b; RIVM, 2021a). A summary of this discussion is provided in Annex B. Because detectable concentrations of PFAS other than the EFSA-4 are observed in the Netherlands (note for instance the concentrations of GenX, PFHxA, PFHpA, PFDA and PFUnDA in ditch water in RIVM (2021b)), it was proposed to use a uniform method to be able to address all policy questions regarding PFAS at a national level.

By combining the TWI with RPFs it is possible to assess the risk to other combinations of PFAS than the four considered by EFSA, as well as to single PFAS. RPFs describe the toxic potency of individual PFAS relative to an index compound and thus take into account differences in potency between PFAS. In the current set of RPFs, PFOA was selected as index compound, and consequently the RPFs describe the toxicity of individual PFAS relative to the toxicity of PFOA. RPFs are available for 23 PFAS, among which GenX, and are based on hepatotoxicity (Bil et al., 2021). The RPF for GenX is 0.06, meaning that this substance is 17 times less toxic than PFOA.

By using RPFs, the exposure to individual PFAS or a combination of PFAS is expressed as 'PFOA equivalents'. This is done by multiplying the exposure to individual PFAS with the relevant RPF and summing the exposure across PFAS. The resulting summed exposure expressed as PFOA-equivalents is then compared with the TWI to assess the risk. A comparable approach with Toxic Equivalency Factors is already being used in mixture risk assessment of dioxins for a considerable time.

GenX was not part of the EFSA opinion and no epidemiological studies are available for this substance. However, an immunotoxicity study in mice and a chronic/carcinogenicity study in rats provide evidence of immunosuppressive effects of GenX. Effects include suppression of the IgM antibody response, increased number of T-lymphocytes, affected CD4+/CD8+ and CD4-/CD8- ratios, decreased absolute and relative (to body weight) spleen weights, and increased albumin/globulin ratio (Annex A). This information supports the use of the TWI based on immunosuppression in combination with the RPF for GenX, and thereby assessment of the summed exposure to GenX and PFOA.

As a remark, the TWI is based on immune effects, while the RPFs are derived from liver effects. Although it was recognized that there are dosing differences for PFOA and PFOS in animal experiments studying the immune response, EFSA stated that they were not able to establish RPFs for immune effects due to a lack of suitable animal studies comparing the effect of different PFAS on the immune response. EFSA therefore assumed equipotency (EFSA, 2020a). It is however known that PFAS are not equipotent with regard to other effects (such as liver effects; Bil et al., 2021). Therefore, RIVM considers it plausible that PFAS are also not equipotent with regard to immune effects.[2] RIVM however recommends that the RPFs derived from liver effects are validated for immune effects in due course. See also Annex B for a more elaborate discussion on this aspect.

Question 2 and 3: Exposure and risk assessment

Concentration GenX and PFOA in dairy products, eggs and fish
GenX and PFOA were analysed in dairy products (milk, cheese and yoghurt), eggs and fish (carp and eel) sampled near the companies Chemours in Dordrecht and Custom Powders in Helmond. Table 1 lists the product concentrations per location as provided by

[2] EFSA is of the opinion that the available RPFs cannot be used for immune effects given the uncertainty about a common mechanism of action of immune and liver effects (EFSA, 2020b). In addition, the RPFs do not take into account the possible differences between PFAS in terms of their distribution across milk and serum. Although RIVM acknowledges these points, RIVM considers that, in view of the information available for relative potencies for other toxicological endpoints, these relative potencies of PFASs are expected to reflect more closely the differences in potency on immune response than the assumption of equipotency made by EFSA (see RIVM comments in EFSA 2020b).

Wageningen Food Safety Research (WFSR). The majority of the concentrations was below the limit of quantification (LOQ). Only the PFOA concentration in one sample of eggs collected 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 individual concentrations of GenX and PFOA (expressed as such and as PFOA-equivalents) and the sum of GenX and PFOA concentrations (expressed as PFOA-equivalents) in dairy products, eggs and fish sampled near the companies Chemours in Dordrecht and Custom Powders in Helmond (data provided by WFSR)

Product and location	n	GenX[1]		PFOA[1]		Sum of Genx and PFOA[1,4]
		Analytical value (ng/g)	PFOA-equivalents[2] (ng/g)	Analytical value (ng/g)	PFOA-equivalents[3] (ng/g)	PFOA-equivalents (ng/g) (LB-UB)
Dordrecht						
Dairy products						
Milk[5]	15	<0.10	<0.006	<0.01	<0.01	0-0.016
Cheese[5]	1	<0.10	<0.006	<0.10	<0.10	0-0.106
Yoghurt[5]	1	<0.10	<0.006	<0.10	<0.10	0-0.106
Eggs[6]	1	<0.25	<0.015	0.140	0.140	0.140-0.155[7]
Helmond						
Dairy products						
Milk[5]	2	<0.10	<0.006	<0.01	<0.01	0-0.016
Eggs[6]	1	<0.25	<0.015	<0.025	<0.025	0-0.040
Fish						
Eel (farmed)	1	<0.10	<0.006	<0.05	<0.05	0-0.056
Carp	1	4.7	0.28	1.3	1.3	1.58

PFOA: perfluoro octanoic acid; LB: lower bound, in which concentrations below the limit of quantification (LOQ) were assumed to equal 0; UB: upper bound, in which concentrations below the LOQ were assumed to equal the value of the LOQ.
[1] Samples with concentrations reported as '<' may contain GenX and PFOA, but the concentrations did not equal or exceed the LOQ of the analytical method.
[2] PFOA-equivalents were obtained for GenX by multiplying the analytical value of GenX by its relative potency factor of 0.06.
[3] PFOA equivalents were obtained for PFOA by multiplying the analytical value by its relative potency factor of 1.
[4] The concentrations of GenX and PFOA, expressed as PFOA-equivalents, were summed.
[5] Cow milk and one sample of goat milk in Dordrecht.
[6] Chicken.
[7] Sum of a quantified amount of 0.140 ng/g as PFOA-equivalents and an amount below the LOQ which was substituted with the value of the LOQ expressed as PFOA-equivalents.

Conversion of GenX and PFOA concentrations to PFOA equivalents
As described in the response to Question 1, RIVM uses the TWI for PFAS in combination with RPFs to assess the risk of the summed exposure to PFAS. To estimate the summed exposure to PFAS, two methods can be applied:

- Summation of the exposure to individual PFAS expressed as PFOA-equivalents (as in the paragraph 'Combined use of TWI with RPFs')
- Summation of the PFAS concentrations expressed as PFOA-equivalents per sample and then calculating the exposure using these summed concentrations.

Following the simple methodology for exposure assessment in the present FO assessment, both methods will provide identical exposure estimates. The order of RPF calculation is only relevant for higher tier exposure assessment taking correlations between substances in samples into account in acute exposure assessments or identifying risk drivers (which PFAS via which foods contribute substantially to exposure). We used the second method based on RPF-adjusted

concentrations to assess the summed exposure to GenX and PFOA. The RPF of GenX is 0.06 and of PFOA is 1 (Bil et al., 2021). This means that the concentration of GenX is multiplied by 0.06 and that of PFOA by 1. The resulting concentrations expressed as PFOA-equivalents were then summed. This was done for GenX and PFOA concentrations in dairy products, eggs and fish.

Table 1 lists the sum concentrations expressed as PFOA-equivalents which were used in the exposure assessment. It should be noted that only the PFOA-equivalents in carp were based on a quantified GenX and PFOA concentration. The eggs sample from Helmond had a quantified PFOA concentration, while the GenX concentration was below the LOQ. Because the majority of samples had a GenX and PFOA concentration below the LOQ, the exposure was assessed for two scenarios:

- The lower bound scenario, in which concentrations below the LOQ were assumed to have a concentration of 0, i.e. samples do not contain PFAS;
- The upper bound scenario, in which concentrations below the LOQ were assumed to equal the value of the LOQ.

FO assessment of 2019
In 2019, calculations were performed to obtain concentrations in products that would lead to an exposure equal to 20% of the TDI ('20% TDI concentrations'). The calculations were based on estimates relevant for acute exposure (high consumption on one exposure day). Those 20% TDI concentrations were used to assess whether the GenX- and PFOA-concentrations analysed were likely to pose a health risk for GenX and for PFOA (RIVM & WFSR, 2019). Considering that the current TWI is a factor 20 lower than the former TDI for PFOA and that a cumulative risk assessment should be performed, no such concentrations were derived in the current assessment. Instead a risk assessment was performed for each product, as was done for carp in 2019 (RIVM & WFSR, 2019).

Point estimates of chronic dietary exposure
As explained in the response to Question 1, the TWI should prevent mothers from reaching a body burden that results in PFAS-levels in breastmilk that would lead to serum levels in the infant that are associated with decreases in vaccination response. Therefore, dietary exposure should preferably be calculated for lactating women and taking into account their exposure from birth onwards. As a first indication of dietary exposure, point estimates of exposure were calculated based on consumption statistics for different age-sex groups available from the DNFCS 2012-2016.

To assess the dietary exposure to GenX and PFOA per product, we combined the summed concentrations of GenX and PFOA, expressed as PFOA equivalents, with the consumption data among 4313 individuals aged 1 to 79 of DNFCS 2012-2016 for these products. 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 data of carp and eel in this survey, the consumption of fish was used as a proxy for both types of fish. Fish consumption included all types of 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 and eel may be consumed. Annex C lists the mean and high (95^{th} percentile) consumption of dairy products, eggs and fish for different age-sex groups for all days in the survey and for only those days on which the consumption of these products was reported ("consumption days").
PFAS may be harmful when ingested at high amounts over a long period of time. Therefore, we used the consumed amounts of dairy product, eggs and fish that best reflect long consumed amounts of these products for the risk assessment. The best estimate for this is the consumed amounts based on 'all days' (irrespective of whether dairy products, eggs or fish were consumed or not), assuming that individuals are not likely to consume locally produced dairy products, eggs or fish caught from the particular fish pond every day. We used the high (95^{th} percentile) consumed amounts to estimate

the summed intake of GenX and PFOA to also protect possible high consumers of these products. The 95th percentile was based on mean consumed amounts across the two consumption days per individual.

Based on the summed concentrations of GenX and PFOA, expressed as PFOA-equivalents, in dairy products, eggs or fish (Table 1) and high consumed amounts of the particular food, the summed intake of GenX and PFOA was estimated per age – sex group using the following equation:

$$Intake = \frac{Consumption \times Concentration}{Body\ weight} * 7$$

Intake	= Intake of PFOA-equivalents in ng/kg bw per week
Consumption	= High (P95) consumption of dairy products, eggs, eel and carp, in gram across two days (Annex C)
Concentration	= Concentration of PFOA-equivalents in carp, eel, milk or eggs in ng/g (Table 1)
Body weight	= Body weight in kg (Annex C)
7	= to extrapolate from a daily intake into a weekly intake

The main difference with the calculation of 2019 for carp is the use of an additional factor 7 to extrapolate daily consumption (for comparison with the TDI, as applicable in 2019) to weekly consumption as the current HBGV is a TWI.

Table 2 and Annex D show the summed intake results of GenX and PFOA, expressed as PFOA-equivalents, for dairy products, eggs and fish. All measurements for milk, either sampled in the Dordrecht area or Helmond area, were below the LOQ and were presented together. It should be noted that the LOQs of PFOA for cheese and yoghurt were a factor 10 higher than that of milk (Table 1). For the exposure calculation, we assumed that the GenX and PFOA concentrations in cheese and yoghurt were similar to that in milk, i.e., < 0.016 ng PFOA-equivalents/kg. For eggs, the exposure was calculated per area, as the sample from Dordrecht was above the LOQ for PFOA, whereas the sample from Helmond was below the LOQ (Table 1). GenX concentrations were below the LOQ in both eggs samples.

Risk characterisation of chronic exposure

For the risk assessment, the summed exposure estimates of GenX and PFOA, expressed as PFOA-equivalents, per product for the different age-sex groups were compared with the TWI of 4.4 ng/kg bw per day. If all exposure estimates per age-sex group are below the TWI, the exposure will not pose a risk, and if they exceed the TWI, the exposure may be of concern. If the exposure exceeds the TWI only for a certain age group (e.g. toddlers), further calculations were performed to assess the exposure for 35-year old lactating women, the age group on which the TWI was based (see section 'TWI for the sum of four PFAS'). For this, 35 exposure years were taken into account. This was done by multiplying the exposure of a certain age group by the number of years represented in that age group (3 years for toddlers, 5 years for other children, 10 years for female adolescents and 17 years for adult women) and divided by 35. It should be noted that this is a conservative estimate, because it is based on summation of P95 dairy consumption. By this conservative estimate, it is assumed that all females are high consumers of dairy products throughout their life up to the age of 35.

For dairy products, only the upper bound intake estimate of the sum of GenX and PFOA of 5.8 ng PFOA-equivalents/kg bw per week for children aged 1-3 years exceeded the TWI. When looking at the exposure of 35-year women from childhood onwards, the upper bound intake estimate was 2.0 ng PFOA-equivalents/kg bw per week, which remains below the TWI. Since the concentration GenX and PFOA was below the LOQ in all dairy products, the upper bound exposure estimates for dairy products were completely driven by the value of the LOQ).

Table 2. Intake of the sum of GenX and PFOA (expressed as PFOA-equivalents) through a high (95th percentile) mean consumption of dairy products and eggs sampled in the Dordrecht area, and for dairy products, eggs, eel and carp sampled in the Helmond area[1]

Age group (year) + sex	LB-UB exposure to the sum of GenX and PFOA[2] ng PFOA-equivalents/kg bw per week				
	Dairy products[3]	Eggs		Eel[6]	Carp[7]
	D/H	D[4]	H[5]	H	H
1-3	0-5.8	2.0-2.2	0-0.68	0-0.41	12
4-8	0-3.7	1.5-1.7	0-0.44	0-0.40	11
9-18; male	0-1.8	0.9-1.0	0-0.26	0-0.11	3.0
9-18; female	0-1.5	1.0-1.1	0-0.28	0-0.27	7.7
19-50; male	0-1.2	0.7-0.8	0-0.19	0-0.41	12
19-50; female	0-1.1	0.8-0.8	0-0.22	0-0.37	10
51-79; male	0-1.1	0.7-0.8	0-0.20	0-0.45	13
51-79; female	0-1.0	0.7-0.7	0-0.19	0-0.41	12
Female, 35 year[8]	0-2.0	-	-	-	-

bw: body weight; D: Dordrecht area; H: Helmond area; LB: lower bound; PFOA: perfluoro octanoic acid; TWI: tolerable weekly intake; UB: upper bound

[1] Exposure was based on a high mean consumption across two days considering all consumption days within the food consumption survey, irrespective of whether the particular food was consumed or not (Table C1 of Annex C).

[2] Exposure presented as the range from lower bound (LB) to upper bound (UB) scenario. In the LB scenario, it was assumed that concentrations below the LOQ were equal to 0. For the UB scenario, it was assumed that concentrations below the LOQ were equal the value of the LOQ. For carp, the LB and UB exposure estimates were the same.

[3] Based on 15 milk samples collected in the Dordrecht and Helmond area. The sum concentration of GenX and PFOA in those samples was below the limit of quantification (LOQ) of 0.016 ng PFOA-equivalents/g (Table 1).

[4] Based on one sample of eggs collected in the Dordrecht area. The sum concentration of GenX and PFOA in this sample was 0.140 ng PFOA-equivalents/g in the LB scenario and 0.155 ng PFOA-equivalents /g in the UB scenario

[5] Based on one sample of eggs collected in the Helmond area. The sum concentration of GenX and PFOA in this sample was below the LOQ of 0.040 ng PFOA-equivalents/g.

[6] Based on one sample of farmed eel collected in the Helmond area. The sum concentration of GenX and PFOA in this sample was below the LOQ of 0.056 ng PFOA-equivalents/g.

[7] Based on 1 sample of carp caught in a pond in the Helmond area. The sum concentration of GenX and PFOA in this sample was 1.58 ng PFOA-equivalents/g.

[8] Weekly intake assessed for 35-year old women based on the exposures presented for the age-sex groups presented in the table and taking into account the number of years represented in the age group. For female adults 17 years if exposure (from the age of 19 up to 35 years) was included. It is calculated by (3*5.8)+(5*3.7)+(10*1.5)+(17*1.1)/35=2.0 ng PFOA-equivalents/kg bw per week.

For the eggs sample from the Dordrecht area (GenX below the LOQ; PFOA 0.140 ng PFOA-equivalents/g), the LB and UB exposure estimates for all age-sex groups were below the TWI (Table 2). This means that if the particular eggs sample was consumed for a prolonged period of time, it would not pose a health risk on the condition that exposure to other (non)dietary sources would be sufficiently low. The same is true for the Helmond eggs sample for which the summed exposure estimates were even lower than for the Dordrecht eggs sample (Table 2). The upper bound exposure estimates for the Helmond eggs sample were all completely driven by the value of the LOQ.

Also for the particular farmed eel sample, LB and UB estimates do not pose a health risk on the condition that background exposure to PFAS from other (non)dietary sources would be sufficiently low. The intake of the sum of GenX and PFOA via carp exceeded the TWI of 4.4 ng/kg bw per week for all age groups, except for male adolescents (Table 2 and Annex D). So consumption of this particular carp, for a prolonged period of time, may pose a health risk.

Discussion

The exposure estimates of the sum of GenX and PFOA present in locally produced dairy products, eggs and eel and locally caught carp described in the current FO assessment should be regarded as first rough estimates, for reasons mentioned below.

Uncertainties in the applied risk assessment approach

The scientific basis of the TWI of PFAS has been discussed previously by RIVM (2020b). After weighing several options for implementation of the TWI, including the default assumption of PFAS equipotency, the use of the TWI together with the RPF method was regarded as the most suitable for the risk assessment of PFAS (Annex B; RIVM, 2021a). By using RPFs in combination with the TWI, it is assumed that other PFAS can also have an adverse effect on the immune system and the RPFs provide an estimate of the degree in which the substances exert this effect relative to PFOA. This consequently takes account of the possibility that exposure to PFAS other than the EFSA-4 can add up and have a cumulative effect. Considering the observed GenX-induced adverse effects on the immune system in rodents (Annex A), exposure to the summed concentrations of GenX and PFOA, expressed as PFOA-equivalents was considered as a reasonable approach.

Although the use of the RPF method together with the TWI has several advantages over the other discussed approaches, it has the uncertainty that the available RPFs were established for liver effects in rats, while the TWI is based on effects on the immune system in humans. It is therefore recommended that the RPFs are validated for immune effects in due course. Moreover, another uncertainty is that the RPFs take no account of the possible differences between PFAS in terms of their distribution across milk and serum. This extrapolation is based on the PBPK modelling for PFOA and PFNA and for PFOS and PFHxS, and is already incorporated in the derivation of the TWI (Annex B).

Although the proposed RPF method is subject to further refinement, RIVM considers that the actual risk can be estimated more accurately if potency differences between PFAS are taken into account and if several PFAS can be included in the risk calculation. By using this approach, the risk associated with exposure to GenX and PFOA is assessed together. The desire to take account of cumulative exposure to substances has been expressed at both national and European level (RLI, 2020; EU, 2020).

Limited number of measurements

As already recognised in the 2019 FO assessment, the number of measurements used in the assessment was limited (RIVM & WFSR, 2019). For egg, eel and carp, there were only one or two samples, which did not provide information on distribution of concentrations of GenX and PFOA in those foods produced in the Dordrecht and Helmond area. It is therefore unclear if the analysed concentrations are representative of all eggs, eel and carp (or other fish) obtained from the Dordrecht and/or Helmond area. Because of this, the intake of GenX and PFOA via eggs, eel, carp or any other fish obtained from the particular areas may be under- or overestimated. No concentration data of carp, eel or other fish from the Dordrecht area were provided to the FO. Therefore, no conclusions can be made for fish in the Dordrecht area. It should be noted that more data for fish sold on the Dutch market and eel caught in several fresh water areas in the Netherlands are available (Zafeiraki et al., 2019). These data also included fish caught near hotspots.

Due to the limited number of the products sampled in the Dordrecht/Helmond area it cannot be concluded whether consumers of locally produced eggs and eel and of locally caught fish may have a higher exposure to PFAS than consumers consuming non-local foods. It should be noted that up-to-date concentration data for the Netherlands are currently not available (see section ‘Background exposure from other foods’).

Measurements below the LOQ

As noted in the 2019 FO assessment, all measurements for dairy products, eel and eggs were below the LOQ, except for one eggs sample (Table 1). The lower bound and upper

bound scenarios provide the extremities of what the exposure could be. If values below the LOQ were assumed to contain no GenX and PFOA, the exposure would be 0 for dairy products, the eggs sample from the Helmond area and the farmed eel sampled from the Helmond area. By assuming that measurements with a value below the LOQ equal the LOQ, so-called upper bound scenario, the exposure will be overestimated based on the available concentrations. Uncertainties around concentrations in samples with analytical results <LOQ can be solved by using more sensitive analytical methods. According to WFSR, an LOQ of 0.0025 ng/g in milk and other foods is currently feasible when instrument performance is optimal (Berendsen et al., 2020), which is lower than those applicable in the 2019 FO assessment (Table 1).

For carp, the estimated exposure would not change as both GenX and PFOA were above the LOQ. For the eggs sample of Dordrecht, the maximum exposure would be 2.0 instead of 2.2 ng PFOA/kg bw per week, which is a minor reduction in exposure, due to the low RPF of GenX.

Background exposure from other foods
The applied methodology to assess the sum intake of GenX and PFOA via the consumption of locally produced dairy products, eggs and fish was based on high consumption estimates of these particular foods combined with a single concentration value. As indicated in the 2019 FO assessment, people living in the vicinity of both companies are not only exposed to GenX and PFOA through the consumption of locally produced dairy products, eggs and/or fish. Also other locally produced foods (home grown vegetables, fruits and potatoes, meat and drinking water; Mengelers et al., 2018; Boon et al., 2019, 2021a,b) or non-locally produced foods contain PFAS (EFSA 2020a; Noorlander et al., 2011) can contribute to the overall exposure to PFAS.

When assessing the PFAS exposure of people living in the vicinity of a hotspot, the background exposure to PFAS should preferably be taken into account. Recently, an indicative background exposure assessment using concentrations of the EFSA-4 in drinking water and foods was performed for the Dutch population according to the RPF approach (Van der Aa et al., 2021). This indicative assessment showed that the exposure to the EFSA-4 exceeded the TWI. It was concluded that up-to-date concentration data in food were needed for a better exposure estimate, as these data were from a 2011 study (Van der Aa, 2021). Such data would allow for an exposure assessment of PFAS for the general population and for people consuming locally produced foods. It could also provide a range of PFAS concentrations in non-locally produced food to which concentrations of locally produced foods can be compared.

The EFSA opinion also showed that intake of the EFSA-4 exceeded the TWI by a large part of the European population. EFSA also recognised the large number of values below the LOQ (EFSA 2021), and concluded that true exposure level for the EFSA-4 is closer to the LB than the UB values. The LOQs of PFAS in food in the EFSA opinion were generally higher than those listed in Table 1. Therefore, the concentration data of EFSA were deemed not useful to perform an exposure assessment of the Dutch population in the present FO.

Exposure to other PFAS
In the studies near Helmond and Dordrecht, only GenX and PFOA were analysed in the dairy products, eggs, eel and carp. However, the presence of other PFAS cannot be excluded. This would mean that the exposure is potentially underestimated, also considering the higher potencies of some compounds (e.g. PFNA, PFDA or PFUnDA).

Exposure from non-food sources
As already mentioned in the 2019 FO assessment, non-food sources (such as consumer products or house dust) are relevant too for the exposure to PFAS (Poothong et al. 2020; Thepaut et al., 2021). Air is another likely source of GenX and PFOA exposure (De Kort et

al. 2019; Poothong et al. 2020). In Helmond, also swimming water was identified as a potential source of exposure (Geraerts et al., 2021; Muller & te Biesebeek, 2018). These sources need to be considered in the risk assessment of these compounds (EFSA, 2020a). Furthermore, an assessment of biomonitoring samples (i.e. blood) could complement such assessments to observe if summed aggregate exposure, reflected in measured serum levels, exceeds the critical serum levels used for deriving the TWI.

Overall conclusion

1) Toxicological starting point

In 2020, the European Food Safety Authority (EFSA) established a tolerable weekly intake (TWI) for the sum of four perfluoroalkyl substances (PFAS; hereinafter called EFSA-4), including perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS)), of 4.4 ng/kg body weight (bw) per week based on a reduced antibody response after vaccination in infants (EFSA, 2020a).

Although EFSA recognized that there were potency differences for PFAS on other toxicological endpoints, EFSA was not able to establish Relative Potency Factors (RPFs) for immune effects due to a lack of suitable studies. Therefore, EFSA assumed equipotency. However, knowing that PFAS are not equipotent for other effects (for example liver effects), RIVM considers it plausible that various PFAS are also not equipotent for their immune effects.

In the Netherlands, PFAS other than the EFSA-4, such as GenX, are detected in environmental media. Therefore, RIVM deemed it necessary to incorporate an additional step prior to being able to apply the EFSA TWI when performing risk assessments. RIVM will use the TWI derived for the EFSA-4 in combination with RPFs derived for liver effects for risk assessment of the combined exposure to PFAS. The RPF for GenX is 0.06, meaning this substance is 17 times less potent compared to PFOA based on hepatotoxicity.

The proposed RPF method is subject to further refinement. Currently research is ongoing at RIVM to further validate the RPFs.

2) Conclusions intake assessment

A simple chronic dietary risk assessment was performed for the summed concentrations of GenX and PFOA, expressed as PFOA-equivalents using RPFs, in products using a lower bound scenario (in which analytical results in samples below the level of quantification (LOQ) equalled 0) and an upper bound scenario (in which concentrations in samples below the LOQ equalled the value of the LOQ). Samples were taken in the areas around the companies Chemours in Dordrecht and Custom Powders in Helmond.

Because EFSA derived a TWI, the weekly chronic exposure to the sum of GenX and PFOA was estimated per product. To investigate whether frequent consumption of large portions of dairy products, eggs or fish locally produced or caught in the Dordrecht or Helmond area would pose a health risk, a point estimate was used. For this, high consumption statistics (95^{th} percentile of consumption) of dairy products, eggs and fish were obtained from the Dutch National Food Consumption Survey of 2012-2016 and multiplied with the summed concentration of GenX and PFOA in the particular products. The maximum weekly intakes for the different age and sex-groups varied between:

- 0-5.8 ng PFOA-equivalents per kg bw for the consumption of dairy products sampled in the Dordrecht and Helmond area. If the exposure was calculated for a 35-year old lactating woman, taking into account her 35 exposure years and hence reflecting the situation on which the TWI was based, the exposure via dairy products varied between 0 and 2.0 ng PFOA-equivalents per kg bw;

- 0.7-2.2 ng PFOA-equivalents per kg bw for the eggs sample collected from the Dordrecht area;
- 0-0.68 PFOA-equivalents per kg bw for the eggs sample collected from the Helmond area;
- 0-0.45 ng PFOA-equivalents per kg bw for one eel obtained from an eel farm in the Helmond area;
- 13 ng PFOA-equivalents per kg bw for one carp caught in a fish pond in Helmond.

It should be noted that the upper bound exposure via dairy products, the eggs sample collected in Helmond and the eel sample was completely driven by the value of the LOQ.

3) Conclusions risk assessment

The conclusions regarding risk assessment for the products of animal origin sampled from the Dordrecht and Helmond area are uncertain because of the small number of samples of which the majority had analytical results below the LOQ, and the lack of background dietary exposure estimates based on recent concentration data.

The following conclusions can be drawn:

- Consumption of dairy products and eggs samples collected in the Dordrecht area and in the Helmond area, and that of the eel sample in the Helmond area for a prolonged period of time would not pose a health risk on the condition that:
    - Background exposure from other (non) dietary sources is sufficiently low;
    - The sampled eggs and eel are representative for all eggs and eel produced or caught in the particular area.
- Consumption of carp from the Helmond area for a prolonged period of time may pose a health risk. Given that only one carp was sampled, it is not clear whether this carp is representative for all carps or other freshwater fish in the Helmond area.

It is noted that the population is also exposed to GenX and PFOA via other dietary and non-dietary sources. When assessing the risk of GenX and PFOA intake from locally produced or caught foods from animal origin, this background exposure should be taken into account. RIVM recently performed an indicative dietary exposure assessment according to the RPF approach, based on data from 2009, which showed that exposure to the EFSA-4 via drinking water and food exceeded the TWI. It was concluded that more recent concentration data in food were needed for an up to date exposure assessment. For drinking water, concentration data were up-to-date.

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Annex A: Toxicity of GenX and PFOA

GenX
The studies by Rushing et al. (2017) and Caverly-Rae (2015)/Craig (2013) were also referred to in the previous version of this FO assessment and were included in ECHA (2019). The studies are highlighted here specifically because this immunotoxicity study in mice (Rushing et al. 2017) and this chronic exposure study in rats (Caverly Rae et al. 2015; Craig 2013) provide evidence of immunosuppressive effects of GenX and therefore support the use of the TWI for this substance as well.

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

Relative liver weights were increased at 10 and 100 mg/kg bw per day (both sexes) and liver peroxisome proliferation (based on increased hepatic acyl CoA oxidase) was found at 10 and 100 mg/kg bw per day in males or at 100 mg/kg bw per day only in females. A suppression of the IgM antibody response (-7.3%) was found at 100 mg/kg bw per day (females only). T-lymphocyte numbers were increased (B-lymphocytes unchanged) at 100 mg/kg bw per day (males only). At this dose also the CD4+/CD8+ and CD4-/CD8- ratios were affected (males only). No effect on spleen weight was found in males but in females absolute and relative spleen weights were decreased at 100 mg/kg bw per day. The NOAEL for immunotoxicity in this study was 10 mg/kg bw per day. The authors of the study conclude that these observations are in line with parameters affected by PFOA, albeit GenX appears to be less potent, and further studies are required to determine the full immunomodulatory profile of GenX and possible synergism with other PFAS.

2-year study in rats
In the 2-year oral rat study according to OECD TG 453 (Caverly Rae et al., 2015, Craig, 2013) Crl:CD(SD) rats, 80 per dose and sex, were exposed to FRD-902 (84% purity) by gavage (water) at 0, 0.1, 1 and 50 mg/kg bw per day (males) or 0, 1, 50 and 500 mg/kg bw per day (females). Interim necropsy was performed on 10 animals after 12 months. The remaining animals were necropsied after 101 weeks (females) or 104 weeks (males). One test substance-associated cause of death/morbidity was inflammation/necrosis of the kidneys, which occurred in seven 500 mg/kg bw per day females and was characterised by papillary necrosis. Females were terminated during Week 101, prior to scheduled termination, due to low survival in all female dose groups, especially control and 50 mg/kg bw per day groups. However, this did not impact the study as this was approximately 2 years of test article exposure. Even though survival among all female groups was low there were no statistically significant differences and survival was comparable among all groups.

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

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

Table A1: Selected haematology parameters presented in percentage change compared to control, measured in rats at 3, 6 and 12 months' time-interval

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

*P < 0.05; **P < 0.01

At 12 months, serum albumin levels increased in males at 1 mg/kg bw per day (Table A2). Serum globulin was increased in females at 50 mg/kg bw per day during the 6 months interval. The changes in albumin and globulin in the mid- and high-dose male and female groups resulted in statistically significant increases in A/G ratio in these groups at all intervals, apart from the 1 mg/kg bw per day dose group at 6 months. Bilirubin levels were statistically significant reduced in females at the mid- and high dose groups at almost all intervals. Furthermore, serum liver enzymes (ALP, ALT, and SDH) were increased in males at 50 mg/kg bw per day. Other observations included decreases in total protein and GGT for females in the high dose group, and increases in BUN for males and females in the high dose group. Also, phosphorus levels were increased for males and females in the high dose groups, as well as chloride, and potassium levels were increased in females in the high dose group.

In females receiving 500 mg/kg bw per day, minimal, statistically significant increases in urine volume and pH and decreases in urine specific gravity (suggestive of a minimal diuresis) were present at both the 6- and 12-month intervals. Although minimal, these changes may be correlative to increased incidences and severity of chronic progressive nephropathy observed microscopically in this dose group at the 1-year interim sacrifice. Females dosed at 500 mg/kg bw per day illustrated increased kidney weights and changes in the kidney, such as increased incidence of tubular dilation, oedema of the renal papilla, transitional cell hyperplasia, tubular and pelvic mineralisation, renal papillary necrosis, and chronic progressive nephropathy (Table A3). A test article-related macroscopic observation included "irregular surface" of the kidneys at interim sacrifice in

one of the 500 mg/kg bw per day dosed females. At terminal sacrifice, this effect was noted in 16/70 females dosed at 500 mg/kg bw per day.

Table A2: Selected clinical chemistry parameters presented in percentage change compared to control, measured in rats at 3, 6 and 12 months' time-interval

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

*P < 0.05; **P < 0.01

Table A3: Incidences of selected histopathological kidney findings for the chronic study in female rats at final sacrifice

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

*Statistically significant from control (P < 0.05)

In high-dosed animals of both sexes, increases in relative liver weight were observed at interim sacrifice. Three males in the highest dose group illustrated minimal focal cystic degeneration and five minimal to mild focal necrosis. For all females, centrilobular hypertrophy was observed at 500 mg/kg bw per day at the 12 month sacrifice. Additional microscopic changes at final sacrifice include increased centrilobular hepatocellular hypertrophy in 7/70 males and 65/70 females and increased centrilobular hepatocellular necrosis in 5/70 males and 7/70 females at 50 and 500 mg/kg bw per day respectively (Table A4). The latter effect was graded as severe mostly (3/5 animals) at the highest dose in males and mild to severe at the middle and high doses in females respectively. Furthermore, males showed a decrease in focal and periportal vacuolisation at 50 mg/kg bw per day. In females, a decrease in centrilobular vacuolisation, panlobular hepatocellular hypertrophy, individual cell hepatocellular necrosis, and angiectasis (i.e. blood- or lymph vessel dilation) were observed at 500 mg/kg bw per day.
Non-neoplastic lesions include hyperplasia of the limited ridge of the nonglandular stomach in 9/70 females and of the squamous cell in the tongue in 13/70 females, as well as an increased incidence of inflammation of the tongue in 13/70 females, dosed at 500 mg/kg bw per day. Moreover, a decreased absolute and relative (to brain) spleen weight was observed in females dosed with 500 mg/kg bw per day at interim sacrifice. No accompanying macroscopic changes were observed for this effect.

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

Table A4: Incidences of selected histopathological liver findings for the chronic study in rats at final sacrifice.

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

*Statistically significant from control (P < 0.05)

PFOA
This study has been published recently, and was not part of the previous version of the FO assessment. Abraham et al. (2020) is the critical study underlying the EFSA (2020a) TWI derivation.

In a cross-sectional study, Abraham et al. (2020) examined the association between internal levels (plasma levels) of PFAS (PFBS, PFHxS, PFOS, PFHxA, PFOA, PFNA, PFDA, PFDoDA, and ADONA) in 1-year old children (N = 101) and an array of biological parameters (among others clinical chemistry, thyroid status, immunoglobulins, white blood cell parameters, red blood cell parameters and lymphocyte subpopulations). Blood plasma samples were taken in the 1990s to study the effect of persistent organic pollutants on the developing child, but were recently re-evaluated to detect PFAS. Limit of quantification (LOQ) in blood plasma was 0.25 ng/mL for all PFAS. Of the 101 children (51 boys, 50 girls), 21 were formula-fed, and 80 children were breastfed for at least 4 months, including 27 children from a dioxin hotspot. The main focus of the study was to evaluate the possible association with PFAS exposure and parameters of immune response to vaccination against Hib, tetanus, and diphtheria. The relation between PFAS levels and effect markers was assessed, among others, by using linear regression analysis and multivariate analysis correcting for exposure to other contaminants.

Levels of PFOA and PFOS in plasma were observed above LOQ in all children, whereas for PFHxS and PFNA levels were below LOQ for 1 and 28 children respectively. The other PFAS (PFBS, PFHxA, PFDA, PFDoDA, and ADONA) were exclusively or predominantly below LOQ. Moreover, also 22 persistent organic pollutants and heavy metals (among which PCBs, lead, and DDE) were detected in all children's plasma. Concentrations of PFOA, PFOS, PFHxS and PFNA were 16.8 +/- 6.6, 15.2 +/- 6.9, 2.1 +/- 1.3, and 0.6 +/- 0.2 ng/mL in breastfed children respectively. In formula-fed children, levels of PFOA (3.8 +/- 1.1 ng/mL), PFOS (6.8 +/- 3.4 ng/mL), PFHxS (1.7 +/- 1.1 ng/mL), and PFNA (0.2 +/- 0.1 ng/mL) were much lower. Statistically significant associations were observed between internal PFOA levels and time since last vaccination-adjusted antibody levels for Hib, tetanus IgG1, and diphtheria. No such associations were observed between PFOS levels and Hib, tetanus IgG1, and diphtheria antibodies. Nor were such associations observed for the other two PFAS (PFNA and PFHxS). Multivariate analysis, correcting for PCBs, also revealed a significant influence of PFOA exposure (and not PFOS, PFNA, or PFHxS) on antibody levels. Additionally, statistically significant inverse associations between PFOA exposure and ex-vivo lymphocyte cytokine production (INFγ) after stimulation with tetanus and diphtheria toxoid, confirming the biological relevance of the observed association. Lastly, positive associations were observed between PFOA exposure and CD45RO+ CD45RA− cells, and PFOA exposure and CD27− cells as percentage of CD8+ T cells, as well as PFOS exposure and CD27− cells as percentage of CD8+ T cells.

The study above reported that an association was only found between PFOA and the effect on the immune system. However, EFSA does not rule out the possibility that this effect may have been caused by the other three PFAS as well (ESFA, 2020)[3]. Therefore, EFSA used the data on internal exposure (plasma levels) to PFOA, PFOS, PFNA and PFHxS and anti-diphtheria and anti-tetanus antibody concentrations to perform dose-response modelling. In doing so, EFSA took the sum of exposure to these four PFAS per individual and expressed this against the individual's immune response. As a pragmatic approach, equipotency of the congeners was assumed at the blood plasma level. This analysis resulted in a lowest $BMDL_{10}$ of 17.5 ng/mL for the sum of the four PFAS based on a decrease in antibody titres against diphtheria. A schematic overview of how this value was further extrapolated to an external intake value, taking into account accumulation of these substances in the body and transfer from mother to child, can be

[3] This association was also significant for the sum of four PFAS. Due to the high correlations among plasma levels of PFOS, PFHxS, PFNA and PFOA it was furthermore considered uncertain whether PFOA drives the association. Such observation would contradict the outcome of animal studies that observe immunosuppression at lower serum levels for PFOS than for PFOA (EFSA 2020a).

found in Appendix A1 of the RIVM memorandum on the implementation of the EFSA-TWI (RIVM, 2021a).

Annex B: RIVM implementation of the EFSA TWI

On 17 September 2020, EFSA issued the opinion 'Risk to human health related to the presence of perfluoroalkyl substances in food' (EFSA 2020a). In this opinion, EFSA derived a sum TWI for exposure to four PFAS, which is significantly lower than the TDI derived by the RIVM in 2016 for PFOA alone (Zeilmaker et al., 2016). RIVM studied the derivation of the EFSA TWI, and decided to replace the RIVM TDI by the EFSA TWI (RIVM, 2020a; RIVM, 2020b). The main argument for this is that EFSA has set the health-based guidance value based on immune effects observed in humans. The EFSA opinion includes studies that show that these effects may occur at lower exposures than previously known, and use of the EFSA TWI therefore provides better protection than the RIVM TDI used earlier.

In the Netherlands, mixtures of PFAS are observed in environmental media (e.g. Gebbink et al., 2017; Zafeiraki et al., 2019; Brandsma et al., 2019). In 2018, the RIVM, on commission of the municipality of Dordrecht, developed an RPF framework (Zeilmaker et al., 2018) to be able to assess the risk of mixtures in environmental media and to derive risk values (i.e. for swim water, wild caught fish, soil or dredging soil (Wintersen et al, 2019; Muller and Smit, 2020). This was followed by a peer reviewed article about the RPF method (Bil et al., 2021). RIVM made EFSA aware of this method during the public consultation of the EFSA 2020 PFAS Opinion, and RIVM additionally referred to their RPF analysis of the NTP toxicity studies (EFSA, 2020b).

Because in the Netherlands detectable concentrations of PFAS other than the EFSA-4 are observed in environmental media as well as there may be occasions when only one PFAS is detected (not necessarily part of the EFSA-4), RIVM deemed it necessary to incorporate an additional step prior to being able to apply the EFSA TWI in practice, when performing risk assessments and to establish risk limits for this group of substances (RIVM, 2021a). RIVM proposed a uniform method for using the EFSA TWI (RIVM, 2021a), which is applicable in various policy contexts (Bulder et al., 2020). The method selected comprises applying the EFSA TWI in combination with RPFs, after several options for implementation of the TWI were weighed and discussed (RIVM, 2021a).

The options were discussed in a panel of RIVM toxicology and epidemiology experts followed by the RIVM management, after which the outcome was approved by the Dutch scientific advisory board on derivation of environmental quality standards for water and air[4]. Below, a summary of the possible options for using the EFSA TWI, line of reasoning and uncertainties for the proposed approach, and an outlook for ongoing and future RIVM research to reduce these uncertainties is provided.

Possible options for using the EFSA TWI:
RIVM drew up six options on how the EFSA TWI could be used (options a to f below). The six options differed in the extent to which they took into account four predefined criteria to achieve a broadly applicable method:

1. The method is applicable to individual PFAS, the EFSA-4, and other PFAS
2. It is desirable to be able to assess PFAS mixtures in different compositions
3. It preferably accounts for differences in potency between PFAS
4. The method is conceptually simple and practically applicable

The six options concerned the following[5]:

a. Divide the EFSA TWI by 4;
b. Filling up the EFSA TWI;
c. Filling up the point of departure;
d. Potency dependent on filling up the EFSA TWI (divide the TWI into separate TWIs on the basis of RPF values);

[4]An independent panel of (eco)toxicologists, environmental scientists and risk assessors from academia, industry and government. The board advices the Dutch ministry of Infrastructure and Water Management regarding the scientific basis of derived environmental quality standards.
[5] See for further explanation of any of these options Appendix B of RIVM (2021).

e. RPF method;
f. Filling up the EFSA TWI + RPF method.

Table B1. Summary of the extent to which different options for the implementation of the EFSA TWI take into consideration criteria 1 to 4

Point to consider	method					
	a	b	c	d	e	f[5]
1) Applicable to PFASs other than the four EFSA PFASs?	No	No	No	No	Yes[2]	Yes[2]
2) Can mixtures of various compositions be assessed?	Yes[1]	No	Yes[1]	Yes[1]	Yes[2]	No/Yes
3) Can different potencies be taken into account?	No	No	Yes, partially[3]	Yes, partially[4]	Yes, partially[4]	No/Yes, partially[4]
4a) Conceptually simple?	Yes	Yes	No	No	Yes	No
4b) Usable in practice?	No	Yes	No	No	Yes	No

[1] Only for the four EFSA PFAS
[2] For the 23 PFASs for which RPFs are available (Bil et al., 2021)
[3] Yes for differences in toxicokinetics, incl. milk/serum ratio, but not for differences in toxicodynamics
[4] For differences in toxicokinetics and dynamics, but excluding differences caused by the milk/serum ratio.
[5] Combination of methods b and e

Line of reasoning for selecting the RPF method:
RIVM selected the RPF method (option e) as the option that best fulfils the above-mentioned criteria. RIVM regarded other options for applying the EFSA TWI as less suitable than the RPF method. This is mainly because they are only applicable to the four EFSA PFAS. In addition, several methods would result in multiple TWIs or risk limits for various combinations of PFAS, which is difficult to interpret conceptually and implement in practice. The advantages of using the RPF method are provided below:

1. **Extension to other PFAS**: the RPF method can be used on the EFSA-4, an additional 19 PFAS, and single PFAS, thereby extending the number of PFAS to be included in risk assessments and allowing for risk assessment of single congeners. The RPFs for 23 PFAS presented in Bil et al. (2021) can be applied to (external) exposure and concentrations (e.g. in drinking water or food products).
2. **Mixtures of various compositions**: the EFSA TWI is dependent on the ratio between PFOA&PFNA on the one hand and PFOS&PFHxS on the other. This means that the EFSA TWI is, in principle, only applicable to mixtures with the same mixture ratio as the ratio in the study by Abraham et al. (2020) on which the EFSA TWI is based. By using the RPF method, the TWI is not dependent on the mixture ratio between various PFAS.
3. **Differences in potency**: EFSA acknowledges in their assessment that in animal studies immunosuppression is observed at lower serum levels for PFOS than for PFOA, but it regarded derivation of RPFs based on immune effects not possible at that moment (EFSA 2020a), and therefore assumed equipotency conform the guidance on combined exposure to multiple chemicals (EFSA, 2019). Nevertheless, it is known that PFAS are not equipotent with regard to the other effects of PFAS (for example liver effects; Bil et al. 2021). Therefore, RIVM considers it plausible that various PFAS are not equipotent with regard to immune effects either.
4. **Simplicity**: the RPF concept is simple and already features in the risk assessment for other chemical substances. The approach is, in fact, already being used with the comparable Toxic Equivalents Factors (TEFs) for dioxins. The practical application of the EFSA TWI as PFOA TWI means that only a single TWI, TDI or risk limit is necessary for a comparison with the sum of PFOA equivalents and this considerably simplifies usage.

5. **Substance specific**: additional substance-specific information can be included at a later stage (provided it is available). In the event of indirect exposure to PFAS in soil and water, for example via fish, vegetables or fruit, substance-specific bioaccumulation factors (BAFs) can be used to assess the risks of PFAS in surface water, groundwater or soil. What is more, adjustments can be made for differences in the distribution across milk and serum if additional information becomes available.

RIVM assumed that PFOA is a logical choice of index substance (with RPF of 1) given that the authors (Abraham et al. 2020) of the underlying study on which the EFSA TWI is based conclude that there is only an association of immune effects with PFOA, and not with the other three PFAS. Examples of how to use the RPF method in different scenarios is provided in RIVM (2021).

RPFs and uncertainties:

1. **RPFs for effects other than liver effects**: one uncertainty of the RPF method is that the available RPFs were established for liver effects in rats, while the EFSA TWI is based on effects on the immune system in humans. Bil et al. (2021) discuss the fact that, in the absence of immune-specific factors from human studies, the RPFs could also be applied to other effects and to humans, but the validation of broad application of the current RPF values is desirable and ongoing.[6]
2. **Toxicokinetics:** another uncertainty is that the RPFs take no account of the possible differences between PFAS in terms of their distribution across milk and serum. This extrapolation remains based on the PBPK modelling for PFOA&PFNA and PFOS&PFHxS already incorporated in the derivation of the EFSA TWI.

Although RIVM acknowledges the above points, RIVM considers that, in view of the available scientific information referred above, it is better justified to account for relative potencies for PFAS than the assumption of equipotency made by EFSA. As already mentioned it is, however, recommended that the RPFs are validated for immune effects in due course.

Concluding remarks

By using the RPFs in combination with the EFSA TWI it is assumed that other PFAS can also have an effect on the immune system and an estimate is given of the degree of this effect per substance. This consequently takes account of the possibility that exposure to different PFAS can add up and have a cumulative effect. This is important to enable risk estimation of several PFAS which occur simultaneously in practice. The desire to take account of cumulative exposure to substances has been expressed at both national and European level (RLI, 2020; EU, 2020).

On the other hand, as already mentioned it should be realized that the available RPFs require validation and that they do not make allowances for all differences between individual PFAS. Furthermore, RPFs are only available for a limited number of PFAS and, as a consequence, this method can only be used to assess the PFAS in question. For the time being, PFAS for which no RPF is available cannot be assessed using this method. If assessment of these PFAS is desirable, an RPF for the PFAS in question will first have to be established, or an individual assessment will have to be carried out based on a substance-specific risk limit or health-based limit value.

The proposed RPF method is not perfect, but RIVM considers that the actual risk can be estimated more accurately if potency differences between PFAS are taken into account and if several PFAS can be included in the risk calculation. The method justifies EFSA's

[6] In 2020, RIVM submitted the results of an (external) RPF analysis to EFSA during public consultation based on recent studies performed by the NTP (NTP 2019a; NTP 2019b), where it discussed the validation of RPFs for adverse effects other than liver effects. The analysis revealed that comparable potency differences exist between PFAS in numerous endpoints such as different organ weights, hormone levels, clinical chemistry, white blood cell parameters and pathology endpoints. Consequently, it is not the case that these potency differences are only observed in conjunction with liver effects. Generally speaking these PFAS potencies are comparable with the findings in Bil et al. (2021). With regard to the EFSA-4, the sequence (from low to high potency) is: PFHxS < PFOA < PFOS < PFNA.

basic principle that children must be protected against immune effects and that several PFAS can cause this effect.

In view of the differences in potency between PFAS and the almost infinite number of different compositions in which PFASs can occur it is, by definition, impossible to determine whether the chosen method is stricter or less strict than the other available methods. Using a method which takes account of 23 instead of 4 PFAS will, however, makes an underestimation of the risk less likely if members of this group of 23 PFAS actually occur simultaneously.

Annex C: Mean and high (95th percentile) consumed amounts of dairy products, eggs and fish

Table C1. Mean and high (95th percentile) consumed amounts of dairy products[1] per age-sex group 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	400	725	407	751	99
4-8	387	789	401	826	96
9-18 man	386	872	415	966	91
9-18 female	301	734	331	849	92
19-50 man	367	933	395	997	93
19-50 female	322	762	347	860	93
51-79 man	380	853	398	897	96
51-79 female	321	688	334	736	97

DNFCS: Dutch National Food Consumption Survey
[1] Based on consumed amounts of dairy products reported in DNFCS 2012-2016.
[2] Based on all days, irrespective of whether dairy products were 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 dairy products 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 dairy products was reported.

Table C2. Mean and high (95th percentile) consumed amounts of eggs[1] per age-sex group 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	6	29	31	70	19
4-8	8	38	37	90	21
9-18 man	9	51	48	125	20
9-18 female	11	54	46	108	24
19-50 man	13	59	55	134	23
19-50 female	12	59	49	100	25
51-79 man	19	63	58	121	32
51-79 female	13	51	52	100	26

DNFCS: Dutch National Food Consumption Survey
[1] Based on consumed amounts of eggs reported in DNFCS 2012-2016.
[2] Based on all days, irrespective of whether eggs 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 eggs 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 eggs was reported.

Table C3. Mean and high (95th percentile) consumed amounts of fish[1] per age-sex group 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.

Annex D: Exposure to the sum of GenX and PFOA via dairy products, eggs, eel and carp

Table D1. Intake of the sum of GenX and PFOA (expressed as PFOA-equivalents) via high (95th percentile) consumption of dairy products with analytical results below the limit of quantification of 0.016 ng PFOA-equivalents/g[1]

Age group (year) + sex	High consumption (gram per day)[2]	Body weight (kg)	LB-UB exposure to the sum of GenX and PFOA (ng PFOA-equivalents/ kg bw per week)
1-3	725	13.9	0-5.8
4-8	789	24.1	0-3.7
9-18; man	872	55.6	0-1.8
9-18; female	734	53.2	0-1.5
19-50; man	933	84.6	0-1.2
19-50; female	762	75.7	0-1.1
51-79; man	853	88.8	0-1.1
51-79; female	688	76.9	0-1.0

bw: body weight; PFOA: perfluoro octanoic acid; TWI: tolerable weekly intake; LB: lower bound; UB: upper bound

[1] Concentration analysed in 15 milk samples (analytical results below to the limit of quantification for milk) in Dordrecht and Helmond (Table 1 of main text).

[2] High consumption based on all consumption days within the food consumption survey, irrespective of whether dairy products were consumed (Annex D, Table D1).

[3] In the lower bound scenario it is assumed that analytical values below the limit of quantification equalled a concentration of 0. In the upper bound scenario it is assumed that analytical values below the limit of quantification equalled the value of the LOQ

Table D2. Intake of the sum of GenX and PFOA (expressed as PFOA-equivalents) via high (95th percentile) consumption of eggs sampled in Dordrecht and Helmond, respectively[1]

Age group (year) + sex	High consumption of Eggs (gram per day)[2]	Body weight (kg)	LB -UB[5] exposure to the sum of GenX and PFOA (ng PFOA-equivalents/ kg bw per week)	
			Dordrecht[3]	Helmond[4]
1-3	29	13.9	2.0-2.2	0-0.68
4-8	38	24.1	1.5-1.7	0-0.44
9-18; man	51	55.6	0.9-1.0	0-0.26
9-18; female	54	53.2	1.0-1.1	0-0.28
19-50; man	59	84.6	0.7-0.8	0-0.19
19-50; female	59	75.7	0.8-0.8	0-0.22
51-79; man	63	88.8	0.7-0.8	0-0.20
51-79; female	51	76.9	0.7-0.7	0-0.19

bw: body weight; PFOA: perfluoro octanoic acid; TWI: tolerable weekly intake

[1] Concentration analysed in 1 eggs sample obtained from the Dordrecht area and 1 eggs sample collected from the Helmond are. Analytical results for GenX were below the limit of quantification (LOQ) and therefore substituted with the value of the LOQ. The analytical result for PFOA in the eggs sample in Helmond were below the LOQ for PFOA and therefore substituted with the value of the LOQ for PFOA. For the eggs sample obtained from Dordrecht, the analytical value was above the LOQ of PFOA (Table 1 main text)

[2]High consumption based on all consumption days within the food consumption survey, irrespective of whether dairy products or eggs were consumed (Annex D, Table D2).

[3] Based on 1 eggs sample collected in the Dordrecht area, with analytical results of GenX below the LOQ and with PFOA concentration of 0.140 ng PFOA-equivalents/g

[4] Based on 1 eggs sample collected in the Helmond area, with analytical results of both GenX and PFOA below the LOQ.

[5] In the lower bound scenario it is assumed that analytical values below the limit of quantification equalled a concentration of 0. In the upper bound scenario it is assumed that analytical values below the limit of quantification equalled the value of the LOQ

Table D3. Exposure to the sum of GenX and PFOA (expressed as PFOA-equivalents) via a high (95th percentile) consumption of fish sampled in Helmond[1]

Age group (year) + sex	High consumption of fish (gram per day)[2]	Body weight (kg)	Exposure to the sum of GenX and PFOA (ng PFOA-equivalents/ kg bw per week)	
			Eel[3] LB-UB	Carp[4]
1-3	14.5	13.9	0-0.41	11.5
4-8	24.5	24.1	0-0.40	11.2
9-18; male	15	55.6	0-0.11	3.0
9-18; female	37	53.2	0-0.27	7.7
19-50; male	88	84.6	0-0.41	11.5
19-50; female	71	75.7	0-0.37	10.4
51-79; male	101	88.8	0-0.45	12.6
51-79; female	80	76.9	0-0.41	11.5

bw: body weight; PFOA: perfluoro octanoic acid; TWI: tolerable weekly intake

[1] Concentration analysed in 1 sample of fish meat of farmed eel in Helmond and of 1 carp caught in a fish pond in Helmond (Table 1 of main text). Analytical results of eel were below the limit of quantification (LOQ) for eel and, therefore, substituted with the value of the LOQ. For carp, the analytical results were above the LOQ.

[2] High consumption based on all consumption days within the food consumption survey, irrespective of whether fish was consumed or not (Annex D, Table D3).

[3] In the lower bound scenario it is assumed that analytical values below the limit of quantification equalled a concentration of 0. In the upper bound scenario it is assumed that analytical values below the limit of quantification equalled the value of the LOQ.

[4] The carp sample had analytical results above the LOQ, therefore UB and LB scenarios resulted in the same exposure estimate.