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Exposure assessment of perfluoroalkyl substances as follow-up on the concerns raised in the recent opinion of EFSA

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Table of contents

LIST OF FIGURES

LIST OF TABLES

List of abbreviations

PERFLUOROALKYL SUBSTANCE ABBREVIATIONS

GROUP OF SUBSTANCES	DEFINITION
4-EFSA-PFAS	The 4 PFAS: PFOA, PFNA, PFHxS, PFOS included in EFSA opinion (EFSA, 2020)
Σ4PFAS	Sum of 4 PFAS (PFOA, PFNA, PFHxS, PFOS)
PFCA	Perfluoroalkyl carboxylates
PFSA	Perfluoroalkyl sulfonates
INDIVIDUAL SUBSTANCES	
Capstone A	1-Propanaminium,N,N-dimethyl-N-oxide-3-[[(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)sulfonyl]amino]-, hydroxide
Capstone B	1-Propanaminium,N-(carboxymethyl)-N,N-dimethyl-3-[[(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)sulfonyl]amino]-, hydroxide
DONA	acid form of ADONA 2,2,3-Trifluor-3-[1,1,2,2,3,3-hexafluor-3-(trifluormethoxy) propoxy]-propionic acid
Major F53B	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid
Minor F53B	11-Chlororeicosafluoro-3-oxaundecane-1-sulfonic acid
FOSA	Perfluorooctane sulphonamide
HFPO-DA	acid form of GenX 2,3,3,3-tetrafluor-2-(heptafluorpropoxy)-propanoic acid
PFBA	Perfluorobutanoic acid
PFPeA	Perfluoropentanoic acid
PFHxA	perfluorohexanoic acid
PFHpA	Perfluoroheptanoic acid
PFOA	**Perfluorooctanoic acid**
PFNA	**Pefluorononanoic acid**
PFDA	Perfluorodecanoic acid
PFUnDA	Perfluoroundecanoic acid
PFDoDA	Perfluorododecanoic acid
PFTrDA	Perfluorotridecanoic acid
PFTeDA	Perfluorotetradecanoic acid
PFBS	Perfluorobutane sulfonic acid
PFPS	Perfluoropentanesulfonic acid
PFHpS	Perfluoroheptane sulfonic acid
PFHxS	**Perfluorohexane sulfonic acid**
PFOS	**Perflorooctane sulfonic acid**
L-PFOS	Linear-PFOS
br-PFOS	Branched-PFOS
tot-PFOS	Total-PFOS
PFNS	Perfluorononane sulfonic acid
PFDS	Perfluorodecane sulfonic acid
PFUnDS	Perfluoroundecane sulfonic acid
PFDoDS	Perfluorododecane sulfonic acid
PFTrDS	Perfluorotridecane sulfonic acid

OTHER ABBREVIATIONS

ABBREVIATION	DEFINITION
ACN	Acetonitrile
AL	Allocation factor
ALT	Alanine transferase
BMD	Benchmark dose
BMDL	Benchmark dose (lower confidence limit)
bw	Body weight
EFSA	European Food Safety Authority
ES	External (MS injection) standard
EU	European Union
EURL	European Union reference laboratory
FCM	Food contact materials
FCS	Food consumption survey
Hib	Haemophilus influenzae type b
HBGV	Health Based Guidance Value
HBM	Human biomonitoring
HR	High resolution
IL	Isotopic labelled
IS	Internal standard
LB	Lower bound
LC	Liquid chromatography
LOQ	Limit of quantification
MB	Middle bound
MeOH	Methanol
ML	Maximum level
MS	Mass spectrometry
MU	Measurement uncertainty
NOAEC	No-Observed-Adverse-Effect Concentration
PBPK model	Physiologically Based Pharmacokinetic model
PP	Polypropylene
PRM	Parallel reaction monitoring
PTFE	Polytetrafluoroethylene
QuEChERS	stands for Quick, Easy, Cheap, Effective, Rugged and Safe
RACE	Rapid Assessment of Contaminant Exposure
RPF	Relative Potency Factor
RSD	Relative standard deviation
RT	Retention time
SC	Sciensano
SPE	Solid-phase extraction
SPADE	Statistical Program to Assess Dietary Exposure
TWA	Time-weighted average
TWI	Tolerable weekly intake
UB	Upper bound
WHO	World Health Organization
WP	Work package
Ww	Wet weight

Summary

EXECUTIVE SUMMARY

CONTEXT

Per- and polyfluoroalkyl substances (PFAS) are manufactured chemicals used (or used to be used) for various applications. Due to their persistent, bioaccumulative and toxic character, they also became environmental contaminants, and exposure to these chemicals may lead to adverse health effects. In 2020, the risks related to PFAS were reassessed by the European Food Safety Authority (EFSA) (EFSA, 2020), resulting in a tolerable weekly intake (TWI) for the sum of PFOS, PFOA, PFHxS and PFNA (cf. 4-EFSA-PFAS), further called ∑4PFAS, of 4.4 ng/kg body weight (bw)/week. Since the last Belgian exposure assessment was carried out only on PFOS and PFOA in 2007-2010, it had to be re-evaluated.

OBJECTIVES

In this project, the dietary exposure of the Belgian population to PFAS were evaluated, followed by a risk assessment. In addition, since it has already been demonstrated that PFAS can be released from food contact materials (FCM), this was further investigated.

MATERIALS AND METHODS

A comprehensive sampling based on a scoring system, reflecting all foods relevant for PFAS exposure and Belgian consumption habits, yielded 283 food samples from the Belgian market belonging to 14 main food groups and 28 FCM. Special attention was given to game meat, offal and egg-containing products. This study did not include own-grown food from private gardens. The selection of PFAS to be analysed, 4-EFSA-PFAS (PFOA, PFNA, PFHxS, PFOS) and also PFBA, PFPeA, PFHxA, PFHpA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHpS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS, Major F53B, Minor F53B, HFPO-DA and DONA) was based on Recommendation (EU) 2022/1431 and Regulation (EU) 2023/915.

Different analytical methods were developed and validated according to Regulation (EU) 2022/1428 and the European Reference Laboratory Guidance document (EURL). A QuEChERS-based extraction was followed by a two-step purification using solid-phase extraction (SPE) with carbon and weak anion-exchange phases. Analysis was performed by ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS). Potential PFAS contaminations in the laboratory were mitigated to achieve very low quantification limits (LOQ). The achieved LOQs ranged from 0.001 to 0.1 µg/kg according to the matrix (i.e. food items) and the PFAS, except for HFPO-DA (maximal LOQ of 1 µg/kg). Afterwards, the methods were applied for the analysis of the selected samples.

The PFAS occurrence data were combined with food consumption data of the most recent Belgian food consumption survey (FSC2014) to perform a dietary exposure assessment according to different approaches. The lower-bound (LB) approach, in which occurrence data below the LOQ are set equal to zero, was considered most relevant, although middle-bound (MB) and upper-bound (UB) assessments were performed as well. A risk assessment for ∑4PFAS (sum of PFOA, PFNA, PFHxS and PFOS) was performed based on the approach applied by EFSA in 2020. A complementary risk assessment was carried out to evaluate the contribution of FCM using the Rapid Assessment of Contaminant Exposure (RACE) tool developed by EFSA.

RESULTS

The concentration of the 25 PFAS was determined in 283 selected food items by applying the validated, sensitive methods. A widespread PFAS contamination in various foodstuffs was demonstrated. Although an average of 1.1 compounds was detected per sample, approximately 57% of the samples contained none of the 25 studied PFAS, about 23% contained only one PFAS, 15% between 2 and 5 PFAS and less than 5% between 6 and 11 PFAS. PFOS was the most detected compound found in 19% of the 283 samples, followed by PFOA (17%). Eight compounds were never detected (i.e. PFTeDA, PFHpS, PFDS, PFUnDS, PFDoDS, PFTrDS, Minor F53B, HFPO-DA). Other PFAS were detected in 1 to 11% of the samples, depending on the PFAS. At least one PFAS was quantified in 74%, 68%, 57% and 53% of the fish, vegetables, water and composite dishes samples, respectively. No PFAS were detected in the nine egg samples independently of their origin (caged, free-range, organic), neither in 5 seasonings and sauces nor in 3 sugar and similar samples. The concentrations of the different PFAS varied from below LOQ to 2.85 µg/kg (i.e. PFTrDA in a crab sample).

In Regulation (EU) 2023/915, maximum levels have been set for specific food groups like fish, meat and eggs. Only one sample exceeded the maximum level of 0.7 µg/kg for PFOA in a crab sample with a concentration of 1.2 µg/kg. Furthermore, indicative levels are mentioned in Recommendation (EU) 2022/1431 for several groups, meaning that further investigation of the causes of the contamination should be carried out when the levels are exceeded. Seven samples of vegetables and fruits (considering a measurement uncertainty of 50%) exceeded the indicative levels of 0.01 µg/kg for PFOA in fruits and vegetables with a maximum concentration of 0.20 µg/kg in oyster mushrooms.

When evaluating the dietary exposure to the individual PFAS, 13 out of 25 targeted PFAS were accepted as sufficiently reliable for the exposure assessment (i.e. < 98% of the data are left-censored). For those, the LB exposure estimates were highest for **PFBA** (mean 5.1 ng/kg bw/week for the total population), followed by a PFAS group of about 10-fold lower exposure estimates: **PFPeA, PFOA, PFHxA and PFOS** (mean 0.36-0.54 ng/kg bw/week for the total population), then a PFAS group of about 30-fold lower estimates than the highest exposure: **PFUnDA, PFBS, PFTrDA and PFHpA** (mean 0.14-0.20 ng/kg bw/week for the total population); finally the lowest exposure estimates were obtained for **PFHxS, PFNA, PFDA and PFDoDA,** with mean values ranging from 0.04 to 0.08 ng/kg bw/week for the total population. For each PFAS, the exposure for children was higher than that for adolescents and adults. The MB dietary exposure to PFOS in Belgium decreased 8-fold compared to a previous Belgian study from 2012, which may reflect the PFOS phase-out in the EU since 2009. The exposure to PFOA, which has been phased out since 2020, also decreased compared to 2012, but to a lower extent (3.3-fold compared to the previous study).

When estimating the **exposure to the Σ4PFAS,** the toxicity potencies were assumed equal, as outlined in the EFSA opinion on PFAS of 2020. The LB mean exposure to Σ4PFAS ranged from 0.93 ng/kg bw/week (adults) to 1.7 ng/kg bw/week (children), while the 95th percentile exposure ranged from 1.8 to 3.5 ng/kg bw/week. PFOA and PFOS dominate the habitual exposure to Σ4PFAS. When comparing the current exposure estimates with a previous estimate by EFSA, the current mean exposures are 3- (adolescents) to 6-fold (children) lower. The major food groups contributing to PFAS exposure were “fish and seafood” (in particular “shrimps and prawns”) and “meat and meat-based products” (in particular “mammals meat”) for all age groups, followed by “water and water-based beverages” (in particular “unbottled water”, used as drinking water and consumed in meals).

Additionally, the dietary exposure estimates were compared to the PFAS blood serum data from the Flemish Environment and Health Study (FLEHS) IV study conducted in Flanders. By limiting the comparison to the adolescent population (due to data compatibility), it could be concluded that the level of PFOS, PFOA, PFNA, and PFHxS in food might broadly reflect their levels in humans.

The obtained exposure assessment for Σ4PFAS was compared to the TWI of 4.4 ng/kg bw/week, related to immune effects, and was not exceeded for the adolescent and adult populations, nor for 98% of the children’s population. Exposures below or at the TWI are considered to be without health risks. Due to the methodology used to derive the TWI, the exceedance of the TWI by children does not automatically

imply a health concern for these children. Even a twofold higher intake than the TWI by children does not result in serum levels higher than the no observed adverse effect concentration (NOAEC) for imunosuppressive effects derived for children by EFSA. In Belgium, the estimated exceedance for children was even less than twofold the TWI. This in turn implies that health concerns for children in Belgium are unlikely. Hence, if only dietary exposure (incl. drinking water) to the sum of PFOS, PFOA, PFNA and PFHxS is considered, health risks are unlikely for the general Belgian population.

Finally, the contribution of FCM to the overall exposure to PFAS was investigated. Six samples out of 28 FCM (21%) showed the presence of four PFAS (i.e. PFPeA, PFHxA, PFHpA, and PFOA) exclusively in FCM made of paper and board and the highest concentration (0.013 µg/kg of PFHxA) was found in a sandwich paper. Interestingly, no migration of PFAS was found in cake moulds, pans or woks with a non-stick coating made of polytetrafluoroethylene (PTFE), regardless of the quality of the item. Based on the evaluation using the RACE tool developed by EFSA, it can be concluded that the presence of PFAS in FCM did not pose any potential risks to consumers.

CONCLUSIONS

Although PFAS are omnipresent in food, only one sample exceeded the maximum levels set by Regulation (EU) 2023/915, and seven samples exceeded the indicative levels stated in Recommendation (EU) 2022/1431. When evaluating the contribution of FCM, it can be seen that six FCM samples made of paper and board contained PFAS, while no PFAS were found in cookware with non-stick coating.

If dietary exposure (incl. drinking water) to Σ4PFAS only is considered, there is no health concern anticipated for most of the Belgian population. The TWI was exceeded for a small fraction of the children, but due to the methodology used to derive the TWI, this does not automatically imply a health concern for these children.

RECOMMENDATIONS

In our study, the combined exposure to PFAS was assessed with the EFSA approach for 4 PFAS. Health risks arising from the combined exposure to PFAS mixtures in food should be further investigated since a combined exposure to other PFAS was demonstrated in our study. To do this, there is a need for an internationally recognized approach for the combined risk assessment for all PFAS detected in food. Given the current exposure levels for the general population, the health risks for people living in polluted areas should be assessed considering as well the consumption of own-grown food and other known PFAS exposure routes, such as air, dust, etc. Finally, the current TWI is derived to protect the most sensitive population group (breastfed infants), but the information for the other ages groups is limited. Additional HBGVs are needed to understand potential health concerns for the general population and specific subpopulations.

UITGEBREIDE SAMENVATTING

CONTEXT

Per- en polyfluoralkylstoffen (PFAS) zijn geproduceerde chemicaliën die voor verschillende toepassingen worden gebruikt (of vroeger werden gebruikt). Door hun persistente, bioaccumulerende en toxische karakter zijn het ook milieuverontreinigende stoffen geworden, en blootstelling aan deze chemische stoffen kan leiden tot schadelijke gezondheidseffecten. In 2020 werden de risico's van PFAS opnieuw beoordeeld door de Europese Autoriteit voor Voedselveiligheid (EFSA) (EFSA, 2020), wat resulteerde in een toelaatbare wekelijkse inname (TWI) voor de som van PFOS, PFOA, PFHxS en PFNA (cf. 4-EFSA-PFAS), verder ∑4PFAS genoemd, van 4,4 ng/kg lichaamsgewicht (lg)/week. Aangezien de blootstelling van de Belgische bevolking enkel werd bepaald voor PFOS en PFOA in de periode 2007-2010, moest deze opnieuw worden geëvalueerd.

OBJECTEVEN

In dit project zal de blootstelling van de Belgische bevolking aan PFAS via de voeding geëvalueerd worden, gevolgd door een risicobeoordeling. Aangezien al is aangetoond dat PFAS kunnen vrijkomen tijdens de verwerking van voeding of uit materialen die met voeding in contact komen (FCM), zal dit verder worden onderzocht.

MATERIAAL EN METHODEN

Eerst werd een uitgebreid staalnameplan ontwikkeld op basis van een scoresysteem, dat alle levensmiddelen weerspiegelt die relevant zijn voor de blootstelling aan PFAS en de Belgische consumptiegewoonten, wat resulteerde in 283 voedingstalen, behorend tot 14 voedingsgroepen en 28 FCM. Er werd speciale aandacht besteed aan vlees van wild, slachtafval en producten die eieren bevatten. Eigen gekweekt voedsel uit privétuinen werd niet in deze studie opgenomen. Vervolgens werd de selectie van PFAS (4-EFSA-PFAS (PFOA, PFNA, PFHxS, PFOS) en ook PFBA, PFPeA, PFHxA, PFHpA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHpS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS, Major F53B, Minor F53B, HFPO-DA and DONA) gebaseerd op de Aanbeveling (EU) 2022/1431 en Verordening (EU) 2023/915.

Verschillende analytische methoden werden ontwikkeld en gevalideerd in overeenstemming met Verordening (EU) 2022/1428 en de richtlijnen van het Europees ReferentieLaboratorium (EURL). Een op QuEChERS gebaseerde extractie werd gevolgd door een tweestapszuivering met behulp van vaste-fase-extractie (SPE) met koolstof en zwakke anionenwisselingsfasen. De analyse werd uitgevoerd met vloeistofchromatografie in combinatie met hoge resolutie massaspectrometrie (LC-HRMS). Potentiële PFAS-contaminaties in het laboratorium werden beperkt om zeer lage kwantificatielimieten (LOQ) te bereiken. De LOQs varieerden van 0,001 tot 0,1 µg/kg, afhankelijk van de matrix (d.w.z. levensmiddelen) en de PFAS, met uitzondering van HFPO-DA (maximale LOQ van 1 µg/kg). Nadien werden deze methoden gebruikt voor de analyse van de geselecteerde stalen.

De bekomen concentratiegegevens werden nadien gecombineerd met de consumptiegegevens van de meest recente Belgische voedselconsumptiepeiling (VCP2014) om de blootstelling aan de ∑4PFAS te evalueren volgens de aanpak van EFSA in hun recente advies volgens verschillende scenarios. Het ondergrensbenadering (LB), waarbij concentratiegegevens onder de LOQ gelijk aan nul worden gesteld, het meest relevant geacht, hoewel er ook evaluaties met de midden- en bovengrenzenbenaderingen (UB) werden uitgevoerd. Op basis van de deze blootstellingsbeoordelingen werd de risicobeoordeling uitgevoerd op basis van de door EFSA in 2020 toegepaste aanpak (voor ∑4PFAS). Er werd een aanvullende risicobeoordeling uitgevoerd om de bijdrage van FCM te evalueren, waarbij gebruik werd gemaakt van de *Rapid Assessment of Contaminant Exposure* (RACE) tool, ontwikkeld door EFSA.

RESULTATEN

De gevalideerde, gevoelige methoden werden toegepast op de 283 geselecteerde voedingsmiddelen en de concentratie van de 25 PFAS werd bepaald, waarbij een wijdverspreide PFAS-verontreiniging in verschillende levensmiddelen werd aangetoond. Hoewel er gemiddeld 1,1 PFAS per staal werd gedetecteerd, bevatte ongeveer 57% geen van de 25 onderzochte PFAS, ongeveer 23% slechts één PFAS, 15% tussen 2 en 5 PFAS en minder dan 5% tussen 6 en 11 PFAS. PFOS was de meest aangetroffen verbinding in 19% van de 283 stalen, gevolgd door PFOA (17%). Acht verbindingen werden nooit gedetecteerd (d.w.z. PFTeDA, PFHpS, PFDS, PFUnDS, PFDoDS, PFTrDS, Minor F53B, HFPO-DA). Andere PFAS werden gedetecteerd in 1 tot 11% van de stalen, afhankelijk van de PFAS. Ten minste één PFAS werd gekwantificeerd in respectievelijk 74%, 68%, 57% en 53% van de stalen vis, groenten, water en samengestelde gerechten. Er werden geen PFAS gedetecteerd in de negen stalen ei, onafhankelijk van hun herkomst (gekooid, scharrel, biologisch), noch in 5 kruiden en sauzen, noch in 3 suiker en soortgelijke stalen. De concentraties van de verschillende PFAS varieerden van onder de LOQ tot 2,85 µg/kg (PFTrDA in een staal van krab).

In Verordening (EU) 2023/915 zijn maximumgehalten vastgesteld voor specifieke voedingsgroepen zoals vis, vlees en eieren. Slechts één monster overschreed het maximumgehalte van 0,7 µg/kg voor PFOA in een staal van krab met een concentratie van 1,2 µg/kg. Verder worden in Aanbeveling (EU) 2022/1431 indicatieve niveaus voor verschillende voedingsgroepen vermeld. Dit betekent dat verder onderzoek naar de oorzaken van de contaminatie moet worden uitgevoerd wanneer deze niveaus worden overschreden. Zeven stalen groenten en fruit overschreden de indicatieve niveaus van 0,01 µg/kg voor PFOA met een maximale concentratie van 0,20 µg/kg in oesterzwammen, waarbij rekening gehouden wordt met een meetonzekerheid van 50%.

Bij het evalueren van de blootstelling van de afzonderlijke PFAS via voeding werden 13 van de 25 onderzochtte PFAS als geschikt beschouwd voor de evaluatie (d.w.z. < 98% van de gegevens zijn *left-censored*). De LB-blootstellingschattingen waren het hoogst voor PFBA (gemiddeld 5,1 ng/kg lg/week voor de totale populatie), gevolgd door een PFAS-groep met ongeveer 10 keer lagere schattingen: PFPeA, PFOA, PFHxA en PFOS (gemiddeld 0,36-0,54 ng/kg lg/week voor de totale populatie), dan een PFAS-groep van ongeveer 30-voudig lagere schattingen in vergelijking met PFBA: PFUnDA, PFBS, PFTrDA en PFHpA (gemiddeld 0,14-0,20 ng/kg lg/week voor de totale populatie); tot slot werden de laagste blootstellingsschattingen verkregen voor PFHxS, PFNA, PFDA en PFDoDA, met gemiddelden tussen 0,04 en 0,08 ng/kg lg/week voor de totale populatie. Voor elke PFAS was de blootstelling voor kinderen hoger dan die voor adolescenten en volwassenen. De blootstelling van de MB aan PFOS via de voeding in België nam 8-voudig af in vergelijking met de eerdere studie uit 2012, wat de uitfasering van PFOS in de EU sinds 2009 kan weerspiegelen. De blootstelling aan PFOA, dat sinds 2020 uitgefaseerd is, daalde ook in vergelijking met 2012, maar in mindere mate (3,3-voudig in vergelijking met de vorige studie).

Voor de evaluatie van de blootstelling aan Σ4PFAS werd verondersteld dat de de toxiciteitspotenties gelijk zijn voor de 4-EFSA-PFAS, zoals opgenomen in het EFSA-advies over PFAS van 2020. De gemiddelde blootstelling aan Σ4PFAS in het LB-scenario varieerde van 0,93 ng/kg lg/week (volwassenen) tot 1,7 ng/kg lg/week (kinderen), terwijl de blootstelling in het 95ste percentiel varieerde van 1,8 tot 3,5 ng/kg lg/week. PFOA en PFOS domineren de blootstelling aan Σ4PFAS. Als we de huidige blootstellingsberekeningen vergelijken met de eerdere berekening van EFSA, zijn de huidige gemiddelde blootstellingen drie- (adolescenten) tot zesmaal (kinderen) lager. De twee belangrijkste voedingsgroepen die bijdragen aan de blootstelling aan PFAS zijn "vis en zeevruchten" (met name "garnalen") en "vlees en vleesbereidingen" (met name "zoogdierenvlees") voor alle leeftijdsgroepen, gevolgd door "water en water-gebaseerde dranken" (met name "kraantjeswater", dat zowel als drinkwater wordt gebruikt als gebruikt wordt voor bereiding van de maaltijden).

Vervolgens werden de verkregen voor Σ4PFAS vergeleken met met de TWI van 4,4 ng/kg lg/week, gerelateerd aan immuuneffecten. Deze werd niet overschreden voor de adolescenten en volwassenen,

noch voor 98% van de kinderen. Daarom worden voor het grootste deel van de Belgische bevolking geen gezondheidsproblemen verwacht door blootstelling aan Σ4PFAS via de voeding. Vanwege de methodologie die is gebruikt om de TWI af te leiden, betekent de overschrijding van de TWI voor een klein deel van de kinderpopulatie niet automatisch dat er een gezondheidsprobleem voor deze kinderen is.

Tot slot werd de bijdrage van FCM aan de totale blootstelling aan PFAS onderzocht. Zes monsters van de 28 FCM (21%) vertoonden de aanwezigheid van 4 PFAS (d.w.z. PFPeA, PFHxA, PFHpA en PFOA) uitsluitend in FCM van papier en karton en de hoogste concentratie (0,013 µg/kg PFHxA) werd gevonden in een sandwichpapier. Interessant genoeg werd er geen migratie van PFAS gevonden in taartvormen, pannen of wokken met een anti-aanbaklaag van polytetrafluorethyleen (PTFE), ongeacht de kwaliteit van het artikel. Op basis van de evaluatie met de RACE tool, ontwikkeld door EFSA, kan worden geconcludeerd dat de aanwezigheid van PFAS in FCM geen potentiële risico's voor de consument oplevert. Het is echter belangrijk op te merken dat het aantal stalen in het onderzoek beperkt is en dat meer onderzoek nodig is om deze bevindingen te bevestigen.

CONCLUSIE

Hoewel PFAS alomtegenwoordig zijn in levensmiddelen, overschreed slechts één staal de maximumgehalten die zijn vastgesteld in Verordening (EU) 2023/915, en zeven groente- en fruitstalen overschreden de indicatieve gehalten die zijn vastgesteld in Aanbeveling (EU) 2022/1431. Bij het evalueren van de bijdrage van FCM blijkt dat zes FCM-stalen van papier en karton PFAS bevatten, maar er werd geen PFAS gededecteerd in kookgerei met anti-aanbaklaag.

Indien enkel rekening wordt gehouden met blootstelling aan Σ4PFAS via de voeding (inclusief drinkwater), wordt er voor het grootste deel van de Belgische bevolking geen gezondheidsprobleem verwacht. De TWI werd overschreden door een klein deel van de kinderenpopulatie, maar vanwege de methodologie die werd gebruikt om de TWI af te leiden, betekent dit niet automatisch dat er een gezondheidsprobleem is voor deze kinderen. Wanneer echter ook de andere PFAS die in de studie zijn opgenomen in een gecombineerde risicobeoordeling zouden worden meegenomen, kunnen gezondheidsproblemen niet worden uitgesloten.

AANBEVELINGEN

In deze studie werd de gecombineerde blootstelling aan PFAS geëvalueerd volgens de methodologie zoals voorgesteld door EFSA voor de 4-EFSA-PFAS. Gezondheidsrisico's door de gecombineerde blootstelling aan mengsels van PFAS in levensmiddelen moeten verder worden onderzocht. Er is behoefte aan een internationaal erkende aanpak voor de gecombineerde risicobeoordeling voor alle PFAS die in levensmiddelen worden aangetroffen. Gezien de huidige blootstellingsniveaus voor de algemene bevolking moet het gezondheidsrisico voor mensen die in verontreinigde gebieden wonen ook worden beoordeeld, rekening houdend met de consumptie van zelfgekweekte voeding, maar ook met alle andere gekende PFAS-blootstellingsroutes zoals lucht, stof, enz. Ten slotte is de huidige TWI afgeleid om de meest gevoelige bevolkingsgroep (zuigelingen die borstvoeding krijgen) te beschermen, maar de informatie voor de andere leeftijdscategorieëen is beperkt. Er zijn aanvullende HBGVs nodig om inzicht te krijgen in potentiële gezondheidsproblemen voor de algemene bevolking en specifieke subpopulaties.

RÉSUMÉ SOMMAIRE

CONTEXTE

Les substances per- et polyfluoroalkyles (PFAS) sont des produits chimiques manufacturés utilisés (ou destinés à être utilisés) pour diverses applications. En raison de leur caractère persistant, bioaccumulable et toxique, elles ont été classifiées comme contaminants environnementaux, et l'exposition à ces produits chimiques peut avoir des effets néfastes sur la santé. En 2020, les risques liés aux PFAS ont été réévalués par l'Autorité européenne de sécurité des aliments (EFSA) (EFSA, 2020), déterminant une dose hebdomadaire tolérable (TWI) pour la somme de PFOS, PFOA, PFHxS et PFNA (cf. 4-EFSA-PFAS), appelée par la suite ∑4PFAS, de 4,4 ng/kg de poids corporel (pc)/semaine. La dernière évaluation de l'exposition de la population belge n'ayant porté que sur le PFOS et le PFOA en 2007-2010, celle ci-doit être réévaluée.

OBJECTIFS

Dans ce projet, l'exposition alimentaire de la population belge aux PFAS a été évaluée, suivie d'une évaluation des risques. De plus, il a déjà été démontré que les PFAS peuvent être libérés par les matériaux en contact avec les aliments (FCM), cette question a été étudiée de manière plus approfondie.

MATÉRIEL ET MÉTHODE

Un plan d'échantillonnage complet basé sur un système de notation, prenant en compte tous les aliments pertinents pour l'exposition aux PFAS ainsi que les habitudes de consommation belges, a été établi. Celui-ci a permis de collecter 283 échantillons d'aliments du marché appartenant à 14 groupes d'aliments principaux et 28 FCM. Une attention particulière a été accordée à la viande de gibier, aux abats et aux produits contenant des œufs. Cette étude n'a pas couvert les aliments cultivés dans les jardins des particuliers. La sélection des PFAS à analyser (4-EFSA-PFAS (PFOA, PFNA, PFHxS, PFOS) ainsi que PFBA, PFPeA, PFHxA, PFHpA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHpS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS, Major F53B, Minor F53B, HFPO-DA et DONA) a été établie sur la base de la recommandation (EU) 2022/1431 et du règlement (EU) 2023/915.

Différentes méthodes analytiques ont été mises au point et validées conformément au règlement (EU) 2022/1428 et au document guide du laboratoire européen de référence (EURL). L'extraction utilisant la méthode QuEChERS, a été suivie d'une purification en deux étapes à l'aide d'extractions en phase solide (SPE) avec des phases stationnaires de carbone et d'échange d'anions faibles. L'analyse a été réalisée par chromatographie liquide à ultra haute performance couplée à la spectrométrie de masse à haute résolution (UPLC-HRMS). Les contaminations potentielles des PFAS dans le laboratoire ont été atténuées afin d'atteindre des limites de quantification (LOQ) très basses. Les LOQ obtenues s'étendaient de 0,001 à 0,1 µg/kg en fonction de la matrice (c'est-à-dire des produits alimentaires) et des PFAS, à l'exception du HFPO-DA (limite de quantification maximale de 1 µg/kg). Les méthodes ont ensuite été appliquées à l'analyse des échantillons sélectionnés.

Les données de présence des PFAS ont été combinées avec les données de la consommation alimentaire de la dernière enquête belge sur la consommation alimentaire (FSC2014) afin de réaliser une évaluation de l'exposition alimentaire selon différentes approches. L'approche de la limite inférieure (LB), dans laquelle les données de présence inférieures à la LOQ sont mises à zéro, a été considérée comme la plus pertinente, bien que des évaluations de la limite moyenne (MB) et de la limite supérieure (UB) aient également été réalisées. Une évaluation des risques pour le ∑4PFAS (somme du PFOA, du PFNA, du PFHxS et du PFOS) a été réalisée sur la base de l'approche appliquée par l'EFSA en 2020. Une évaluation complémentaire des risques a été réalisée pour évaluer la contribution du FCM à l'aide de l'outil Rapid Assessment of Contaminant Exposure (RACE) mis au point par l'EFSA.

RÉSULTATS

La concentration des 25 PFAS a été déterminée dans 283 denrées alimentaires sélectionnées en appliquant des méthodes sensibles et validées. Une contamination généralisée par les PFAS dans les diverses denrées alimentaires a été démontrée. Bien qu'une moyenne de 1,1 composé ait été détectée par échantillon, environ 57 % des échantillons ne contenaient aucun des 25 PFAS étudiés, environ 23 % ne contenaient qu'un seul PFAS, 15 % entre 2 et 5 PFAS et moins de 5 % entre 6 et 11 PFAS. Le PFOS est le composé le plus détecté dans 19 % des 283 échantillons, suivi par le PFOA (17 %). Huit composés n'ont jamais été détectés (PFTeDA, PFHpS, PFDS, PFUnDS, PFDoDS, PFTrDS, Minor F53B, HFPO-DA). D'autres PFAS ont été détectés dans 1 à 11 % des échantillons, selon le PFAS. Au moins un PFAS a été quantifié dans 74 %, 68 %, 57 % et 53 % des échantillons de, respectivement, poisson, légumes, eau et plats composés. Aucun PFAS n'a été détecté dans les neuf échantillons d'œufs, quelle que soit leur origine (en cage, en liberté, biologique), ni dans les cinq assaisonnements et sauces, et ni dans les trois échantillons de sucre et similaires. Les concentrations des différents PFAS variaient de inférieure à la LOQ à 2,85 µg/kg (pour le PFTrDA dans un échantillon de crabe).

Le règlement (EU) 2023/915 fixe des limites maximales pour des groupes d'aliments spécifiques tels que le poisson, la viande et les œufs. Un seul échantillon a dépassé la limite maximale de 0,7 µg/kg pour le PFOA avec une concentration de 1,2 µg/kg dans un échantillon de crabe. De plus, la recommandation (EU) 2022/1431 mentionne des niveaux indicatifs pour plusieurs groupes, ce qui signifie qu'une enquête plus approfondie sur les causes de la contamination doit être menée lorsque les niveaux sont dépassés. Sept échantillons de légumes et de fruits (en tenant compte d'une incertitude de mesure de 50%) ont dépassé les niveaux indicatifs de 0,01 µg/kg pour le PFOA dans les fruits et légumes, avec une concentration maximale de 0,20 µg/kg dans des pleurotes.

Lors de l'évaluation de l'exposition alimentaire aux différents PFAS, 13 des 25 PFAS ciblés ont été jugés suffisamment fiables pour l'évaluation de l'exposition (signifiant que < 98 % des données sont *« left-censored*»). Pour ces données individuelles, les estimations de l'exposition à la LB étaient les plus élevées pour le **PFBA** (moyenne de 5,1 ng/kg pc/semaine pour la population totale), suivi d'un groupe de PFAS dont les estimations de l'exposition étaient environ 10 fois inférieures : **PFPeA, PFOA, PFHxA et PFOS** (moyenne de 0,36 à 0,54 ng/kg pc/semaine pour la population totale), puis un groupe de PFAS dont les estimations étaient environ 30 fois inférieures à l'exposition la plus élevée : **PFUnDA, PFBS, PFTrDA et PFHpA** (moyenne de 0,14 à 0,20 ng/kg pc/semaine pour la population totale) ; enfin, les estimations d'exposition les plus faibles ont été obtenues pour le **PFHxS, le PFNA, le PFDA et le PFDoDA**, avec des valeurs moyennes allant de 0,04 à 0,08 ng/kg pc/semaine pour la population totale. Pour chaque PFAS, l'exposition des enfants était plus élevée que celle des adolescents et des adultes. L'exposition alimentaire de la MB au PFOS en Belgique a été divisée par 8 par rapport à une précédente belge de 2012, ce qui peut refléter l'élimination progressive du PFOS dans l'UE depuis 2009. L'exposition au PFOA, qui a progressivement été éliminé à partir de 2020, a également diminuée par rapport à 2012, mais dans une moindre mesure (3,3 fois par rapport à l'étude précédente).

Lors de l'estimation de l'**exposition aux Σ4PFAS**, les potentiels de toxicité ont été supposées égales, comme indiqué dans l'avis de l'EFSA sur les PFAS de 2020. L'exposition moyenne des LB aux Σ4PFAS était comprise entre 0,93 ng/kg pc/semaine (adultes) et 1,7 ng/kg pc/semaine (enfants), tandis que l'exposition du 95ème percentile était comprise entre 1,8 et 3,5 ng/kg pc/semaine. Le PFOA et le PFOS sont les principaux constituants de l'exposition classique à la Σ4PFAS. Si l'on compare les estimations actuelles de l'exposition avec une estimation antérieure de l'EFSA, les expositions moyennes actuelles sont de 3 (adolescents) à 6 fois inférieures (enfants). Les principaux groupes d'aliments contribuant le plus à l'exposition aux PFAS étaient le "poisson et les fruits de mer" (en particulier les "crevettes") et la "viande et les produits à base de viande" (en particulier la "viande de mammifères") pour tous les groupes d'âge, suivi par "l'eau et les boissons à base d'eau" (en particulier "l'eau non embouteillée", utilisée comme eau de boisson et consommée pendant les repas).

En outre, les estimations de l'exposition alimentaire ont été comparées aux données sur les PFAS dans le sérum sanguin de l'étude FLEHS IV (Flemish Environment and Health Study) menée en Flandre. En limitant la comparaison à la population adolescente (en raison de la compatibilité des données), on peut conclure que les niveaux de PFOS, PFOA, PFNA et PFHxS dans l'alimentation peuvent largement refléter leurs niveaux chez l'homme.

L'évaluation de l'exposition obtenue pour la Σ4PFAS a été comparée au TWI de 4,4 ng/kg pc/semaine, liée aux effets immunitaires, n'était pas dépassée ni pour les populations d'adolescents et d'adultes, ni pour 98 % de la population d'enfants. Par conséquent, pour la majeure partie de la population belge, aucun problème de santé lié à l'exposition alimentaire à la Σ4PFAS n'est envisagé. En raison de la méthodologie utilisée pour calculer du TWI, le dépassement du TWI pour la petite fraction de la population infantile n'implique pas automatiquement un problème de santé pour ces enfants.

Enfin, la contribution des FCM à l'exposition globale aux PFAS a été étudiée. Six échantillons sur 28 FCM (21%) ont révélé la présence de quatre PFAS (à savoir PFPeA, PFHxA, PFHpA et PFOA) exclusivement dans des FCM en papier et en carton, la concentration la plus élevée (0,013 µg/kg de PFHxA) ayant été trouvée dans un papier sandwich. Il est intéressant de noter qu'aucune migration de PFAS n'a été constatée dans les moules à gâteaux, les poêles ou les woks dotés d'un revêtement antiadhésif en polytétrafluoroéthylène (PTFE), quelle que soit la qualité de l'article. L'évaluation réalisée à l'aide de l'outil RACE mis au point par l'EFSA permet de conclure que la présence de PFAS dans les FCM ne présente aucun risque potentiel pour les consommateurs.

CONCLUSIONS

Bien que les PFAS soient omniprésents dans les aliments, un seul échantillon dépassait les niveaux maximaux fixés par le règlement (UE) 2023/915, et sept échantillons de légumes et de fruits dépassaient les niveaux indicatifs indiqués dans la recommandation (UE) 2022/1431. Si l'on évalue la contribution des FCM, on constate que six échantillons de FCM en papier et en carton contiennent des PFAS, tandis qu'aucun PFAS n'a été trouvé dans des ustensiles de cuisine à revêtement antiadhésif.

Si l'on considère l'exposition alimentaire (y compris l'eau de boisson) uniquement pour la Σ4PFAS, aucun problème de santé n'est à prévoir pour la majeure partie de la population belge. Le TWI a été dépassé pour une petite fraction des enfants, mais en raison de la méthodologie utilisée pour calculer le TWI, cela n'implique pas automatiquement un problème de santé pour ces enfants.

RECOMMENDATIONS

Dans notre étude, l'exposition combinée aux PFAS a été évaluée selon l'approche de l'EFSA pour 4 PFAS. Les risques pour la santé découlant de l'exposition combinée à des mélanges de PFAS dans les aliments devraient faire l'objet d'études plus approfondies étant donné qu'une exposition combinée à d'autres PFAS a été démontrée dans notre étude. Pour ce faire, il est nécessaire d'adopter une approche internationalement reconnue pour l'évaluation combinée des risques de tous les PFAS détectés dans les aliments. Compte tenu des niveaux d'exposition actuels de la population générale, il convient d'évaluer les risques pour la santé des personnes vivant dans des zones polluées en tenant compte également de la consommation d'aliments cultivés sur place, et en considérant aussi toutes les autres voies connues d'exposition aux PFAS, telles que l'air, la poussière, etc. Enfin, le TWI actuel est calculé pour protéger le groupe de population le plus sensible (les nourrissons allaités), mais l'information pour les autres catégories est limité. D'autres HBGV sont nécessaires pour comprendre les problèmes de santé potentiels pour la population générale et des sous-populations spécifiques.

Problem

Per- and polyfluoroalkyl substances (PFAS) are industrial chemicals encompassing thousands of chemicals. They have been produced since the 1950s and are used (or used to be used) for various applications. Due to their persistent, bioaccumulative, and toxic character, they have become environmental contaminants, and exposure to these chemicals may lead to adverse health effects (Buck et al., 2011). Significant research efforts in the last two decades have focused on subgroups of PFAS, namely perfluoroalkyl sulfonates (PFSA) and carboxylates (PFCA).

Human exposure to these chemicals can occur through multiple exposure pathways, of which food has been identified as a dominant source. Moreover, food of animal origin has been shown to contain the highest concentrations of PFSA/PFCA (Hlouskova et al., 2013). In 2018, EFSA published an Opinion on the perfluorooctane sulfonic acid (PFOS) and the perfluorooctanoic acid (PFOA) and set two separate tolerable weekly intakes (TWIs), namely 13 ng/kg body weight (bw)/week for PFOS and 6 ng/kg bw/week for PFOA (EFSA, 2018a). In 2020, the risks related to PFAS were re-evaluated by EFSA (EFSA, 2020). The TWIs have been modified, and the opinion's scope was extended to other PFAS, including perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS). PFOA, PFOS, PFHxS, and PFNA (4-EFSA-PFAS). Together, they represented approximately half of the lower bound (LB) exposure of PFAS for which occurrence data were available. Because derivation of relative potency factors was not possible, the EFSA opinion assumed equal, weight-based potency. Consequently, a TWI for the sum of these 4-EFSA-PFAS was established at 4.4 ng/kg bw/week. Since a higher exposure of 12 ng/kg bw/week for the sum of PFOA and PFOS was determined for the Belgian population in 2007-2010 (Bervoets et al., 2012), this exposure should urgently be re-evaluated. This project will provide information on the dietary exposure of the Belgian population to PFOS, PFOA, PFHxS and PFNA and the sum of these 4-EFSA-PFAS (i.e. Σ4PFAS). Since occurrence data on contributions from other PFSA (C4-C13), PFCA (C5-C14), and PFAS substitutes (DONA, F53B and HFPO-DA) are currently not available in Belgium, this project aims to fill this gap.

Even if the diet was identified as the predominant exposure pathway in most studies (EFSA, 2020), dust ingestion, indoor air inhalation, and dermal exposure may also contribute substantially to exposure (non-dietary exposure), particularly at the individual level (Fu et al., 2015; Liu et al., 2019; Poothong et al., 2020). Food contact materials (FCM) were also highlighted in a study conducted by the RIVM (National Dutch Institute for Public Health and the Environment) and confirmed in the recent EFSA opinion (Bokkers et al., 2018; EFSA, 2020). Other recent studies have also reported the contribution of food processing, packaging and polytetrafluoroethylene (PTFE) cookware to the exposure(Choi et al., 2018; Jogsten et al., 2009; Schaider et al., 2017). However, the results of previous studies were inconclusive (Begley et al., 2005; Gebbink et al., 2013). Therefore, the impact of FCM on exposure to PFAS will be investigated further in this project.

Research objectives

This research project aims to answer the following research questions :

1/ What is the occurrence of PFAS (*i.e.* PFOA, PFOS, PFNA, PFHxS, 4-EFSA-PFAS sum and other relevant PFAS) in the Belgian food chain? What are their concentrations?
In the past, most studies focussed on the occurrence of PFOA and PFOS, although many more PFAS exist. Based on several similar effects in animals, toxicokinetics, and observed levels in the blood, the EFSA CONTAM panel performed a risk assessment for the sum of 4-EFSA-PFAS (∑4PFAS) (EFSA, 2020). Therefore, monitoring these PFAS using sufficiently sensitive analytical methods is essential. The Commission recommended a LOQ of 1 µg/kg for monitoring PFAS in food (Commission Recommendation 2010/161/EU, 2010). However, the new TWI of 4.4 ng/kg bw/day mentioned in the EFSA opinion would necessitate a much lower LOQ (up to 0.001 µg/kg for individuals PFAS in fruits and vegetables). Therefore, more sensitive analytical methods must be developed and validated. According to EFSA, the highest mean occurrence values (for the ∑4PFAS) were found in edible offal from game animals, followed by fish, eggs, meat, drinking water, fruits, and vegetables (EFSA, 2020). However, this should be investigated further for the Belgian market.

2/ What is the exposure of the Belgian population to PFAS? What are the risks?
Dietary exposure will be assessed by combining the occurrence data with consumption data from the Belgian food consumption survey (FCS 2014) (Bel et al., 2016). Afterwards, the results will be compared with the TWI of 4.4 ng/kg bw/week, as mentioned in the EFSA opinion (EFSA, 2020).

3/ What are the potential sources of PFAS contamination in food?
An important additional source of exposure to PFAS may be the use of FCM. However, only minimal information is available in the scientific literature, and the results were inconsistent (Begley et al., 2005; Gebbink et al., 2013). Therefore, this should be investigated further and considered in the overall exposure evaluation.

To summarize, the main objective of this project is to evaluate the exposure (and corresponding risk) of the Belgian population to PFAS, complemented by an investigation of food contamination sources (e.g. FCM). An overview of the different work packages (WP) is given in Figure 1.

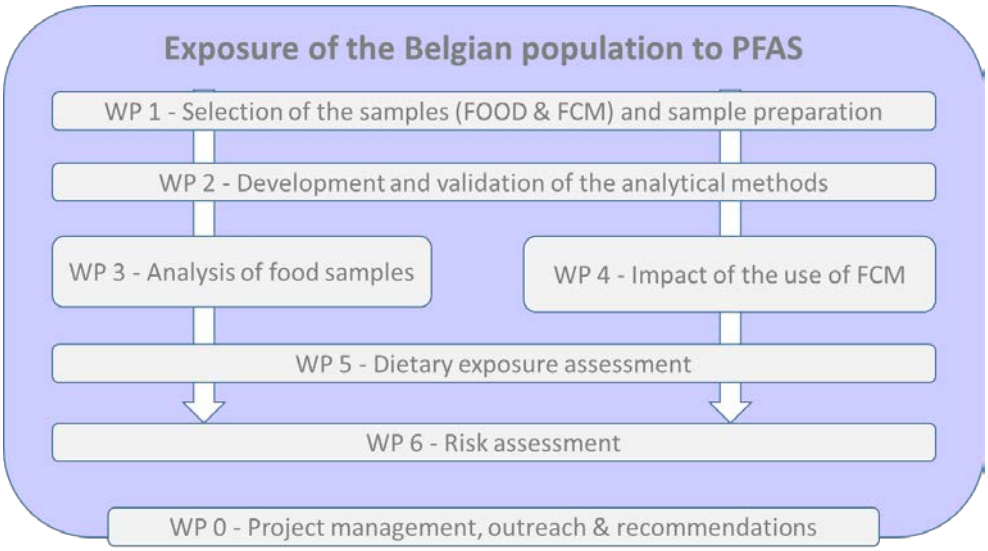

Figure 1: Workflow of the different work packages of the project

Materials & methods

SAMPLING AND SAMPLE PREPARATION (WP1)

Sampling

The comprehensive sample collection in the FLUOREX project is exposure-driven. The selection was initially based on main food groups (equivalent to the FoodEx level 1) and was further refined into subgroups (equivalent to the FoodEx level 3) to ensure the coverage of all foods relevant for PFAS exposure.
Sampling priorities were first set for the 21 groups at the main food classification according to the following criteria, thereby focussing on the 4-EFSA-PFAS:

(i) **Risk probability** for the PFAS based on the EFSA opinion (EFSA, 2020) as a result of the available occurrence data, including concentration and frequency data (*risk*)
(ii) **Contribution to exposure** according to the Belgian consumption level of food items per food group (very high, high, moderate, low and very low) (Bel et al., 2016). Contribution levels are defined using the percentile consumption quantity and frequency of consumption (*exp*)
(iii) and **food group variability** based on the number of food groups on level 3 included in each level 1 according to EFSA classification and matched in the FCS of 2014(*var*) (Bel et al., 2016)

A weighting factor (Wf) was also attributed for each criterion and the total score was calculated for each of the 21 FoodEx groups.
Next, similar prioritisations were carried out within each selected main group at the subgroup level (i.e. FoodEx level 3). At this refinement level, the following criteria have been set:

(i) **Risk probability** based on a literature review (Augustsson et al., 2021; Ghisi et al., 2019; Herzke et al., 2013; Hlouskova et al., 2013; Stahl et al., 2014; Pasecnaja et al., 2022; Teunen et al., 2021; Sznajder-Katarzyńska et al., 2019; Zafeiraki et al., 2016; D'Hollander et al., 2015; Bervoets et al., 2012; EFSA, 2020) including detailed information from the EFSA opinion at the subgroup level for the **4-EFSA-PFAS** as a result of the available occurrence data, including concentration and frequency data.
(ii) **Exposure frequency** according to the National Belgian consumption survey of food items per subgroup (FSC2014) (Bel et al., 2016) (very high, high, moderate, low and very low).
(iii) **Exposure consumption** according to the National Belgian consumption survey of food items per food group at the subgroup level (very high, high, moderate, low and very low) (Bel et al., 2016).
(iv) **Additional risk probability** based on a literature review (Augustsson et al., 2021; Ghisi et al., 2019; Herzke et al., 2013; Hlouskova et al., 2013; Stahl et al., 2014; Pasecnaja et al., 2022; Teunen et al., 2021; Sznajder-Katarzyńska et al., 2019; Zafeiraki et al., 2016; D'Hollander et al., 2015; Bervoets et al., 2012; EFSA, 2020) including detailed information from EFSA opinion at the subgroup level **for other PFAS** as a result of the available occurrence data, including concentration and frequency data.

A weighting factor (Wf) was also attributed to each criterion.

Products containing ingredients of animal origin (e.g. eggs) are specifically relevant for PFAS exposure. Since egg-containing products are distributed over different main food groups, a specific strategy for egg-containing products has been developed using the results of other projects like Nutritrack[1] and MultiExpAdd[2]. Data on food items in the Belgian market in 2020 was collected for these two projects,

[1] NUTRITRACK project - Food monitoring system to track the nutritional quality of food products on the Belgian market, financed by SPF Health, 2018- no end date
[2] MultiExpAdd project - Determination of the predominant (co-)occurrences and single and combined exposures of food additives in the diet of different groups of the Belgian population, financed by SPF Health, 2020-2021

considering the newly launched products from the GNDP Mintel Database. This collection covers more than 80% of all available food products. Products containing eggs either in high percentages or as a significant ingredient, were selected. Hence, 30 additional egg-containing products across various food groups were specifically selected. Further, some supplementary samples were attributed to diverse food groups based on the specific demand for FPS Health, Food Chain Safety, and Environment.

The brands to be purchased for individual samples were selected based on market share data and market analysis (e.g. Euromonitor) to provide the top-selling brands for each food item when relevant. Based on market data from the GAIN report (Nielsen Grocery Universe, 2017). Special attention was paid to selecting game animals and offals during sampling. About 20% of all selected samples were organic products. Consequently, sampling resources were dedicated to providing food samples most representative of Belgian consumption habits.

Preparation of samples for analysis

Challenges regarding contamination

The presence of PFAS in the environment is ubiquitous. Therefore, specific attention should be paid to laboratory background contamination. During the sample manipulation and analysis, specific attention was paid to reducing the PFAS contamination within the laboratory to a minimum.

Analytical sample portion

According to the Commission Recommendation (EU) 2022/1431 on the monitoring of perfluoroalkyl substances in food (Commission Recommendation (EU) 2022/1431, 2022), only the edible part was taken as an analytical sample. The totality of the purchased food products was used to prepare the analytical sample portion, between 250 g to 1 kg for the majority of the samples, with a few exceptions for expensive samples (e.g. dried morels).

Regarding fish and meat foodstuffs, only the edible part was taken. The whole meat part of canned fish or seafood was analysed, and the liquid in the can was removed. For the whole fish (trout and carp), the head, skin, tail and offals were removed. Similarly, the head, carcass, and tail were removed for the crustacean and shell from molluscs (mussel). Only the white meat was taken for analysis for the whole crab sample. Meat and skin were separately analyzed for one whole chicken item and one item of chicken filet with skin.

For fruits and vegetables (including starchy roots and tubers and fruit-based baby food meals), the chopped samples were washed with water (assessed periodically and found to be free-PFAS), except freeze-dried samples (e.g. morels, penny bun), prepared samples (grated carrot), freeze samples (spinach), jarred samples (green beans), and samples with non-edible skin (banana, pineapple, avocado, mango, kiwi and passion fruits). The juice from jarred samples (green beans) was removed before homogenisation. The external part of the onion, leek roots, apple core, strawberry green part, grape stem, peduncles, and stones were removed prior to homogenisation. The citrus fruits (lemon, orange) were homogenised with the skin. The potatoes were homogenised with skin except for the baby potatoes.

The egg samples were homogenised as a whole, combining the egg yolk and the egg white for each sample (only one egg of each sample was kept in stock).

Concerning milk and dairy products, the juice was removed from the mozzarella sample.

Analytical sample preparation

All the analytical samples were milled and/or homogenized. When applicable, the samples were chopped using a stainless steel knife (e.g., for fish, meat, fruit, and vegetables), then evenly milled with

a mixer Resch 200 (Verder Scientific GmbH & Co, Aartselaar, Belgium). Other samples (e.g., cereal-based products, composite dishes, dairy products) were directly milled. Before analysis, liquids, sauces, spreads, and baby food samples were homogenized. The fruit and vegetable samples (including fruit-based baby food meals, starchy roots and tubers) were chopped, lyophilized and then milled. The milled samples were aliquoted in polypropylene (PP) containers and stored in a freezer until analysis.

DEVELOPMENT AND VALIDATION OF THE ANALYTICAL METHODS (WP2)

Analytical Method

Standards

Mixtures of non-labelled PFAS (PFAC-MXC (2000 ng/mL; PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxDA, PFODA, L-PFBS, L-PFPeS, L-PFHxS, L-PFHpS, L-PFOS, L-PFNS, L-PFDS, L- PFDoDS) and PFAC-MXF (2000ng/mL; HFPO-DA, DONA, Major F53B, Minor F53B)) and isotopically labelled internal standard (IL-IS) PFAS (2000 ng/mL; MPFAC-C-ES (MPFBA, M5PFPeA, M5PFHxA, M4PFHpA, M8PFOA, M9PFNA, M6PFDA, M7PFUnDA, MPFDoDA, M2PFTeDA, M3PFBS, M3PFHxS, M8PFOS) and MPFAC-C-IS (2000 ng/mL; M3PFBA, M2PFOA, MPFDA, MPFOS)), as well as individual non labelled PFAS (50 µg/mL; PFTrDS, PFUnDS) and individual IL-IS PFAS (50 g/mL; MHFPO-DA), were obtained from Wellington Laboratories (Ontario, Canada).

Sample weight

Milled and homogenized samples were weighted in Falcon tubes 50 mL as follows: 5.00 g ± 0.02 g for all the matrices except 10.00 g ± 0.05 g for liquid (milk, soft, alcohol); equivalent of 25 g ± 0.1 g fresh weight for the lyophilized fruits and vegetables (including starchy roots and tubers and fruit-based baby food meals); 100.00 g ± 0.05 g for water samples (in 2 tubes).

Sample extraction

Extraction of PFAS from the samples was based on a QuEChERS method. Before the extraction, the samples were fortified with 2 ng of IL-IS PFAS solution. Milli-Q water was added to the Falcon tubes as follows: 10 mL for all the matrices except 15 mL for freeze-dried fruits and vegetables (including starchy roots and tubers and fruit-based baby food meals). No water was added to liquid samples (milk, soft, alcohol). The samples were agitated for 10 min. Afterwards, 10 mL of ACN (NH_4OH, 1%) was added to all samples, and they were agitated for 20 min. Then 0.5 g of NaCl and 4 g of $MgSO_4$ were added, and samples were shaken vigorously by hand for 1 min. The samples were centrifuged for 10 min at 10000 rpm at 5 °C. The water samples were not subjected to this extraction step.

Sample clean-up

The first clean-up step was performed using SPE SupelClean ENVI-carb – PSA 500 mg/300 mg for the liver and SupelClean Envicarb 500 mg or Bond Elut Carbon S 500 mg for all other matrices. The water samples were not subjected to this first purification step. The extract (upper phase) was loaded onto the cartridge and the eluate was collected. The elution continued with 6 mL of MeOH with 2% acetic acid. The eluate was evaporated up to ±10 mL, and the volume was adjusted to 50 mL with Milli-Q water. The pH was then adjusted to 3 using formic acid.

The second clean-up step was based on SPE with SPE Bond Elut PFAS WAX 500 mg for all the matrices and SPE Strata WAX/GCB 200 mg/50 mg for water. After loading the extract, the SPE columns were washed with 6 mL of Milli-Q water with 20 mM ammonium acetate and 6 mL of MeOH with 2% acetic acid. The elution of PFAS was achieved with 12 mL of MeOH/NH_4OH (95/5; v/v).

After the purification, the eluates were evaporated under a nitrogen stream at 50 °C to near dryness (<100 µl). Two ng of the external (MS injection) standard (ES) were added, and the volume was brought up to 500 µl with MeOH. The solution was vortexed for 30 sec, centrifuged at 4000 rpm for 5 min at 5 °C, and transferred to a 0.2 µm PP (polypropylene) auto-filtrating vial prior to LC-HRMS analysis.

Instrumental analysis

Analysis of PFAS was performed with a UPLC Vanquish™ (ThermoFisher Scientific, San Jose, CA, USA) coupled to a Q-Exactive Focus™ Orbitrap mass spectrometer (ThermoFisher Scientific) with a heated electrospray interface (HESI) operated in negative mode.

The LC system was equipped with an Acquity™ reversed-phase isolator column (2.1 x 50 mm) installed after the LC pump and before the injection valve to offset background contaminants from the LC pump, degasser, and mobile phases. For the LC separation of PFAS, an Acquity UPLC BEH C18 (2.1 x 100 mm, 1.7 µm) analytical column (Waters) with a Vanguard Acquity™ BEH C18 (2.1 x 5 mm, 1.7 µm) precolumn (Waters) were used at 55 °C. The mobile phases A and B, 20 mM ammonium acetate in water/MeOH (96/4; v/v) and in MeOH/water (96/4; v/v), respectively, were used at a flow rate of 0.4 mL/min. The injection volume injected was 5 µl. The LC gradient started with an isocratic hold of 1 min at 20% of mobile phase B, increasing linearly for 8.5 min up to 100% of mobile phase B, followed by an isocratic hold for 1 min at 100% mobile phase B before going back to the initial percentage in 0.1 min and kept for 2.4 more minutes at 20% of mobile phase B for a total run of 13 minutes.

The mass spectrometer was operated in the full scan–parallel reaction monitoring (FS-PRM) mode. PRM mode acquiring MS/MS scans based on an inclusion list covering all native, IS and ES analytes as a triple quadrupole. The resolution was set 35 000 for the FS mode and 17500 for the PRM mode. All data acquisition and analysis were performed using TraceFinder 5.1 software.

Method validation

Validation set-up

Different food samples were selected for the method validation experiments to represent a wide variety of each food group. The target matrices were grouped according to their physico-chemical properties: (i) animal-origin tissue (including “fish and seafood” (FIS), “meat and meat products” (MEA) (except organs)), (ii) livers, (iii) eggs, (iv) fruits and vegetables, and (vi) water. The validation was performed in triplicate (n=3) at minimum 3 different fortification levels. Control samples at the LOQ and higher levels were performed for the food groups cereals, alcohols, dairy products, composites dishes and chocolate spreads.

Validation criteria

The limit of quantification (LOQ) was defined as the lowest fortification level meeting the identification requirements and analytical performance criteria for recovery and precision.
Trueness and precision were assessed for each matrix group by repeated analyses (n=3) of fortified samples at minimally three concentration levels, performed by different analysts. Trueness (bias) and precision (RSD_{RW}) were calculated as described by ISO 5725-2 guidelines.
IS recoveries were controlled to ensure good extraction efficiency.
The identification criterion for chromatography was the ratio of the chromatographic RT of the analyte and the IS (i.e. relative RT of the analyte) corresponding to that of the calibration standard with a maximum deviation of 1%. The identification and confirmation criteria for mass spectrometry were the presence of two (or three) ions with mass accuracy ≤ 5 ppm.
A calibration curve consisted of points at least 5 different fortification levels with a deviation of the back-calculated concentration from the true concentration for each calibration point below 20%. The matrix

effect, i.e. the difference of response from a standard in matrix extract and a standard in solvent using IS, was evaluated for three matrices (beef, liver, and eggs).
The expanded measurement uncertainty was calculated using both the precision (RSD_{RW}) and the laboratory bias based on the data generated during the validation as follows:

$$U = k \times u = |bias| + 2\,RSD_{RW}$$

where
U is the expanded measurement uncertainty, k is the coverage factor of the 95% confidence interval (k = 2), and u is the relative standard uncertainty.

Reference material analysis

Seven recent reference materials from the interlaboratory studies on PFAS in food organized by the European Union Reference Laboratory (EURL) were analyzed. The EURL accuracy criteria (z-score <|2| with a fitness-for-purpose-based standard deviation for proficiency assessment, σ_p set at 20%) were evaluated for the PFAS present in wheat flour, fish fillet, liquid whole egg, fish meal, pork liver, feed and milk powder.

ANALYSIS OF THE FOOD SAMPLES (WP3)

All samples selected in WP1 were analysed using the analytical methods validated in WP2. During the analysis, a quality control plan was implemented to ensure the quality and reliability of the results. For each batch of samples, the daily method's performance was monitored.

IMPACT OF THE USE OF FCM (WP4)

Sampling

The sampling was designed to analyse samples with non-sticky coating or resistance to moisture and oil. A previous research project (MIGRACARTO, financed by FPS Health, Food Chain Safety and Environment) focussed on takeaway articles and straws. Therefore, the FCM selected in the framework of FLUOREX focused on different types of articles where PFAS could be expected, such as pans, muffin cups etc. Different brands and qualities were selected for articles like pans, woks and cake moulds.

Sample preparation and analysis

The experiments were performed according to the guideline on testing conditions for kitchenware articles in contact with foodstuffs developed by the EURL for FCMs (Beldi et al., 2023) and the royal decree on coating and varnishes intended to come into contact with food (Arrêté Royal, 2016). When article filling (e.g., pans, bakeware) or immersion (e.g., dough hook) was possible, the intact article was used. If the article could not be filled or immersed (e.g. muffin cups), one dm^2 was cut and immersed in the extraction solvent or simulant. After the migration or extraction experiments, 25 mL of the aqueous fraction extract was combined with 25 mL of the organic fraction for the analysis of PFAS. The organic phase was dried under nitrogen, and the sample was reconstituted in 50 mL of the associated aqueous simulant. The extract was then purified using SPE with Bond Elut PFAS WAX, 500mg SPE cartridges (Agilent).
Finally, the eluates were dried until 100 µL at 50°C, 400 µl of MeOH was added, and the extract was transferred to a PP vial. The final extract was then analysed by LC-HRMS using the analytical method described above to analyse food. Twelve PFAS (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFPeS, PFHxS, PFOS and HFPO-DA) were targeted and quantified using a calibration curve prepared in methanol ranging from 0.2 ng/mL up to 40 ng/mL.

Evaluation of the additional exposure related to FCM

To evaluate the impact of FCM on the exposure to PFAS, a ***scenario*** is performed on the sum of the 4-EFSA-PFAS (∑4PFAS) (see chapter “Exposure assessment for PFAS”).

Since no specific harmonized EU legislation exists for FCM made of paper and board, a risk assessment was performed using the RACE Tool of EFSA (Fürst et al., 2019). The RACE tool was initially developed to support risk managers in deciding whether a RASFF notification should be made. The EFSA Working Group created a universal approach for risk evaluation. The RACE Tool is a decision tree that suggests a quick and reliable way to evaluate risks. The risk evaluation is based on assessing toxicological properties and dietary exposure. The outcome is determined by comparing the exposure level to a toxicological reference point (in this case, the TWI), resulting in “no risk”, “low probability of adverse health effects or low concern for public health”, “potential risk”, or “risk”. The terminology depends on the available toxicological data (such as TDI or TTC) for the investigated PFAS. When a “potential risk” or “risk” is indicated, other investigations should be conducted as a concern for consumers is highlighted. In this study, the potential risks were assessed for children, adolescents and adults using consumption hypotheses formulated based on the assumed frequency of use according to personal opinions gathered from various colleagues of Sciensano as the consumption data from the national survey are inappropriate.

DIETARY EXPOSURE ASSESSMENT (WP5)

Food consumption data

The exposure evaluation is realized using national representative food consumption data from the second Belgian food consumption survey (FCS2014) for persons aged between 3 and 64 years. The objectives, concept and methodology of the food consumption survey have been described elsewhere (Bel et al., 2016).

Dietary assessment in adolescents and adults (> 10 years) was performed by the 24-h dietary recall method, carried out on two non-consecutive days, using GloboDiet© (former EPIC-Soft), a computerised 24-h recall program. Dietary assessment in children (3 to 9 years old) was done using two self-administered non-consecutive one-day food diaries followed by a GloboDiet completion interview with a proxy respondent. Pre-defined coded lists of foods, recipes, facets and descriptors are used in Globodiet©. Facets and descriptors describe foods and recipes in more details. Facets characterize different aspects of the dietary item, such as the cooking method used, preservation method or the applied packaging material (e.g. canned food). Descriptors are pre-defined answers for the facets, e.g. grilled, fried or boiled for the facet ‘cooking method’. Furthermore, the food items are linked to the FoodEx2 classification (https://www.efsa.europa.eu/en/data/data-standardisation).

Two sets of data of the FCS were used for the dietary exposure assessment: a data set containing consumption quantities of mixed recipes (composite dishes such as e.g. tuna salad with tuna and mayonnaise as the main ingredients; “Mixed recipe” dataset) and a data set in which the mixed recipes were disaggregated into ingredients (e.g. mayonnaise and tuna; “Food & ingredient of mixed recipe” dataset). The latter dataset also contains consumed quantities of foods that are not part of a recipe. Both datasets can be linked to each other based on the identification number of the individuals, the interview day, the place of consumption and the time of consumption.

Occurrence data

A dataset with analytical results for 283 food samples was submitted for the PFAS exposure assessment. The dataset contained analytical results for 4-EFSA-PFAS (PFOA, PFNA, PFHxS, PFOS (total)) and also, PFBA, PFPeA, PFHxA, PFHpA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFBS, PFPeS, PFHpS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS, Major F53B, Minor F53B, HFPO-DA and

DONA. Data related to baby food samples (n = 10) were not used for the exposure assessment. A large amount of data was left-censored. For 8 PFAS (PFTeDA, PFHpS, PFDS, PFUnDS, PFDoDS, PFTrDS, Minor F53B and HFPO-DA), all results were left-censored. For these PFAS, no exposure calculations were performed. For four PFAS (PFPeS, PFNS, Major F53B and DONA), 98.9 to 99.6% of the analytical results were left-censored. Given the low reliability of exposure estimates based on such data, it was decided not to perform exposure assessments for these PFAS either. Hence, occurrence data for 13 individual PFAS (PFOA, PFNA, PFHxS, PFOS, PFBA, PFPeA, PFHxA, PFHpA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFBS) were used in the exposure assessment. For PFBA, no analytical data were available for the meat, fish and seafood, eggs and water samples. The missing data were substituted by data from available reports of EFSA (EFSA, 2020) and VMM (VMM, 2022). Considering left-censored analytical data, the substitution method as recommended by WHO (WHO, 2011) and EFSA was used for non-quantified results (i.e. analytical result < LOQ) (EFSA, 2010). In the present report, analytical data below the LOQ were set equal to zero (lower-bound approach; LB) and the LOQ (upper-bound approach, UB) because more than 60% of the data were left-censored (WHO 2009). The LOQ values depended on the PFAS and the food matrix in which the PFAS was measured and varied between 0.001 ng/g and 1 ng/g.

Matching of occurrence data to consumption data

In order to perform the PFAS exposure and risk assessment, the data on dietary consumption and analytical concentration levels of the different PFAS expressed in ng/g had to be coupled.

In the sampling phase, food groups corresponding to FoodEx2 Level 3 were selected according to the criteria described earlier. Within each food group one or more food products were sampled and they were manually linked to their corresponding FoodEx2 Level 3 Exposure Hierarchy Code and Term Code (e.g. sample “Atlantic Salmon filet” linked to “Diadromous fish”, with a Term Code A028E and Exposure Hierarchy Code: Z0007.0001.0002). Analytical results were then subsequently linked. These corresponding groups (FoodEx2 Level 3) were also used as aggregation groups. Mean PFAS concentrations were calculated per aggregation group. The calculated mean PFAS concentrations were subsequently assigned to all consumed food items belonging to the same FoodEx Level 3 food group in the FCS2014.

Occurrence data for tap water were linked to “Unbottled water” in the FCS2014. This group does not only include the consumption of tap water as drinking water, but also the consumption of water used to prepare meals, if it is supposed to be consumed (e.g. as soup, absorbed when preparing risotto, …). Water used to prepare some types of chocolate milk (either water-based or from a vending machine) or lemonade from syrup (either prepared at home or industrially) is included as well. This was a part of a disaggregation methodology in the FCS2014 applied on the composite dishes in order to report the consumption data to EFSA. Hereby specific recipes were used and when water was one of the main ingredients, it was reported. Consequently, it is taken into account in the exposure assessment. However, if water is considered to be drained during food preparation, it is not included (e.g. water used to prepare pasta, to boil vegetables or potatoes, etc.). Liquid coffee and tea were considered to be ingredients of a drink. Hence, the exposure assessment does not include the water used to prepare liquid coffee or tea. It can also not be included based on the consumption of liquid coffee/tea and PFAS levels in unbottled water, as no PFAS were analysed in coffee powder or tea leaves (which may contain PFAS as well) and because the processing factors for different PFAS are unknown for coffee/tea preparation.

Exposure assessment for PFAS

For the exposure assessment, Σ4PFAS was evaluated assuming equipotency of the 4-EFSA-PFAS following the recommendation of EFSA (EFSA, 2020).

The exposure assessments were performed for the Belgian population aged 3-64 years using the FCS2014 food consumption database. Only respondents with two completed 24-h dietary recalls and available measured body weight were included in the exposure assessments (FCS2014: n=3096; 1529 men and 1567 women). The exposure assessment was performed for three age groups: children (3-9 years), adolescents (10-17 years) and adults (18-64 years).

For each individual in the FCS2014, the exposure to the different PFAS on each interview day was calculated using following equation:

$$Y_i = \sum_{k=1}^{n} \frac{X_{k,i} \cdot C_k}{bw_i}$$

where: Y_i is the daily PFAS intake of a given individual i expressed in ng/kg bw/d; n is the number of food items consumed by individual i, bw_i is the measured body weight of individual i (kg); $X_{k,I}$ is the amount of the food item k consumed on that day (g/d); C_k is the concentration of the concerned PFAS in food item k (ng/g). To calculate the weekly intake, the daily intake was multiplied by 7.

To assess the long-term average intake (habitual or usual intake) from these short-term measurements, the data require statistical modelling in order to take into account between-person and within-person variations. Statistical modelling mitigates the limitation of short-term food consumption data better than averaging over two 24-h recalls per individual.

The habitual intake distributions were estimated by the Statistical Program to Assess Dietary Exposure (SPADE) (Dekkers et al., 2014). SPADE is freely available as an R package called SPADE.RIVM (version 4.1.00). The habitual intake distribution is modelled as a function of age. To ensure representative results for the Belgian population and for the different seasons and interview days (week versus weekend days), weighting factors were used. The habitual intake distribution was weighted for age, sex, province, season and day of the week. Based on the habitual intake distributions, different percentiles of habitual intake could be derived. In this report, the 50th percentile (median intake), the 95th percentile (high level intake) as well as the mean habitual intake are reported for each exposure scenario.

In the lower-bound exposure scenario, the 1-part model for daily intakes was used for PFOA, $PFOS_{tot}$, Σ4PFAS, PFBA, PFPeA, PFHxA, and PFBS. The 2-part model for episodical intakes was used for PFNA, PFHxS, PFHpA, PFDA, PFUnDA, PFDoDA and PFTrDA. In the upper- and middle-bound exposure scenarios, the 1-part model was used for all PFAS.

Uncertainty in the habitual intake distribution was quantified with ready for use bootstrap (n=200) which provided reliable confidence intervals with a 0.05 significance level (Dekkers et al., 2014). All calculations are performed in the Open Source R Studio Software (version 2023.03.0).

Statistics

The Kruskal-Wallis one-way analysis of variance by ranks (Ostertagová et al., 2014) was used for comparing PFAS exposure among different age populations (children, adolescents, adults), different sexes (male, female) and different regions (Flemish region, Walloon, Brussels capital region). If the Kruskal-Wallis statistic was significant, the eta-squared measure (η^2) was computed. The eta-squared estimate assumes values from zero to one, and multiplied by 100% indicates the percentage of variance in PFAS exposure that is explained by the independent variable (Tomczak et al., 2014). The Bonferroni adjustment on the pairwise Wilcoxon rank sum test was selected to perform pairwise comparisons. The Grubbs test was applied to verify whether the highest value in the PFAS exposure datasets could be identified as an outlier (applied when testing regional differences). The calculations were performed using the Open Source R Studio Software (version 2023.03.0). Statistical analyses were performed on the individual, unweighted lower-bound exposure data. The significance level for all tests was set at $\alpha = 0.05$.

RISK ASSESSMENT (WP6)

Introduction

In 2018, EFSA published an opinion on PFOA and PFOS and derived separate TWI for these compounds based on their effects in humans. An increase in serum cholesterol levels (PFOA, PFOS (adults)) and decrease in antibody response at vaccination (PFOS (children)) were the critical effects, but reduced birth weight and increased prevalence of high serum levels of the liver enzyme alanine aminotransferase (ALT) were considered as well in the opinion (EFSA, 2018a). Two years later, the EFSA CONTAM Panel performed a risk assessment for the sum of four PFAS (PFOA, PFNA, PFHxS and PFOS) because of similar effects in animals, toxicokinetics and observed levels in human blood (EFSA, 2020). Furthermore, at the EU level, these four PFAS were the most prominent in serum of adults. The CONTAM Panel concluded that effects on the immune system (decrease in immune response), observed at the lowest serum PFAS levels in both animals and humans, are critical for the risk assessment.

Two critical studies have been considered for the derivation of the Health Based Guidance Value (HBGV). A study with children on the Faroe Islands, which showed various associations between the serum levels of individual PFAS, but also the sum of PFOA, PFNA, PFHxS and PFOS, and antibody titres against diphtheria and tetanus (Budtz-Jørgensen et al., 2018; Grandjean, Heilmann, Weihe, Nielsen, Mogensen, & Budtz-Jørgensen, 2017; Grandjean, Heilmann, Weihe, Nielsen, Mogensen, Timmermann, et al., 2017). A No-Observed-Adverse-Effect Concentration (NOAEC) of 27.0 ng/mL was identified for the sum of these 4-EFSA-PFAS at 5 years of age and the antibody titres against diphtheria at 7 years. A second, more recent, study with children from Germany showed an inverse association between serum levels of PFOA, but also the sum of 4-EFSA-PFAS, and antibody titres against haemophilus influenzae type b (Hib), diphtheria and tetanus in serum sampled from 1-year-old children, predominantly breastfed (Abraham et al., 2020). A lowest benchmark dose ($BMDL_{10}$) of 17.5 ng/mL at the age of 1 year was derived for the sum of 4-EFSA-PFAS, based on the inverse association between serum levels of the sum of these 4-EFSA-PFAS and antibody titres against diphtheria.

This $BMDL_{10}$ of 17.5 ng/mL (critical serum concentration at 1 year of age in breastfed children) was used as a starting point to estimate a corresponding daily intake by mothers. Such intake would result in a serum level of 6.9 ng/mL in the mother at 35 years of age. Using a Physiologically Based Pharmacokinetic model (PBPK model), and assuming 12 months of breastfeeding, it was estimated that the $BMDL_{10}$ in infants corresponds to an intake by the mother of 0.63 ng/kg bw/day for the sum of the 4-EFSA-PFAS. The daily intake of 0.63 ng/kg bw/day was used as the starting point, and a **group TWI** of 7 x 0.63 = **4.4 ng/kg bw/week** for the sum of 4-EFSA-PFAS was established. EFSA thereby relied on the assumption that all four PFAS were equipotent for immunotoxic effects in humans. No additional uncertainty factors were applied because the $BMDL_{10}$ was based on infants, which are expected to be a sensitive population group. Moreover, a decreased vaccination response was considered to be a risk factor for disease rather than a disease. This TWI should prevent that mothers reach a body burden that results in levels in milk that would lead to serum levels in the infant associated with a decrease in vaccination response. As a result, the higher exposure of breastfed infants is taken into account in the derivation of the TWI and the intake of infants should therefore not be compared with this TWI.

The EFSA opinion indicated that this group TWI (**4.4 ng/kg bw/week**) is protective for the other potential critical endpoints (increase in serum cholesterol, reduced birth weight and high serum levels of ALT) considered in the previous opinion on PFOS and PFOA ((EFSA, 2018b, 2020).

Risk evaluation

Based on the available exposure assessment and health-based guidance value, a risk assessment based on the approach applied by EFSA in 2020 was performed. For this risk assessment, it is assumed that an individual consumed the foods and drinks considered in the exposure assessment during their whole life, and that the PFAS were present at the calculated mean concentrations throughout their life.

The risk assessment is performed for the Σ4PFAS by comparing the exposure for the general Belgian population and different age populations with the group TWI (**4.4 ng/kg bw/week**). Exposures below or at the TWI are considered to be without appreciable risk to health. Exposures above the TWI should, however, be interpreted cautiously due to the methodology used to derive the TWI.

Uncertainty analysis

By performing the PFAS exposure and risk assessments, various assumptions were made. Some limitations of the approach were thereby identified, which are described as uncertainties. The characterisation and expression of uncertainties is done qualitatively according to the EFSA guidance documents (EFSA, 2018a, 2018c, 2019). Each source of uncertainty is thereby accompanied by a definition, which refers to the cause of uncertainty (amount and quality of evidence), and is related to the degree of uncertainty. The symbols '+' and '–' are used as a pair of ordinal scales, indicating the direction of uncertainty and the magnitude of the uncertainty. However, the sources of uncertainty related to the risk assessment were only described without indication of the direction or magnitude of the uncertainty, as this kind of information often needs further research.

Results

SAMPLING AND SAMPLE PREPARATION (WP1)

Selection and collection of samples

Based on the scoring system results, the number of samples attributed to each food group is presented in Table 1. It was decided to collect 283 individual items distributed across fourteen main food groups as follows: 45 “meat and meat products” (MEA), 43 “fish and other seafood” (FIS), 35 “grains and grain-based products” (GRA), 38 “vegetables and vegetable products” (VEG), 33 “fruit and fruit products” (FRU), 17 “milk and dairy products” (MIL), 15 “water and water-based beverages” (WAT), 14 “composite dishes” (COM), 13 “alcoholic beverages” (ALC), 10 “food products for young population” (YNG), 9 “eggs and egg products” (EGG), 5 “seasoning, sauces and condiments” (SSC) and 3 “starchy roots and tubers” (STA) and 3 “sugar and similar” (SUG). Other food groups were not selected for the current analysis.

In order to have a representative exposure assessment, at least 3 samples were selected for each chosen subgroup. About 20% of organic products (56 samples) were selected. In total, 74 subgroups represented the possible variety of dietary sources of PFAS. Eggs were assigned to two subgroups (“Whole eggs” and ”Hardened egg products”), and “flowering brassica” represented three subgroups (“Head brassica”, ”Broccoli group” and “Cauliflowers”).

Table 1. Sampling strategy and sampling plan at the main group level

Code group	Code name	Name of the FoodEx Level 1 group	Selection criteria			Total Score	Number of samples	Additional egg-containing product samples	Additional samples
			Risk probability (*risk*)*	Contribution to exposure (*exp*)*	Food group variability (*var*)*				
Z0007	FIS	Fish, seafood, amphibians, reptiles and invertebrates	high (3)	moderate (2)	high (3)	0.83	43	/	/
Z0006	MEA	Meat and meat products	moderate (2)	high (3)	high (3)	0.79	43	/	2
Z0005	FRU	Fruit and fruit products	high (3)	moderate (2)	moderate (2)	0.75	33	/	/
Z0002	VEG	Vegetables and vegetable products	low (1)	high (3)	Very high (4)	0.73	33	/	5
Z0001	GRA	Grains and grain-based products	low (1)	high (3)	Very high (4)	0.73	25	10	/
Z0008	MIL	Milk and dairy products	low (1)	high (3)	moderate (2)	0.58	17	/	/
Z0014	ALC	Alcoholic beverages	low (1)	high (3)	low (1)	0.51	12	1	/
Z0013	WAT	Water and water-based beverages	low (1)	high (3)	low (1)	0.51	15	/	/
Z0009	EGG	Eggs and egg products	high (3)	low (1)	very low (0)	0.50	9	/	/
Z0010	SUG	Sugar and similar, confectionery and water-based sweet desserts	low (1)	moderate (2)	moderate (2)	0.48	0	/	3
Z0019	SSC	Seasoning, sauces and condiments	low (1)	moderate (2)	low (1)	0.41	0	5	/
Z0018	COM	Composite dishes	low (1)	moderate (2)	low (1)	0.41	0	14	/
Z0011	-	Animal and vegetable fats and oils and primary derivatives thereof	low (1)	moderate (2)	low (1)	0.41	0	/	/
Z0015	-	Coffee, cocoa, tea and infusions	very low (0)	high (3)	low (1)	0.38	0	/	/
Z0003	STA	Starchy roots or tubers and products thereof, sugar plants	low (1)	moderate (2)	very low (0)	0.33	0	/	3
Z0004	-	Legumes, nuts, oilseeds and spices	low (1)	low (1)	low (1)	0.31	0	/	/
Z0012	-	Fruit and vegetable juices and nectars (including concentrates)	very low (0)	moderate (2)	low (1)	0.28	0	/	/
Z0016	YNG	Food products for young population	moderate (2)	very low (0)	very low (0)	0.27	0	/	10
Z0017	-	Products for non-standard diets, food imitates and food supplements	very low (0)	low (1)	very low (0)	0.10	0	/	/
Z0020	-	Major isolated ingredients, additives, flavours, baking and processing aids	very low (0)	very low (0)	very low (0)	0.00	0	/	/
Z0021	-	Other ingredients	very low (0)	very low (0)	very low (0)	0.00	0	/	/

* Numbers in brackets represent the numerical value associated with each selection criteria

DEVELOPMENT AND VALIDATION OF THE ANALYTICAL METHODS (WP2)

Method validation

Identification criteria

The chromatography and mass spectrometry identification criteria were assessed in all the validation samples.

Linearity and matrix effect

Analytical standards of PFAS at fourteen concentrations between 0.1 and 200 ng/mL were prepared to assess the working range of the analytical method. The corresponding calibration curves were constructed by plotting the analyte/IS area ratio against the analyte concentration level. Calibration curves were best fitted to a quadratic model, and weighting factor 1/X was applied to minimize back-calculation errors at low concentrations. The curves showed a good correlation for all target compounds within the tested interval, with back-calculated concentrations lower than ± 20%.

Responses in neat solvent and matrix were compared to assess the matrix effect in three matrices (i.e. eggs, liver and chicken meat). The matrix effects were fully compensated (<20%) for all PFAS using the IS listed in Table 1 for these three matrices.

Limits of quantification (LOQ)

For LOQ estimation, the lowest validated level approach was adopted. It corresponds to the level at which identification criteria, trueness and precision are met. Most LOQs were set at 0.015 or 0.05 µg/kg. LOQs were lowest for water (0.001-0.010 µg/L) and fruits and vegetables (0.002-0.005 µg/kg) but higher for eggs and liver (0.1 µg/kg) (Table 2, Table 3). However, the LOQ for HFPO-DA was higher and set at 1 µg/kg for some matrices.

The LOQs required in the EU regulation for the 4-EFSA-PFAS were fulfilled for the regulated matrices/PFAS combinations and were generally 5 times lower than the required level in each food group (Commission Implementing Regulation (EU) 2022/1428, 2022). The LOQ required for food products for the young population, was only met for PFHxS (0.015 µg/kg) and would have to be decreased by a factor of 2 (PFOS, PFOA) or 3 (PFNA) to meet Commission recommendation criteria (Commission Recommendation (EU) 2022/1431, 2022).

In each batch, the blank-level contributions of all PFAS were checked and should be less than 50% of the determined LOQ or LOQ was set accordingly.

IS recoveries

IS recoveries were calculated for each validation batch. Most values were within the acceptable range of 30-140%, and the PFAS/matrix combinations with recovery rates falling outside this range were excluded from the validation or analysed by screening.

Trueness and precision

Most fortified sample recoveries met the 80 to 120% guidance criterion for the 4-EFSA-PFAS and 65 to 135% for the other PFAS, except for PFTrDA and PFUnDS in eggs (Table 3). As good precision was observed, a systematic recovery correction based on validation data was applied to real samples to compensate for these two lowest recoveries.

Good precision was observed for all food matrices analyzed at all levels. RSD_{RW} were $\leq$ 20% and $\leq$ 25% for the 4-EFSA-PFAS and the other PFAS, respectively, except for PFTrDS in animal-origin tissue and PFUnDS in the liver. For these two PFAS/matrix combinations, only an estimated value of the LOQ was possible as the performance criteria were not met.

In addition, due to high RSD_{RW}, out-of-the-range recoveries and/or identification criteria outside the range, three PFAS could not be validated in eggs (PFTeDA, PFDoDS, PFTrDS) and five in liver (PFTrDA, PFTeDA, PFDoDS, PFTrDS and HFPO-DA). The lack of isotopic labelled IS for long-chain PFAS to compensate for losses during extraction and matrix effects could partially explain these results (PFDoDS, PFTrDS, and PFTrDA).

Expanded measurement uncertainty

Expanded measurement uncertainty (MU) was assessed for different matrices during the validation. The obtained values of MU were below 30% for the 4-EFSA-PFAS and most other PFAS. Higher uncertainties ranging from 45 to 60% were obtained for the long-chain sulfonic PFAS (PFNS, PFDS, PFUnDS, PFDoDS) and Minor F53B in animal-origin tissue, eggs, and liver as well as for PFTrDA and DONA in the liver.

Application to EURL interlaboratory study samples

Seven EURL interlaboratory study samples (wheat flour, fish fillet, liquid whole egg, fish meal, pork liver, compound feed and milk powder.) were analyzed as reference material. Z-scores are calculated with a standard deviation for proficiency assessment $\sigma_{p,}$ defined as 20%. All z-scores are acceptable (<|2|) except for PFUnDA in the fish fillet sample (z-score = 2.8, questionable range). Successful analysis of these EURL samples demonstrates that acceptable measurements in real samples could be expected.

Table 2. Method performance by matrix group (water and fruits and vegetables)

Compounds	Water				Fruits and vegetables			
	Accuracy (%) Mean ± RSD			LOQ µg/L	Accuracy (%) Mean ± RSD			LOQ µg/kg ww
	Very low level	Low level	Medium level		Very low levels	Low level	Medium level	
	0.0010 µg/L	0.010 µg/L	0.10 µg/L		0.002, 0.005 µg/kg	0.010 µg/kg	0.05 µg/kg	
PFBA	-	-	-	n.v.	-	-	72 ± 26	0.05
PFPeA	122 ± 5	94 ± 7	97 ± 9	0,0010	82 ± 8	94 ± 6	97 ± 7	0.002
PFHxA	93 ± 9	86 ± 6	91 ± 10	0,0010	119 ± 19	103 ± 9	97 ± 8	0.002
PFHpA	100 ± 12	92 ± 8	98 ± 10	0,0010	97 ± 13	98 ± 7	97 ± 7	0.002
PFOA	96 ± 20	94 ± 8	97 ± 9	0,0010	118 ± 22	105 ± 10	99 ± 8	0.005
PFNA	106 ± 13	93 ± 8	99 ± 9	0,0010	92 ± 11	99 ± 8	98 ± 9	0.002
PFDA	113 ± 7	98 ± 14	99 ± 10	0,0010	95 ± 11	101 ± 8	100 ± 10	0.002
PFUnDA	117 ± 10	91 ± 10	98 ± 9	0,010	72 ± 15	94 ± 11	99 ± 10	0.005
PFDoDA	117 ± 6	94 ± 9	99 ± 10	0,0010	74 ± 22	92 ± 18	96 ± 12	0.005
PFTrDA	116 ± 13	98 ± 19	105 ± 22	0,010	70 ± 56	55 ± 10	64 ± 27	0.005*
PFTeDA	120 ± 12	98 ± 11	101 ± 11	0,0010	87 ± 12	80 ± 10	97 ± 9	0.005
PFBS	113 ± 7	92 ± 7	98 ± 9	0,0010	99 ± 13	99 ± 8	97 ± 7	0.002
PFPeS	120 ± 9	90 ± 7	98 ± 9	0,0010	93 ± 9	102 ± 11	102 ± 9	0.002
PFHxS	118 ± 7	91 ± 9	98 ± 9	0,0010	104 ± 12	101 ± 9	98 ± 8	0.002
PFHpS	123 ± 11	85 ± 8	94 ± 8	0,0010	109 ± 13	102 ± 10	100 ± 7	0.002
PFOS	117 ± 11	90 ± 8	97 ± 9	0,0010	101 ± 15	100 ± 8	100 ± 10	0.002
PFNS	113 ± 13	87 ± 8	98 ± 12	0,0010	85 ± 15	90 ± 13	87 ± 19	0.002
PFDS	117 ± 7	85 ± 5	94 ± 11	0,0010	85 ± 21	78 ± 17	73 ± 33	0.002
PFUnDS	107 ± 17	87 ± 7	96 ± 18	0,0010	61 ± 35	59 ± 16	64 ± 47	0.005*
PFDoDS	99 ± 22	78 ± 13	81 ± 14	0,0010	62 ± 68	42 ± 7	49 ± 46	0.005*
PFTrDS	105 ± 9	76 ± 12	78 ± 16	0,0010	62 ± 68	42 ± 6	47 ± 37	0.005*
Major F53B	107 ± 9	86 ± 11	102 ± 16	0,0010	100 ± 12	96 ± 11	91 ± 12	0.002
Minor F53B	112 ± 11	80 ± 7	94 ± 15	0,0010	70 ± 22	71 ± 17	67 ± 41	0.005
HFPO-DA	-	-	-	n.v.	-	102 ± 12	100 ± 9	0.01
DONA	109 ± 11	94 ± 9	103 ± 10	0,0010	99 ± 14	95 ± 7	97 ± 8	0.002

The analysis were performed at each level for each matrix group in triplicate (n=3). n.v. not validated. *screening. Bold: a recovery correction was applied

Table 3. Method performance by matrix group (animal origin tissues, eggs and liver)

Compounds	Animal origin tissue				Eggs			Liver		
	Accuracy (%) Mean ± RSD			LOQ µg/kg ww	Accuracy (%) Mean ± RSD		LOQ µg/kg ww	Accuracy (%) Mean ± RSD		LOQ µg/kg ww
	Low levels	Medium levels	High levels		Medium levels	High levels		Medium levels	High levels	
	0.015 / 0.025 µg/kg	0.05 / 0.1 / 0.3 µg/kg	0.5 / 1 / 2 µg/kg		0.05 / 0.1 / 0.2 / 0.3 µg/kg	0.5 / 1 µg/kg		0.1 / 0.2 / 0.3 µg/kg	0.5 / 1 µg/kg	
PFBA	-	-	-	n.v.	-	-	n.v.	-	-	n.v.
PFPeA	-	98 ± 4	101 ± 3	0.05	109 ± 5	105 ± 6	0.1	110 ± 9	107 ± 5	0.1
PFHxA	-	102 ± 4	100 ± 5	0.05	111 ± 7	107 ± 6	0.1	108 ± 12	99 ± 5	0.1
PFHpA	95 ± 22	95 ± 5	98 ± 5	0.015	107 ± 6	107 ± 6	0.1	108 ± 8	106 ± 6	0.1
PFOA	111 ± 22	102 ± 9	101 ± 5	0.015	112 ± 7	107 ± 7	0.1	90 ± 22	105 ± 11	0.1
PFNA	113 ± 11	93 ± 17	100 ± 5	0.015	100 ± 13	109 ± 10	0.1	99 ± 14	105 ± 5	0.1
PFDA	-	93 ± 11	102 ± 7	0.05	110 ± 9	107 ± 8	0.1	115 ± 20	100 ± 5	0.1
PFUnDA	99 ± 19	102 ± 9	102 ± 5	0.015	106 ± 6	106 ± 5	0.1	114 ± 10	108 ± 8	0.1
PFDoDA	-	100 ± 3	103 ± 4	0.05	107 ± 7	106 ± 6	0.1	114 ± 13	107 ± 3	0.1
PFTrDA	-	96 ± 8	103 ± 14	0.05	**54 ± 10**	**55 ± 9**	0.1	84 ± 24	85 ± 22	n.v.
PFTeDA	-	91 ± 11	103 ± 12	0.3	-	-	n.v.	-	-	n.v.
PFBS	105 ± 15	102 ± 6	101 ± 4	0.015	108 ± 6	109 ± 6	0.1	100 ± 13	103 ± 7	0.1
PFPeS	89 ± 16	101 ± 4	105 ± 8	0.015	121 ± 6	113 ± 4	0.1	109 ± 6	107 ± 6	0.1
PFHxS	98 ± 15	102 ± 5	100 ± 4	0.015	107 ± 6	107 ± 7	0.1	107 ± 6	106 ± 5	0.1
PFHpS	79 ± 23	97 ± 4	96 ± 6	0.015	99 ± 6	97 ± 9	0.1	103 ± 8	102 ± 5	0.1
PFOS	-**	90 ± 17	99 ± 5	0.015	95 ± 24	105 ± 3	0.1	101 ± 20	103 ± 9	0.1
PFNS	86 ± 27	94 ± 13	94 ± 11	0.015	75 ± 9	78 ± 11	0.1	95 ± 13	101 ± 6	0.1
PFDS	77 ± 30	99 ± 7	86 ± 14	0.015	68 ± 30	59 ± 12	0.1	85 ± 21	100 ± 8	0.1
PFUnDS	-	98 ± 14	85 ± 16	0.05	**35 ± 9**	**38 ± 12**	0.1	-	75 ± 26	0.5*
PFDoDS	-	90 ± 20	78 ± 21	0.05	-	-	n.v.	-	-	n.v.
PFTrDS	-	74 ± 41	69 ± 27	0.05*	-	-	n.v.	-	-	n.v.
Major F53B	90 ± 20	103 ± 13	99 ± 10	0.015	101 ± 6	107 ± 5	0.1	104 ± 9	108 ± 6	0.1
Minor F53B	-	97 ± 22	91 ± 18	0.05	67 ± 8	74 ± 9	0.1	82 ± 22	84 ± 23	0.1
HFPO-DA	-	-	106 ± 8	1	-	112 ± 4	1	-	-	n.v.
DONA	103 ± 12	105 ± 14	99 ± 14	0.015/0.05	89 ± 7	100 ± 9	0.1	97 ± 11	105 ± 23	0.1

The analysis were performed at each level for each matrix group in triplicate (n=3). n.v. not validated. *Estimated value. **No matrix blank was available. Bold: a recovery correction was applied

ANALYSIS OF THE FOOD SAMPLES (WP3)

General overview of the results

PFAS detections

PFOS was the most detected compound found in 19% of the 283 samples, followed by PFOA (17%). Short-chain carboxylic acid compounds PFBA, PFPeA and PFHxA were detected in 11, 10 and 8% of the 283 samples, respectively. Long-chain carboxylic acid compounds PFUnDA, PFNA and PFTrDA were detected in 9, 8 and 6% of the samples, respectively. Eight compounds were never detected (i.e. PFTeDA, PFHpS, PFDS, PFUnDS, PFDoDS, PFTrDS, Minor F53B, HFPO-DA), while other PFAS like PFHpA, PFDA, PFBS, PFDoDA, PFHxS, Major F53B, PFNS, PFPeS, and DONA were detected in 0.4 to 5% of the samples (Figure 2). It should be noted that PFBA was analysed in only 167 samples due to contamination issues. However, it still has a detection rate of 18% among these samples.

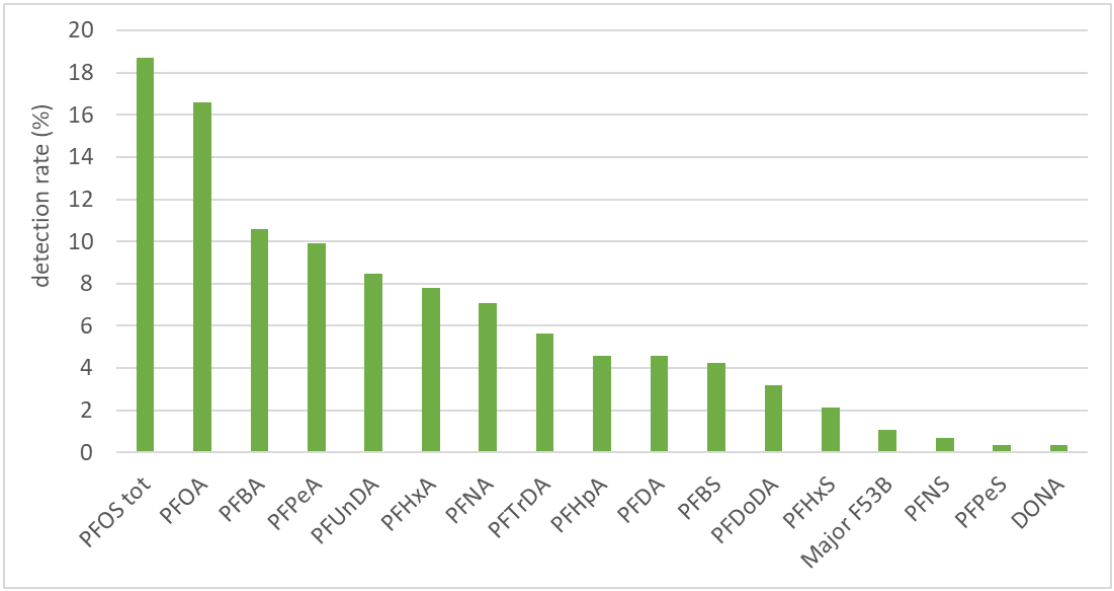

Figure 2. Percentage of detection of the different PFAS in the food samples.

In 123 samples out of 283, at least one PFAS was detected, with an average of 1.1 PFAS per sample. About 23% of the samples contained only one PFAS, 15% contained 2 to 5 PFAS, and less than 5% contained 6 to 11 PFAS (Figure 3).

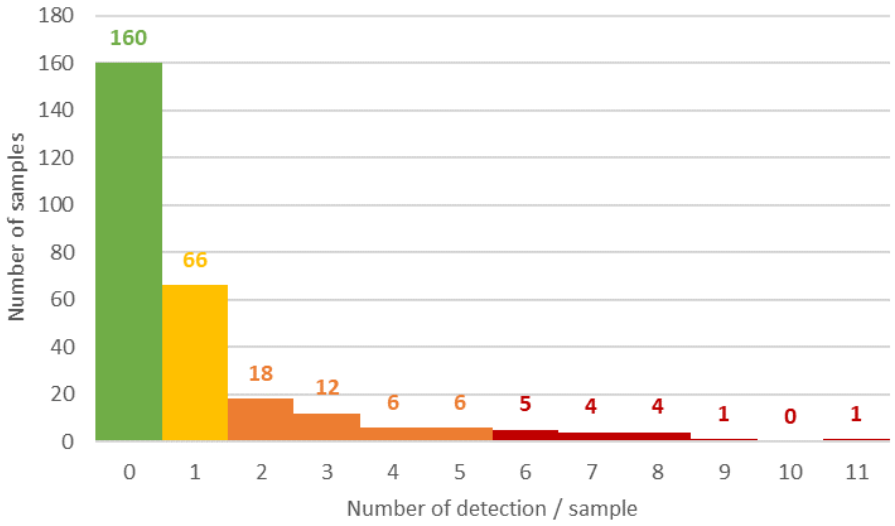

Figure 3: Overview of the number of PFAS detections in the samples.

In 3 food groups, no PFAS were detected, i.e. “eggs and egg products” (EGG, nine samples), “seasoning sauces and condiments” (SSC, five samples) and “sugar and similar, confectionery and water-based sweet desserts” (SUG, three samples), while most PFAS detections were observed in the food groups “fish and other seafood” with at least one PFAS detection in 74% of the samples. Moreover, at least one PFAS was detected in 68% of the samples of the food group “vegetables and vegetable products” (VEG), 57% for the “composite dishes” (COM) and 53% for the “water and water-based beverages” (WAT). An overview is given in Table 4.

Table 4. Percentage of detection of at least one of the 4-EFSA-PFAS and at least one of the 25 analyzed PFAS in each of the groups.

	Number of samples	Detection of at least one of the 4-EFSA-PFAS % of sample	Detection of at least one of the 25 analyzed PFAS % of sample
Fish and seafood (FIS)	43	70	74
Vegetables and vegetable products (VEG)	38	47	68
Water and water-based beverages (WAT)	15	40	53
Composite dishes (COM)	14	29	57
Meat and meat products (MEA)	45	36	38
Milk and dairy products (MIL)	17	24	24
Food products for young population (YNG)	10	20	30
Grains and grain-based products (GRA)	35	17	17
Fruit and fruit products (FRU)	33	6	45
Starchy roots or tubers (STA)	3	0	33
Alcoholic beverages (ALC)	13	0	23
Eggs and egg products (EGG)	9	0	0
Seasoning, sauces and condiments (SSC)	5	0	0
Sugar and similar (SUG)	3	0	0

Furthermore, a distinction could be made between the PFAS covered by the recent EFSA opinion (i.e. 4-EFSA-PFAS) and the 25 PFAS analysed in the study (EFSA, 2020). At least one of the 25 PFAS included in the study was detected in 123 samples, while 88 samples contained at least one of the 4-EFSA-PFAS. In the food groups “alcoholic beverages” (ALC) and “starchy roots and tubers” (STA), only other PFAS than the 4-EFSA-PFAS were detected. The most detected PFAS for these samples were PFBA, PFPeA, PFHxA, PFHpA and PFBS. However, only the 4-EFSA-PFAS were detected in the food groups “grain and grain-based products” (GRA) and “milk and dairy products” (MIL). For the other food groups, the presence of PFAS was always associated with at least one of the 4-EFSA-PFAS.

PFAS concentrations

The results are expressed in µg/kg ww (wet weight), which will be noted as µg/kg for ease of reading. Quantified results below the validated LOQ but within the calibration curve range and meeting all quality criteria will be included when interpreting the results.

In total, 302 PFAS detections occurred in 123 samples, but the concentrations varied widely. The highest concentration (i.e. 2.9 µg/kg) was measured for PFTrDA in a crab sample. In addition, five other detections were higher than 1.0 µg/kg, i.e. 1.1 µg/kg of PFUnDA in a crab sample, 1.7 µg/kg of PFTrDA in crab sample and 1.2 µg/kg of PFOA in another crab sample, 1.8 µg/kg of PFOS in a grey shrimp sample and 2.5 µg/kg of PFBA in a red pepper sample.
Furthermore, 81 detections between 0.1 and 1 µg/kg were measured, while 124 detections ranged from 0.015 to 0.1 µg/kg. Finally, 87 detections were in the range of 0.001 to 0.015 µg/kg. However, it should be noted that the number of detections is also highly influenced by the varying LOQs throughout the different PFAS and matrices.

Since the recent EFSA opinion focussed on the sum of 4 PFAS (i.e. PFOS, PFOA, PFNA, PFHxS) (EFSA, 2020), specific attention was given to the sum of the 4-EFSA-PFAS results. This sum varied from below LOQ to 2.7 µg/kg for a crab sample. Moreover, the sum of 25 PFAS ranged from below LOQ

to 5.36 µg/kg for a crab sample. The relative contribution of the sum of the concentrations of the 4-EFSA-PFAS to the sum of the 25 analysed PFAS ranged from 0 to 100%, with an average of 52%.

Maximum levels (ML) and indicative level exceedances

Commission Regulation (EU) 2023/915 sets out maximum levels (ML) for FIS, MEA and EGG groups (Commission Regulation (EU) 2023/915, 2023). Only one sample of these groups exceeded the maximum level, i.e. a crab sample with a PFOA content of 1.2 µg/kg , while the maximum level is set at 0.7 µg/kg with an MU of 30%. A more detailed discussion will be given in the paragraphs describing the results per food group.

Indicative levels were included in Recommendation (EU) 2022/1431 for other groups. More specifically, an indicative level of 0.010 µg/kg for the individual PFAS was given for FRU, VEG, STA, YNG, and MIL (Commission Recommendation (EU) 2022/1431, 2022). Several exceedances were reported for VEG and FRU. An overview is given in Table 5. For the other groups, no exceedances were noted.

Table 5. Exceedances of the indicative level stated in Recommendation (EU) 2022/1431 for PFOA in VEG and FRU.

Sample	Group	Farming type	Origin	PFOA (µg/kg)	Indicative level exceedance considering MU of 50%
spinach, fresh	spinach-type leaves	organic	IT	0.011	no
green beans, jarred	beans (with pods) and similar-	conv.	n.d.	0.013	no
endives, grown in open ground	witloofs and similar-	conv.	BE	0.014	no
carrot, fresh	carrots and similar-	organic	UE	0.016	no
broccoli	flowering brassica-	organic	NL	0.019	no
red pepper	Solanaceae	conv.	NL	0.026	yes
leek, bulk	leeks and similar-	organic	ES	0.031	yes
spinach, frozen	spinach-type leaves	organic	BE	0.031	yes
white mushrooms	Fungi	organic	BE	0.032	yes
brussels sprouts, cleaned	flowering brassica-	conv.	BE	0.039	yes
green oak-leaf lettuce	lettuces and salad plants	conv.	BE	0.065	yes
strawberries	berries and small fruits	organic	BE	0.084	yes
oyster mushroom	Fungi	organic	BE	0.20	yes

A specific Directive (EU) 2020/2184 for exists for water, with an ML of 0.50 µg/L for “PFAS total” and 0.10 µg/L for the sum of 20 individual PFAS (i.e. PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFHpS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFUnDS, PFDoDS, and PFTrDS) (Directive (EU) 2020/2184, 2020). The quantified concentrations in all samples were at least 3 and 15 times lower than these limits, respectively.

Finally, no maximum or indicative levels exist for the other food groups (e.g. ALC, COM, GRA, MIL other than milk, SSC, SUG and WAT other than water).

Detected PFAS of interest in each food group studied

Within 25 studied PFAS, three main groups can be distinguished, i.e. perfluoroalkyl carboxylic PFAS (PFAC) with 11 compounds, perfluoroalkyl sulfonic PFAS (PFSA) with 10 compounds and perfluoroalkyl substitutes with 4 compounds. The percentage of detection for each PFAS in each food group under investigation is shown in Table 6.

For vegetable-based products (VEG, FRU, GRA, STA, ALC (wine)), only short-chain PFAC ($n \leq 9$), short-chain PFSA ($n = 4$) and sporadically DONA were detected (Table 6). PFAC with a chain length from 4 to 8 carbons occurred the most in these vegetable-based products.

In the water samples, PFAC ($n \leq 8$) were most often present, together with PFBS. The long-chain PFAS were never detected.

Table 6. Detect PFAS of interest in each food group studied (in %).

a/ carboxylic group

Group name (number of sample)	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
Eggs and egg products (9)	na										na
Seasoning and sauces (5)											
Sugar and similar (3)											
Grains and grain-based products (35)					17						
Food products for young population (10)	10	10			20						
Alcoholic beverages (13)	23										
Fruit and fruit products (33)	30	21	9.1	3.0	6.1						
Starchy roots or tubers (3)		33	33								
Milk and dairy products (17)											
Vegetables and vegetable products (38)	32	32	26	11	42	2.6					
Water and water-based beverages (15)	na	53	40	42	50						
Composite dishes (14)	29				7.1		7.1	14		7.1	
Meat and meat products (45)	na		6.7	4.4	6.7	4.4	2.2	2.2	2.2	2.2	
Fish and seafood (43)	na			2.3	26	40	26	49	19	33	

b/ sulfonic group

	PFBS	PFPeS	PFHxS	PFHpS	PFOS tot	PFNS	PFDS	PFUnDS	PFDoDS	PFTrDS
Eggs and egg products (9)									na	na
Seasoning and sauces (5)										
Sugar and similar (3)										
Grains and grain-based products (35)										
Food products for young population (10)										
Alcoholic beverages (13)										
Fruit and fruit products (33)	3.0									
Starchy roots or tubers (3)										
Milk and dairy products (17)					24					
Vegetables and vegetable products (38)	11									
Water and water-based beverages (15)	50	8.3	25		8.3					
Composite dishes (14)					21					
Meat and meat products (45)	2.2		4.4		36					
Fish and seafood (43)			2.3		65	4.7				

c/ substitute group

	Major F53B	Minor F53B	HFPO-DA	DONA
Eggs and egg products (9)				
Seasoning and sauces (5)				
Sugar and similar (3)				
Grains and grain-based products (35)				
Food products for young population (10)				
Alcoholic beverages (13)				
Fruit and fruit products (33)				
Starchy roots or tubers (3)				
Milk and dairy products (17)				
Vegetables and vegetable products (38)				2.6
Water and water-based beverages (15)			na	
Composite dishes (14)				
Meat and meat products (45)				
Fish and seafood (43)	7.0			

The number of samples per food group is given within the brackets, whereas the percentage of detected PFAS of interest in each food group studied is given in the coloured cells, n.a.: not analysed.

For animal-based products (FIS, MEA, COM containing fish or meat), PFAC (n = 6-13 carbons) was detected, and the long-chain PFAC (n ≥ 10) were detected only in these groups. PFOS was mainly detected in these animal-based products, i.e. 65% of the FIS samples, 36% of the MEA samples, 24% of the MIL samples and 21% of the COM samples containing fish or meat. Other PFSA (i.e. PFBS, PFHxS and PFNS) and Major F53B (substitute group) were sporadically detected in these groups.

Organic products

During the sampling, specific attention was given to organic products (i.e. 20% of the samples). The organic samples were included in different groups (FIS, MEA, GRA, VEG, FRU, MIL, ALC, YNG, EGG, SSC, STA and SUG). However, the wide variation in the results within each group, in combination with the limited sample number, hampers the interpretation of the results. No significant difference between organic and conventional products was observed, but this interpretation should be considered cautiously.

Occurrence of PFAS in the different food groups

The concentrations of PFAS are expressed in µg/kg ww and, for ease of reading, are reported here as µg/kg. For liquid (water, soft drinks, alcoholic beverages and milk samples), results are given in µg/L.

Fish and other seafood (FIS)

For the 43 samples of the “fish and seafood group” (FIS), at least one PFAS concentration was above the LOQs in 73% of the samples. The concentrations ranged from <LOQ to 2.85 µg/kg (Table 7, Figure 4). It should be mentioned that PFBA was not analyzed in this food group.

The highest number of compounds detected was 11 PFAS for a cooked crab sample, followed by 10 PFAS in a fish roe (salmon eggs), 9 PFAS in 2 canned crab samples, trout eggs, tarama (containing 19% of fish eggs) and grey shrimps, while 8 PFAS were detected in a sample of langoustine.

The highest detection frequency was observed for PFOS (65%), followed by PFUnDA (49%), PFNA (40%), PFTrDA (33%) and PFOA (26%), whereas no detection was observed for 13 out of 24 analysed PFAS (PFPeA, PFHxA, PFTeDA, PFBS, PFPeS, PFHpS, PFDS, PFUnDS, PFDoDS, PFTrDS, Minor F53B, HFPO-DA, DONA). PFOS was quantified in at least one sample of each subgroup, while PFUnDA was quantified in all subgroups except for processed or preserved seafood.

Among all the samples, the highest concentrations were found in the subgroups of crabs and sea-spiders, fish roe, and shrimps and prawns. The highest quantifiable PFAS levels were measured for PFTrDA and PFUnDA in canned king crab (2.9 µg/kg and 1.1 µg/kg, respectively), PFOA and PFNA in a sample of cooked crab (1.2 µg/kg and 0.9 µg/kg, respectively), PFUnDA in salmon eggs (0.92 µg/kg) and PFOS in grey shrimps (1.8 µg/kg). Among these highest concentrations, only PFOA content of 1.2 µg/kg in a crab sample exceeded the maximum levels set at 0.7 µg/kg.

In the subgroups composed exclusively of marine fish (marine, processed or preserved fish), the PFAS detection frequency was higher than in “diadromous” and “freshwater fish”. This could be related to the higher number of farmed fish in the latter two subgroups (100% and 40%, respectively) compared to the marine fish subgroup (no farmed fish).

Differences in PFAS profiles and concentrations were observed for farm-raised and wild-caught fish. The average number of compounds for the 14 farm-raised fish was 1.07 ± 1.90, while that for the 26 wild-caught fish (this information was missing for 3 fish samples) was 3.42 ± 2.87. The average concentration for farm-raised fish was 0.092 ± 0.21 µg/kg, while that for wild-caught fish amounted to 0.92 ± 1.5 µg/kg.

For farmed fish, no differences were observed between 10 EU-farmed and 4 non-EU farmed fish, and the high detection was owing to trout eggs (7 PFAS) and eel (3 PFAS), while other samples contained

either no PFAS or only PFOS. The fish which were farmed in the EU were mainly salmon and river fish (eel, carp, trout), whereas non-EU farmed fish were pangasius and shrimp.
Among the shrimps and prawns subgroup, the 2 farmed samples contained either no PFAS or only PFUnDA at 0.050 µg/kg, respectively, whereas the 3 wild-caught samples contained between 3 to 9 PFAS at concentrations for the sum of 24 PFAS ranging from 0.169 to 3.2 µg/kg.

Among the wild-caught samples, 12 were from the Atlantic Ocean, 6 from the Pacific Ocean, while for the other 8 samples, the finishing location was either not defined or the fish caught in different locations was mixed or was caught in other locations such as the Indian Ocean, Tanzania Lake and Russia. No notable differences in concentrations, type or profile of PFAS were found between the samples from the Atlantic and the Pacific Oceans. Shrimp, cod and mussels came from the Atlantic Ocean, whereas tuna and pollock were from the Pacific Ocean, and among the 3 crabs, one was caught in the Atlantic and the Pacific Oceans, and another one was from Russia.

Altogether, a significant variation in detection frequency was observed in some subgroups where the number of samples was limited (3 to 7 samples). However, these results could help target specific subgroups (fish roe, crabs and sea-spiders, shrimps and prawns, and diadromous fish), for which more samples might be needed to draw a sound conclusion.

“Fish and seafood” appears to be the most studied food group with regard to PFAS presence, as a considerable number of literature reports from different sampling years are available. These studies indicated generally higher concentrations and more diverse patterns of detected PFAS compared to other food groups. The FLUOREX results were also in line with these statements.

Although the EFSA Opinion (2020) based its evaluations on publications from about a decade ago or older, it is still important to mention that for PFOS, PFOA and a number of other PFAS, “fish and other seafood” was found to be the most important contributor to the mean LB exposure (EFSA, 2020). The highest mean PFOS levels were reported for carp, eel, roach, perch, bream, barbel and sardine. Regarding FLUOREX results, the highest concentration in fish and seafood was found not for PFOS but for PFTrDA.

Table 7. Percentage of detection and descriptive statistics for PFAS concentrations (µg/kg) for FIS group at subgroup.

		Freshwater fish (n=5)		Diadromous fish (n=7)		Marine fish (n=5)		Fish roe (n=3)		Crabs (n=3)		Shrimps and prawns (n=5)		Mussels (n=3)		Squids, cuttlefishes, octopuses (n=3)		Processed or preserved fish (inc processed offal) (n=6)		Processed or preserved seafood (n=3)	
PFAS	LOQ	Min **Max** *mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *mean)*	Det %	Min **Max** *mean)*	Det %	Min **Max** *(mean)*	Det %
PFBA	n.v.	-	n.a	-	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.
PFPeA	0.05	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFHxA	0.05	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFHpA	0.015	-	0	-	0	-	0	-	0	<LOQ **0.071** *(0.02)*	33	-	0	-	0	-	0	-	0	-	0
PFOA	0.015	-	0	-	0	-	0	<LOQ **0.067** *(0.022)*	33	0.14 **1.2** *(0.53)*	100	<LOQ **0.18** *(0.047)*	40	<LOQ **0.063** *(0.030)*	67	-	0	<LOQ **0.021** *(0.0062)*	33	<LOQ **0.017** *(0.0057)*	33
PFNA	0.015	-	0	-	0	<LOQ **0.054** *(0.016)*	40	<LOQ **0.56** *(0.20)*	67	0.077 **0.90** *(0.42)*	100	<LOQ **0.28** *(0.12)*	60	<LOQ **0.056** *(0.028)*	67	<LOQ **0.032** *(0.011)*	33	<LOQ **0.076** *(0.028)*	67	-	0
PFDA	0.05	-	0	-	0	<LOQ **0.047** *(0.013)*	40	<LOQ **0.20** *(0.085)*	67	0.12 **0.22** *(0.17)*	100	<LOQ **0.44** *(0.11)*	40	<LOQ **0.049** *(0.016)*	33	-	0	<LOQ **0.038** *(0.0063)*	17	-	0
PFUnDA	0.015	<LOQ **0.065** *(0.013)*	20	<LOQ **0.11** *(0.015)*	14	<LOQ **0.15** *(0.087)*	80	<LOQ **0.92** *(0.34)*	67	0.29 **1.1** *(0.77)*	100	<LOQ **0.42** *(0.15)*	60	<LOQ **0.062** *(0.026)*	67	<LOQ **0.049** *(0.016)*	33	<LOQ **0.21** *(0.079)*	67	-	0
PFDoDA	0.05	-	0	-	0	-	0	<LOQ **0.12** *(0.050)*	67	0.060 **0.21** *(0.15)*	100	<LOQ **0.17** *(0.059)*	40	-	0	-	0	<LOQ **0.041** *(0.0069)*	17	-	0
PFTrDA	0.05	-	0	<LOQ **0.083** *(0.012)*	14	<LOQ **0.30** *(0.060)*	20	0.048 **0.26** *(0.13)*	100	0.25 **2.9** *(1.6)*	100	<LOQ **0.72** *(0.17)*	40	<LOQ **0.061** *(0.020)*	33	-	0	<LOQ **0.11** *(0.018)*	17	<LOQ **0.12** *(0.055)*	67
PFTeDA	0.3	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFBS	0.015	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFPeS	0.015	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFHxS	0.015	-	0	-	0	-	0	-	0	<LOQ **0.15** *(0.049)*	33	-	0	-	0	-	0	-	0	-	0
PFHpS	0.015	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
Tot-PFOS	0.015	<LOQ **0.19** *(0.055)*	60	<LOQ **0.17** *(0.031)*	43	0.016 **0.19** *(0.090)*	100	0.033 **0.47** *(0.30)*	100	0.51 **0.71** *(0.59)*	100	<LOQ **1.8** *(0.38)*	60	0.049 **0.21** *0.13*	100	<LOQ **0.050** *(0.017)*	33	<LOQ **0.26** *(0.059)*	33	<LOQ **0.14** *(0.076)*	67
L-PFOS	0.015	<LOQ **0.18** *(0.052)*	60	<LOQ **0.16** *(0.030)*	43	0.016 **0.18** *(0.086)*	100	0.029 **0.45** *(0.28)*	100	0.47 **0.71** *(0.57)*	100	<LOQ **1.7** *(0.36)*	60	0.049 **0.20** *(0.13)*	100	<LOQ **0.050** *(0.017)*	33	<LOQ **0.23** *(0.054)*	33	<LOQ **0.14** *(0.076)*	67
B-PFOS	0.015	-	0	-	0	-	0	<LOQ **0.033** *(0.018)*	67	<LOQ **0.038** *(0.013)*	33	<LOQ **0.075** *(0.015)*	20	-	0	-	0	<LOQ **0.022** *(0.0037)*	17	-	0
PFNS	0.015	-	0	-	0	-	0	<LOQ **0.033** *(0.011)*	33	-	0	-	0	-	0	-	0	<LOQ **0.026** *(0.0043)*	17	-	0
PFDS	0.015	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFUnDS	0.05	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFDoDS	0.05	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFTrDS*	0.05	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
Major F53B	0.015	-	0	-	0	-	0	<LOQ **0.018** *(0.006)*	33	<LOQ **0.060** *(0.036)*	67	-	0	-	0	-	0	-	0	-	0
Minor F53B	0.05	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
HFPO-DA	1	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
DONA	0.05	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0

Det% = detection frequency. n.v. not validated. n.a. not analysed. *Estimated value. Mean is calculated on LB approach: results for measurements that lead to the conclusion that the analyte content is below LOQ are replaced by 0.

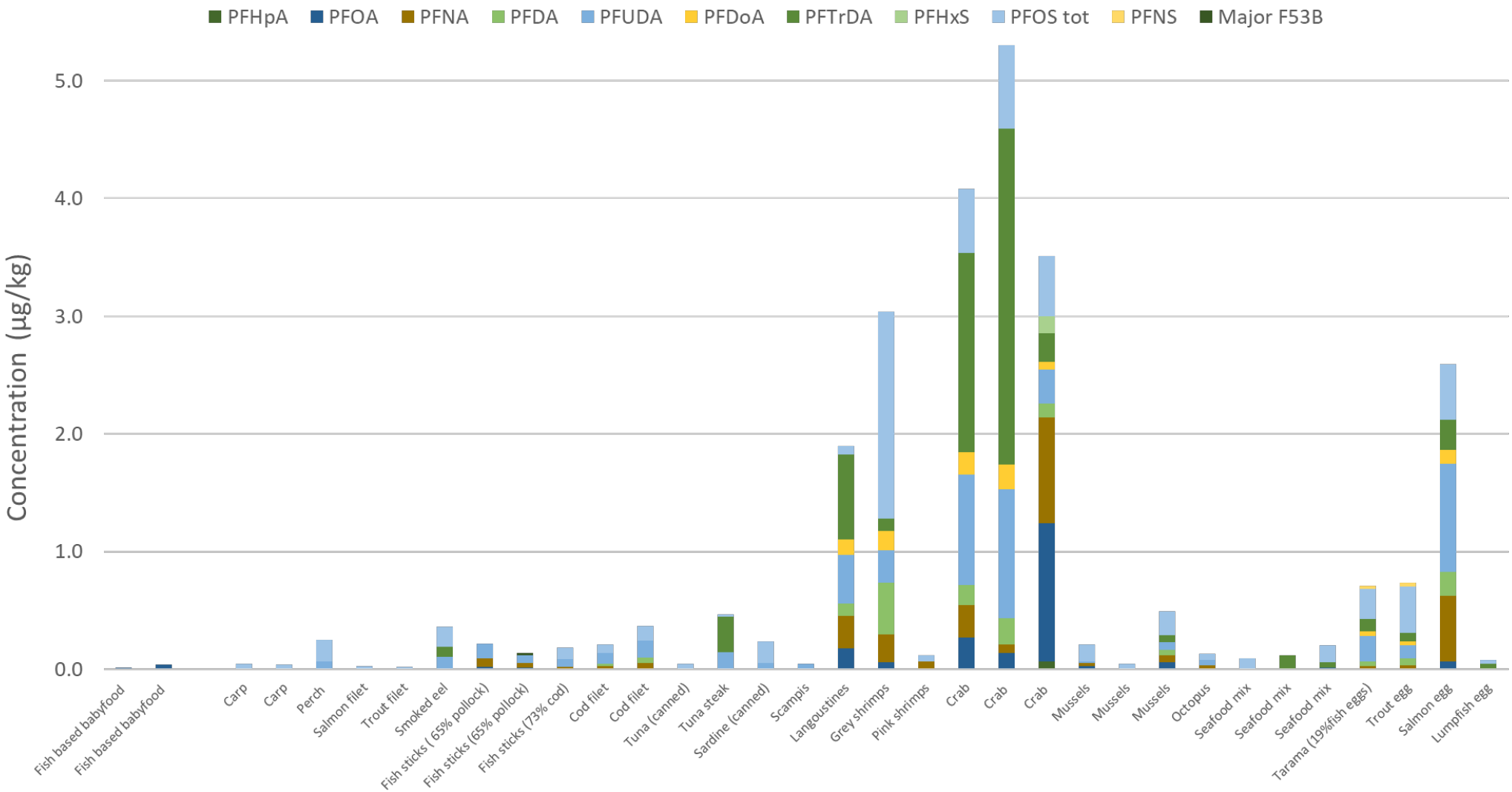

Figure 4. Individual results for the 32 FIS and 2 fish-based YNG samples with PFAS quantified values

Meat and meat products (MEA)

Among the 45 samples in the MEA group, PFOS was the most detected compound in 36% of the samples (16 out of 45). An overview of the results is given in Table 8 and Figure 5. No PFAS were found in the three subgroups: “bird fresh meat”, “raw cured (or seasoned) meat”, and “cooked cured (or seasoned) meat”. In the four subgroups of “mammals liver”, “fresh raw sausages”, “preserved or partly preserved sausages”, and “offals (other than liver-like)”, only PFOS was detected, except for a black pudding pork offal, in which PFHxA was detected close to the LOQ.

In the subgroups “mammals meat” and “liver-based spreadable texture specialities”, more PFAS were detected, and more particularly in only three samples (out of 18), i.e. a wild boar-based pâté and a pork pâté in the group “liver-based spreadable-textured specialities liver” and a stew of wild pork in the group “mammal meat”. The stew of wild pork contained 11 PFAS (i.e. PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFHxS and PFOS) was the sample with the highest number of PFAS in this project, while the wild boar-based pâté contained 6 PFAS (i.e. PFHxA, PFHpA, PFOA, PFNA, PFHxS and PFOS) and was in the top 5% of the most contaminated samples. The pork pâté contained only PFOA and PFOS.

The highest concentration of PFOS was detected in a veal liver (0.20 µg/kg) and a “liver-based spreadable-textured speciality”, i.e. liver-based pork pâté (0.20 µg/kg). Within the subgroup “liver-based spreadable-textures specialities”, PFOS was detected in 78% of the samples (i.e. 7 out of 9 samples), while the liver content in these samples varied between 14 and 38% (with two samples with an unspecified liver content). Interestingly, no PFAS were detected in the two foie gras samples.

According to Regulation (EU) 2023/915, a maximum level (ML) of 5.0 µg/kg was set for PFOS in the meat of game animals, and an ML of 0.3 µg/kg for the meat of bovine animals, pigs and poultry. Except for the detection of 0.13 µg/kg for the wild boar stew, all the other detections for the mammal meat samples were below 0.05 µg/kg and thus well below the authorized ML. Moreover, the content PFOA, PFNA, PFHxS and the sum of 4-EFSA-PFAS concentrations of the wild boar stew were all below the ML of Regulation (EU) 2023/915, i.e. 0.14 µg/kg (ML PFOA = 3.5 µg/kg), 0.042 µg/kg (ML PFNA = 1.5 µg/kg), 0.043 µg/kg (ML PFHxS = 0.6 µg/kg) and 0.36 µg/kg (ML 4-EFSA-PFAS = 9.0 µg/kg). The Regulation (EU) 2023/915 sets the ML for PFOS at 6.0 and 50 µg/kg and for the 4-EFSA-PFAS at 8.0 and 50 µg/kg for offal of bovine animals, pig and poultry, and for offal of games animals, respectively. All the detections are well below this level, with a maximum for PFOS and for the 4-EFSA-PFAS of 0.20 µg/kg for the veal liver.

According to the most recent EFSA opinion, the highest concentrations for meat samples were reported for PFOS, PFOA, PFDA and PFNA in the group of “edible offal from game animals” with lower bound mean concentrations of 214 µg/kg, 5.5 µg/kg, 5.8 µg/kg and 10 µg/kg, respectively ((EFSA, 2020). However, only three samples were included in FLUOREX in the subgroup “offals (other than liver-like)”, and only PFHxA and PFOS were found at much lower concentrations. For example, the mean concentration for PFOS was 0.010 µg/kg in the FLUOREX samples. However, when comparing the results for PFOS in the sub-group “liver-based spreadable-textured specialities” to the group of pastes, pâtés and terrines in the recent EFSA opinion (EFSA, 2020), it was noticed that this value was 0 in the EFSA opinion. At the same time, PFOS was found in 78% of the samples (i.e. 7 out of 9) with a mean lower bound concentration of 0.056 µg/kg. Overall, it is very challenging to compare the results of FLUOREX with the EFSA opinion since the groups are defined differently.

Compared to PERFOOD (Bervoets et al., 2012), the results follow the same trend, meaning that there was no detection of PFAS in bird fresh meat (chicken) and the highest concentration was found for PFOS in liver and pâté with a mean concentration of 2.6 µg/kg, although the high mean concentration originated from a very contaminated pâté. If this sample is discarded, the mean concentration for PFOS was 0.23 µg/kg, which is still a factor of 4 higher than the results of FLUOREX.

Table 8. Percentage of detection and descriptive statistics for PFAS concentrations (µg/kg) for MEA group at subgroup level

		Mammals meat (n=9)		Bird fresh meat (n=6)		Mammals liver (n=1)		Offals (other than liver)-like (n=3)		Raw cured (or seasoned) meat (n=6)		Cooked cured (or seasoned) meat (n=3)		Fresh raw sausages (n=3)		Preserved or partly preserved sausages (n=5)		Liver-based spreadable-textured specialities (n=9)	
PFAS	LOQ	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %
PFBA	n.v.	-	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.
PFPeA	0.05	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFHxA	0.015	<LOQ **0.031** *(0.0034)*	11	-	0	-	0	<LOQ **0.020** *(0.0067)*	33	-	0	-	0	-	0	-	0	<LOQ **0.021** *(0.0023)*	11
PFHpA	0.015	<LOQ **0.15** *(0.016)*	11	-	0	-	0	-	0	-	0	-	0	-	0	-	0	<LOQ **0.056** *(0.0062)*	11
PFOA	0.015	<LOQ **0.14** *(0.016)*	11	-	0	-	0	-	0	-	0	-	0	-	0	-	0	<LOQ **0.073** *(0.010)*	22
PFNA	0.015	<LOQ **0.042** *(0.0047)*	11	-	0	-	0	-	0	-	0	-	0	-	0	-	0	<LOQ **0.018** *(0.0020)*	11
PFDA	0.05	<LOQ **0.022** *(0.0024)*	11	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFUnDA	0.015	<LOQ **0.057** *(0.0063)*	11	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFDoDA	0.05	<LOQ **0.049** *(0.0054)*	11	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFTrDA	0.05	<LOQ **0.11** *(0.012)*	11	-	0	-	n.a.	-	0	-	0	-	0	-	0	-	0	-	0
PFTeDA	0.3	-	0	-	0	-	n.a.	-	0	-	0	-	0	-	0	-	0	-	0
PFBS	0.015	<LOQ **0.019** *(0.0021)*	11	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFPeS	0.015	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFHxS	0.015	<LOQ **0.043** *(0.0048)*	11	-	0	-	0	-	0	-	0	-	0	-	0	-	*.0*	<LOQ **0.053** *(0.0059)*	11
PFHpS	0.015	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
Tot-PFOS	0.015	<LOQ **0.13** *(0.024)*	56	-	0	0.20 **0.20** *(0.20)*	100	<LOQ **0.031** *(0.010)*	33	-	0	-	0	<LOQ **0.027** *(0.0090)*	33	<LOQ **0.045** *(0.0090)*	20	<LOQ **0.20** *(0.056)*	78
L-PFOS	0.015	<LOQ **0.12** *(0.021)*	56	-	0	0.16 **0.16** *(0.16)*	100	<LOQ **0.022** *(0.0073)*	33	-	0	-	0	<LOQ **0.019** *(0.0063)*	33	<LOQ **0.038** *(0.0076)*	20	<LOQ **0.17** *(0.045)*	78
B-PFOS	n.a.	-	n.a.	-	n.a.	n.a.	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.	-	n.a.
PFNS	0.015	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFDS	0.015	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFUnDS	0.05	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFDoDS	0.05	-	0	-	0	-.	n.a.	-	0	-	0	-	0	-	0	-	0	-	0
PFTrDS*	0.05	-	0	-	0	-	n.a.	-	0	-	0	-	0	-	0	-	0	-	0
Major F53B	0.015	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
Minor F53B	0.05	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
HFPO-DA	1	-	0	-	0	-	n.a.	-	0	-	0	-	0	-	0	-	0	-	0
DONA	0.015	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0

Det% = detection frequency. n.v. not validated. n.a. not analysed. *Estimated value. Mean is calculated on LB approach: results for measurements that lead to the conclusion that the analyte content is below LOQ are replaced by 0.

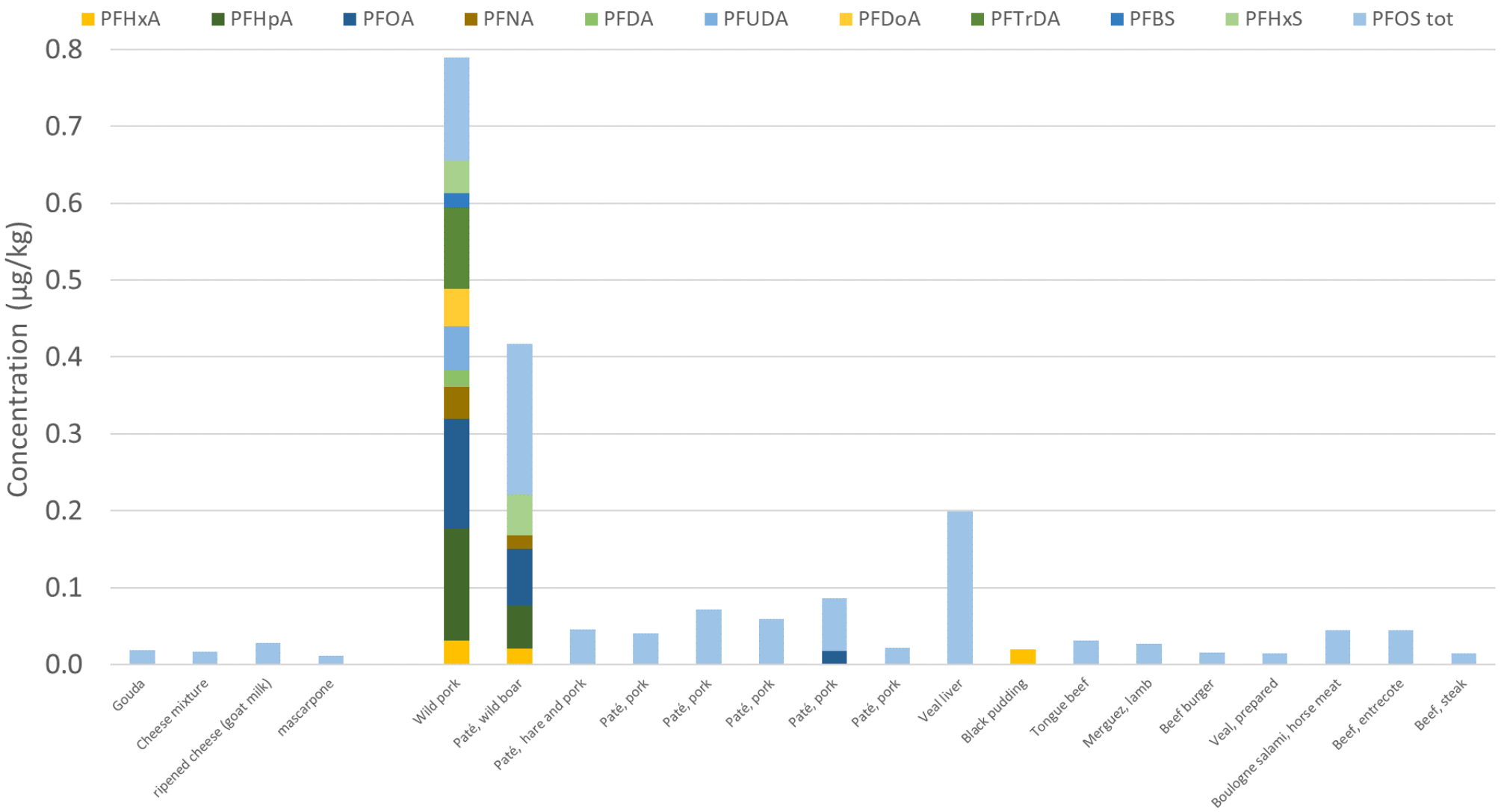

Figure 5. Individual results for the 4 MIL and 17 MEA samples with PFAS quantified values

Milk and dairy products (MIL)

Seventeen samples of “milk and dairy products” were collected for the project. Among the 25 analyzed PFAS, only PFOS was detected (Figure 5). The level of PFOS in a mascarpone sample (uncured cheese based on cow milk) was 0.012 µg/kg, while the average level in 2 samples of ripened cheese based on cow milk was 0.018 µg/kg. The highest value of PFOS (0.028 µg/kg) was measured in a ripened cheese-based” on goat milk. Milk and yoghurt were free from PFAS. The PFAS levels detected in “milk and dairy samples of the FLUOREX project were generally lower than those reported in most of the other studies.

Vegetables and vegetable products (VEG) and Starchy roots and tubers (STA)

In FLUOREX, 38 samples of “vegetables and vegetable products” (VEG) and 3 samples of starchy roots and tubers (STA) were collected for analysis. An overview of the detected PFAS and the descriptive statistics are given in Table 9. Among the 41 samples analyzed, 66% (27 samples) contained at least one PFAS above the LOQ level.

The most frequently detected PFAS were PFOA (39%), PFPeA (32%), PFBA (29%) and PFHxA (27%). Besides that, the occurrence data also showed single detections of DONA (0.0041 µg/kg in bulk leek), PFOS (0.0027 µg/kg in celeriac) and PFNA (0.0024 µg/kg in dried true morels), and the presence of PFBS in 4 samples (average conc. 0.044 µg/kg) and PFHpA also in 4 samples (average conc. 0.009 µg/kg). The highest concentrations found for the sum of 25 PFAS were 2.7 µg/kg in red pepper, 1.6 µg/kg in fresh spinach and 0.53 µg/kg in broccoli. The highest concentrations for a single PFAS were measured for PFBA (2.5 µg/kg) in red pepper, 0.75 µg/kg for PFHxA in fresh spinach and 0.52 µg/kg for PFBA in broccoli. The highest number of PFAS present in a sample at the same time was 6 PFAS in fresh spinach. Samples of red pepper and another fresh spinach had 5 PFAS, while samples of dried true morels, jarred green beans and bulk leek contained 4 PFAS in a single sample. All collected samples of “flowering brassica” (5 samples) and “spinach-type leaves” (3 samples) contained at least one PFAS.

For “starchy roots and tubers”, one out of two potato samples analyzed without the peel contained PFPeA (0.0059 µg/kg) and PFHxA (0.0033 µg/kg) at concentrations close to the LOQ of the method. No PFAS were detected in the baby potato (Charlotte) sample analyzed with the peel.

The average detection frequency in 15 organic products (1.87 ± 1.88) was not significantly different from that in 26 conventional products (1.36 ± 1.55). However, the results should be interpreted with caution since the number of samples in each subgroup was different, and other parameters, such as origin, should be taken into account.

Comparing the detected PFAS values to the indicative limits of the Commission Recommendation (EU) 2022/1431, it can be observed that the concentrations of PFOA in 12 out of 38 VEG samples were above the indicative level, including 7 detections still exceeding this level with MU of 50% taken into account Table 5. Such maximum PFOA concentration (0.20 µg/kg) was measured in oyster mushrooms and exceeded the indicative level of 0.010 µg/kg for vegetables by 20 times. Other PFAS mentioned in this Recommendation, namely PFOS, PFNA and PFHxS, were either not detected or were below the indicative limit.

Table 9. Percentage of detection and descriptive statistics for PFAS concentrations (µg/kg) for the groups VEG and STA at subgroup level

		Lettuces and salad plants (n=3)		Spinach-type leaves (n=3)		Celeries and similar (n=3)		Fungi (n=6)		Flowering brassica (n=5)		Beans (with pods) and similar (n=3)		Witloofs and similar (n=3)		Leeks and similar (n=3)		Solanacea (n=3)		Carrots and similar (n=3)		Onions and similar (n=3)		Potatoes and similar (n=3)	
PFAS	LOQ	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *mean)*	Det %	Min **Max** *mean)*	Det %	Min **Max** *(mean)*	%Det	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	%Det
PFBA	0.05	<LOQ **0.063** *0.021*	33	<LOQ **0.22** *0.13*	67	<LOQ **0.11** *0.069*	67	<LOQ **0.053** *0.0088*	17	<LOQ **0.52** *0.11*	40	<LOQ **0.11** *0.04*	33	-	0	<LOQ **0.068** *0.023*	33	<LOQ **2.5** *0.85*	67	-	0	-	0	-	0
PFPeA	0.002	<LOQ **0.0028** *0.00093*	33	<LOQ **0.47** *0.16*	67	-	0	<LOQ **0.0036** *0.00093*	33	<LOQ **0.014** *0.0059*	80	<LOQ **0.0058** *0.0019*	33	-	0	-	0	<LOQ **0.18** *0.062*	67	-	0	-	0	<LOQ **0.059** *0.0020*	33
PFHxA	0.002	-	0	<LOQ **0.75** *0.25*	67	-	0	<LOQ **0.010** *0.0021*	33	<LOQ **0.0083** *0.0021*	40	<LOQ **0.0050** *0.0017*	33	-	0	<LOQ **0.0024** *0.00080*	33	<LOQ **0.019** *0.0070*	67	-	0	-	0	<LOQ **0.0033** *0.0011*	33
PFHpA	0.002	-	0	<LOQ **0.025** *0.010*	67	-	0	<LOQ **0.0020** *0.00033*	17	-	0	<LOQ **0.0046** *0.0015*	33	-	0	-	0	-	0	-	0	-	0	-	0
PFOA	0.005	<LOQ **0.065** *0.022*	33	<LOQ **0.031** *0.014*	67	-	0	<LOQ **0.196** *0.039*	50	<LOQ **0.039** *0.012*	40	<LOQ **0.013** *0.0043*	33	<LOQ **0.014** *0.0077*	67	<LOQ **0.031** *0.010*	33	<LOQ **0.026** *0.011*	67	<LOQ **0.016** *0.0053*	33	<LOQ **0.056** *0.0019*	33	-	0
PFNA	0.002	-	0	-	0	-	0	<LOQ **0.0024** *0.00040*	17	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFDA	0.002	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFUnDA	0.005	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFDoDA	0.005	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFTrDA	0.005	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFTeDA	0.005	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFBS	0.002	-	0	<LOQ **0.092** *0.043*	67	<LOQ **0.023** *0.0077*	33	-	0	-	0	-	0	-	0	-	0	<LOQ **0.021** *0.0070*	33	-	0	-	0	-	0
PFPeS	0.002	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFHxS	0.002	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFHpS	0.002	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
Tot-PFOS	0.002	-	0	-	0	<LOQ **0.0027** *0.00090*	33	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
L-PFOS	0.002	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
B-PFOS	0.002	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFNS	0.002	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFDS	0.005	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFUnDS	0.005	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFDoDS	0.005	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
PFTrDS	0.005	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
Major F53B	0.002	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
Minor F53B	0.005	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
HFPO-DA	0.01	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0
DONA	0.002	-	0	-	0	-	0	-	0	-	0	-	0	-	0	<LOQ **0.0041** *0.0014*	33	-	0	-	0	-	0	-	0

Det% = detection frequency. Mean is calculated on LB approach: results for measurements that lead to the conclusion that the analyte content is below LOQ are replaced by 0.

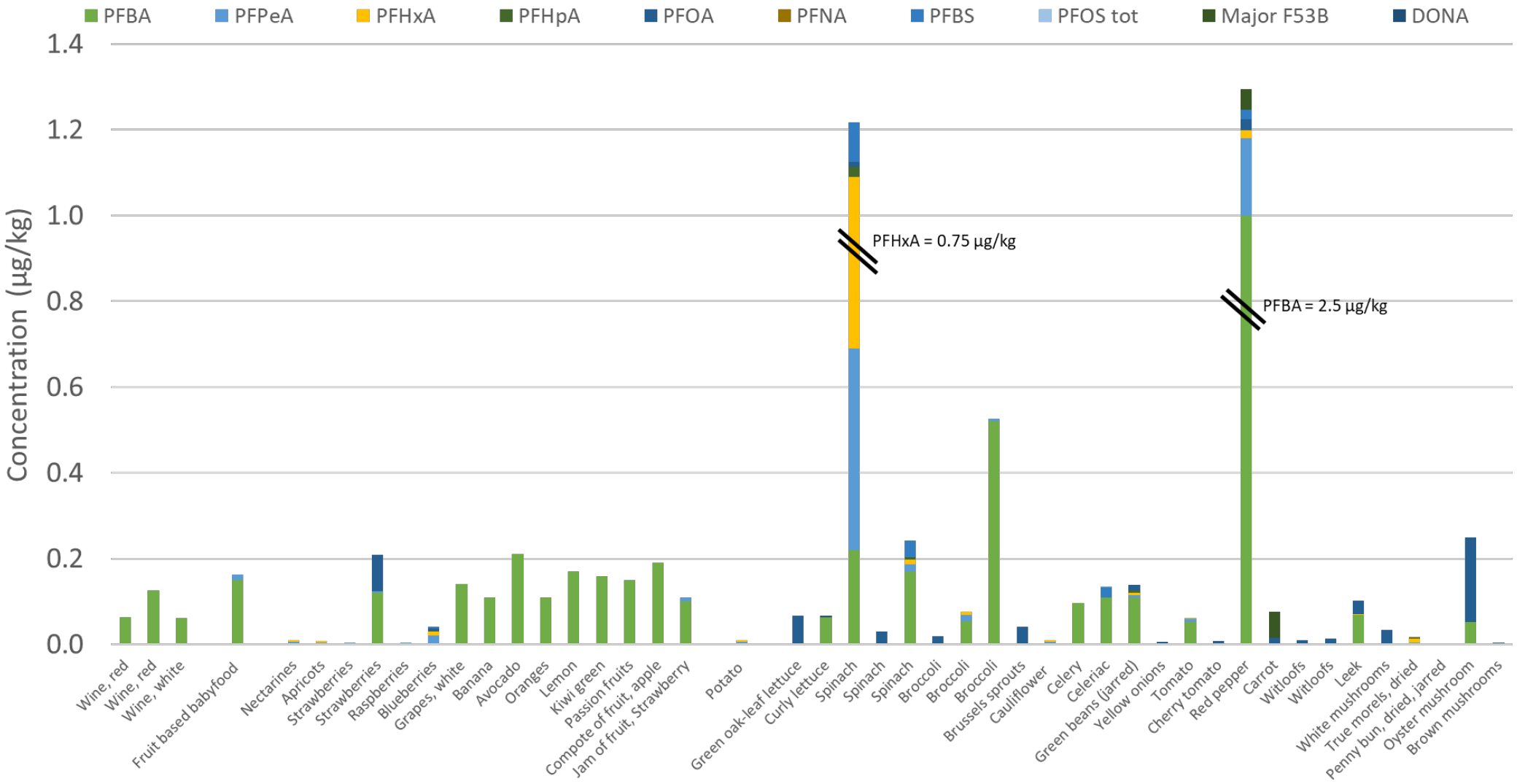

Figure 6. Individual results for the 3 ALC, 1 fruit-based YNG, 15 FRU, 1STA and 26 VEG samples with PFAS quantified values

Alcoholic beverage (ALC)

For the FLUOREX project, 13 samples of alcoholic beverages were purchased. Among the analyzed PFAS, only PFBA was found at quantifiable levels (Figure 6). PFBA was detected in 3 out of 4 wine samples at an average level of 0.063 µg/L and a maximum concentration of 0.127 µg/L in red wine. No PFAS were detected in beer, unsweetened spirits and liqueur (advocaat with 26% of eggs).

According to the EFSA opinion of 2020, no PFOS and PFNA were detected in alcoholic beverages, while PFOA and PFHxS were found in 1 out of 6 samples at average concentrations of 0.010 µg/L and 0.006 µg/L, respectively (EFSA, 2020). PERFOOD project reported that alcoholic drinks contained PFBA, PFHxA and PFHxS. Similarly to the FLUOREX results, only PFBA was found in wine. In general, the PFAS concentrations were low, between 0.023 µg/L and 0.055 µg/L (Bervoets et al., 2012). It was speculated that, due to the observed pattern, the contamination possibly originated from the production process rather than from the raw materials of these alcoholic beverages.

Fruits and fruit products (FRU)

In 33 “fruits and fruit products”, only the short-chain PFAS were found (n ≤ 8) (Table 10, Figure 6). The frequency of detection among all the samples was the highest for PFBA (30%, 10 out of 33 samples), while other PFAS were detected at lower frequencies, namely 21% for PFPeA, 9% for PFHxA, 6% for PFOA, 3% for PFHpA and 3% for PFBS. The concentration range in the positive samples varied from 0.022 µg/kg (for PFHpA in blueberries) to 0.21 µg/kg (for PFBA in avocado). In the subgroup of “pome fruits”, no PFAS were detected. Regarding the other subgroups, PFBA was present in at least 2 samples of each of the 6 subgroups. In the subgroups “miscellaneous fruits with inedible peel, small”, “miscellaneous fruits with inedible peel, large”, and “citrus fruits”, only PFBA was detected. Only PFBA and PFPeA were present in the subgroup “fruit/vegetable spreads and similar, while “stone fruits” contained only PFPeA and PFHxA.

“Berries and small fruits” was the subgroup with the highest number of positive samples (83%, 5 out of 6 samples), and this was also the only subgroup containing PFBS. Among the subgroup “berries and small fruits”, but also among the group “fruit and fruits products”, blueberries contained the highest number of PFAS, namely PFPeA, PFHxA, PFHpA, PFOA and PFBS. Such a pattern of contamination of “berries and small fruits” could be attributed to the volatility of PFCA, pointing towards contamination of products with PFAS via the air rather than the soil, and the surface contact of berries is higher compared to other fruits. Similarly, other studies only on vegetables suggested that higher PFAS concentrations in plants than expected from soil could be come from airborne PFAS and atmospheric deposition, mainly on plant leaves (Liu et al., 2019; Seo et al., 2019).

Among the PFAS mentioned in the Commission Recommendation (EU) 2022/1431 for fruits, in FLUOREX, only PFOA was detected in 2 samples, and the concentration of one (strawberries, 0.084 µg/kg) exceeded the indicative level of 0.010 µg/kg.

Table 10. Percentage of detection and descriptive statistics for PFAS concentrations (µg/kg) for FRU group at subgroup level.

		Pome fruits (n=6)		Berries and small fruits (n=6)		Miscellaneous fruits with inedible peel, large (n=5)		Miscellaneous fruits with inedible peel, Small (n=4)		Stone fruits (n=5)		Citrus fruits (n=3)		Fruit/vegetable spreads and similar (n=4)	
PFAS	LOQ	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %
PFBA	0.10	-	0	<LOQ **0.14** *0.043*	33	<LOQ **0.21** *0.06*	40	<LOQ **0.16** *0.078*	50	-	0	<LOQ **0.17** *0.093*	67	<LOQ **0.19** *0.073*	50
PFPeA	0.002	-	0	<LOQ **0.020** *0.0056*	67	-	0	-	0	<LOQ **0.0058** *0.0019*	40		0	<LOQ **0.010** *0.0025*	25
PFHxA	0.002	-	0	<LOQ **0.010** *0.0016*	17	-	0	-	0	<LOQ **0.0047** *0.0016*	40		0		0
PFHpA	0.002	-	0	<LOQ **0.0022** *0.00037*	17	-	0	-	0	-	0		0		0
PFOA	0.005	-	0	<LOQ **0.084** *0.015*	33	-	0	-	0	-	0		0		0
PFNA	0.002	-	0	-	0	-	0	-	0	-	0		0		0
PFDA	0.002	-	0	-	0	-	0	-	0	-	0		0		0
PFUnDA	0.005	-	0	-	0	-	0	-	0	-	0		0		0
PFDoDA	0.005	-	0	-	0	-	0	-	0	-	0		0		0
PFTrDA	0.005	-	0	-	0	-	0	-	0	-	0		0		0
PFTeDA	0.005	-	0	-	0	-	0	-	0	-	0		0		0
PFBS	0.002	-	0	<LOQ **0.0041** *0.00068*	17	-	0	-	0	-	0		0		0
PFPeS	0.002	-	0	-	0	-	0	-	0	-	0		0		0
PFHxS	0.002	-	0	-	0	-	0	-	0	-	0		0		0
PFHpS	0.002	-	0	-	0	-	0	-	0	-	0		0		0
Tot-PFOS	0.002	-	0	-	0	-	0	-	0	-	0		0		0
L-PFOS	0.002	-	0	-	0	-	0	-	0	-	0		0		0
B-PFOS	0.002	-	0	-	0	-	0	-	0	-	0		0		0
PFNS	0.002	-	0	-	0	-	0	-	0	-	0		0		0
PFDS	0.005	-	0	-	0	-	0	-	0	-	0		0		0
PFUnDS	0.005	-	0	-	0	-	0	-	0	-	0		0		0
PFDoDS	0.005	-	0	-	0	-	0	-	0	-	0		0		0
PFTrDS	0.005	-	0	-	0	-	0	-	0	-	0		0		0
Major F53B	0.002	-	0	-	0	-	0	-	0	-	0		0		0
Minor F53B	0.005	-	0	-	0	-	0	-	0	-	0		0		0
HFPO-DA	0.01	-	0	-	0	-	0	-	0	-	0		0		0
DONA	0.002	-	0	-	0	-	0	-	0	-	0		0		0

Det% = detection frequency. Mean is calculated on LB approach: results for measurements that lead to the conclusion that the analyte content is below LOQ are replaced by 0.

Grains and grain-based products (GRA)

A total of 35 samples of “grains and grain products” were collected for the project. Among the 25 analyzed PFAS, only PFOA was detected. The 6 contaminated samples were 2 biscuits (cookies with chocolate taste, speculoos), 2 breakfast cereals (extruded grains with chocolate powder (wheat flour), rice crisps), 1 cereal grain (white long-grain rice) and 1 leavened bread (soft bagels) (Figure 7). The measured concentrations ranged from 0.017 µg/kg to 0.137 µg/kg. The highest concentration was found in a sample of speculoos biscuits. It is important to mention that the bagel sample having a level of PFOA (0.017 µg/kg) just above the method’s LOQ had eggs listed as one of the ingredients.

Although the number of reports on the occurrence of PFAS in food has increased in recent years, data on PFAS in “grains and grain-based products” remains scarce. Low concentrations of PFAS in grains and grain products were previously reported in a similar project PERFOOD (2007-2012). In that study, only 3 PFAS, namely PFOA, L-PFOS and PFTeDA, were detected in some samples of biscuits, cakes and pastry, and the range of concentrations was from 0.017 µg/kg to 0.050 µg/kg. PFOA was found at low concentrations (<0.070 µg/kg) in most samples of grains, flours and bread. PFDoDA was detected in bread and spaghetti.

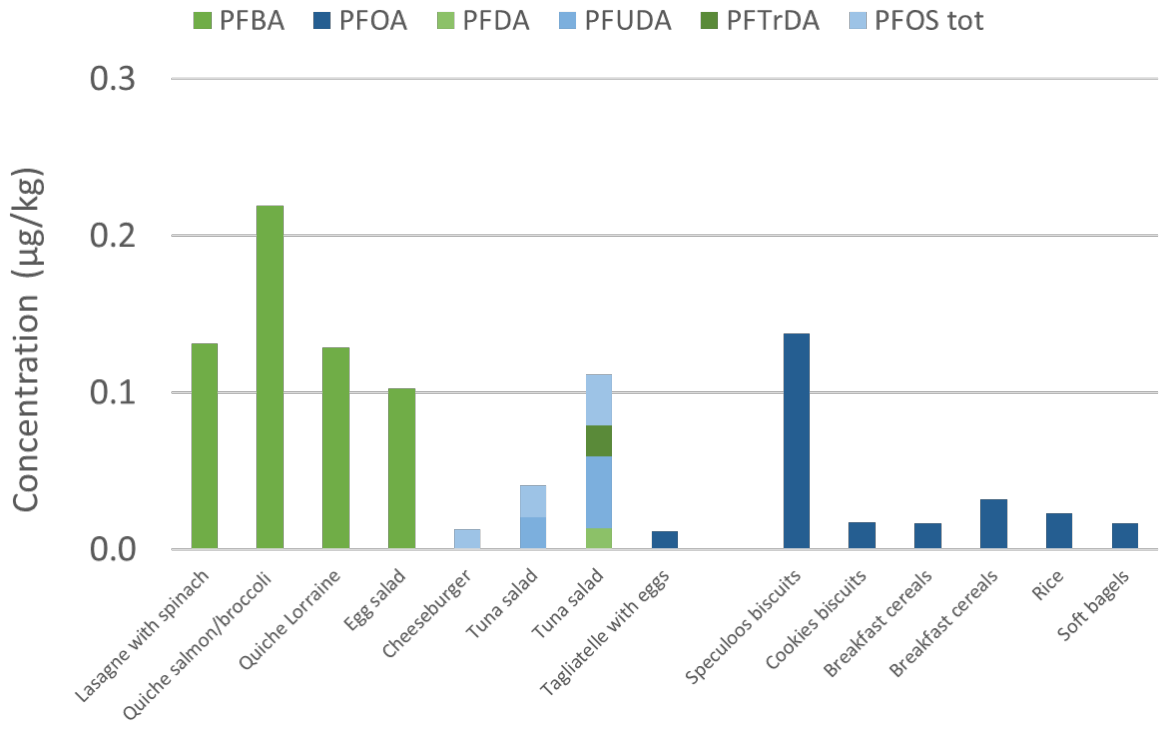

Figure 7. Individual results for the 8 COM and 6 GRA samples with PFAS quantified values

Composite dishes (COM)

In 14 food products collected in the group of “composite dishes”, 8 samples had detectable levels of PFAS (Table 10, Figure 7). The detected PFAS were PFBA, PFOA, PFDA, PFUnDA, PFTrDA and PFOS, and the range of concentrations was from below the LOQ (0.012 µg/kg) for PFOA in tagliatelle (contains 20% of fresh eggs) to 0.219 µg/kg for PFBA in a quiche containing 7% of salmon and 12.4% of eggs (Table 11). Generally, among the detected PFAS, PFBA had the highest frequency of occurrence (29%) and the highest levels. Besides quiche with salmon, PBFA was also detected in quiche Lorraine (12.4% of eggs), egg salad (containing 70% of eggs), and lasagne with spinach and pork meat (containing 2.5% of eggs). Notably, all the detections of long-chain carboxylic acid-PFAS (PFDA, PFUnDA and PFTrDA) were observed in one or both salads, with 41% and 44% of tuna. These 2 tuna salads also contained PFOS at the levels of 0.020 µg/kg and 0.032 µg/kg.

Occurrence data for PFAS in composite dishes is very limited. EFSA report of 2012 mentioned that there was only one quantified result, 0.01 µg/kg of PFOA, in a prepared salad sample (EFSA, 2012).

Water and water-based beverages (WAT)

In total, 12 water samples were selected based on their location, container material, and type of water (i.e. sparkling or tap water). Six commercial bottles of water from different brands were collected: four from geographically distant Belgian sources, one from France and one from Italy. In addition, six tap water samples were collected: two from each of the Belgian regions (i.e. Brussels, Flanders and Wallonia).

At least 1 PFAS was detected in 7 out of 12 water samples. Only sulfonic or carboxylic molecules with short carbon chains (≤ 8 carbons) were quantified. No PFAS with chains longer than eight carbons nor PFAS substitutes were quantified (Table 11, Figure 8). The most frequently found PFAS were PFPeA, followed by PFOA, PFBS, PFHxA and PFHpA. The individual detected concentrations ranged from 0.0010 µg/L to a maximum of 0.0058 µg/L.

Among the selected samples, PFAS detections were more frequent in tap (83%) than in bottled water samples (33%). All but one of the detections in bottled water (PFPeA, 0.0012 µg/L) came from the single glass container bottle without knowing whether the container could contribute to this result. No noticeable difference was observed between carbonated and still waters. When comparing the results to the occurrence date used in the most recent EFSA opinion (EFSA, 2020), PFNA was not detected in the FLUOREX samples, while it was detected in 99% of the samples considered by EFSA, while the results were comparable for the other PFAS (i.e. PFOA, PFOS and PFHxS).

No noticeable difference was observed in the presence of a filter or a softener. Next, a very small test was conducted to evaluate the potential impact of using a filter or a softener. After passing a water sample through a Brita filter or after carbonation by a Sodastream machine, the PFAS sum content (0.018 µg/L) decreased to 0.0065 and 0.014 µg/L respectively. This single test should be taken cautiously, and the measurement uncertainty (50%) should be considered before misinterpreting these results.
The Directive (EU) 2020/2184 sets a concentration limit of 0.50 µg/L for “PFAS total” and 0.10 µg/L for the sum of 20 individual PFAS (i.e. PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFHpS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFUnDS, PFDoDS, and PFTrDS) (Directive (EU) 2020/2184, 2020). The quantified concentrations in all samples are at least 3 and 15 times lower than these limits, respectively. Among the six tap water samples, the sum of 20 PFAS ranged from 0.013 to 0.029 µg/L, except for one sample, which showed no quantifiable results. However, it should be noted that the drinking water directive should be applied to drinking water before entering the house, but the samples were taken inside the house. Therefore, the results should be interpreted with caution.

Not only were water samples analysed in this group, but also, a limited number of soft drinks were included. It is important to note that the LOQ for soft drinks is about eight times higher than that of water samples. In 1 sample (out of 3), PFPeA (0.011 µg/L) and PFHxA (0.0090 µg/L) were found. To our knowledge, this was the first time soft drinks were analysed for PFAS.

Table 11. Percentage of detection and descriptive statistics for PFAS concentrations for COM (µg/kg) and WAT (µg/L) groups at subgroup level

		Salads (n=5)		Pasta and rice (or other cereal-based) dishes (n=2)		Pasta and similar (n=1)		Dishes excl. pasta or rice dishes, pizza, sandwiches (n=3)		Sandwiches, pizza and other stuffed bread-like cereal products (n=3)			Bottled water (n=6)		Unbottled water (n=6)			Soft drinks (n=3)	
PFAS	LOQ COM	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	LOQ Water	Min **Max** *(mean)*	Det %	Min **Max** *(mean)*	Det %	LOQ Soft drinks	Min **Max** *(mean)*	Det %
PFBA	0.1	<LOQ **0.10** *0.021*	20	<LOQ **0.13** *0.065*	50	-	0	-	0	<LOQ **0.22** *0.12*	67	n.v.	-	n.a.	-	n.a.	0.05	-	0
PFPeA	0.015	-	0	-	0	-	0	-	0	-	0	0.001	<LOQ **0.012** *0.00038*	33	<LOQ **0.0054** *0.0030*	83	0.008	<LOQ **0.011** *0.0037*	33
PFHxA	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	<LOQ **0.0058** *0.0037*	83	0.008	<LOQ **0.0090** *0.0030*	33
PFHpA	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	<LOQ **0.0035** *0.0017*	83	0.008	-	0
PFOA	0.015	-	0	-	0	0.012 **0.012** *0.0039*	100	-	0	-	0	0.001	<LOQ **0.0023** *0.00038*	17	<LOQ **0.0045** *0.0024*	83	0.008	-	0
PFNA	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
PFDA	0.015	<LOQ **0.013** *0.0027*	20	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
PFUnDA	0.015	<LOQ **0.046** *0.013*	40	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
PFDoDA	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
PFTrDA	0.015	<LOQ **0.020** *0.0039*	20	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
PFTeDA	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
PFBS	0.015	-	0	-	0	-	0	-	0	-	0	0.001	<LOQ **0.0018** *0.00030*	17	<LOQ **0.0056** *0.0024*	83	0.008	-	0
PFPeS	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	<LOQ **0.0010** *0.00017*	17	0.008	-	0
PFHxS	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	<LOQ **0.0054** *0.0016*	50	0.008	-	0
PFHpS	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
Tot-PFOS	0.015	<LOQ **0.032** *0.010*	40	-	0	-	0	<LOQ **0.013** *0.0043*	33	-	0	0.001	-	0	<LOQ **0.0019** *0.00032*	17	0.008	-	0
L-PFOS	0.015	<LOQ **0.020** *0.0039*	20	-	0	-	0	-	0	-	0	0.001	-	0	<LOQ **0.0011** *0.00018*	17	0.008	-	0
B-PFOS	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	n.a.	-	n.a.	0.008	-	0
PFNS	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
PFDS	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
PFUnDS	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
PFDoDS	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
PFTrDS	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
Major F53B	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
Minor F53B	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
HFPO-DA	1.000	-	0	-	0	-	0	-	0	-	0	n.v.	-	n.a.	-	n.a.	0.05	-	0
DONA	0.015	-	0	-	0	-	0	-	0	-	0	0.001	-	0	-	0	0.008	-	0
Sum of 20 PFAS												0.037	<LOQ **0.0052** *0.0011*	33	<LOQ **0.029** *0.015*	83			

Det% = detection frequency. Mean is calculated on LB approach: results for measurements below LOQ are replaced by 0. The sum of 20 PFAS refers to the Directive (EU) No. 2020/2184.

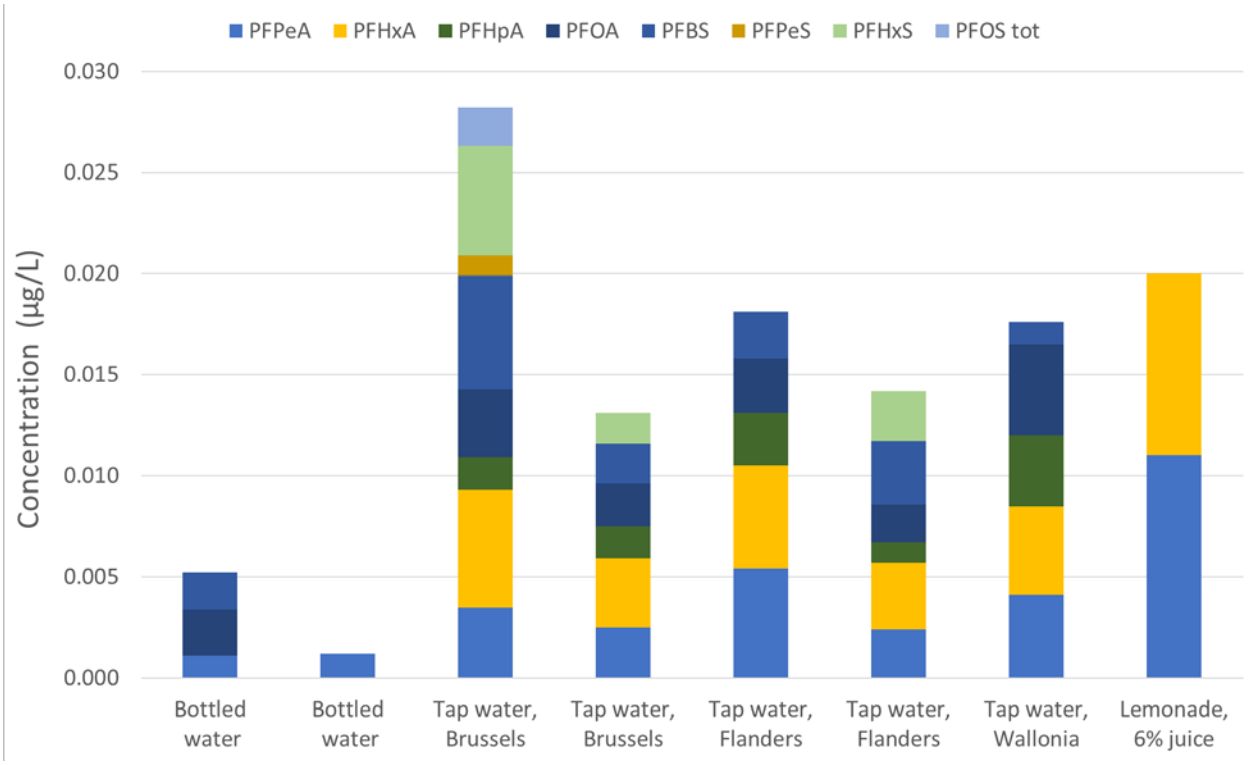

Figure 8. Individual results for the 8 WAT samples with PFAS quantified values

Food products for young population (YNG)

Ten samples of "food products for young population" (YNG) showed measurable levels of PFBA (0.150 µg/kg) and PFPeA (0.013 µg/kg) in 1 fruit-based meal, and PFOA (0.018 µg/kg in a fish-based meal with 9.5% of herring hake and 0.044 µg/kg in a fish-based meal with 8% of trout) (Figure 4 and Figure 6). Other samples from this food group, including meat-based meals with up to 9% of meat, were free from PFAS. As the detected levels of PFOA did not exceed the indicative level of 0.050 µg/kg specified in the Commission Recommendation (EU) 2022/1431, no further investigation of the causes of the contamination should be carried out (Commission Recommendation (EU) 2022/1431, 2022).

Eggs and egg products (EGG)

Nine samples of commercial eggs were collected, of which seven samples originated from supermarkets in Belgium, while the remaining two samples (i.e. bottled egg yolk) were from Benelux. None of the egg samples contained PFAS above the LOQ level, regardless of the type of farming (organic, conventional) or the type of production (free-range, free-cage). However, the short-chain supply representing local food production nor own-grown food from private gardens was considered during sampling. Previous data also indicated no significant differences between organic and conventional eggs (Chiumiento et al., 2023).

Although in this project, no PFAS were measurable in eggs, according to the EFSA Opinion (EFSA, 2020), the group of "Eggs and egg products" contributed the most, among a number of other foods, to the PFOS and PFOA exposure via the diet. Generally, PFOS in the egg is distributed mainly in the egg yolk, while no PFOS is detected in the egg white (Wang et al., 2008). In the FLUOREX project, the whole egg (the yolk combined with the egg white) was analyzed.

The focus of the FLUOREX project was on commercial eggs as the most common channel of egg distribution to consumers. However, it is worth mentioning that PFAS levels in home-produced eggs can be much higher.

Among the 30 egg-containing products coming from groups other than EGG, there was no PFAS detected in 5 SSC and 1 ALC samples. In addition, only 1 out of 10 GRA samples contained PFOA and 12 out of 14 COM samples contained at least 1 PFAS. More detailed information is provided directly in the group section.

Seasoning, sauces and condiments (SSC)

The group of "seasonings, sauces, and condiments" was comprised of 5 "savoury sauces", more specifically, 3 products of mayonnaise, 1 béarnaise, and 1 cocktail sauce. All the samples contained eggs in their composition. No PFAS were detected in these foodstuffs.

A previous report presented the same findings for savoury sauces as in the FLUOREX project (EFSA, 2020). In the PERFOOD project, PFOA was detected in the different sauces analyzed, but not in mayonnaise. L-PFOS was found in béchamel sauce, mushroom sauce and mayonnaise. For the latter product, the measured concentration was 0.009 µg/kg.

Sugar and similar, confectionery and water-based sweet desserts (SUG)

For the "sugar and confectionary" group, 3 samples were purchased; these were chocolate and hazelnut spreads of different brands. No PFAS were detected.

Other analytical information

PFOS total – branched / linear proportion

Due to its production process, PFOS typically occur as a mixture of linear (L-PFOS) and branched (br-PFOS) isomers. Regulation (EU) 2023/915 (Commission Regulation (EU) 2023/915, 2023) and recommendation (EU) 2022/1431 (Commission Recommendation (EU) 2022/1431, 2022) state that the quantification of PFOS (total-PFOS) should include both L-PFOS and br-PFOS. However, the quantification of br-PFOS is not straightforward due to the lack of standards and the problematic separation of the isomers by chromatography (Figure 9). In this study, br-PFOS is quantified with the linear standard (native and ILIS), which is the most pragmatic approach and in accordance with the EURL guidance document (EURL for halogenated POPs in feed and food, 2022).

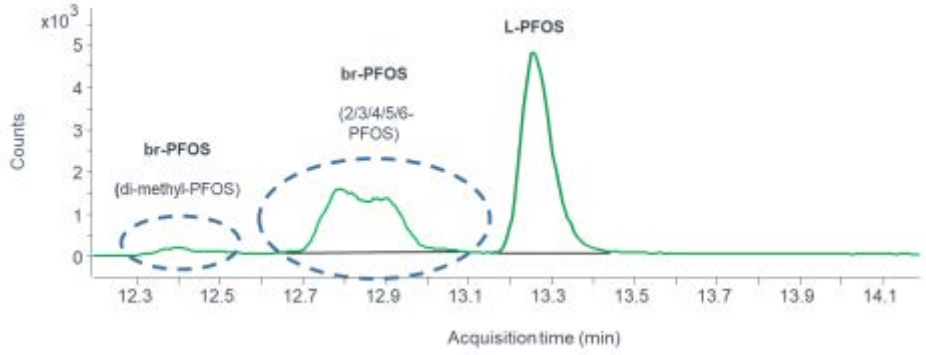

Figure 9. Typical spectra of the extracted-ion chromatogram (mass transition m/z 499 ➔ 80) of linear PFOS (L-PFOS) and its branched isomers (br-PFOS) of a technical PFOS standard (by the Guidance Document on Analytical Parameters for PFAS in Food and Feed, (EURL for halogenated POPs in feed and food, 2022).

Although total PFOS was reported in 53 FIS, MEA, MIL, COM, VEG and WAT samples, only 48 samples from 4 groups (FIS, MEA, MIL and WAT) contained br-PFOS. The mean percentage of L-PFOS varied from 58% in the WAT group to 95% in the FIS group, with an RSD of less than 8% in all groups (Table 12), meaning that the mean percentage of branched PFOS varied from 5% in FIS to 42% in WAT. The

mean percentage of L-PFOS in MEA is 79%. The two highest percentages of L-PFOS are found in products containing wild mammals (89% in wild pork and 86% in liver-based pâté). In the MIL group, samples with a part of br-PFOS belong to the ripened cheese sub-group with an average percentage of L-PFOS content of 71%.

Table 12. Descriptive statistics for the percentage of L-PFOS (linear PFOS) in total-PFOS in different food groups (FIS, MEA, MIL and WAT).

Food group	L-PFOS in total PFOS	
	mean ± RSD (%)	min - max (%)
FIS (n = 28)	95 ± 5	83 - 100
MEA (n = 16)	79 ± 8	70 - 89
MIL (n= 3)*	71 ± 6	67 - 76
WAT (n = 1)	58 ± 0	58 - 58

* only ripened cheese in the MIL group

IMPACT OF THE USE OF FCM (WP4)

Sampling

A total of 28 FCM were purchased on the Belgian market, including 2 cake moulds, 2 pans, 3 woks, 6 muffin cups, 5 sandwich/bread papers,3 baking papers, 1 dough hook, 1 roasting bag, 2 chicken bags, 1 baking foil and 2 popcorn bags. Different brand qualities were chosen for each item for pans, cake moulds, and woks.

Analytical results

In the manufacturing of FCM, PFAS can be used to provide a non-sticky surface and resistance to water, oil, and fat, granting them protection against various types of food. Given their harmful health effects, examining the potential migration of these PFAS was essential. The results are shown in Figure 10.

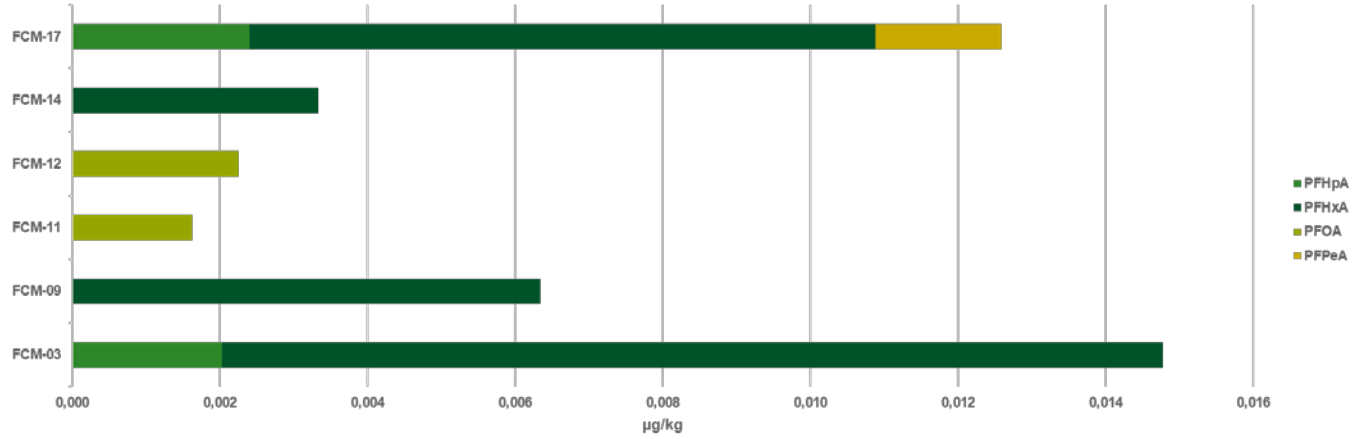

Figure 10. PFAS concentration in FCM samples (only samples with a detection are represented)

Out of the 12 PFAS that were tested, PFPeA, PFHxA, PFHpA, and PFOA were found in six samples that were made exclusively of paper and board materials (such as sandwich papers, muffin cups, air fryer papers, and chicken bags). However, the concentrations of these chemicals were quite low, with the highest concentration (0.013 µg/kg) found in a sandwich paper (FCM-03) for PFHxA. PFPeA was found in only one sample (sandwich paper) at 0.002 µg/kg, while PFHxA was present in four samples (chicken bag, sandwich bag, 2 sandwich papers) with concentrations ranging from 0.003 µg/kg (sandwich bag) up to 0.013 µg/kg (sandwich paper). Furthermore, PFHpA was also found in two samples (sandwich papers) at 0.002 µg/kg, and PFOA was detected in two samples (muffin cup and airfrier paper) at 0.002 µg/kg. Interestingly, no migration of PFAS was highlighted in cake moulds, pans, or wok with a non-stick coating made of PTFE, regardless of the quality of the article.

Finally, based on the concentration found in this current study, it could be concluded that intentional use of these chemicals is unlikely and that the contamination is most likely due to environmental background contamination.

Risk assessment related to FCM

Among the 4-EFSA-PFAS, only PFOA was detected in the FCM samples. Since no specific harmonized EU legislation exists for FCM made of paper and board, a risk assessment was performed using the RACE Tool of EFSA (Fürst et al., 2019). In this study, the potential risks were assessed for children, adolescents and adults using consumption hypotheses formulated based on the assumed frequency of use according to personal opinions gathered from various colleagues of Sciensano. Next, the results were comapred with the TWI of 4.4 ng/kg bw/week for ∑4PFAS, determined by EFSA (EFSA, 2019). Among those PFAS, PFOA exclusively was detected in two samples. The exposure determined per sample was then compared to this TWI. None of the samples were found to exert potential risk for the consumers, no matter the considering population (Table 13).

Table 13. Risk level for PFAS according to FCM risk scenario

	PFAS scenario		
Samples	**Children**	**Adolescents**	**Adults**
FCM-11 (Airfrier paper)	no risk	no risk	no risk
FCM-12 (Muffin cup)	no risk	no risk	no risk

DIETARY EXPOSURE ASSESSMENT (WP5)

Exposure of the Belgian population to PFAS (WP5)

Figure 11 shows the mean and 95th percentile habitual exposure estimates for the different PFAS and summed PFAS for Belgian children, adolescents and adults for the mean lower-bound exposure scenario. As the LB exposure scenario is likely to be more realistic than the UB exposure scenario, only results of the LB exposure scenario are further discussed.

For each PFAS, the exposure for children was higher than that for adolescents and adults. The ranking of the PFAS according to the level of exposure was similar for the three age groups and for the total population, irrespective of whether it is based on mean, median or 95th percentile exposure. The exposure estimates were highest for PFBA (mean 5.1 ng/kg bw/week for the total population), followed by PFPeA, PFOA, PFHxA and PFOS, which showed about 10-fold lower estimates (mean 0.36-0.54 ng/kg bw/week for the total population). The exposure estimates for PFUnDA, PFBS, PFTrDA and PFHpA were about 30-fold lower than those for PFBA, with mean values ranging from 0.14 to 0.20 ng/kg bw/week for the total population. Lowest exposure estimates were obtained for PFHxS, PFNA, PFDA and PFDoDA, with mean values ranging from 0.04 to 0.08 ng/kg bw/week for the total population, which is about 80-fold lower than the estimates for PFBA. The mean MB exposure estimates for PFOS and PFOA, respectively 0.97 and 1.03 ng/kg bw/week for adults, were considerably lower than the assessment of Cornelis et al. (Cornelis et al., 2012). In 2012, the average MB dietary intake of PFOS for adults equalled 24 ng/kg bw/day (which corresponds to a weekly intake of 168 ng/kg bw/week, and the dietary PFOA intake was estimated at 6.1 ng/kg bw/day (equalling 43 ng/kg bw/week). Within the PERFOOD project (Bervoets et al., 2012), which dates from around the same period as the Cornelis study, the MB dietary intake of PFOS and PFOA was estimated at 80 and 33 ng/day. Recalculated on a weekly basis and for a 70-kg person, this corresponds to a dietary intake of 8.0 ng/kg bw/week for PFOS and 3.3 ng/kg bw/week for PFOA. Despite the discrepancy between both previous studies, it can be concluded that the PFOS exposure substantially (8-fold and 170-fold compared to the PERFOOD study and the study by Cornelis and coworkers, respectively) reduced over more than a decade (2012 vs 2023), which may be explained by the ban on PFOS in the European Union since 2009 (Directive

2006/122/EC, 2006) and an improvement in the analytical techniques (in particular the reduction in LOQ's, which have an impact on the calculation of the MB exposure estimates). PFOA has only been phased out since 2020 (Commission Regulation (EU) 2017/1000, 2017), which might explain the smaller reduction in exposure (3.3-fold and 40-fold compared to the PERFOOD study and the study by Cornelis and coworkers, respectively) since 2012.

The mean summed LB exposure to PFOA, PFOS, PFNA and PFHxS (assuming equipotency, Σ4PFAS) ranged from 0.93 ng/kg bw/week (adults) to 1.7 ng/kg bw/week (children), while the 95th percentile exposure ranged from 1.8 to 3.5 ng/kg bw/week (Table 14). The median exposure estimates were similar to the mean exposure estimates. The habitual exposure to Σ4PFAS is dominated by PFOA and PFOS. Table 14 demonstrates the effect of the bounding approach, applied to handle left-censored data, on the habitual exposure estimates for Σ4PFAS. When values below the quantification limit are substituted by the quantification limit (upper-bound or UB-approach), the exposure estimates are on average 7-fold higher than when those values are substituted by zero (lower-bound or LB-approach). If these values are substituted by half of the quantification limit (middle-bound or MB-approach), the estimates are on average 4-fold higher than for the lower-bound approach. The exposure estimates are likely underestimated by the LB-approach, but even more likely to be overestimated by the UB- approach because matrices in which a PFAS is not detected may truly not contain this PFAS. The EFSA CONTAM Panel considered as well that calculated LB exposures are likely more realistic than UB exposure estimates (EFSA, 2020).

Table 15 provides the chronic LB exposure estimates to PFOA, PFOS, PFNA, PFHxS and their sum provided by EFSA (2020) (EFSA, 2020). The exposure estimates were based on consumption data from the Belgian food consumption survey of 2004 (for adolescents and adults) and from the Flanders preschool dietary survey of 2002-2003 for other children (3-9 years). The occurrence data were based on monitoring results in food samples and drinking water from 16 EU countries, including Belgium. When comparing the current exposure estimates, with those from EFSA, the current mean exposures are 2- (PFOA) to 13-fold (PFNA) lower than the previous estimates. The 95th percentile exposures are a factor 2 (PFOA) to 19 (PFOS) lower than the EFSA estimates. For the sum of the 4 PFAS, the current mean exposures are a factor 3 (adolescents) to 6 (children) lower than the EFSA estimates, and the 95th percentile exposures are 5- (adolescents) to 9-fold (children and adults) lower than the EFSA estimates. Possible explanations for the considerably lower current exposure estimates are the use of more recent food consumption data (FCS 2014) and the use of the most recent occurrence data that are (1) obtained with more sensitive analytical methods and (2) specific for Belgium.

Table 14. Mean, 50th (P50) and 95th percentile (P95) habitual exposure estimates (ng/kg bw/week) for the sum of PFOA, PFOS, PFNA and PFHxS (Σ4PFAS), calculated according to the lower-bound, middle-bound and upper-bound exposure scenarios.

	Mean ng/kg bw/week	**P50** ng/kg bw/week	**P95** ng/kg bw/week
Lower-bound approach			
Whole population (3-64 y)	1.0	0.90	2.2
Children (3-9 y)	1.7	1.5	3.5
Adolescents (10-17 y)	1.1	1.0	2.2
Adults (18-64 y)	0.93	0.83	1.8
Middle-bound approach			
Whole population (3-64 y)	4.0	3.6	7.5
Children (3-9 y)	7.2	6.7	12
Adolescents (10-17 y)	4.7	4.5	7.3
Adults (18-64 y)	3.4	3.3	5.5
Upper-bound approach			
Whole population (3-64 y)	7.0	6.2	13
Children (3-9 y)	13	12	21
Adolescents (10-17 y)	8.2	7.9	13
Adults (18-64 y)	5.9	5.7	9.4

Table 15. Lower-bound mean and 95th percentile (P95) exposure estimates(ng/kg bw/week) for PFOA, PFOS, PFNA and PFHxS and their sum estimated by EFSA (EFSA, 2020). The values are calculated by multiplying the data provided in Annex A (Table A5) of the EFSA report, which are expressed in ng/kg bw/day), by 7 days.

PFAS	Age group*	Mean	P95
		ng/kg bw/week	ng/kg bw/week
PFOA	Other children	1.70	4.25
PFOA	Adolescents	0.88	2.22
PFOA	Adults	1.12	2.67
PFOS	Other children	5.69	22.0
PFOS	Adolescents	2.29	7.27
PFOS	Adults	3.17	11.6
PFNA	Other children	1.35	3.05
PFNA	Adolescents	0.18	0.56
PFNA	Adults	0.23	0.61
PFHxS	Other children	1.09	2.59
PFHxS	Adolescents	0.41	1.10
PFHxS	Adults	0.53	1.24
Σ4PFAS	Other children	9.83	30.5
Σ4PFAS	Adolescents	3.77	10.2
Σ4PFAS	Adults	5.07	16.0

* "Other children" refers to children other than infants, toddlers and adolescents.

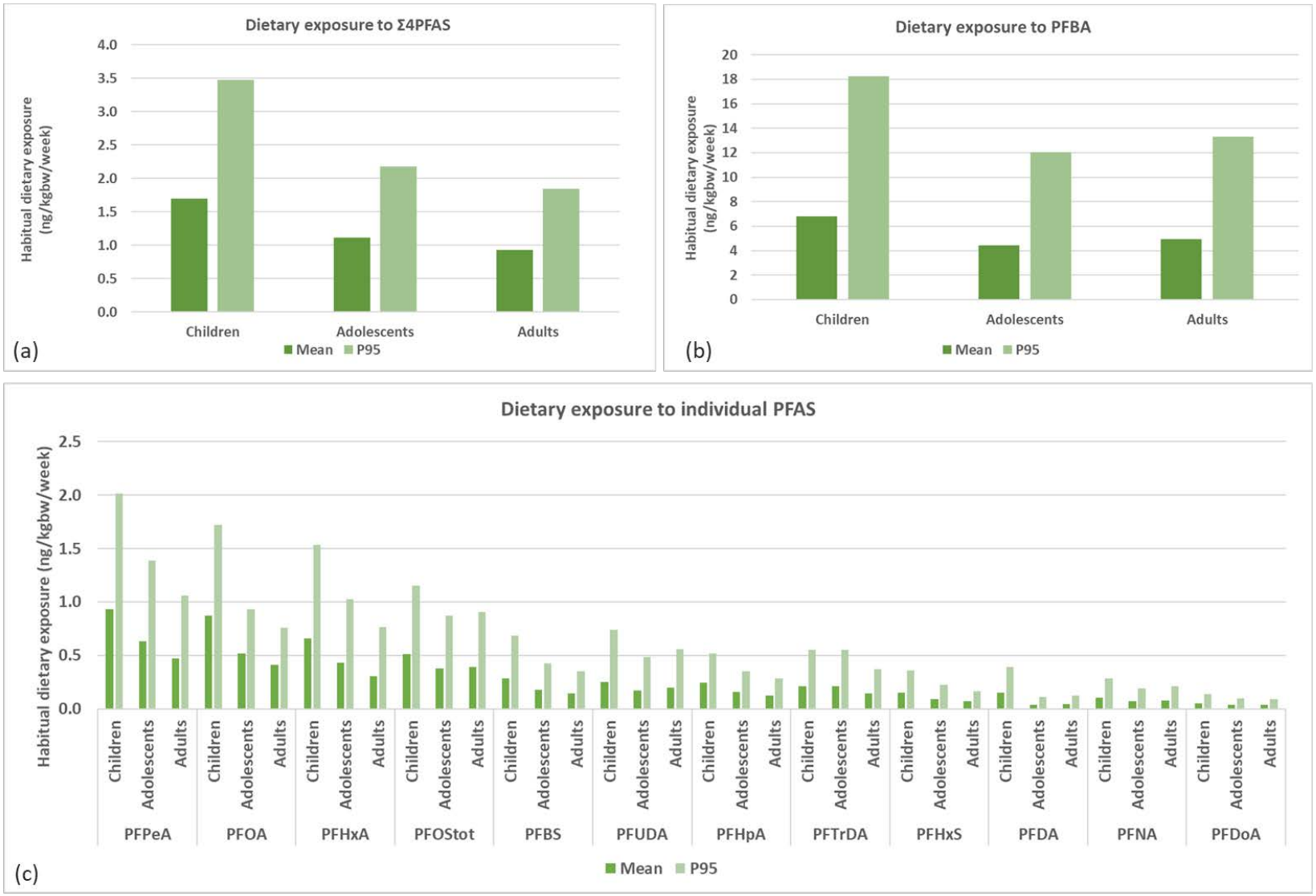

Figure 11. Mean and 95th percentile (P95) habitual exposure estimates for (a) the sum of PFOA, PFOS, PFNA and PFHxS (Σ4PFAS, assuming equipotency) (b) PFBA and (c) the other individual PFAS.

Food groups contributing to exposure

Figure 12 shows for Σ4PFAS the major (FoodEx Level 1) food groups contributing to the mean lower-bound exposure for adults, adolescents and children. The top three of the food groups for all age groups consist of “Fish and seafood”, “Meat and meat products”, followed by “Water and water-based beverages”.

At a more detailed level (FoodEx Level 3), the top three food groups contributing to the mean Σ4PFAS exposure are “Mammals meat” (20%), “Shrimps and prawns” (15%) and “Unbottled water” (15%) for adults, “Mammals meat” (20%), “Unbottled water” (19%)” and “Shrimps and prawns” (9.6%) for adolescents, and “Unbottled water” (21%)”, “Mammals meat” (16%) and “Biscuits” (11%) for children.

In the EFSA opinion of 2020, “Fish meat” and “Fruit and fruit products” were the main contributing food groups to Σ4PFAS exposure for all population groups of the Member States for which the exposure was assessed. The group “Eggs and egg products” was also a main contributor for all population groups except infants, based on detected levels for PFOS and PFOA in a limited number of samples. Detailed contribution data for Belgium are only available for adults (and toddlers, but they are not considered in this report): “Fish and seafood” contributed 47% to the mean LB exposure, followed by “Fruit and fruit products” (17%) and “Meat and meat products” (12%). Drinking water contributed for only 5% to the mean exposure. The difference between the main contributing food groups in the current study and the EFSA opinion is mainly due to differences in the occurrence data and the aggregation of data: in the EFSA opinion a mean value of 0.069 ng/g was assigned to all fruit and fruit products (at FoodEx Level 1), while in the current assessment, a mean value of 0.015 ng/g was assigned to berries and small fruit, while zero was assigned to the other types of fruit at FoodEx Level 3. For drinking water, a mean value of 0.004 ng/g was applied in the EFSA opinion for the food group “Drinking water”. In the current assessment, the food group “Water and water-based beverages” included bottled water (mean Σ4PFAS concentration 0.004 ng/g), as well as unbottled water (0.043 ng/g) and soft drinks (0 ng/g). The contribution of unbottled water boosted the contribution of water and water-based beverages to the mean Σ4PFAS exposure.

The FLUOREX project targeted representative food samples with the final goal to calculate the exposure and to characterise the risk in relation to the TWI for Σ4PFAS. For this, the main food groups that contribute to that estimated exposure were determined. Although, exposure estimates for each individual PFAS were calculated, the additional analyses to identify main contributor food group(s) for each PFAS were recognised but were not performed at this point. However, in the PERFOOD project (Bervoets et al., 2012), the contribution per each group for two main PFAS, PFOA and PFOS was reported. The food groups that contributed most to the dietary intake of PFOS (MB exposure scenario) were mushrooms (35%), apples (19%) and vegetables (non-specified) (12%). The large contribution of apples could be attributed to a single sample (out of five apple samples) collected from a commercial producer located within a hotspot location, while the contribution of vegetables (non-specified) was influenced by the extreme high values that were found in mushrooms. Other important contributors were potatoes (3.4%), crustaceans (3.4%) and bovine meat (2.9%). The most important contributing food groups to PFOA intake were potatoes (31%), apples (21%) and fish (13%) (Bervoets et al., 2012). As food samples in the FLUOREX project are collected in the major supermarkets, the commercial short-chain supply, which represents a more local food production and in which higher occurrence data may occur, may be underrepresented.

Socio-demographic differences in PFAS exposure

The effect of age on the dietary exposure to Σ4PFAS was large, with 20% of the variance in exposure explained by the age group. The exposure was significantly higher for children compared to adolescents and adults, which can be explained by their higher food consumption per unit bodyweight. No significant differences in exposure could be detected among adults and adolescents. There were no significant differences in dietary Σ4PFAS exposure among the three regions. However, if the highest exposure value, which was an outlying value according to the Grubbs test, was removed from the dataset, the difference in exposure to Σ4PFAS between the Flemish region and the Walloon region became

significant. The effect of the region on the dietary exposure was, however, small (< 1% of variance explained). Regional differences in PFAS exposure were also observed when comparing human biomonitoring results of PFOS, PFOA, PFHxS and PFNA between populations living in the North and the South of Belgium (Pirard et al., 2020).

The differences in exposure to Σ4PFAS between males and females were as well significant but small, with less than 1% of the variance in exposure that was explained by the difference in sex.

It should be noted, however, that statistical analyses were performed on the individual, unweighted exposure data and not on the habitual exposure data. The significance of socio-demographic differences may differ for the habitual exposure, but this cannot be tested statistically.

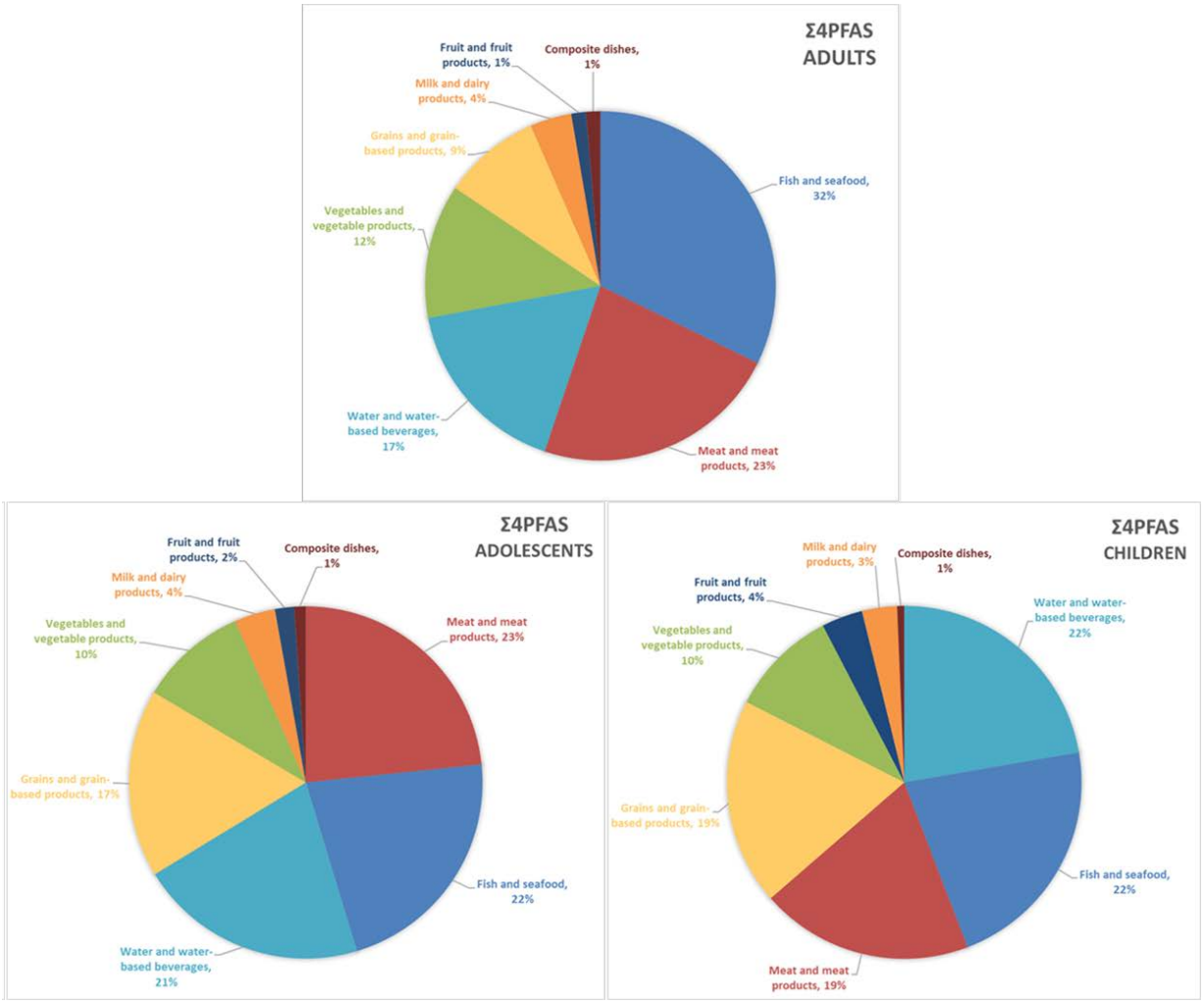

Figure 12: Major contributing food groups to lower-bound Σ4PFAS exposure for adults, adolescents and children.

RISK ASSESSMENT (WP6)

EFSA (2020) risk characterization approach (Σ4PFAS)

The distributions of the Σ4PFAS exposure estimates (Figure 13; lower-bound approach) show that the TWI of 4.4 ng/kg bw/week was not exceeded for the adolescent and adult populations. Hence, for most of the Belgian population, there are no health concerns anticipated related to dietary exposure to the sum of PFOA, PFNA, PFOS and PFHxS. The TWI is exceeded by 2.2% of the children's population due to dietary exposure (incl. exposure through drinking water). Due to the methodology used to derive the TWI, this does not automatically imply a health concern for these children. The TWI was derived to prevent mothers from reaching a body burden (6.9 ng Σ4PFAS/mL in serum at the age of 35) that would lead to serum levels in their breastfed infant associated with decreases in vaccination response (17.5 ng Σ4PFAS/mL at the age of 1). The latter serum concentration was the lowest $BMDL_{10}$ obtained after BMD modelling for the sum of 4-EFSA-PFAS based on an association with reduction in antibody titres against diphtheria. A study on children in the Faroe Islands, showed associations between Σ4PFAS and immunosuppressive effects as well (Grandjean et al., 2012). A NOAEC serum level of 27 ng/mL at the age of 5 was derived from this study (EFSA, 2020). However, exposure at the TWI results in serum levels lower than the derived NOAEC making it difficult to evaluate the impact of the exceedance of the TWI by children, as stated by EFSA. Even a twofold higher intake than the TWI by children did not result in serum levels higher than this NOAEC of 27.5 ng/ml (EFSA, 2020). Although dietary intakes corresponding to this NOAEC have not been calculated, it is unlikely that the Belgian children exceeding the TWI according to the current exposure estimate would reach the NOAEC serum level as the estimates are far less than twofold the TWI. Exposure estimates for the adolescent and adult populations did not exceed the TWI. Hence, for most of the Belgian population, there are no health concerns anticipated related to dietary exposure to the sum of 4-EFSA-PFAS.

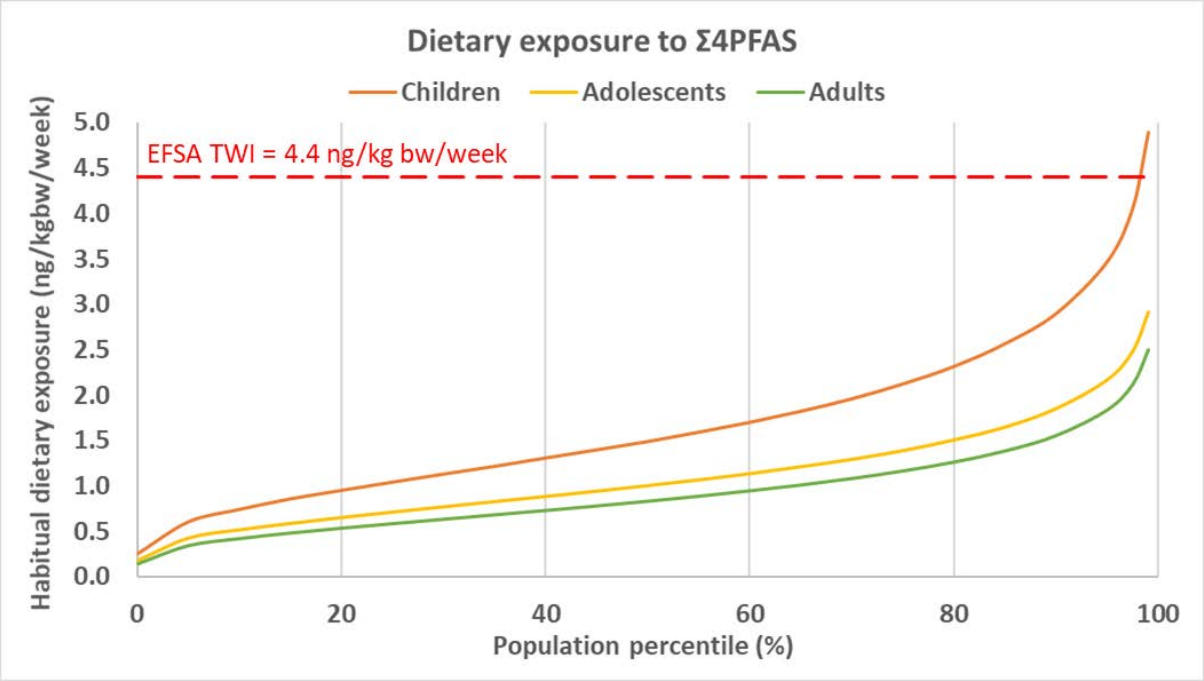

Figure 13. Distribution of the dietary Σ4PFAS exposure estimates for Belgian children, adolescents and adults (lower-bound approach).

Comparison to Biomonitoring data

The estimated exposure for ∑4PFAS and the data obtained from the Flemish biomonitoring study (FLEHS IV), reported in Richterová et al. (2023), were correlated. The median-LB dietary intake estimates were used assuming equipotency and also after adjusting them by using relative potency factors (RPFs) related to liver toxicity (external RPF) (Table 16). In case of biomonitoring data,

adjustment was done using internal RPFs calculated for relative liver weight based on time-weighted average (TWA) (Bil et al., 2023). Due to limited data availability, the comparison was only done for the adolescent population.

Table 16. Comparison of dietary exposure estimates (ng/kg bw/week) and exposure levels (ng/mL) in adolescents (FLEHS IV as presented in Richterova et al.)

PFAS	P50-LB DietExpo ng/kg bw/day (Fluorex)	RPF adjusted estimate*	PFAS exposure levels (µg/L) (Richterová et al., 2023)	PFAS exposure levels RPF adjusted	Internal RPF (Bil et al., 2023)
PFOA	0.48	**0.48**	2.2	2.20	1
PFOS tot	0.32	**0.64**	1.1	5.50	5
PFHxS	0.08	**0.05**	0.49	0.29	0.6
PFNA	0.05	**0.51**	0.32	1.28	4
Total	0.93	1.67	4.11	9.27	

*The external RPF are provided in Table 6 in Materials and methods.

Richterová et al. observed a higher frequency of seafood and fish and eggs consumption to be significantly associated with higher levels of PFOS and PFNA in teenagers (Richterová et al., 2023). The latter was not the case in our study since PFAS were not detected in eggs and egg-related products purchased in the supermarkets, whereas in Richterová et al., the main link was established towards locally produced eggs (Richterová et al., 2023). On the other hand, seafood as a group was identified as one of the major contributors to the total exposure in all age groups. This type of comparison deserves more attention and elaboration in the future.

A Kruskal-Wallis test was performed to determine if the contribution of median exposure estimates (P50) for each PFAS to Σ4PFAS was comparable to the contribution of each PFAS, as measured P50 blood serum level, to Σ4PFAS in blood serum (Figure 14). The test revealed that assuming equal potency for 4-EFSA-PFAS, the contributions of each PFAS to the total sum (of either PFAS measured in food, or those measure in serum blood) were comparable ($H = 1.5$, $p > 0.05$) after being adjusted with RPFs. This comparable contributions to the total sum, imply that there was no statistically significant difference in the contribution of four PFAS (expressed as median exposure estimates) to their sum among dietary exposure and measured blood serum levels. It may also imply that PFAS level in food might broadly reflect their levels in humans.

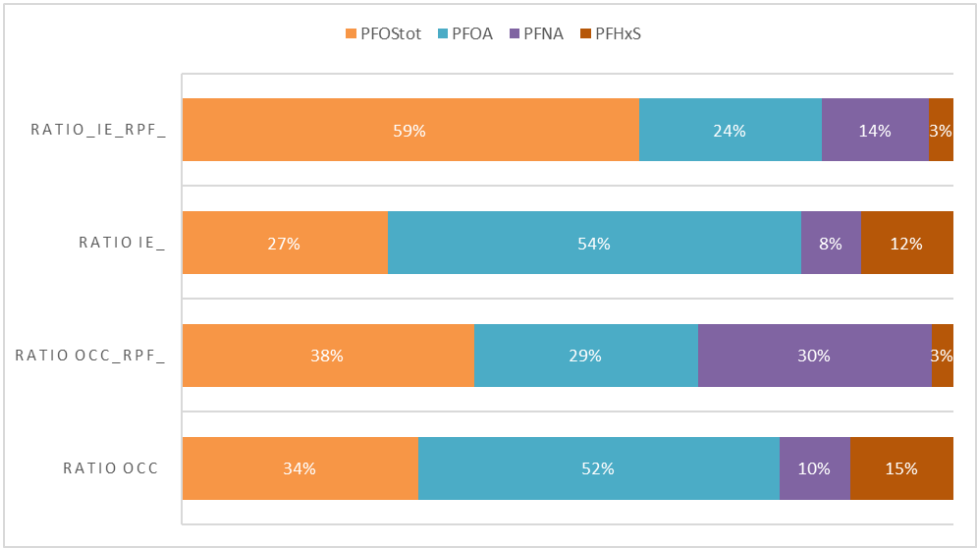

Figure 14. Contribution of each PFAS (out of 4) to the total ∑4PFAS exposures assuming equal potency for dietary exposure (Ratio_OCC) without using or with using the RPF (Ratio_OCC_RPF) approach for dietary exposure estimates presented by Zeilmaker et al. (Zeilmaker et al., 2018), and contribution of each PFAS to the internal ∑4PFAS exposure expressed in serum levels, without RPFs (Ratio_IE) or with internal RPFs reported by Bil et al. (2023) (Ratio_IE_RPF) *dietary exposure estimates represent the P50 of the LB-approach.

Uncertainty analysis

Uncertainties related to the exposure assessment

The exposure estimates are associated with a number of uncertainties. Table 17 identifies the uncertainties that may affect the exposure assessment and uses a pair of ordinal scales to describe the degree of uncertainty (magnitude and direction). The groups of the ordinal scales (plus or minus, and low, medium or high expressed by the number of signs) specify an order without specifying the magnitude of the difference between the groups and were indicated based on the experts knowledge and experience.

In general, the identified uncertainties result in a (slight) underestimation of the exposure to Σ4PFAS lower-bound scenario, but the level of uncertainty is reduced compared to the exposure estimates performed by EFSA for the Belgian population for the following reasons:

- Application of more recent food consumption data.
- Application of more sensitive analytical methods to obtain PFAS occurrence data.
- Because of day-to-day variations in food intake data of individuals, intake data of a large number of days are ideally needed to determine the long-term or habitual exposure of an individual to a substance. The feasibility to collect such long-term data for a large number of individuals is, however, limited. The use of 2x24h recalls to estimate long-term exposure is an acceptable methodology but still may overestimate the exposure for high percentiles. Therefore, a statistical modelling method that accounts for within-individual variation, is applied to determine the habitual (or usual) intake based on short-term food consumption data. Statistical modelling mitigates the limitation of short-term food consumption data better than averaging over two 24-hour recalls per individual.
- Foods representative for the Belgian market have been sampled. The sample selection strategy thereby included considerations about expected concentrations of PFAS, consumption amounts of food, and data gaps as well.
- Aggregation of data at a more detailed FoodEx level (level 3) for all food groups instead of aggregation at FoodEx level 1, 2 or 3.

Table 17. Qualitative evaluation of the degree of uncertainty from different sources in the PFAS exposure estimation.

Source of the uncertainty	Direction and magnitude of uncertainty[$]
Consumption data	
Consumption data collected in 2014 for the Belgian population (FCS_2014) were used. The consumption pattern may have changed since then.	-/+
Use of 2x24h recalls to estimate long-term exposure.	-/+
Occurrence data	
A dataset of 273 data was used for the exposure estimation.	-/+
Food samples were collected in major supermarkets. The commercial short-chain supply, representing a more local food production and in which higher PFAS occurrence data may occur, may be underrepresented.	-
Large amount of left-censored data:	
Use of lower-bound (LB) for left-censored data	--
Use of upper-bound (UB) for left-censored data	+++
Use of a (fixed) mean concentration (instead of the concentration data distribution) per aggregated food subgroup.	-/+
Extrapolation of mean value to all other food items within the same aggregated food subgroup	-/+
The 74 aggregated food subgroups cover 71% of the consumed food items in the FCS_2014. However, as the sample selection strategy included considerations about consumption amounts of food, this source of uncertainty may be negligeable.	(-)
Matching of occurrence data to consumption data	
Aggregation of occurrence data and extrapolation to consumptions of other food items within the same food subgroup	-/++
Representativity of the sampled food items	-/+
No extrapolation of aggregated mean concentrations to other food groups (e.g. occurrence data for unbottled water are not matched to consumptions of coffee or tea drinks).	-
Exposure assessment	
Categorization of food items into FoodEx food groups impacts the calculation of the contribution of each food group to the exposure	-/+
Use of semi-probabilistic modelling to estimate habitual exposure	-/+
Exposure via other sources (dust, soil, air, consumer products) not included	---
Exposure via locally produced food (e.g. eggs, vegetables, fruit) not included	--
Σ4PFAS exposure: assumption of dose addition and equipotency for the 4 PFAS	--/+

[$]The minus sign ("-") represents an uncertainty with potential to cause underestimation of the exposure, the plus sign ("+") represents an uncertainty with potential to cause overestimation of the exposure. The number of signs represents the magnitude of the uncertainty (1 = low, 2 = medium, 3 = high).

Uncertainties related to the risk characterisation

Table 18 provides the sources of uncertainty related to the risk assessment in a descriptive way, without indication of the direction or magnitude of the uncertainty as this kind of information often needs further research.

Limiting the risk assessment to a selection of 4 PFAS and to only dietary exposure, likely underestimates the risk. Although it is believed that food could be the major source of exposure to PFAS, it is not the only one and by characterising the risk as 100% from only one source leads to underestimation.

Table 18. Description of the sources of uncertainty in the risk characterisation for PFAS mixtures in Belgian population.

Sources of the uncertainty
EFSA (2020) risk characterisation approach (Σ4PFAS)
Limiting the risk of exposure to only four PFAS, although they are often considered the most prominent.
The assumption of dose addition.
The assumption of equipotency for the 4 PFAS.
Assuming that dietary exposure represents a main source of exposure.
The methodology used to derive TWI considered protective effect for children

Conclusions and recommendations

Conclusions

During the FLUOREX project, a comprehensive sampling yielding 283 samples, reflecting all foods relevant for PFAS exposure and Belgian consumption habits, was realized. In addition, special attention was given to game meat, offal and egg-containing products. Note that this study did not include exposure to the food grown in private gardens (including those of people living in areas considered as polluted (hotspots). Next, an analytical method for quantifying 25 PFAS was developed and validated. Potential PFAS contaminations from the laboratory material, reagents, and environment were mitigated to achieve very low limits of quantification (LOQ), defined as the lowest validated level. The achieved LOQs ranged from 0.001 to 0.1 µg/kg according to the matrix and the PFAS, except for HFPO-DA (maximal LOQ of 1 µg/kg).

The developed method was applied for the analysis of the selected samples. However, not all PFAS could be analysed in all samples (e.g. PFBA in 167 out of 283 samples). The obtained occurrence data demonstrated widespread PFAS contamination in food. Although an average of 1.1 compounds were detected per sample with a total of 302 detections in the project, approximately 57% of the samples contained none of the 25 studied PFAS. PFOS was the most detected compound found in 19% of the 283 samples, followed by PFOA (17%). Eight compounds were never detected (i.e. PFTeDA, PFHpS, PFDS, PFUnDS, PFDoDS, PFTrDS, Minor F53B, HFPO-DA). Other PFAS were detected in 1 to 11% of the samples, depending on the PFAS. The concentrations of the different PFAS varied from below LOQ to 2.85 µg/kg (i.e. PFTrDA in a crab sample). Since EFSA focussed on four PFAS (i.e. PFOS, PFOA, PFNA and PFHxS, i.e. 4-EFSA-PFAS), a distinction could be made between these 4-EFSA-PFAS and the other PFAS included in the analytical scope. The contribution of the 4-EFSA-PFAS to the overall PFAS concentration varied from 0 to 100%, with an average of 53%. In 35 samples (i.e., about 28% of the samples with PFAS detection), mainly plant-based products, only PFAS other than those regulated were found (i.e. PFBA, PFPeA, PFHxA, PFHpA and PFBS). For animal-based products, regulated PFOS and PFOA were mainly detected, together with long-chain carboxylic PFAS (n=9-13 carbons) (e.g. PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA).

In Regulation (EU) 2023/915, maximum levels have been set for specific food groups like fish and seafood, meat and eggs. Only one sample (crab) exceeded the maximum level of 0.7 µg/kg for PFOA with a concentration of 1.2 µg/kg. Furthermore, indicative levels are mentioned in Recommendation (EU) 2022/1431 for several foodstuffs, indicating that further investigation of the causes of the contamination should be carried out when the levels are exceeded. Seven fruit and vegetable samples exceeded the indicative levels of 0.01 µg/kg for PFOA in fruits and vegetables with a maximum concentration of 0.20 µg/kg in oyster mushrooms, when taking into account the measurement uncertainty of 50%.

The occurrence data were combined with consumption data from the most recent food consumption survey to estimate the dietary exposure to PFAS and associated health risks. For the majority of the Belgian population, it can be concluded that no health concerns are estimated related to dietary exposure to the sum of 4-EFSA-PFAS (∑4PFAS). For 2.2% of the children, the tolerable weekly intake (TWI) related to immune effects, was exceeded, but due to the methodology used to derive the TWI, this does not automatically imply a health concern for these children. Health concerns are unlikely since the exceedance was less than two-fold the TWI. Furthermore, the current dietary exposure to ∑4PFAS is lower than the previous estimate by EFSA in 2020. The most important food groups contributing to the exposure are “fish and seafood”, “meat and meat products” and “water and water-based beverages”.

Finally, the contribution of FCM to the overall exposure to PFAS was investigated and revealed that PFAS are only present in FCM made of paper and board. In contrast, samples with PTFE coating did not release any of the targeted PFAS, regardless of the quality of the product. The concentrations

discovered in the study suggest that the presence of PFAS is unintentional and most likely due to environmental background contamination. The subsequent risk assessment did not highlight any potential concerns for consumers related to the use of FCM.

Recommendations

Since PFAS contamination is ubiquitous, reaching the very low LOQs targeted by the Recommendation (EU) 2022/1431 is necessary to guarantee an accurate dietary exposure assessment. During the analysis, it was noticed that PFAS were often not detected in food, and as a result, the use of left-censored data for the exposure assessment is critical. However, the non-detection of PFAS does not imply that exposure to these PFAS does not exist or is not possible in the future. Monitoring these PFAS in food further and providing efforts to improve the LOQs is recommended. However, this is very challenging for specific food groups like fruits and vegetables.
Furthermore, the sampling in FLUOREX focused on the supermarkets whereas food grown in private gardens (including those of people living in in areas considered as polluted (cf. hotspots)) were not considered. As a result, the health concerns for people living in polluted areas could be underestimated and should thus be assessed considering the consumption of locally grown food.

Although EFSA developed a TWI for the ∑4PFAS, based on the occurrence data it is obvious that the exposure of the Belgian population is not limited to these 4-EFSA-PFAS. Unfortunately, there is no internationally harmonized approach for the combined risk assessment for all PFAS detected in food. The available methodologies should be urgently further investigated and validated by the scientific community to provide a comprehensive risk assessment of to all PFAS in food.

. Although the current TWI is suitable for risk management because it protects the most sensitive population, namely breastfed infants, it is less relevant to assess the health risks for an exposed population when it is exceeded. Additional health-based guidance values are needed to understand potential health concerns for the general population and specific subpopulations.

Moreover, food is not the only source of PFAS exposure. Therefore, it would be interesting to establish appropriate allocation factors to the various PFAS sources (e.g. air, dust, etc.) when evaluating the potential exceedances of the HBGV. However, more data are needed to attribute adequate allocation factors.
Finally, the study focussed on the main exposure route for PFAS, namely dietary exposure. Based on the first comparative analysis of biomonitoring study results, it can be implied that the PFAS level in food might broadly reflect their levels in humans. Comparison of the dietary exposure estimates to the levels measured in human matrices (human biomonitoring, HBM) deserves more attention and elaboration in the future. HBM may also be particularly relevant to validate the combined exposure and risk assessment from multiple sources since they provide valuable insights into the actual levels of contaminants in individuals.

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