PERFLUOROOCTANE SULFONATE PRECURSORS AS INDIRECT SOURCES OF INTERNAL EXPOSURE TO PERFLUOROOCTANE SULFONATE (PFOS)

BY

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ABSTRACT

Perfluorooctane sulfonate (PFOS) is a perfluoroalkyl substance with extensive historical use. Its persistence, bioaccumulation, toxicity and health concerns led to its incorporation in the Stockholm Convention as a persistent organic pollutant (POP) in 2009. Direct exposure to PFOS has been widely reported in different environmental and biological samples, and recent human biomonitoring studies indicate that levels are in decline. However, certain uncertainties remain when estimating its body burdens: indirect exposure to so called PFOS-precursor compounds – such as perfluorooctane sulfonamides (FOSAs) and perfluorooctane sulfonamidoethanols (FOSEs) –, followed by *in vivo* metabolism could contribute to the current levels of PFOS in human biological samples.

To evaluate the direct and the indirect contribution to PFOS overall exposure, sample preparation and instrumental HPLC-MS/MS methods for the analysis of PFOS – linear and sum of branched isomers – and suspected PFOS precursors – three FOSAs, two FOSEs and three FOSAAs – in dust, food and serum samples were developed and validated. These methods were applied to 57 vacuum cleaner dust samples, 113 solid food composites, 121 liquid food composites and 60 serum samples from a well-established Norwegian cohort. Indoor environment questionnaires, food diaries and food frequency questionnaires were compared to the reported concentrations of PFOS – linear and branched – and PFOS precursors. Daily intakes for total PFOS and PFOS precursors via dust and food ingestion were estimated under different scenarios, and compared with reported internal exposure

levels of PFOS. Finally, for a better understanding of the link between external and internal exposure, a qualitative study of the *in vitro* metabolism of two PFOS precursors – MeFOSA and MeFOSE – was conducted.

All the dust samples were positive for, at least, one of the analysed pollutants, with average concentrations of 77 ng/g for ΣPFAS – average daily intakes of 4.51 pg/kg bw/day in mean scenario, and 59.66 pg/kg bw/day in high scenario –, presenting a profile dominated by EtFOSA, followed by and EtFOSE and MeFOSE. Still, diet was identified as the most important pathway of external exposure, with reported average daily intakes of 0.24 ng/kg bw/day in median bound scenario, dominated by FOSEs exposure. Conversely, for internal exposure PFOS was the most detected analyte (87 %), in an average concentration of 5.3 ng/mL and contributing more than 90 % to the overall internal exposure to PFASs. These results supported the initial hypothesis of the contribution of PFOS precursors to indirect exposure to PFOS, also in line with the conducted *in vitro* metabolism assays, which revealed the fast conversion of PFOS precursors to PFOS when they were incubated with human liver microsomes.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
Å	Amstrongs
AFFF	Aqueous fire-fighting foam
Alam	Alamatechin
AM	Arithmetic Mean
amu	Atomic Mass Unit
A-TEAM	Advanced Tools for Exposure Assessment and Biomonitoring
BD	Bedroom
BfR	German Federal Institute for Risk Assessment
bw	Body Weight
C18	Octadecyl carbon chain bonded silica
CAS Nr	Chemical Abstracts Service number
CCD	Cetral Composite Design
CDC	US Center for Disease Control and Prevention
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
DF	Detection Frequency
DF	Detection Frequency
DoE	Design of Experiments
ECF	Electro-chemical fluorination
EDI	Estimated Daily Intake
EF	Enantiomeric Fraction
EFSA	European Food Safety Authority
ESI	Electrospray Ionisation
EtAc	Ethyl Acetate
EtFOSA	Ethyl perfluorooctane sulfonamide
EtFOSAA	Ethyl perfluorooctane sulfonamidoacetic acid
EtFOSE	Ethyl perfluorooctane sulfonamidoethanol
FD	Food Diary
FFQ	Food Frequency Questionnaire
FOSA	Perfluorooctane sulfonamide
FOSAA	Perfluorooctane sulfonamidoacetic acid
g	Gram
GM	Geometric Mean
h	Hour

H_2O	Water
HCl	Hydrochloric acid
HCOOH	Formic acid
HLCyt	Human Liver Cytosols
HLM	Human Liver Microsomes
HPLC	High Performance Liquic Chromatography
HRMS	High Resolution Mass Spectrometry
IEQ	Indoor Environment Questionnaire
IS	Mass labelled standard
IVM	Institute for Environmental Studies
KMO test	Kaiser-Meyer-Olkin (KMO) test for sampling adequacy
Kow	Partition coeffitient octanol/water
L	Litre
LOD	Limit Of Detection
LOQ	Limit Of Quantification
LR	Living room
MeCN	Acetonitrile
MeFOSA	Methyl perfluorooctane sulfonamide
MeFOSAA	Methyl perfluorooctane sulfonamidoacetic acid
MeFOSE	Methyl perfluorooctane sulfonamidoethanol
MeOH	Methanol
min	Minute
mM	mili molar
MOE	Margin of Exposure
MPA	Mobile Phase A (aqueous)
MPB	Mobile Phase B (organic)
MS	Mass Spectrometry
MS/MS	Tandem mass spectrometry
n	Number of cases included in a specific study
Na_2SO4	Sodium sulphate
NaAc	Sodium acetate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
ND	Not detected
NH_4Ac	Ammonium acetate
NH_4COOH	Ammonium formate
NH_4OH	Ammonium hydroxide
NHANES	National Health and Nutrition Examination Surveys
NIPH	Norwegian Institute of Public Health
NIST	National Institute of Standards and Technology

n-PFOS	Linear PFOS
NR	Not Reported
NR	Not Reported
PAP	3'-phosphoadenosine-5'-phosphosulfate
PCA	Principal Components Analysis
PFAS	Perfluoroalkyl substance
PFC	Perfluorochemical
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFSA	Perfluorinated Sulfonamide
POP	Persistent organic pollutant
POSF	Perfluorooctane sulfonyl fluoride
PP	Polypropylene
psi	Pounds per Square Inch
PTFE	Polytetrafluoroethylene
Q1	25th percentile
Q3	75th percentile
QA/QC	Quality assurance / quality control
QqQ	Triple quadrupole mass spectrometer
QTOF	Quadrupole - Time of Flight
Rec	Recovery
rpm	Revolutions Per Minute
RRF	Relative Response Factor
RRT	Relative Retention Time
RSD	Relative Standard Deviation
Sad	Mass labelled standard addition solution
SceBRA	Scenario-Based Risk Assessment
SD	Standard Deviation
SNUR	Significant New Use Rules
SoA	State of Art
SPE	Solid Phase Extraction
SRM	Standard Reference Materials
SS	Suspect Screening
Subs	Substrate
SULT	Sulfotransferase
TDI	Tolerable Daily Intakes
TEA	Triethylamine
THF	Tetrahydrofuran
TS	Target Screening

UDPGA	Uridine 5'-diphospho-glucuronic Acid
UGT	Uridine 5'-diphospho-glucuronosyltransferase
UHPLC	Ultra High Performance Liquid Chromatography
USEPA	US Environmental Protection Agency
V	Volts
VU	Vrije Universiteit Amsterdam
WAX	Weak Anion Exchange
WS	Working solution
XIC	Extracted Ion Chromatogram
ΣBr-PFOS	Sum of branched PFOS isomers
ΣPFAS	Sum of PFOS and PFOS precursors measured in this study
ΣPFOS	Sum of linear and branched PFOS isomers

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1. INTRODUCTION

1.1 OVERVIEW

Perfluoroalkyl substances (PFASs) are a family of synthetic compounds characterised by a fully fluorinated hydrophobic linear carbon chain, to which are attached different hydrophilic functional groups (Fromme *et al.*, 2009). These chemicals have been manufactured since the late 1940s by 3M Company (3M, 1999) as well as other companies like Dupont, and have been produced and used in commercial products and industrial processes for over 60 years (Lindstrom, Strynar and Libelo, 2011). PFASs possess low molecular polarisability, short C–F bond length, and large C–F bond binding energy. Such characteristics govern the oil and water repellency, physical and chemical stability, and surfactant properties of PFASs (Zushi, Hogarh and Masunaga, 2011). These properties mean that PFASs have found wide use in a variety of applications, with historic production peaking at the end of the 20th century in North America and Europe (Paul, Jones and Sweetman, 2009). In an environmental context however, the strong C–F bond means that PFASs are resistant to thermal, chemical and biological degradation (Kissa, 2001) and are capable of bioaccumulation and long-range environmental transport, exemplified by their detection in the Arctic (Chaemfa *et al.*, 2010; Sonne, 2010; Zhao, Wong and Wong, 2012). As a result, perfluorooctane sulfonate (PFOS) and its salts, as well as perfluorooctane sulfonyl fluoride (POSF) – raw material employed for the synthesis PFOS and other perfluoroalkyl substances, represented in *Figure 01* – were in 2009 listed as persistent organic pollutants (POPs) under the Stockholm Convention (Stockholm-Convention, 2009). POSF can degrade to PFOS directly or indirectly

through chemical or enzymatic hydrolysis, and hence POSF-derived products (other PFASs which synthesis require the use of POSF as raw material) can be degraded ultimately to PFOS (Zhao *et al.*, 2016).

The main applications of PFOS and PFOS derivatives included uses in inks, varnishes, waxes, fire-fighting foams, metal plating and cleaning products, coating formulations (for walls, furniture, carpeting, food packaging), lubricants, water and oil repellents for leather, paper and textiles (3M, 2000). Before 2003, POSF was used as a raw material for the synthesis of PFOS (among other perfluorooctane sulphonamide derivates) (Buck *et al.*, 2011). However, 3M Company replaced POSF derivate products with perfluorobutane sulfonate (PFBS) after 2003, because the former was considered harmful to the environment (Renner, 2006).

Over the last 15 years, a substantial weight of evidence has emerged concerning environmental contamination with PFOS, consequent human exposure, and its effects. This chapter reviews this evidence, and summarises recent developments that exploit the relative abundance of branched chain PFOS isomers to provide valuable insights into the environmental fate and behaviour of PFOS and its precursors.

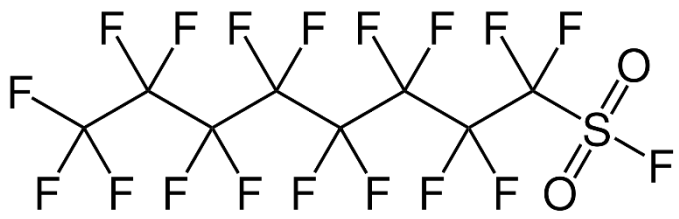

***Figure 01*.** POSF structure, main substance employed as raw material for the synthesis of some long chain PFASs

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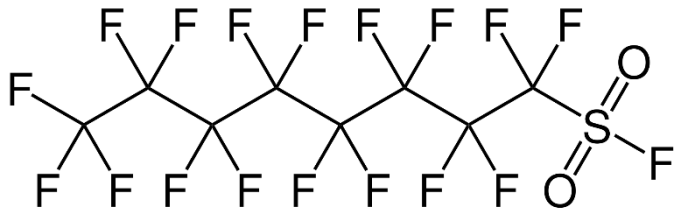

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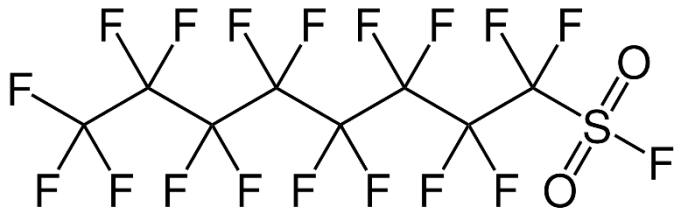

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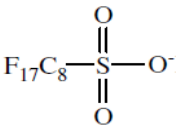
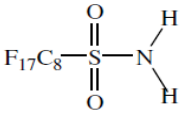
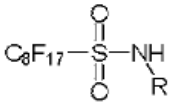
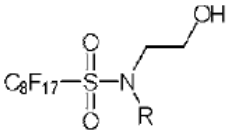
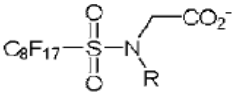
1.2 SOURCES, PRODUCTION AND APPLICATIONS

The history of perfluorochemicals (PFCs) production – hence PFASs and PFOS related substances – is difficult to portray accurately due to the nature of the available information and the successive changes in regulations and production lines (Lindstrom, Strynar and Libelo, 2011). Anyway, it is known that the 3M Company was the first main producer of perfluorooctane sulfonyl fluoride (POSF), product employed afterwards as the main raw material for the synthesis of PFOS and most of the perfluorooctane sulfonyl substances. The 3M company started POSF production – and subsequently, PFOS production as a second step-– in 1949 until its curtailment in 2002, with the total cumulative production estimated to be approximately 96,000 tons in the peak years between 1970 and 2002, with further 26,500 tons of solid unwanted or wastes (Olsen *et al.*, 2005; Paul, Jones and Sweetman, 2009). The 3M Company largest production sites were based in the US and Belgium, but another 6 plants were also located in Europe (4 in EU member states), 6 in Asia (of which 4 were in Japan) and one in South America (Paul, Jones and Sweetman, 2009). According to the US Environmental Protection Agency (US EPA), in addition to 3M Company, around 20 non-US companies were producing and supplying POSF (and PFOS). In 2002, the 3M Company discontinued its production; but other companies mostly based in Southeast Asia commenced its manufacture to meet existing market demands, with an estimated 1,000 tons being produced annually since 2002 (Paul, Jones and Sweetman, 2009).

Table 01. List of PFOS and its main precursors

CAS number	Common name	Name	Molecular formula	Structure
NA	PFOS anion	Perfluoro-1-octanesulfonate	$C_8F_{17}SO_3^-$	
754-91-6	FOSA	Perfluoro-1-octanesulfonamide	$C_8H_2F_{17}NO_2S$	
4151-50-2	N-EtFOSA	N-ethylperfluoro-1-octanesulfonamide	$C_{10}H_6F_{17}NO_2S$	 R = methyl for MeFOSA R = ethyl for EtFOSA
31506-32-8	N-MeFOSA	N-methylperfluoro-1-octanesulfonamide	$C_9H_4F_{17}NO_2S$	
1691-99-2	N-EtFOSE	2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	$C_{12}H_{10}F_{17}NO_3S$	 R = methyl for MeFOSE R = ethyl for EtFOSE
24448-09-7	N-MeFOSE	2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	$C_{11}H_8F_{17}NO_3S$	
2806-24-8	FOSAA	Perfluorooctane sulfonamidoacetate	$C_{10}H_4F_{17}NO_4S$	 R = methyl for MeFOSAA R = ethyl for EtFOSAA R = H for FOSAA
2355-31-9	N-MeFOSAA	N-methylperfluoro-1-octanesulfonamidoacetic acid	$C_{11}H_6F_{17}NO_4S$	
2991-50-6	N-EtFOSAA	N-ethylperfluoro-1-octanesulfonamidoacetic acid	$C_{12}H_8F_{17}NO_4S$	

N/A = Not applicable. R = Substituent

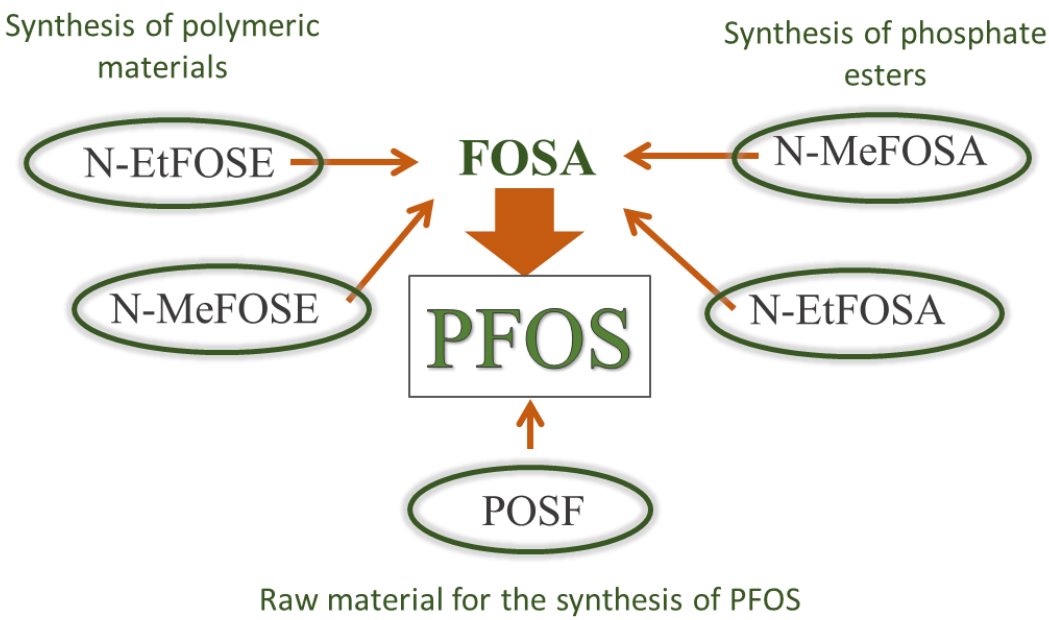


Figure 02. Sources of PFOS and PFOS precursors according to their synthesis processes

PFASs synthesis routes have been well described by Lehmler et al. (Lehmler *et al.*, 2010). The two main processes are electro-chemical fluorination (ECF) (3M, 1999), and telomerisation (Schulz *et al.*, 2011), with POSF synthesised via ECF. In this process, a straight chain hydrocarbon is reacted with H and F atoms and electricity to substitute all of the hydrogen atoms with fluorine (Kissa, 2001) in a reaction common for different chain length as shown in *Equation 01*, where *n* is the number of carbons in the alkyl chain. Reaction yields are chain-length dependent, being lower as the number of carbons increases. This constitutes the main process of POSF synthesis (around 12 % yield), generating about 70 % of the straight chain product with the remainder comprised of branched and cyclic isomers (Paul, Jones and Sweetman, 2009). POSF can then be used in a series of reactions via N-methyl and N-ethyl perfluorooctane sulfonamide (N-MeFOSA and N-EtFOSA) to yield N-methyl and N-ethyl perfluorooctane sulfonamidoethanols (N-MeFOSE and N-EtFOSE),

which historically were used to produce polymeric materials and phosphate esters respectively, and used on surface coatings for textiles and paper products (see *Figure 02*). (Olsen *et al.*, 2005; Paul, Jones and Sweetman, 2009; D'Eon J and Mabury, 2011). It is important to note here that a number of possible PFOS isomers exist in POSF based mixtures (in which process PFOS impurities are present between 0.1 and 5 % (Paul, Jones and Sweetman, 2009) due to the nature of the ECF process itself). The isomer composition of the commercial PFOS products can be up to 30 % of total PFOS. Moreover, some of these isomers (specifically those that are branched chain) are chiral, with the result that the environmental fate and behaviour of PFOS may vary according to its isomeric and enantiomeric composition.

Equation 01 $C_nH_{2n+1}SO_2F + (2n+1)F^- \rightarrow C_nF_{2n+1}SO_2F + (2n+1)H^+(4n+2)e^-$

The major applications of POSF derivatives have been: 1) in carpets to impart stain and dirt repellence, 2) in apparel to provide water repellence, 3) in paper and packaging to afford oil and grease repellence, 4) in performance chemicals such as hydraulic fluids for aviation, and 5) in aqueous fire-fighting foams (AFFFs). AFFFs are perhaps the most prominent method of widespread environmental dispersal, with use for oil drilling and military fire-fighting practice (Paul, Jones and Sweetman, 2009).

All compounds produced from POSF are widely referred to as "PFOS equivalents" or just "PFOS", due to their collective potential to degrade or transform into PFOS (Paul, Jones and Sweetman, 2009). In contrast, PFOS itself is extraordinarily stable in the

environment, with no known natural mechanism of degradation. Hence, regulatory bodies have been working to reduce the production and use of some PFASs (Zushi, Hogarh and Masunaga, 2011). The 3M Company, together with the US EPA resolved to decrease the production of PFOS and related compounds between 2000 and 2002 (3M, 2008). At the same time, Significant New Use Rules (SNUR) were also put in place (2000, 2002, and 2007) in the US, designed to restrict the production and use of materials that contained PFOS or its various precursors. The US EPA then worked with eight leading chemical companies in the 2010-2015 PFOA Stewardship Program to reduce emissions and residual content of perfluorooctanoic acid (PFOA) and long-chain PFCs by 95 % by 2010, with the long-term goal to work towards elimination of long-chain PFCs by 2015 (USEPA, 2010).

Within the EU, PFOS and its derivatives are regulated in the market or only used as a substance or constituent of preparations listed as permissible (Parliament, 2006). Under this directive, PFOS may still be used in applications that are deemed un-substitutable, including photolithographic processes, photographic coatings, mist suppressants for non-decorative hard chromium (VI), plating/wetting agents in controlled electroplating systems (pollution prevention and control are required), and hydraulic fluids for aviation. Such regulation started within the EU in June 2008 (Zushi, Hogarh and Masunaga, 2011).

The presence of PFOS in the environment has been attributed to two major sources: direct and indirect (Prevedouros *et al.*, 2006; Armitage *et al.*, 2009; Paul, Jones and Sweetman, 2009). Direct sources are derived from the manufacture and application of PFOS and POSF (Paul, Jones and Sweetman, 2009). By comparison, indirect sources are a consequence of chemical reaction impurities or breakdown of so-

called precursors such as MeFOSE and EtFOSE (see *Table 01*), represented in *Figure 02*. It has been estimated that 85 % of indirect emissions occur via release from consumer products during use and disposal (3M, 2000).

1.3 HEALTH CONCERNS

General toxicological findings associated with laboratory animals exposed to PFOS include hepatomegaly and hepatic peroxisome proliferation, liver, testicular (Leydig cell), and pancreatic (acinar cell) tumours, reproductive and developmental deficits, neurotoxicity, and immunotoxicity (DeWitt *et al.*, 2012).

Most of the reported studies concerning PFOS toxicity have been conducted on mice, with subsequent extrapolation to humans of observed murine effects complicated by interspecies variability in toxicokinetics. Adverse effects attributed to PFOS in rodents include decreased body weight, increased liver weight, and a steep dose-response curve for mortality (Seacat *et al.*, 2003), as well as an increase in hepatocellular and follicular cell adenomas at high exposure levels (3M, 2002).

Human studies carried out on workers occupationally exposed to PFASs have generally yielded inconsistent results. While such workers have circulating blood levels of PFASs that are hundreds of times those of non-occupationally exposed individuals (Olsen *et al.*, 2003; Steenland, Fletcher and Savitz, 2010), it is difficult to determine conclusive results in these studies (either positive or negative) because sample populations are small, historical exposure levels are uncertain, individuals often have had simultaneous exposures to other compounds, and they may have pre-existing conditions that complicate evaluations (Fletcher *et al.*, 2013).

Compared to PFOS, studies of PFOA exposed workers are more numerous. Several studies have shown a positive association between PFOA exposure and cholesterol, which could have implications for the development of cardiovascular disease (Zobel and Olsen, 2012). PFOA has also been associated with elevated uric acid levels, which may in turn lead to hypertension and cerebrovascular disease (Olsen *et al.*, 2003; Sakr *et al.*, 2007; Costa, Sartori and Consonni, 2009; Lindstrom, Strynar and Libelo, 2011).

Based on the toxicological evidence available to date, chronic exposure guidelines are being developed for PFOS and PFOA by the US EPA and other jurisdictions for water and food, but little has been done thus far for other PFASs. A review of current global guidelines and regulations can be found in Zushi et al. (Zushi, Hogarh and Masunaga, 2011), and some especially pertinent illustrative examples are discussed briefly here. The continuing uncertainty surrounding the human health impacts of PFASs is reflected in the disparity between the values promulgated by different jurisdictions. The risk from PFOS for human adults has been evaluated as low based on the Margin of Exposure (MOE), derived from the ratio of the provisional tolerable daily intakes (pTDI) and the level of intake (Zushi, Hogarh and Masunaga, 2011). Fromme et al. (Fromme *et al.*, 2009) estimated the average (and high end) daily intake of PFOS and PFOA, including the indirect contribution from their precursors, as 1.6 (11.0) and 2.9 (12.7) ng/kg bw/day, respectively. These exposures are comfortably lower than the pTDIs for the general adult population of 100 ng/kg bw/day for PFOS and 3,000 ng/kg bw/day for PFOA, promulgated by the German Federal Institute for Risk Assessment (BfR) and the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) respectively.

Moreover, the USEPA issued provisional short-term health advisories for PFOS (200 ng/L) and PFOA (400 ng/L) in drinking water, on the assumption that short-term consumption below these levels will safeguard public health (USEPA, 2009).

In a parallel approach to limit values for external exposure via ingestion of food and water, the Biomonitoring Commission of the German Federal Environmental Agency used the 95th percentile concentration values of two German studies (Midasch, Schettgen and Angerer, 2006; Fromme, Schlummer, *et al.*, 2007), to establish reference values for PFOA and PFOS in plasma of children and adults. These reference values specify a maximum permissible presence of PFOS of 10 µg/L for children, 20 µg/L for adult females, and 25 µg/L for adult males (Wilhelm *et al.*, 2009).

1.4 HUMAN EXPOSURE TO PFOS AND PFOS PRECURSORS

PFOS is one of the most reported PFASs in literature due to its past extensive use, its high detection frequencies, its high persistence and its inclusion in 2009 as POP under the Stockholm Convention. The first report of the presence of PFOS and other PFASs in samples of human blood purchased from biological supply companies emerged in 2001 (Hansen *et al.*, 2001), although the first paper regarding the presence of organofluorine compounds in biological samples dates from 1968 (Taves, 1968a, 1968b). Since then, a considerable database inclusive biomonitoring and human exposure to PFASs has emerged.

Traditionally, just direct exposure to PFOS was considered, and after the 3M Company phase out and the incorporation of PFOS and its salts to the list of

persistent organic pollutants in 2009 decreasing levels in human biomonitoring data were expected to occur. Different papers published recently clearly show that pattern (Olsen *et al.*, 2008, 2012; Glynn *et al.*, 2012; Nost *et al.*, 2014), but an underlying concern started to grow: Why levels of PFOS are decreasing but they are not according to what theoretical models predicted?. According to Olsen et al. (Olsen, Burris, *et al.*, 2007), PFOS half-life is serum was estimated to be 4.8 years, so as a consequence of its restriction after 2002, its external and internal exposure levels tend to decline, as recent longitudinal studies from different countries revealed (Nost *et al.*, 2014; Toms *et al.*, 2014). Still, its ubiquity in biological samples 15 years after it was phased-out, such as serum, reveal additional external sources of exposure besides the direct exposure to small POSF productions in some countries, and the legacy PFOS still present in soil, dust, water, and wildlife.

As a consequence of that concern, newer studies starting hypothesise on an additional source of PFOS exposure: Human exposure to PFOS – as for many other environmental pollutants – can occur via two different routes as described by Prevedouros et al. (Prevedouros *et al.*, 2006; Ross, Wong and Martin, 2012): a) direct exposure to PFOS when it occurs via one or multiple exposure pathways (diet, inhalation, contact) to the target pollutant, and b) indirect exposure to PFOS when it happens via exposure to their precursors and subsequent biotransformation of these precursors to PFOS in the body. The following section will summarise past and present on human exposure to both, PFOS and PFOS precursors.

1.4.1 Direct Pathways of Human Exposure

Non-occupational exposure – direct and indirect – to PFOS mainly occur via the ingestion of food and drinking water, as well via inhalation and dust ingestion.

Overall, based on the exposure models and reviews published to date (Trudel *et al.*, 2008; Vestergren *et al.*, 2008; Fromme *et al.*, 2009; D'Eon J and Mabury, 2011; Ericson Jogsten *et al.*, 2012) food contaminated via bioaccumulation, has been suggested by several authors as the principal pathway of direct human exposure to PFOS (Fromme, Schlummer, *et al.*, 2007; Trudel *et al.*, 2008; Vestergren *et al.*, 2008; Fromme *et al.*, 2009; Kärrman *et al.*, 2009; Herzke *et al.*, 2013; D'Hollander *et al.*, 2015).

In 2012, Ericson Jogsten et al. (Ericson Jogsten *et al.*, 2012) reported diet as the main pathway of PFOS exposure for adults and toddlers from Catalonia, Spain (constituting more than 70°% of the daily total intake). Ingestion of water was identified as the second most important human exposure pathway, with inhalation of air and ingestion of dust considered negligible (< 0.5 % of the total intake) in all scenarios except in the worst case scenario for toddlers, where the relative contribution was 4 %. An alternative Scenario-Based Risk Assessment approach (SceBRA) was used in the studies of Trudel et al. (Trudel *et al.*, 2008) and Vestergren et al. (Vestergren *et al.*, 2008). The SceBRA is a more complete risk assessment screening tool – compared to these just including risk quotients and ratios of exposure concentrations – by the inclusion of the number of exposed people. This new term allows differentiate among a larger number of situations and establish if a certain risk is restricted to concrete situations or it is widespread, as well as establishing relations between the number of exposed population and the risk

quotients (Scheringer *et al.*, 2001). Trudel et al. (Trudel *et al.*, 2008) and Vestergren et al. (Vestergren *et al.*, 2008) reported food ingestion as one of the most important pathways under three different exposure scenarios (low, intermediate and high), although there was some divergence between the two studies about the absolute contribution of diet. Moreover, house dust ingestion was identified as a significant direct exposure pathway in both studies (though different absolute values of its proportional contribution to overall exposure were reported); while for some other pathways, e.g. direct hand contact with carpets treated with products containing PFOS and subsequent oral ingestion, assessment of their importance differs substantially between studies. Future evaluations of the relative contributions of different pathways to overall exposure to PFOS will benefit from recent and on-going improvements in analytical techniques that permit detection of PFOS in foodstuffs and other exposure matrices at lower levels.

Diet

Available food data is broad and diverse. Several studies are focused in a reduced group of food items, mainly on these more likely to contain high levels of PFOS due to their high protein content, as meat, fish or dairies (Malinsky, Jacoby and Reagen, 2011; Chung and Lam, 2014; D'Hollander *et al.*, 2015), while a few are mainly focused in different ones where PFOS could be present due to atmospheric deposition or uptake from soils or water (Herzke *et al.*, 2013; D'Hollander *et al.*, 2015). Many others cover a wider range of food items in order to better estimate daily exposure to PFOS, but differentiating among them and reporting concentrations product by product or for groups of them (Ericson, Martí-Cid, *et al.*,

2008; Ballesteros-Gomez, Rubio and van Leeuwen, 2010; Noorlander *et al.*, 2011; Guerranti *et al.*, 2013). Other studies estimate exposure to PFOS as total diet or duplicated diet, where a mixture of different food items are analysed and reported together with a nutritional study of the mixture (Fromme, Schlummer, *et al.*, 2007; Kärrman *et al.*, 2009). Finally, some publications report levels just as concentration per item or mixture of items, while some others report daily intakes.

As an overall, PFOS exposure though food ingestion has been widely studied but it became a very complex task to resume, due to the very different ways of presenting the data: part of the available information comes from the analysis of individual food items, while part of it comes from groups of food commodities, or from whole diet composites. Data can also come from raw or from cooked commodities – as individual cooked items or as a whole meal –. Moreover, there is no harmonisation on how to report the data, being sometimes presented as concentration per food item or meal, some others as daily intakes (body weight corrected or not), and usually supported by different types of food questionnaires: food diaries, food frequency questionnaires, including food packaging and cooking utensils or not.

Drinking Water

Data concerning concentrations of PFOS in drinking water are rather limited, and all published studies report concentrations in the ng/L range (see *Table 02*). Initially, Saito et al. (Saito *et al.*, 2004) reported PFOS concentrations in tap water from Japan to fall between 0.1 and 12.0 ng/L. Later studies (Skutlarek, Exner and Färber, 2006; Lange *et al.*, 2007; Tanaka *et al.*, 2008; Ericson *et al.*, 2009) have reported higher

concentrations however; up to 58 ng/L and 143 ng/L PFOS in tap water from Spain (Ericson *et al.*, 2009) and Japan (Tanaka *et al.*, 2008) respectively. Overall, PFOS is one of the most frequently detected PFASs – together with PFOA – in drinking water, with detection frequencies varying between 40 and 100 % in published papers. In 2013, Eschauzier et al. (Eschauzier *et al.*, 2013) hypothesised the link between the difference of tap water coming from ground water (usually PFASs free) and the one coming from surface water, often reporting background contamination. Reassuringly, maximum values reported in drinking water to date, fall below the US EPA's short term advisory limit concentration for drinking water of 200 ng/L PFOS.

Table 02. Comparison of reported PFOS concentrations and ranges in drinking water (ng/L)

Authors	Country	n	% DF	A/GM	Median[1]	Range
(Ericson, Nadal, *et al.*, 2008)	Spain	4	100	0.57[1] (GM)	0.59	0.39-0.87
(Ericson *et al.*, 2009)	Spain	40	87	3.72 (GM)	0.51	<0.12-58.12
(Kim, Kho, *et al.*, 2011)	Korea	15	NR	NR	NR	<0.33-11.00
(Loos *et al.*, 2007)	Italy	6	100	8.1 (A)	NR	6.20-9.70
(Saito *et al.*, 2004)	Japan	30	67	0.7-12.5[2] (GM)	0.65	<0.10-12.00
(Skutlarek, Exner and Färber, 2006)	Germany	37	35	2.09[1] (GM)	1	<1.00-22.00
(Takagi *et al.*, 2008)	Japan	26	96	1.51 (GM)	1.9	<0.16-22.00
(Tanaka *et al.*, 2008)	Japan	NR	NR	NR	NR	<0.01-143.0

1) For concentrations < LOQ, the value was assumed = 1/2 LOQ. 2) Estimated in 6 different areas

DF: Detection frequency. A: Average. GM: Geometric mean. NR: Not reported

Table 03. Comparison of reported PFOS concentrations and ranges in indoor dust (ng/g)

Authors	Country/Microenvironment Category	n	% DF	Average	Median [1]	Range
(Björklund, Thuresson and De Wit, 2009)	Sweden / Houses	10	100	49 [2]	39	15-120
	Sweden / Apartments	38	79	175 [2]	85	<8.0-1,100
	Sweden / Offices	10	100	144 [2]	110	29-490
	Sweden / Daycare centres	10	100	38 [2]	31	23-65
	Sweden / Cars	5	60	18 [2]	12	<8.0-33
(Ericson Jogsten *et al.*, 2012)	Spain / Houses	10	100	2.1	2.2	0.13-12.0
(Fraser *et al.*, 2013)	Czech Republic / Offices	31	55	14.6 [3]	NR	6.8-98.2
	Czech Republic / Homes	30	27	26.9 [3]	NR	14.1-280
	Czech Republic / Vehicles	13	16	15.8 [3]	NR	10.1-280
(Goosey and Harrad, 2011)	UK / Cars	20	100	132	97	20-1,500
	UK / Classrooms	42	100	640.7	980	22-3,700
	UK / Houses	45	100	144.7	450	3.5-7,400
	UK / Offices	20	100	182.5	370	20-1,000
	Australia / Houses	20	100	187	170	6.5-8,100
	Canada / Houses	19	100	157.8	140	42-1,300
	France / Houses	10	100	193.8	160	54-1,700
	Germany / Houses	10	100	188.9	170	47-1,000
	Kazakhstan / Houses	9	80	12.5	59	<0.03-130
	Thailand / Houses	20	100	19.5	16	3-130
	USA / Houses	10	100	318.1	310	110-930
(Kato, Calafat and Needham, 2009)	Australia / Houses	39	74	NR	480	<2.6-18,000
(Kubwabo *et al.*, 2005)	Canada / Houses	67	67	443.7	37.8	2.3-5,065
(Moriwaki, Takata and Arakawa, 2003)	Japan / Houses	16	100	39.5	25	15.0-2,500
(Shoeib *et al.*, 2016)	Egypt / Houses	17	58	NR	0.29	0.23 -2.16
(Strynar and Lindstrom, 2008)	USA / Houses (102) and child day care centres (10)	112	95	761	201	<8.9-12,100
(Tian *et al.*, 2016)	Korea / Houses	17	93	13.7 [2]	11.4	0.7-52.1
(Xu *et al.*, 2013)	Germany / Houses	31	100	97.1 [2]	NR	32.2-2456

1) For concentrations < LOQ, the value was assumed = 1/2 LOQ. 2) Arithmetic mean. 3) Geometric mean.

DF: Detection frequency. NR: Not reported. N = number of cases.

Table 04. Comparison of reported PFOS concentrations and ranges in indoor and outdoor air (pg/m^3)

Authors	Country	Source	n	% DF	Mean	Median[1]	Range
(Barber *et al.*, 2007)	Norway	Indoor air	4	0	NR	NR	<LOD
(Ericson Jogsten *et al.*, 2012)	Spain	Indoor air	10	33	0.3	0.1	<0.13-67.0
(Goosey and Harrad, 2012)	UK	Indoor air	20	90	12.4	11.5	<1.0-400.0
	UK	Indoor air	12	100	49.4	55	12.0-89.0
(Shoeib *et al.*, 2011)	Canada	Indoor air	39	0	<LOD	<LOD	<LOD
(Barber *et al.*, 2007)	UK	Outdoor air	2	NR	NR	NR	<LOD-46
	UK	Outdoor air	10	NR	NR	NR	<LOD-1.6
(Dreyer and Ebinghaus, 2009a)	Germany	Outdoor air	117	0	<LOD	<LOD	<LOD
	Germany	Outdoor air	121	0	<LOD	<LOD	<LOD
(Genualdi *et al.*, 2010)	Diff. Countries	Outdoor air	20	50	NR	NR	2.03-149.5
(Goosey and Harrad, 2012)	UK	Outdoor air	10	70	1.5	1.6	<0.1-6.1
(Shoeib *et al.*, 2011)	Canada	Outdoor air	6	0	<LOD	<LOD	<LOD

1) For concentrations < LOQ, the value was assumed = 1/2 LOQ. N = number of cases.

DF: Detection frequency. NR: Not reported. LOD = Limit of detection

Table 05. Index of published research articles monitoring PFOS precursors in food commodities

Authors	Analytes	Country	n	Matrix
(Van Leeuwen *et al.*, 2009)	PFOS, PFOSA	Netherlands	Intercomparison	Fish
(van Leeuwen *et al.*, 2009)	PFOS, PFOSA	Netherlands	Intercomparison	Water, fish
(Gebbink *et al.*, 2015)	PFOS, FOSA, FOSAA, EtFOSAA, MeFOSAA	Sweden	130	Diet
(Ullah *et al.*, 2014)	PFOS, FOSA, EtFOSAA, MeFOSAA	Sweden	21	Fish
(Tittlemier, Pepper and Edwards, 2006)	FOSA, EtFOSA, MeFOSA	Canada	151	Diet

N = number of cases.

Table 06. Index of published research articles monitoring PFOS precursors in indoor dust

Authors	Analytes	Country	n	Matrix
(Haug, Huber, Schlabach, *et al.*, 2011)	PFOS,PFOSA, EtFOSA, MeFOSA, MeFOSE	Norway	41	Household dust
(Shoeib *et al.*, 2011)	PFOS, EtFOSA, MeFOSA, EtFOSE, MeFOSE	Canada	152	Household dust
(Goosey and Harrad, 2011)	PFOS, FOSA, EtFOSA, MeFOSA, EtFOSE, MeFOSE	Australia	20	Household dust
		Canada	20	Household dust
		France	9	Household dust
		Germany	10	Household dust
		Kazakhstan	9	Household dust
		Thailand	20	Household dust
		UK	45	Household dust
		US	10	Household dust
		UK	20	Cars
		UK	42	Classrooms
		UK	20	Offices
(Lankova *et al.*, 2015)	PFOS, FOSA, EtFOSA, MeFOSA	Czech Republic	18	Household dust
(Fraser *et al.*, 2013)	PFOS, EtFOSE, MeFOSE	US	30	Home. Exposed workers
		US	31	Offices. Exposed workers
		US	13	Cars. Exposed workers
(Kato, Calafat and Needham, 2009)	PFOS, PFOSA, EtFOSA, MeFOSA, EtFOSE, MeFOSE	UK	9	Household dust
		Australia	10	Household dust
		Germany	10	Household dust
		US	10	Household dust

N = number of cases.

Indoor Air and Dust

In addition to drinking water; relatively recent investigations show the indoor environment is a potentially important contributor to human exposure to PFASs including PFOS (Fromme *et al.*, 2009; D'Hollander *et al.*, 2010; Goosey and Harrad, 2011; Haug, Huber, Schlabach, *et al.*, 2011). The first paper concerning PFOS contamination of indoor dust was published in 2003, by Moriwaki et al. (Moriwaki, Takata and Arakawa, 2003) (*Table 03*). Sixteen samples of house dust were analysed, containing concentrations of PFOS between 11 and 2,500 ng/g. Since then, similar studies have been carried out in Canada, Japan, Sweden, USA, Australia, the

UK, Egypt, Germany, Czech Republic, and Spain, with wide variation in concentrations found. While Bjorklund et al. (Björklund, Thuresson and De Wit, 2009) reported concentrations of PFOS in dust from 10 houses in Sweden in 2009 to range between 15 and 120 ng/g, Tian et al. (Tian *et al.*, 2016) reported average of 13.7 ng/g for Korean houses and even much lower data was reported by Shoeib et al. (Shoeib *et al.*, 2016) about Egyptian indoor environments with a median of 0.29 ng/g. Strynar and Lindstrom (Strynar and Lindstrom, 2008) and Kato et al. (Kato, Calafat and Needham, 2009) reported substantially higher concentrations, ranging between 8.9 and 12,100 ng/g in the USA, and 2.6 and 18,000 ng/g in Australia. Median concentrations further reflect international variations, being 38 ng/g for the Swedish study, and 201 ng/g and 480 ng/g for the Canadian and Australian surveys respectively. Moreover, Goosey and Harrad (Goosey and Harrad, 2011) also reported statistically significant differences ($p < 0.05$) between concentrations of PFOS in dust from different countries. Specifically, UK, Australia, Canada, France, Germany, and US > Kazakhstan; and UK, Australia, Canada, and US > Thailand. They attributed such differences to lower use of products containing PFASs in Kazakhstan and Thailand compared to Europe, North America, and Australia.

Moreover, recent studies have reported concentrations of PFOS and other PFASs in indoor air (principally vapour phase, but with some particulate phase compounds incorporated) (Goosey and Harrad, 2011; Ericson Jogsten *et al.*, 2012; Shoeib *et al.*, 2016). In these, PFOS was the most prevalent PFASs, with a wide range of concentrations between countries (for example, lower values detected in Spain, higher in the UK). The frequency of detection for PFOS in indoor air is more variable than for dust (in air the range is from 0 % to 100 % c.f. 60°% to 100 % for dust).

Outdoor Air

Outdoor air has also been studied, sometimes in conjunction with indoor air. Shoeib et al. (Shoeib *et al.*, 2005) reported PFASs concentrations in outdoor air were 1 or 2 orders of magnitude lower than in indoor air, as data from more recent studies in *Table 04* corroborate. This is consistent with the hypothesis that substantial indoor sources of PFOS exist, with the result that indoor air likely exerts an appreciable influence on outdoor atmospheric contamination. While this would logically lead to higher atmospheric concentrations of PFOS in conurbations due to higher urban building densities; Barber et al. (Barber *et al.*, 2007) reported higher detection frequencies of PFASs (including PFOS) than expected in outdoor air from rural areas. Such findings suggest the environmental distribution of PFASs is complex, and that indoor environments are not the only driver influencing outdoor contamination.

1.4.2 Indirect Sources of Human Exposure

POSF-derived substances may be metabolised *in vivo* to PFOS, constituting a substantial indirect source of human exposure to PFOS. The so called "POSF-derived substances" in general are a mixture of compounds with structures with the general formula C8F17SO2NRR', that are referred to generically as "PFOS-precursors" (or "PreFOS" in some literature, such as Asher et al. (Asher *et al.*, 2012)). The main PFOS-precursor substances and its salts were listed in *Table 01*.

It has been shown that some of these called PFOS-precursors are degraded to PFOS by *in vivo* metabolic processes (Xu *et al.*, 2004; Martin *et al.*, 2010; Chen *et al.*, 2015). Some PFOS-precursors like N-EtFOSA and N-EtFOSE, have shown low conversion

factors < 1 % in rats and trout (Tomy *et al.*, 2004; Xu *et al.*, 2004), have been studied in earthworms (Zhao *et al.*, 2016) or have not yet previously reported (as N-MeFOSA, presented in later chapters). However, in 2003, Seacat et al. (Seacat *et al.*, 2003) reported a conversion factor to PFOS of up to 20 % in a study where rats were exposed long term to N-EtFOSE; an observation confirmed subsequently by Xie et al. (Xie *et al.*, 2009). In 2015, Chen et al. (Chen *et al.*, 2015) studied both, *in vitro* and *in vivo* (in carps) isomeric biotransformation of FOSA to PFOS, both tests moving towards the idea of the biotransformation of FOSA to PFOS taken place in the liver of the fish, differing from what was the previously observed in rats and monkeys. That same study also revealed the isomeric differences in the metabolism, showing a decrease of the percentage of the branched isomers of FOSA at the end of the incubation, while that percentage increased during the period of time the incubation was carried out.

Although the reported levels of PFOS-precursors are generally lower and their physicochemical properties differ from those of PFOS, a variety of them have been detected in water (Dreyer and Ebinghaus, 2009b), in indoor and outdoor air (Taniyasu *et al.*, 2005; Jahnke *et al.*, 2007), in food (Tittlemier, Pepper and Edwards, 2006; Gebbink *et al.*, 2015), and in live organisms (from mussels to bald eagles) and water-bird eggs (Kannan *et al.*, 2005; Wang *et al.*, 2008). One of the most measured PFOS-precursors is perfluorooctane sulfonamide (FOSA), which is a stable intermediate in the pathway of PFOS-precursor degradation to PFOS (Martin *et al.*, 2010). Perfluorinated sulfonamide based products (PFSAm) are also important, as their production is associated with the presence of FOSAs and FOSEs as degradation or residual products. Positive correlations between the concentrations of FOSA and

PFOS have been found in biological samples (e.g. (Martin *et al.*, 2004)) suggesting that FOSA, and maybe other PFOS-precursors, can be important contributors to body burdens of PFOS in animal species (Asher *et al.*, 2012)(see *Table 05* and *Table 06*).

As mentioned above, recent papers have examined the utility of human exposure models to evaluate the contribution of indirect exposure pathways to human body burdens of PFOS (Vestergren *et al.*, 2008; Fromme *et al.*, 2009; D'Eon J and Mabury, 2011; Gebbink, Berger and Cousins, 2015). Such studies are still quite limited in number, but their general consensus is that the significance of indirect sources in driving human body burdens of PFOS should be taken into account, or even had hitherto been underestimated (e.g. (D'Eon J and Mabury, 2011)). This becomes even more important in the wake of the 3M Company phase out, as while direct sources of PFOS exposure are expected to decrease in the general population, indirect sources stemming from continued use of PFOS-precursors remain (see *Figure 03*). Vestergren et al. (Vestergren *et al.*, 2008) suggested the relative contributions of direct and indirect exposure were dependent on the level of exposure. For them, while under low and intermediate exposure scenarios – when the four main exposure pathways were considered (diet, water, dust and air) –, direct dietary exposure appeared the principal pathway, intake of PFOS under a high-end exposure scenario was dominated by indirect precursor exposure via indoor dust (41 – 68 %), and indoor air (10 – 19 %). The study of Gebbink et al. (Gebbink, Berger and Cousins, 2015) considered comparable pathways of exposure to those studied by Vestergren et al: According to Gebbink et al., low exposure scenario was clearly dominated by direct exposure to PFOS through diet (≈ 86 %), followed by indirect

exposure to through air (≈ 8 %). Intermediate exposure scenario was also dominated by direct exposure through diet but in a smaller percentage (≈ 65 %), while direct exposure through dust (≈ 10 %), indirect exposure through air (≈ 10 %), and direct exposure through water (≈ 5 %) increased their relative contribution. Finally, high exposure scenario showed ≈ 60 %.of direct exposure from diet (≈ 50 %), dust (≈ 12 %) and water (≈ 10 %), while indirect exposure constituted more than 30 %, divided between dust and air, being this scenario the one with higher proportion of indirect exposure (PFOS precursors exposure) contribution. However, total exposure in the Gebbink et al. study was 1-2 orders of magnitude lower, with indirect exposure to PFOS making higher and lower contributions to overall exposure under low (11 %) and high (33 %) exposure scenarios respectively than estimated previously. Gebbink et al. attributed the differences between their observations and those of previous studies, to their use of recent data reporting lower levels of PFOS and PFOS-precursors in human diet (Ullah *et al.*, 2014; Gebbink *et al.*, 2015). Furthermore, other reasons such as the use of more recently published biotransformation factors describing the conversion of precursors, as well as the development of more sensitive analytical methods – with the quantification of PFOS precursors in much lower concentrations, or even in detectable levels that not sensitive instruments would not be able to reach – were identified as causes of the lower exposure estimates. Moreover, D'Eon and Mabury (D'Eon J and Mabury, 2011) critically reviewed the contribution of PFOS precursors to observed body burdens of PFOS, and suggested that studies to date may underestimate the contribution of such indirect exposure. This was principally due to the fact that such studies consider indirect exposure to occur only as a result of

exposure to PFOS precursors present as impurities or residual products from the manufacture of PFOS, but do not include exposure arising from manufacture and use of the precursors themselves.

In summary, studies to date suggest strongly that indirect exposure to PFOS makes an important contribution to human body burdens, and these relative contributions might be strongly exposure-dependent. However, such studies are not yet conclusive. For example, estimates of the contribution of such exposure varies between 10 % and 70 % of the daily intake of PFOS in the studies of Verstergren et al. (Vestergren *et al.*, 2008) and Gebbink et al. (Gebbink, Berger and Cousins, 2015) (based on the three different scenarios) and Fromme et al. (Fromme *et al.*, 2009). Such variation is attributable to inherent uncertainties in pivotal parameters such as the estimated efficiency of precursor metabolism to PFOS. At the current time, efforts must focus on addressing: 1) the lack of data on the toxicokinetics of various PFOS-precursor compounds in animals, 2) the difficulty in extrapolating rodent data to humans, and 3) the fact that many commercially relevant PFOS precursors have yet to be determined in any sample (Martin *et al.*, 2010). Overall, the uncertainties associated with studies to date, highlight a clear need for alternative approaches, and a small but growing number of studies suggests that exploitation of the chiral properties of some PFOS isomers and their precursors may constitute one such approach (Wang *et al.*, 2009; Liu *et al.*, 2015).

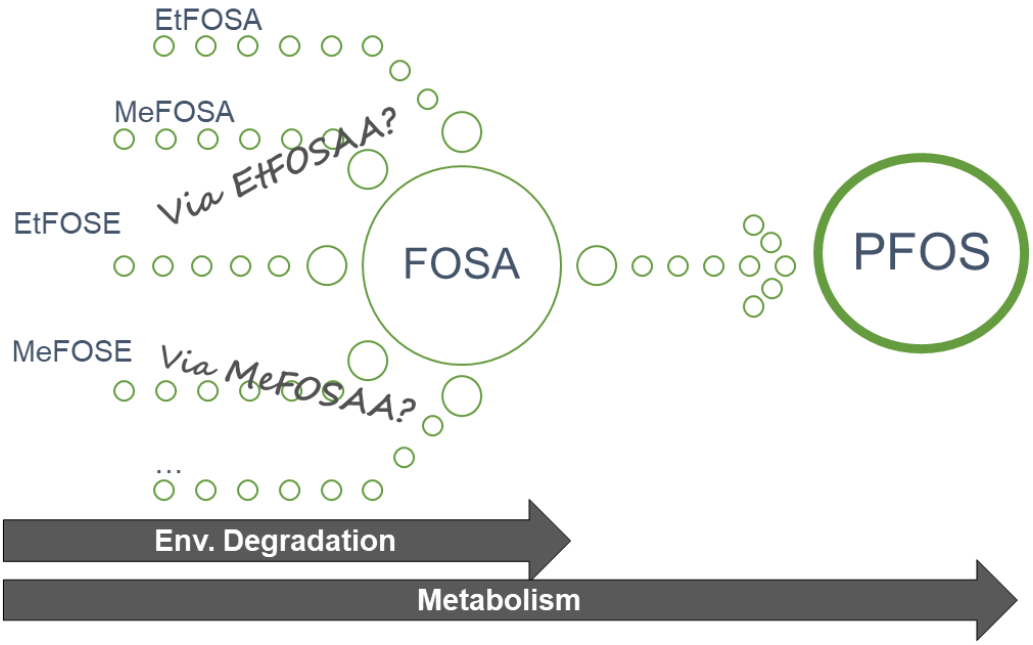


Figure 03. Schematic overview of the processes leading to PFOS from PFOS precursors exposure

1.5 HUMAN BIOMONITORING DATA

With respect to human biomonitoring, concentrations of PFASs in human blood (whole blood, plasma and serum) in the general population have been reviewed recently (Fromme *et al.*, 2009; Angerer *et al.*, 2011) (*Table 07*). Most human biomonitoring studies are not carried out on whole blood, but on serum. The first reported concentrations of PFOS in blood were published by Hansen et al. (Hansen *et al.*, 2001). This study showed 100 % of the blood samples contained PFOS at concentrations ranging from 6.7 to 81.5 ng/mL. Following this seminal report, concern about how PFOS enters and remains in the human body increased, leading to the publication of a number of studies, each based on the analysis of a large number of blood samples. Amongst the most relevant of these are those of Calafat et al. and Kato et al. (Calafat, Kuklenyik, *et al.*, 2007; Calafat, Wong, *et al.*, 2007; Kato *et*

al., 2011) in the North American population, which each discuss results from the National Health and Nutrition Examination Surveys (NHANES) carried out by the US Center for Disease Control and Prevention (CDC), and published in the Fourth National Report on Human Exposure to Environmental Chemicals (CDC, 2009a, 2009b). In these reports, the presence of a range of chemical contaminants is studied in blood and urine from the general population of the USA. The PFOS measurements reported in the two papers from Calafat et al. refer to the NHANES results from 1999-2000 and 2003-2004, and are based on 1,562 and 2,094 serum samples, with a detection frequency (DF) > 96 % for PFOS in both studies, and geometric means of 21.1 and 20.7 ng/mL respectively. One of the studies (Calafat, Wong, *et al.*, 2007), also reported that geometric mean PFOS levels declined by 32 % between 1999/2000 and 2003/2004. Moreover, the most recent (2007/2008) NHANES results (Kato *et al.*, 2011), indicate that PFOS concentrations continue to decline (exemplified by a geometric mean of 13.2 ng/mL). This follows an earlier report (Olsen, Mair, *et al.*, 2007) of a decrease on PFOS levels in human blood in the general American population, from a geometric mean of 33.1 ng/mL in samples collected in 2000, to 15.1 ng/mL in samples collected in 2005. A second study (Olsen *et al.*, 2008) based on a large number of human blood samples (around 600), highlighted that the observed ≈ 60 % decline in PFOS was consistent with its elimination half-life and the time period since the phase-out of PFOS by 3M Company in 2000–2002. Combined, these studies suggest that restrictions on the production and use of PFOS have led to reductions in human exposure in the US, although it remains in the environment, wildlife and the US population (CDC, 2009a). Other US studies document similar PFOS concentrations in blood, but cannot provide evidence of a temporal trend.

Specifically, Hansen et al. (Hansen *et al.*, 2001), as well as Olsen et al. (Olsen *et al.*, 2005), published results in which median PFOS concentrations were 26.2 and 34.7 ng/mL for samples taken in the late 1990s/early 2000s (exact sampling dates not given) and 1974/1989 respectively. This apparent increase in human exposure in the immediate aftermath of the 2002 voluntary cessation of production by 3M Company may be attributed to variation in the respective populations sampled in the two studies.

An important point is that – in line with Taniyasu et al., (Taniyasu *et al.*, 2003) – the values in *Table 07* include data for both serum and whole blood. This approach is preferred here to the alternative format employed by others (e.g. (Kannan *et al.*, 2004; Yeung *et al.*, 2006)) whereby concentrations in whole blood were converted to concentrations in serum by multiplying whole blood concentrations by 2, to allow comparison across different studies. This conversion becomes even more sensitive when analysing PFOS precursors, due to their different distribution between serum and blood (Martin *et al.*, 2010). Notwithstanding the influence of serum versus whole blood basis concentrations, examination of the global database between 2004 and 2007, reveals some differences in both median and maximum PFOS concentrations in human blood recorded in different studies shown in *Table 07*. Likely causes of these between-study variations in the concentrations of PFOS include international variations in use and exposure, as well as variations between sampled populations in lifestyle, age, ethnicity, and gender (Kato *et al.*, 2011). While such differences in absolute concentrations of PFOS exist, they are not as marked as those observed for other halogenated persistent organic pollutants like polybrominated diphenyl ethers (Hites, 2004).

On the other side, PFOS precursors – including FOSAs beyond FOSA, FOSEs and FOSAAs – have been much less reported, as *Table 08* shows.

Table 09 reveals that, in addition to blood, human milk is being monitored increasingly. This shift towards monitoring milk may be attributed to its less invasive nature, greater sample availability and mass, recent improvements in the sensitivity and accuracy of ultra-trace analytical techniques (although these are likely still worse than for serum), and the dual role of human milk as an indicator of both the donor's body burden, and dietary intake of nursing infants. Of course, this is offset to some degree by the fact that human milk as a biomonitoring tool is restricted to a specific sector of the population. Moreover, comparing *Table 07 and Table 09*, it is apparent that concentrations of PFOS in human blood exceed those in human milk. Several studies of human milk have been carried out since the first published reports. Most such studies show DF > 90 %, except those of Bernsmann and Fürst (Bernsmann and Fürst, 2008) (DF of 66 % in Germany), and Guerranti et al. (Guerranti *et al.*, 2013), in which the detection frequency was below 50 % (DF of 41 % in Italy). Median concentrations range from 0.04 to 0.33 ng/mL, except for the study of Roosens et al. (Roosens *et al.*, 2010) for the Flemish general population, who reported a median concentration an order of magnitude higher than other studies (2.9 ng/mL). Some of the samples reported by Roosens et al. were collected from donors living near a PFOS production facility, for which the authors also reported high concentrations of PFOS in serum. Elevated concentrations of PFOS had also been reported previously in biota from the same location by Dauwe et al. (Dauwe *et al.*, 2007).

Table 07. Comparison of reported PFOS concentrations and ranges in human blood or serum (ng/mL)

Authors	Country	Matrix	n	% DF	Mean	Median[1]	Range
(Ericson *et al.*, 2007)	Spain	Whole blood	48	100	7.64	7.6	0.76-16.2
(Fromme, Schlummer, *et al.*, 2007)	Germany	Plasma	356	100	13.5	13.7	2.1-55.0
(Hansen *et al.*, 2001)	USA	Serum	65	100	25.519	25.7	6.7-81.5
(Haug, Huber, Becher, *et al.*, 2011)	Norway	Serum	41	100	6.9	NR	2.3-15.0
(Holzer *et al.*, 2008).	North Rhine	Plasma	80	NR	NR	4.3	1.5-26.2
(Kannan *et al.*, 2004).	USA	Serum	46/29[2]	91/93[2]	32.5/32.9[2]	28.9/26.2[2]	<1.3-124
	USA	Whole blood	11/19[2]	100/100[2]	66/73.2[2]	81/72[2]	11-164
	USA	Plasma	70	100	42.8	42	16-83
	Colombia	Whole blood	25/31[2]	100/100[2]	8.0/8.5[2]	7.3/8.1[2]	4.6-14
	Brazil	Whole blood	17/10[2]	100/100[2]	10.7/13.5[2]	8.4/12.7[2]	4.3-35
	Italy	Serum	8/42[2]	87.5/90.5[2]	4.4/4.3[2]	3.5/4.2[2]	<1-10.3
	Poland	Whole blood	15/10[2]	100/100[2]	33.3/55.4[2]	33.8/40.9[2]	16.0-116
	Belgium	Plasma	4/16[2]	100/100[2]	11.1/16.8[2]	10.4/17.6[2]	4.5-27.0
	India	Serum	11/34[2]	55/50[2]	2.3/1.7[2]	2.5/1.3[2]	<1-3
	Malaysia	Whole blood	7/16[2]	100/100[2]	11.7/13.2[2]	12.7/13.1[2]	6.2-18.8
	Korea	Whole blood	25/25[2]	100/100[2]	15.1/27.1[2]	11.3/18.3[2]	3.0-92
	Japan	Serum	13/25[2]	100/100[2]	20.1/14.1[2]	18.3/12.4[2]	4.1-40.3
(Kärrman, van Bavel, *et al.*, 2006)	Sweden	Whole blood	66	100	16	17.1	1.7-37.0
(Kärrman, Mueller, *et al.*, 2006)	Australia	Serum	40	NR	21.3	20.8	12.7-29.5
(Kärrman, Langlois, *et al.*, 2007)	Sweden	Serum	12	100	20.7	18.7	8.2-48.0
(Calafat, Kuklenyik, *et al.*, 2007; Calafat, Wong, *et al.*, 2007; Kato *et al.*, 2011)	USA	Serum (years 99-00)	1,562	100	30.4	NR	NR
		Serum (years 03-04)	2,094	99.9	20.7	NR	NR
		Serum (years 05-06)	2,120	99.9	17.1	NR	NR
		Serum (years 07-08)	2,100	99.8	13.2	NR	NR
(Midasch, Schettgen and Angerer, 2006)	Germany	Plasma	105	100	NR	22.3	6.2-131.0
(Olsen *et al.*, 2005)	USA	Serum	178	NR	30.1	29.5	NR
		Plasma	178	NR	33.3	34.7	NR
(Yeung *et al.*, 2006)	China	Serum	85	NR	NR	52.7	NR

1) For concentrations < LOQ, the value was assumed to = 1/2 LOQ. 2) Separate female/male data reported for this study.
DF: Detection frequency. NR: Not reported. N = number of cases.

Table 08. Index of published research articles monitoring PFOS precursors in human biological matrices

Authors	Analytes	Country	n	Matrix
(Glynn *et al.*, 2012)	PFOS, FOSA	Sweden	413	Serum
(Yeung *et al.*, 2013)	PFOS, FOSAA, EtFOSAA, MeFOSAA	Germany	420	Blood
(Lee and Mabury, 2011)	PFOS, FOSAA, EtFOSAA, MeFOSAA	US	50	Serum
(Gebbink, Glynn and Berger, 2015)	PFOS, FOSA, FOSAA, EtFOSAA, MeFOSAA	Sweden	30	Serum
	PFOS, PFOSA, PFOSAA, FOSAA, EtFOSAA	US	238	Serum
(Van Leeuwen *et al.*, 2006)	PFOS, PFOSA	Netherlands	Intercomparison	Whole blood
(Fraser *et al.*, 2013)	PFOS, PFOSA, EtFOSE, MeFOSE	US	31	Home. Exposed workers

N = number of cases.

In contrast to blood and milk, only a small number of papers have reported concentrations of PFASs in other human matrices such as: liver, seminal plasma, and umbilical cord blood (Olsen *et al.*, 2003; Inoue *et al.*, 2004; Kuklenyik *et al.*, 2004; So *et al.*, 2006; Apelberg *et al.*, 2007; Kärrman, Ericson, *et al.*, 2007; Midasch *et al.*, 2007; Guruge *et al.*, 2011).

Scientific understanding of the origins of and influences on the presence of PFOS in humans is complicated by a number of factors (Lindstrom, Strynar and Libelo, 2011). Just as environmental degradation of PFOS precursors constitutes an important indirect source of PFOS contamination of the ambient environment; external exposure to PFOS precursors followed by *in vivo* metabolism, has been identified as a potentially substantial indirect contributor to human body burdens of PFOS (Trudel *et al.*, 2008; Vestergren *et al.*, 2008). Such indirect pathways are distinct from direct exposure via human contact with and uptake of PFOS itself. Moreover, PFOS (as well as other long chain PFASs) tend to accumulate in the human body with an estimated half-life of around 5 years (Olsen, Burris, *et al.*, 2007). This slow elimination from the human body hampers efforts to determine how changes

in lifestyle, diet, or other exposure-related factors influence blood levels. Notwithstanding this, while age has been suggested to exert little influence on circulating PFASs concentrations, with inconsistent results in cross-sectional studies (Harada *et al.*, 2007; Haug, Thomsen and Becher, 2009b), age associations could be consistent with dietary exposure in a post phase out situation (Nost *et al.*, 2014). However, as highlighted above, gender and ethnicity do seem to influence the accumulation of some compounds. In a recent paper, Kato et al. (Kato *et al.*, 2011) attributed differences in human body burdens between ethnic groups to ethnic differences in exposure related to lifestyle, the use of products containing PFASs, and diet. Meanwhile, gender-related differences in body burden (lower concentrations in women than men) have been attributed to physiological differences (i.e. accumulation and elimination), as well as pregnancy, lactation and menstruation (Harada *et al.*, 2004).

1.6 ISOMER PATTERNS OF PFOS AND ITS PRECURSORS

Historically, ΣPFOS has been quantified together (see *Table 02* to *Table 04, Table 07* and T*able 09*). Recently however, new approaches (discussed further below) have been have been suggested as biomarkers of exposure and applied in efforts to differentiate between direct exposure to PFOS and PFOS-precursor exposure (Benskin, Holt and Martin, 2009; Martin *et al.*, 2010).

Table 09. Comparison of reported PFOS concentrations and ranges in human breast milk (ng/mL)

Authors	Year	Country	n	% DF	Mean	Median[1]	Range
(Antignac *et al.*, 2013)	2013	France	48	90	0.092	0.075	<0.050-0.330
(Bernsmann and Fürst, 2008)	2008	Germany	203	66	NR	0.082	0.05-0.284
(Fromme *et al.*, 2010)	2010	Germany	201	72	NR	0.04	<0.030-0.110
(Guerranti *et al.*, 2013)	2013	Italy	49	41	0.85	NR	<1.020-4.280
(Haug, Huber, Becher, *et al.*, 2011)	2011	Norway	19	100	0.093	0.087	0.004-0.250
(Kadar *et al.*, 2011)	2011	France	30	100	NR	0.074	0.024-0.171
(Kārrman *et al.*, 2010)	2010	Spain	10	100	0.12	0.11	0.070-0.220
(Kim, Lee, *et al.*, 2011)	2011	Korea	17	100	0.061	NR	0.032-0.130
(Liu *et al.*, 2010)	2010	China	24	100	0.046	0.049	0.006-0.137
(Llorca *et al.*, 2010)	2010	Spain	20	95	0.071	0.084	0.028-0.865
(Mosch *et al.*, 2010)	2010	Germany	20	95	NR	0.049	<0.030-0.195
(Roosens *et al.*, 2010)	2010	Belgium	22	NR	NR	2.9	<0.400-28.2
(Sundstrom *et al.*, 2011)	2011	Sweden	202	100	0.156	0.206	0.088-0.151
(Tao *et al.*, 2008)	2008	USA	45	96	NR	0.106	<0.032-0.617
		Cambodia	24	100	0.067	0.04	0.017-0.327
		Vietnam	40	100	0.076	0.058	0.017-0.393
		Indonesia	20	100	0.084	0.067	0.025-0.256
		Philippines	24	100	0.098	0.104	0.027-0.208
		Malaysia	13	100	0.121	0.111	0.049-0.350
		India	39	85	0.046	0.039	<0.011-0.120
		Japan	24	100	0.232	0.196	0.140-0.523
(Thomsen *et al.*, 2010)	2010	Norway	68	NR	NR	0.11	0.028-0.36
(Volkel *et al.*, 2008).	2008	Germany	19	100	0.116	0.113	0.028-0.239
		Germany	38	100	0.126	0.123	0.033-0.309
		Hungary	13	100	0.317	0.33	0.096-0.639

1) For concentrations < LOQ, the value was assumed = 1/2 LOQ. 2) 20 pools of human milk

DF: Detection frequency. NR: Not reported. N = number of cases.

Table 10. Linear versus branched chain composition profiles and enantiomer fractions (EFs) of PFOS and its precursors in various matrices

Authors	Country	Study	Matrix	n	Analytes
(Asher *et al.*, 2012)	Canada	Lake	Aquatic Species	67	FOSA (≈57 % linear)
					PFOS (>90 % linear)
			Water	2	PFOS (70 % linear)
			Sediment	3	PFOS (>90 % linear)
(Beesoon *et al.*, 2011)	Canada	Human	Dust	18	PFOS (≈70 % linear)
			Serum	20	PFOS (≈64 % linear)
			Cord serum	20	PFOS (≈54 % linear)
(Benskin, Bataineh and Martin, 2007)	Canada	Human	Serum	14	PFOS (≈80 % linear)
(Haug, Thomsen and Becher, 2009b)	Norway	Human	Serum	57	PFOS (53-78 % linear)
(Olsen *et al.*, 2008)	Canada	Niagara/Lake	Fish	22	PFOS (88-93 % linear)
			Water	NR	PFOS (43-56 % linear)
(Kärrman, Langlois, *et al.*, 2007)	Sweden	Human	Serum/blood	17	PFOS (68 % linear)
	UK			13	PFOS (59 % linear)
	Australia			40	PFOS (59 % linear)
(Ross, Wong and Martin, 2012)	Canada	Animals	Blood	8	FOSA (≈78 % linear)
			Blood	8	PFOS (≈77 % linear)
			Heart	8	FOSA (≈93 % linear)
			Fat	8	FOSA (≈86 % linear)
(Sharpe *et al.*, 2010)	Canada	-	Fish	NR	PFOS (>70 % linear)
(Wang *et al.*, 2011)	Canada	Animals	Rats	3	1mPFOS (EF≈0.5)
		Human	Serum	8	1mPFOS (EF=0.43)
		Human	Serum	7	1mPFOS (EF=0.35-0.43)
(Zhang *et al.*, 2013)	China	Human	Serum	129	PFOS (48 % linear)

N = number of cases. EF = Enantiomeric fraction

1.6.1 Isomer Profiles

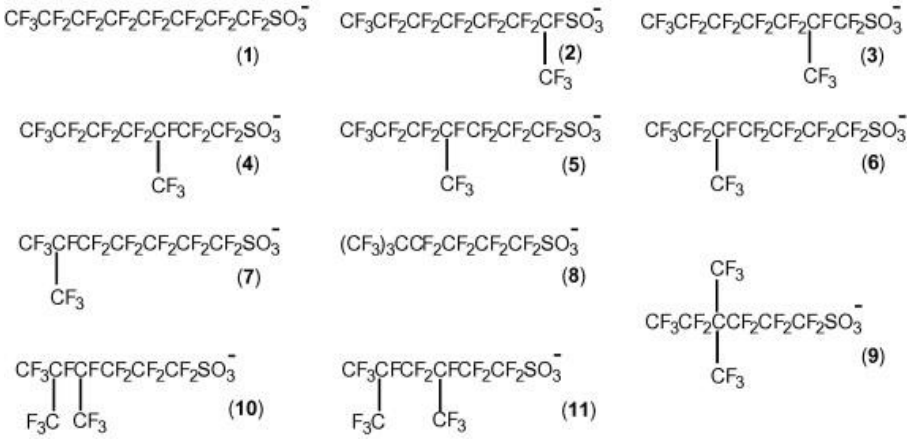


Figure 04. Structure of linear (n) PFOS isomer (1), and branched (br) FOS isomers (2 to 11)

As described above, the processes via which PFOS precursors (i.e. FOSAs and FOSEs) are manufactured are expected to produce about 70 % of the linear isomer, with the remaining 30 % made up of a mixture of various branched chain isomers (see *Figure 04*). In contrast, due to preferential retention of linear PFOS in humans and rats, PFOS isomer profiles in animal species are expected to comprise < 30 % branched chain isomers. While this holds true for species such as fish and gulls for which ≥ 90 % of PFOS is the linear isomer (Houde *et al.*, 2008; Gebbink and Letcher, 2010; Asher *et al.*, 2012) (*Table 10*); in some human samples, the proportion of branched chain isomers can be 40-50 % (Kärrman, Langlois, *et al.*, 2007; Beesoon *et al.*, 2011; Zhang *et al.*, 2013; Liu *et al.*, 2015). Moreover, an *in vitro* study using human microsomes has showed branched chain FOSAs to be preferentially metabolised to PFOS relative to linear FOSA (Benskin, Holt and Martin, 2009). This provides further evidence that precursor exposure may account for human PFOS isomer profiles that are enriched in branched chain isomers. This enriched profile in some human

samples has been hypothesised as providing evidence of precursor exposure. Moreover, observed temporal and within-population variations in the relative abundance of branched chain PFOS isomers in humans (Kärrman, Langlois, *et al.*, 2007; Haug, Thomsen and Becher, 2009b), may be at least partly attributable to concomitant variations in precursor exposure. In fact, the study of temporal trends by Liu et al. (Liu *et al.*, 2015), shows the percentage of branched isomers in the Swedish population has increased from 32 to 45 % between 1996 and 2010, suggesting that exposure to PFOS precursors is becoming more important compared to direct exposure, as predicted by the theoretical models discussed earlier.

Current evidence to support the PFOS precursors exposure hypothesis is not clear-cut however (Ross, Wong and Martin, 2012). While excretion in rats of branched chain FOSAs exceeded that of the linear isomer; a corresponding increase in the relative abundance of the sum of branched chain PFOS isomers was not observed in the same animals. More detailed analysis of the relative abundance of individual branched chain isomers in this study suggests a more complex situation. While the relative abundance in the studied rats of one branched isomer (5m-PFOS), increased relative to its abundance in a commercial PFOS mixture; that of another (1m-PFOS) decreased (Ross, Wong and Martin, 2012). This may point to a need to monitor relative abundances of individual branched chain isomers rather than the sum of all such isomers, to provide more conclusive insights into the relative contribution of precursor exposure. This conclusion is supported by the study of Gebbink et al. (Gebbink, Berger and Cousins, 2015), where an estimated isomeric pattern of 84 % linear PFOS was calculated for exposure via water, diet, air and dust, that contrasts

with isomer patterns observed in human serum samples (Beesoon et al., 2011; Haug et al., 2009; Benskin et al., 2007; Zhang et al., 2013). The potential feasibility of such a detailed isomer-specific approach is demonstrated by a study of PFOS isomer distributions in gull eggs from spatially distinct breeding colonies throughout the Laurentian Great Lakes (Gebbink and Letcher, 2010). In this study, eight individual branched chain PFOS isomers were detected in gull eggs, with spatial variations in the contribution of linear PFOS in eggs highlighted as potentially at least partly attributable to location-specific variations in the PFOS precursor exposure.

1.7 EXTRACTION AND ANALYTICAL DETERMINATION

In terms of sample preparation, the extraction techniques are usually determined – together with the physical and chemical properties of the analytes of interest – by the nature of the matrix of interest, as there are many substances which can cause interferences during analysis, and they differ matrix from matrix. A second aspect to be considered when analysing PFASs is their capability of being retained on glassware, as Martin et al. reported (Martin *et al.*, 2004), fact which could lead to potential losses when the sample is stored or processed.

1.7.1 Sample Preparation

The extraction and analysis of PFASs, including PFOS, FOSAs, FOSEs and FOSAAs in the same extraction method in such variable matrices as dust, food composites and blood can be a challenging task. PFOS has been analysed and reported extensively (see *Tables 02* to *04, Table 07* and *Table 09*), while FOSAs, FOSEs and FOSAAs have

been just reported in a reduced number of publications, as *Table 05, Table 06* and *Table 08* showed.

A large number of papers reporting PFOS analysis have been published. It is commonly selected as representative long chain fluorinated compound to include in traditional targeted analysis of PFASs (Apelberg *et al.*, 2007; Dauwe *et al.*, 2007; Guruge *et al.*, 2011; van Asselt *et al.*, 2011; Cai *et al.*, 2012; Glynn *et al.*, 2012; Kubwabo, Kosarac and Lalonde, 2013). On the other side, available data of the PFOS precursors described along the introduction chapter and included in this study is rather limited.

For dust sample preparation, ultrasonic solid-liquid extraction with different organic solvents as methanol, acetone or dichloromethane (Goosey and Harrad, 2011; Huber, Haug and Schlabach, 2011; Shoeib *et al.*, 2011) have been reported, followed by clean-up steps based on SPE purification, by the use of activated carbon (Shoeib *et al.*, 2011; Fraser *et al.*, 2013) or weak anion exchange sorbents (Goosey and Harrad, 2011). Newer and more efficient instrumentation for the automatisation of the sample preparation has been also reported, as on-line solid phase extraction (SPE) by Kato et al. (Kato *et al.*, 2011).

For food sample preparation, extractions methods have been described for fish tissue by the use of ultrasonic solid-liquid extraction with acetonitrile and methanol, followed by freezing and SPE clean-up steps (Ullah *et al.*, 2014), or directly evaporated and injected into the system (Van Leeuwen *et al.*, 2009). For a Swedish diet study, Gebbink et al. (Gebbink *et al.*, 2015) described a method where the samples were extracted by solid-liquid extraction and the clean-up step was carried out by the use of weak anion exchange SPE cartridges. On the other side, Tittlemier

et al. (Tittlemier, Pepper and Edwards, 2006) described an extraction method for food composites based on an hexane:acetone extraction, followed by acidified silica purification.

For blood extraction, Olsen et al. reported in 2004 a method based on methyl-tert-butyl-ether serum extraction (Olsen *et al.*, 2004). After it, some other extractions have been described for PFOS precursors analysis: same procedure as for food extraction was described by Gebbink et al (Gebbink, Glynn and Berger, 2015), while other authors as Yeung et al. or Lee et al. (Lee and Mabury, 2011; Yeung *et al.*, 2013) reported plasma data extracted by ion-pair extraction. On the other hand, Fraser et al. (Fraser *et al.*, 2013) employed on-line SPE for the serum analysis.

1.7.2 Analytical Determination

High performance liquid chromatography (HPLC) and gas chromatography (GC), both coupled to mass spectrometry (MS), are nowadays two of the most powerful tools employed for the analytical determination of environmental pollutants. They both combine the advantages of the analytical separation – HPLC or GC – with the selectivity, sensitivity and accuracy that MS determination provides. These techniques can provide accurate qualitative and quantitative information.

In the targeted analysis of PFOS and PFOS precursors, the chromatographic separation has been dominated by liquid chromatography, commonly by the use of reversed phase bonded silica columns, specially C18 columns (Olsen *et al.*, 2004; Yeung *et al.*, 2013; D'Hollander *et al.*, 2015; Gebbink *et al.*, 2015). The chromatographic separation has been usually followed by mass spectrometry

determination, as all the methods from *Table 05, Table 06* and *Table 08* show. Electrospray negative ionisation (ESI $^{-}$) has been employed through the years as the main source and ionisation mode for the analysis of PFASs, partly due to the need of strong ionisation energy that atmospheric-pressure chemical ionisation (APCI) sources cannot provide, and well as to the functional groups characteristic from this family of organic pollutants and their easy ionisability as [M^{-}] moiety,

1.8 SUMMARY

PFOS is an environmental pollutant which has been widely studied. Significant manufacture of both PFOS and PFOS precursors continues today; e.g., PFOS production has increased in China since 2002 (with higher reported levels of PFOS in some regions of China than in the US, despite the small production volumes in China compared to reported 3M production (Olsen et al., 2012)), while PFOS and PFOS-precursors are still being manufactured in Europe and Asia for certain applications (UNEP, 2010; Paul et al., 2009; Zhang et al., 2013).

This introduction has highlighted the potential insights into PFOS environmental fate that may be gained from better knowledge of the isomer specific behaviour of both PFOS and its precursors. Despite this, at the current time, only a few papers have been published reporting the relative abundance of both linear and branched PFOS isomers in the environment. In part, this is likely due to the fact that reference standards for branched chain isomers have only recently become available, and to the challenging nature of existing analytical methods for their measurement, exacerbated by the usually very low concentrations of individual branched chain

isomers in environmental and biological samples. Furthermore, while variations in precursor exposure may explain variations in PFOS isomer profiles; other factors such as gender and pregnancy may also be influential. Despite these obstacles, exploiting the isomer patterns of PFOS and its precursors offers new opportunities to gain insights into their environmental fate and behaviour.

Given the potential rewards, further development, validation, and carefully targeted application of analytical methods for the determination of branched signatures of PFOS isomers are necessary. They will not be a trivial task; but they constitute urgent research priorities.

2. AIMS AND OBJECTIVES

The aim of this chapter is to define the overall concept of this thesis: objectives, hypothesis, and approaches to achieve the established goals.

This work presented in this thesis is part of a multidisciplinary FP7 project (A-TEAM: advanced tools for exposure assessment and biomonitoring), so the aims and objectives of the project will be also presented, as well as where the work undertaken in this thesis fits in the overall picture of the project.

2.1 IADVANCED TOOLS FOR EXPOSURE ASSESSMENT AND BIOMONITORING: THE A-TEAM PROJECT

The A-TEAM is a Marie Curie Initial Training Network (ITN) project which main research goal was to further understanding of how and to what extent consumer chemicals enter humans, and of how such chemicals could be best monitored in our indoor environment, diet, and bodies (*Figure 05*). The A-TEAM vision was that such enhanced understanding of the underpinning science would lead to more effective approaches to monitoring human exposure to chemicals within Europe, thereby improving assessment of risk associated both with recent and current-use consumer chemicals, as well as those under development, and leading ultimately to more sustainable approaches to the use of chemicals. Eight partners from five different countries were involved: University of Birmingham and University of Reading from the UK, University of Antwerp and Flemish Institute for Technological Research from Belgium, University of Amsterdam from The Netherlands, Swedish

Environmental Research Institute and Stockholm University from Sweden, and Norwegian Institute of Public health from Norway.

The principal objectives of the A-TEAM project were to provide robust scientific information that would allow better understanding of:

- The identification at an early stage of chemicals likely to accumulate in Europeans.
- To monitor chemicals in the external environment in a way that best reflects what accumulates in the body.
- The relative importance of different exposure pathways to overall exposure for selected consumer chemicals of toxicological concern.
- How contact with chemicals in our external environment translates into their presence in our bodies and how best to monitor this presence.

To achieve these objectives, four families of environmental contaminants widely employed in different applications in consumer products were selected and studied by the different partners involved in the project:

- Perfluoroalkyl substances (PFASs), used in consumer applications that exploit their strong surfactant properties such as surface treatment agents.
- "Emerging" brominated flame retardants (EBFRs), employed to impart fire safety to consumer goods and materials.
- Organophosphate esters (OPEs) used both, as plasticisers and flame retardants.
- Phthalate esters (PEs) used as plasticisers.

2.2 PFOS PRECURSORS AS INDIRECT SOURCES OF INTERNAL EXPOSURE TO PFOS WITHIN THE A-TEAM PROJECT

Within the whole project context, the aim of the research project this thesis is addressed to, was to increase the knowledge base and experience of the PhD candidate in different research areas (i.e. analytical and environmental chemistry, and exposure assessment) that are relevant to the challenge of achieving accurate and easily applicable approaches to monitoring human exposure to perfluoroalkyl substances (PFASs).

This research proposal was designed to assess the extent to which PFOS precursors such as perfluorooctane sulfonamides (FOSAs) and sulfonamidoethanols (FOSEs) contribute significantly to human body burdens of PFOS.

The project also involved:

- Exposure to complementary methods of PFASs analysis and *in vitro* methods for studying contaminant metabolism.
- Training in monitoring human exposure to PFASs.
- Collaboration with other research teams to incorporate precursor metabolism into PFASs PK exposure modelling.

To achieve the overall project aim, the development and the validation of analytical methods for the determination of concentrations and branched signatures of PFOS isomers was required. These techniques meant to be applied to a variety of appropriate samples to provide insights into the contribution of PFOS precursors to human body burdens of PFOS. This included the development and the application of

LC-MS/MS techniques to determine concentrations and signatures of PFOS isomers and relevant PFOS precursors in environmental samples. In this way, the hypothesis that human exposure to PFOS precursors makes a significant contribution to human body burdens of PFOS would be tested.

2.3 AIMS AND OBJECTIVES OF THIS PhD THESIS PROPOSAL

The aims of this thesis are: 1) to characterise external exposure to PFOS, FOSAs, FOSEs and FOSAAs – suspected to be PFOS precursors –, 2) to characterise internal exposure to PFOS, and 3) to link external exposure to PFOS precursors to internal exposure to PFOS by the use of *in vitro* studies (see *Figure 6*).

External exposure examined in this research include the two most important routes of external exposure to environmental pollutants: diet – by the study of solid and liquid food ingestion, including drinking water –, and indoor dust, while internal exposure includes human serum samples as representative matrix. *In vitro* study include the incubation of selected PFOS precursors with human liver microsomes.

The main hypothesis of this thesis is:

"PFOS precursors – such as FOSAs and FOSEs – contribute significantly to current body burdens of PFOS, as indirect sources of external exposure"

To test the main hypothesis, I will develop analytical methods for the analysis of selected PFASs suggested to be PFOS precursors, such as FOSAs, FOSEs and FOSAAs, and for PFOS branched isomers. I will also develop sample preparation methodologies for the suitable analysis of the selected PFASs in solid food

composites, liquid food composites and serum. I will also report their concentration in the representative matrices. I will study the *in vitro* metabolism of selected PFOS precursors and propose pathways for their internal conversion to PFOS. Finally, I will combine all the exposure data and I will evaluate whether the levels at which PFOS and PFOS precursors are present in external matrices contribute to the body burdens of PFOS.

Figure 05. Graphical overview of the main objectives of the A-TEAM project

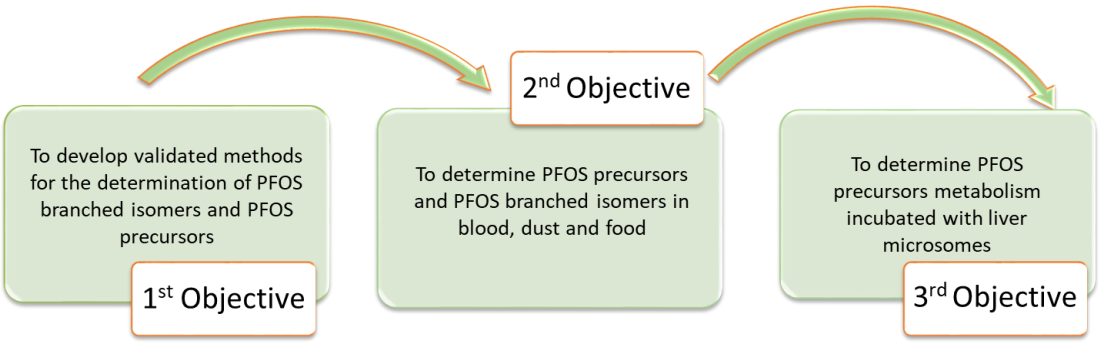


Figure 06. Graphical overview of the main goals of the present thesis project

Within the framework of the main hypothesis, more specific hypotheses are proposed:

1. Indoor home dust is a significant source of exposure to long chain PFASs such as PFOS, FOSAs and FOSEs.
2. FOSAs and FOSEs contribute to the overall external exposure to PFASs via indoor dust ingestion.
3. Diet is a significant source of exposure to long chain PFASs such as PFOS, FOSAs, FOSAAs and FOSEs.
4. FOSAs, FOSEs and FOSAAs contribute to the overall external exposure to PFASs via food consumption.
5. PFOS is ubiquitous in serum.
6. PFOS branched isomer ratios in serum differ from the reported for environmental external exposure.
7. FOSAs and FOSEs are rapidly metabolised to PFOS.

3. METHODOLOGY

3.1 INTRODUCTION

All samples for this study were collected as part of the A-TEAM project cohort, which is composed of 61 participants, all of them workers at the Norwegian Institute of Public Health (NIPH) in Oslo, Norway. The overall objective of the A-TEAM project was to enhance knowledge of external and internal human exposure to certain consumer chemicals, PFASs included. The sampling campaign was conducted between November 2013 and April 2014, and each set of samples was collected over the same 48 hour period, in two consecutive visits (one per day). Indoor air, personal air, floor dust, settled dust, hand wipes, food, drinks, blood, urine, saliva and finger nail samples were collected to cover the main external and internal exposure media. Detailed protocols are provided later in this chapter. Questionnaires were also collected, including information related to sample collection, lifestyle, frequented indoor and outdoor environments, food records and lifestyle habits of each participant. The samples were aliquoted and stored in pre-cleaned polypropylene (PP) containers at NIPH, and shipped to the laboratories involved in their analysis (Papadopoulou *et al.*, 2016). At Birmingham, 57 house dust from vacuum cleaner bags, 113 solid food composites, 121 drink composites and 60 blood serum samples were received.

The selected compounds among the PFASs to be monitored are: PFOS, perfluorooctane sulfonamides (FOSA, MeFOSA and EtFOSA), sulfonamide alcohols (MeFOSE and EtFOSE), and sulfonamide acetic acids (FOSAA, MeFOSAA and

EtFOSAA) listed in *Table 11*. PFOS was chosen due to its persistence in the environment, its long half-life in the human body (around 5 years) (Olsen, Mair, *et al.*, 2007), and its stability as end-product resulting from degradation and metabolism of other PFASs like FOSAs, FOSEs and FOSAAs (Vestergren *et al.*, 2008; Plumlee, McNeill and Reinhard, 2009; Zhao *et al.*, 2016). PFOS, FOSAs and FOSEs have been added to different products and formulations: inks, varnishes, waxes, fire-fighting foams, metal plating and cleaning products, coatings (for walls, furniture, carpeting, food packaging), lubricants, water and oil repellents for leather, paper and textiles (3M, 2000) and its presence in environmental and biomonitoring samples has also been reported (Noorlander *et al.*, 2011; Gebbink *et al.*, 2015; Gebbink, Berger and Cousins, 2015; Miralles-Marco and Harrad, 2015; Shoeib *et al.*, 2016), FOSAAs were included due to their presence in food and biological matrices as oxidation products of FOSEs (Xie *et al.*, 2009; Benskin *et al.*, 2013).

Many analytical papers including a wide list of representative PFASs have been published in the last 15 years (Wolfa and Reagenb, 2011; Tang *et al.*, 2014; Pérez-Ortega *et al.*, 2016; Zacs and Bartkevics, 2016; Harrington, 2017), revealing PFOS – together with PFOA – to be one of the most relevant PFASs due to its high detection levels and frequencies. In contrast, the number of publications and available methods for the determination of PFOS precursors is markedly lower. Moreover, most such papers include only FOSA as an intermediate metabolite, with only a sub-set including FOSAs, FOSEs and FOSAAs together (see *Table 05, Table 06* and *Table 08*) (Kato, Calafat and Needham, 2009; Goosey and Harrad, 2012; Fraser *et al.*, 2013; Gebbink, Glynn and Berger, 2015; Lankova *et al.*, 2015).

The extraction method initially used for dust samples was adapted from existing methods (Taniyasu *et al.*, 2005; Young and Tran, 2006; Goosey and Harrad, 2011). On it, acetone was used as the extraction solvent because PFOS was found to be more soluble in polar organic solvents (Takagai and Igarashi, 2002). For the remaining matrices –solid food, liquids and serum – the extraction method were modified and specifically adapted to the nature of every matrix, in order to remove matrix specific interferences without analyte looses. The clean-up step for dust samples was first adapted and validated from a previously reported method (Goosey and Harrad, 2011), to be later applied to the rest of the matrices included in this study. A basic description of the complete sample treatments are given in *Figure 07* to *Figure 10*. The analytical methods were modified from previously published methods (Goosey and Harrad, 2011; Benskin *et al.* 2007). An evaluation and optimisation of all parameters and conditions to enhance method sensitivity and specificity in target matrices was conducted.

3.2 SAMPLING CAMPAIGN

The sampling campaign protocol was approved by the Regional Committee for Medical and Health Research Ethics in Norway (2013/1269).

The participants were contacted around two weeks before the date of the first visit, and PP pre-cleaned containers of different sizes were distributed to them, together with instructions on how to weigh and collect some of the samples. They were also requested to complete and return the following forms and questionnaires: a) Consent form to be signed prior to the collection of the samples, where the

participants agreed to the collection and use of these samples; b) Food diary (FD), containing detailed information about the individual food sample content (ingredient by ingredient), amount, packaging materials, cooking methods and utensils, etc. used for every meal collected during the two consecutive days of food sample collection period; c) Food frequency questionnaire (FFQ), containing generic information about the average consumption and frequency of a wide range of food items (n = 225) of the participants during the last whole year (Brantsæter *et al.*, 2008); and d) Indoor environment questionnaire (IEQ), which collected information about house and other frequent indoor environments, lifestyle, habits, daily used products as well as basic personal information.

Finally, three appointments were scheduled with every participant. These comprised: a) two visits in two consecutive days to their homes to collect all the samples but blood; and b) one extra appointment with a research nurse at NIPH to collect blood samples.

3.2.1 Dust Samples

Participants were told when contacted to not discard their vacuum cleaner bags until the sampling appointment. During the second visit, the bags were collected from the vacuum cleaner by the researchers, wrapped in aluminium foil and kept in a plastic bucket at room temperature. In case the participants needed to replace the bag before the appointment, they had to wrap it themselves in aluminium foil and keep it at room temperature until its collection.

The bags were cut with scissors and tweezers and the dust was sieved with a 500 μm mesh sieve. 2 g of sieved dust were placed in 30 mL pre-cleaned PP containers and kept at 4 °C until they were shipped to Birmingham.

For blanks, six empty pre-cleaned 30 mL PP containers were sent to Birmingham. Once there, 2 g of clean sodium sulphate were added to each bottle, shaken for 2 minutes and stored together with the dust samples. The purpose of these blanks was the identification of potential cross-contamination from the PP bottles, as well as from the storage places.

3.2.2 Food Samples

Food samples were divided in two groups: Solid and liquid.

The food sampling protocol was established following duplicate diet method (Shim, Oh and Kim, 2014) during a period of two consecutive days. In it, a duplicate portion of all food consumed by the participant over a specific period of time is weighed (by the use of a kitchen scale measuring ± 0.1 g), recorded in a food diary, and mixed. In this study, participants received instructions on how to collect the food samples, and four bottles were given to them: Two for solid food samples (relating to day 1 and day 2 of the sampling), and another two for the liquid food samples (also relating to day 1 and day 2 of the sampling); as well as two food diaries to complete relating to day 1 and day 2. Samples and food diaries were collected during the second visit to be homogenised within the following 24 hours.

Table 11. Perfluorinated analytes: native and mass labelled standards

Name	Abbreviation	CAS Nr	Molecular formula	Monoisotopic mass	Molecular weight
Potassium perfluoro-1-octanesulfonate (K salt)	PFOS	2795-39-3	$C_8F_{17}KO_3SK$	537.89337	538.2
Perfluoro-1-methylheptasulfonate (Na salt)	1m-PFOS	NA	$C_8F_{17}KO_3SNa$	NA	522.1
L-PFOSK with branched isomers	br-PFOS	NA	$C_8F_{17}KO_3SK$	NA	538.2
Perfluoro-1-octanesulfonamide	FOSA	754-91-6	$C_8H_2F_{17}NO_2S$	498.95349	499.1
N-methylperfluoro-1-octanesulfonamide	MeFOSA	31506-32-8	$C_9H_4F_{17}NO_2S$	512.96912	513.2
N-ethylperfluoro-1-octanesulfonamide	EtFOSA	4151-50-2	$C_{10}H_6F_{17}NO_2S$	526.98480	527.2
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	MeFOSE	24448-09-7	$C_{11}H_8F_{17}NO_3S$	556.99536	557.2
2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	EtFOSE	1691-99-2	$C_{12}H_{10}F_{17}NO_3S$	571.01099	571.3
Perfluoro-1-octanesulfonamidoacetic acid	FOSAA	2806-24-8	$C_{10}H_4F_{17}NO_4S$	556.95896	557.2
N-methylperfluoro-1-octanesulfonamidoacetic acid	MeFOSAA	2355-31-9	$C_{11}H_6F_{17}NO_4S$	570.97461	571.2
N-ethylperfluoro-1-octanesulfonamidoacetic acid	EtFOSAA	2991-50-6	$C_{12}H_8F17NO4S$	584.99023	585.2
Perfluoro-1-(1,2,3,4-13C4) octanesulfonate (Na salt)	PFOS-IS	NA	$^{13}C_4{}^{12}C_4F_{17}KO_3SNa$	NA	526.1
Perfluoro-1-(13C8)octanesulfonamide	FOSA-IS	NA	$^{13}C_8H_2F_{17}NO_2S$	NA	507.1
N-methyl-d3-perfluoro-1-octanesulfonamide	MeFOSA-IS	NA	$C_9D_3HF_{17}NO_2S$	NA	516.2
2-(N-methyl-d3-perfluoro-1-octane-sulfonamido)ethan-d4-ol	MeFOSE-IS	NA	$C_{11}D_7HF_{17}NO_3S$	NA	564.3
N-methyl-d3-perfluoro-1-octane-sulfonamidoacetic acid	MeFOSAA-IS	NA	$C_{11}D_3H_3F_{17}NO_4S$	NA	574.2

NA: Not applicable. CAS Nr: Chemical Abstract Service number

All the solid food samples were weighed and homogenised in a food processor (Robot-coupe Blixer 3) for 2 minutes. Then, 100 g were weighed, placed in 250 mL pre-cleaned PP containers and stored at - 20 °C until they were shipped to Birmingham. All the liquid food samples were homogenised by shaking for 1 minute. Then, 100 g were weighed and stored following the same procedure as solid food samples.

For solid food blanks, six pre-cleaned 2 L PP bottles were each filled with 100 g of diatomaceous earth, shaken for 1 minute, emptied into the food processor, homogenised for 2 minutes and placed in 250 mL pre-cleaned PP containers. The purpose of these blanks was the identification of potential cross-contamination from the food processor, as well as from the storage places.

For liquid food blanks, six empty pre-cleaned 2 L PP bottles and six empty PP pre-cleaned 125 mL containers were sent to Birmingham. Once there, 100 mL deionised water were added to each 2 L PP bottle, shaken for 1 minute and placed in the 250 mL PP containers. The purpose of these blanks was the identification of potential cross-contamination from the PP bottles, as well as from the storage places.

3.2.3 Blood Samples

Blood samples were collected by a research nurse at the NIPH and placed in 10 mL plastic Vacutainer® serum tubes. The samples were left to coagulate for 1 hour and centrifuged at 2,500 rpm for 15 minutes. The supernatant serum fractions were transferred into 2 mL PP cryogenic vials and stored at - 80 °C until they were

shipped to Birmingham. Once there, the samples were kept at -20 °C until their analysis.

For blanks, six empty pre-cleaned 2 mL tubes were sent to Birmingham. Once there, 2 mL of calf serum were added to each tube, shaken for 2 minutes and stored together with the blood samples at - 20 °C. The purpose of these blanks was the identification of potential cross-contamination from tubes, as well as from the storage places.

3.2.4 Sample Storage

All the material used during the sampling campaign (including PP bottles and containers) was rinsed with methanol, dried at room temperature and kept closed or wrapped in aluminium foil until its use.

Dust samples were shipped to Birmingham at room temperature and stored at 4°°C until their analysis. Food samples were sent to Birmingham in expanded polystyrene containers filled with dry ice. Once in Birmingham, liquid food samples were placed in the freezer at – 20 °C until their analysis, while the solid food samples were weighed, freeze-dried, weighed again and homogenised with a coffee blender prior to storage at – 20 °C in 125 mL pre-cleaned glass containers until their analysis. Serum samples were sent to Birmingham in expanded polystyrene containers filled with dry ice. They were stored there at – 20 °C until their analysis. All blanks were stored together with the corresponding samples.

All samples were removed from the fridge (4 °C) or freezer (- 20 °C) and allowed to reach room temperature before commencing sample treatment. Any remaining sample was replaced in the fridge or freezer until the end of the project.

The concentrated extracts to be injected into the high performance liquid chromatography coupled to tandem (triple quadrupole) mass spectrometer (HPLC-MS/MS(QqQ)) system were kept in low volume amber glass screw neck vials (200 µL) with screw neck cap and PTFE/silicone septum, purchased from Waters® Corp. These vials were stored at – 20 °C before the analysis. Once the samples were analysed, the screw cap was replaced and the vials were stored back at – 20 °C until the end of the project.

3.3 STANDARDS AND REAGENTS

All standards were supplied by Wellington Laboratories Inc. Individual stock solutions, working solutions (WS) and mass labelled standard addition solutions (Sad) were prepared in methanol (*Table 12* and *Table 13*), and stored in Certan® vials (Supelco), which have a very low solvent evaporation rate that maintains standard integrity, at -20 °C. HPLC grade methanol (MeOH), acetone and hexane, analytical grade iso-octane and tetrahydrofuran (THF) were provided by Fisher Scientific UK Ltd, while trielthylamine (TEA) was supplied by Sigma Aldrich. Ammonium acetate (NH_4Ac), ammonium formate (NH_4COOH), ammonium hydroxide (NH_4OH), sodium acetate (NaAc), hydrochloric acid (HCl), formic acid (HCOOH), sodium sulphate (Na_2SO_4) and celite chemicals were purchased from Fisher Scientific UK Ltd. Newborn calf serum was provided by Gibco Life

Technologies Ltd. Nitrogen used for evaporation was supplied by BOC Gases. Oasis WAX (weak anion exchange) cartridges for SPE were supplied by Waters® Corp.

Calibration curves were prepared by addition of increasing amounts of native standards, followed by a constant amount of mass labelled standards (internal calibration).

Native standards were employed in the validation stages – spiking multiple samples at different levels – and in QA/QC – spiking the matrices prior the extraction process – when no standard reference materials were available.

Mass labelled standards were added to all the samples – spiked with native standards or not – prior the extraction process as recovery standards.

3.4 SAMPLE PREPARATION

The initial sample preparation method (extraction and clean-up steps), which was applied to the dust samples was previously reported by Goosey and Harrad (Goosey and Harrad, 2011), being itself a combination of the extraction originally developed by Taniyasu et al. (Taniyasu *et al.*, 2005), some tips from an application note from Waters® (Young and Tran, 2006) and based on the sample preparation developed by Kubwabo et al. (Kubwabo *et al.*, 2005). For the rest of the matrices included in this research, the extraction methods were modified and validated according to each matrix requirements, keeping in common the subsequent solid phase extraction as clean-up step for all of them. Explanations about the specific sample preparation procedures and validation measures are detailed in the following sections.

Table 12. Native standards, working solutions (WS) for the calibration curve preparation, and mass labelled standard solutions for the analysis of PFOS precursors

Analyte	Ci (µg/mL)	C [1] (ng/mL)	WS (IS) (ng/mL)			WS (low) (ng/mL)			WS (high) (ng/mL)			Sad IS (ng/mL)		
			Vc (mL)	Vf (mL)	$C_{(IS)}$	Vc (mL)	Vf (mL)	$C_{(low)}$	Vc (mL)	Vf (mL)	$C_{(high)}$	Vc (mL)	Vf (mL)	$C_{Sad\ IS}$
PFOS	50.0	49,000				0.2		980	0.5		4,900			
FOSA	50.0	49,000				0.2		980	0.5		4,900			
MeFOSA	50.0	49,000				0.2	10	980	0.5	5	4,900			
EtFOSA	50.0	49,000				0.2		980	0.5		4,900			
MeFOSE	50.0	49,000				0.2		980	0.5		4,900			
EtFOSE	50.0	49,000				0.2		980	0.5		4,900			
FOSAA	50.0	49,000				0.2		980	0.5		4,900			
MeFOSAA	50.0	49,000				0.2	10	980	0.5	5	4,900			
EtFOSAA	50.0	49,000				0.2		980	0.5		4,900			
PFOS-IS	50.0	49,000	0.5		2,450									196
FOSA-IS	50.0	49,000	0.5	10	2,450									196
MeFOSA-IS	50.0	49,000	0.5		2,450							2	25	196
MeFOSE-IS	50.0	49,000	0.5		2,450									196
MeFOSAA-IS	50.0	49,000	0.5	10	2,450									196

1) Corrected by the purity of the commercial standard. Ci= Initial concentration of the commercial standard (not purity corrected). WS= Working solution. Sad = Addition solution. IS = Mass labelled standard. Vc = Volume of the purity corrected solution. Vf = Final volume. C = Concentration

Table 13. Native standards, working solutions (low and high) for the calibration curve preparation, and mass labelled standard solutions for the analysis of PFOS branched isomers

Abrev.	Ci (µg/mL)	C [1] (ng/mL)	BrWS (IS) (ng/mL)			BrWS (low) (ng/mL)			BrWS (high) (ng/mL)			Sad IS (ng/mL)		
			Vc (mL)	Vf (mL)	$C_{(Br\text{-}IS)}$	Vc (mL)	Vf (mL)	$C_{(Br\text{-}low)}$	Vc (mL)	Vf (mL)	$C_{(Br\text{-}high)}$	Vc (mL)	Vf (mL)	$C_{Sad\ IS}$
br-PFOS	50.0	49,000				0.2	10	980	0.5	5	4,900			
PFOS-IS	50.0	49,000	0.5	10	2,450							2	25	196

2) Corrected by the purity of the commercial standard. Ci= Initial concentration of the commercial standard (not purity corrected). BrWS= Working solution for branched isomers. Sad = Addition solution. IS = Mass labelled standard. Vc = Volume of the purity corrected solution. Vf = Final volume. C = Concentration

3.4.1 Dust

The dust sample preparation is summarised in *Figure 07*. As mentioned above, this method was applied to dust samples by (Goosey and Harrad, 2011) having been originally reported by other authors (Kubwabo *et al.*, 2005; Taniyasu *et al.*, 2005; Kubwabo, Kosarac and Lalonde, 2013).

For the extraction, 5 mL of acetone were added to a 15 mL centrifuge tube containing 100 mg of sieved dust (previously spiked with the mixture of mass labelled standards employed as recovery standards, shaken and let rest for an hour). The mixture was shaken for 5 min, followed by 10 min sonication and 5 min centrifugation (3,500 rpm). This process was repeated 3 times, collecting all the supernatants together in a new glass tube (15 mL as final volume). 3 drops of iso-octane were added to the solution and it was evaporated until an approximate volume of 0.5 mL under nitrogen stream. Then, 9 mL of a solution of 0.2 % HCOOH in water was added to the content of the glass tube. At this step, if some particulate matter was visible, 1 g of celite was added to the mixture and it was filtered through a grade 1 filter.

For the SPE clean-up, Waters® Oasis WAX cartridges (6 cc, 150 mg, 30 μm) and a Phenomenex® SPE 24-Position vacuum manifold coupled to a Charles Austen, Capex L2X diaphragm pump, at a pressure of 20 kPa, were used. The WAX cartridges contain a mixed phase sorbent which is able to retain strong acids, as well as perfluoroalkyl sulfonates. The cartridges were first conditioned with 4 mL of 0.1 % NH_4OH in methanol, followed by 4 mL of methanol and 4 mL of 0.1 % HCOOH in water. The samples were then loaded into the cartridge. They were washed with 4 mL of 25 mM acetate buffer adjusted to pH 4 and vacuum was applied for 30 min, to

ensure all the water was removed. The samples were eluted with 4 mL of methanol, followed by 4 mL of 0.1 % NH_4OH in methanol.

The extract was finally evaporated under a mild nitrogen stream, and reconstituted in 150 µL of methanol.

3.4.2 Solid Food

For the solid food sample preparation, different solvents were tested as extraction solvents: acetone (as for dust), acetonitrile (generic solvent), methanol (Padilla-Sánchez and Haug, 2016) and a mixture of THF:H_2O (70:30 v/v) (Ballesteros-Gomez, Rubio and van Leeuwen, 2010; Luque *et al.*, 2012). Additionally, due to the complexity of the matrix represented by the 24 h duplicate diet homogenates; more specific clean-up steps to ensure an efficient lipid removal, such as fat precipitation by cooling the sample, previously reported for substances where acid wash with sulphuric acid was not suitable (Castillo, González and Miralles, 2011), and the use of active carbon (Ballesteros-Gomez, Rubio and van Leeuwen, 2010) were also tested.

As representative matrix for all the tests, dry cat food (Purina One® adult) was crushed and spiked with native and mass labelled standards in triplicate at two different levels. Recoveries and relative standard deviation (% RSD) were compared for all the solvents and the proposed additional clean-up steps, together with the clarity of the final extracts. The results corresponding to these tests showed that: a) fat precipitation by cooling after the extraction step decreased slightly the recoveries for all the experiments carried out, but the clarity of extracts increased

substantially; b) the use of active carbon as a post clean-up step was discarded due to the poor recoveries (up to 0 %) for FOSEs; and c) acetone and acetonitrile were the most effective extraction solvents, with recoveries ranging between 65-110 % and RSD (%) < 30 % for all the analytes included in the method. Acetone was chosen due to its lower vapour pressure and its consequent faster evaporation after the extraction step. The sample treatment finally applied to the solid food samples is summarised in *Figure 08* and detailed below.

For the extraction, 5 mL of acetone were added to a 15 mL centrifuge tube containing 2.5 g of dry food (previously spiked with the mixture of mass labelled standards, shaken and allowed to rest for an hour). The mixture was shaken for 5 min, followed by 10 min sonication and 5 min centrifugation (3,500 rpm). This process was repeated 3 times, collecting all the supernatants together in a new PP tube (15 mL as final volume). The solution was kept overnight in the fridge at 4 °C. The morning after, the sample was transferred to an ice bath, fast filtered through a grade 1 filter and rinsed with 1 mL of cold acetone. 3 drops of iso-octane were added to the solution and it was evaporated until an approximate volume of 0.5 mL under nitrogen stream. Then, 9 mL of a solution of 0.2 % HCOOH in water was added to the content of the glass tube and the extracted is ready for the clean-up step.

The SPE clean-up, evaporation and reconstitution steps remained as explained above for dust samples.

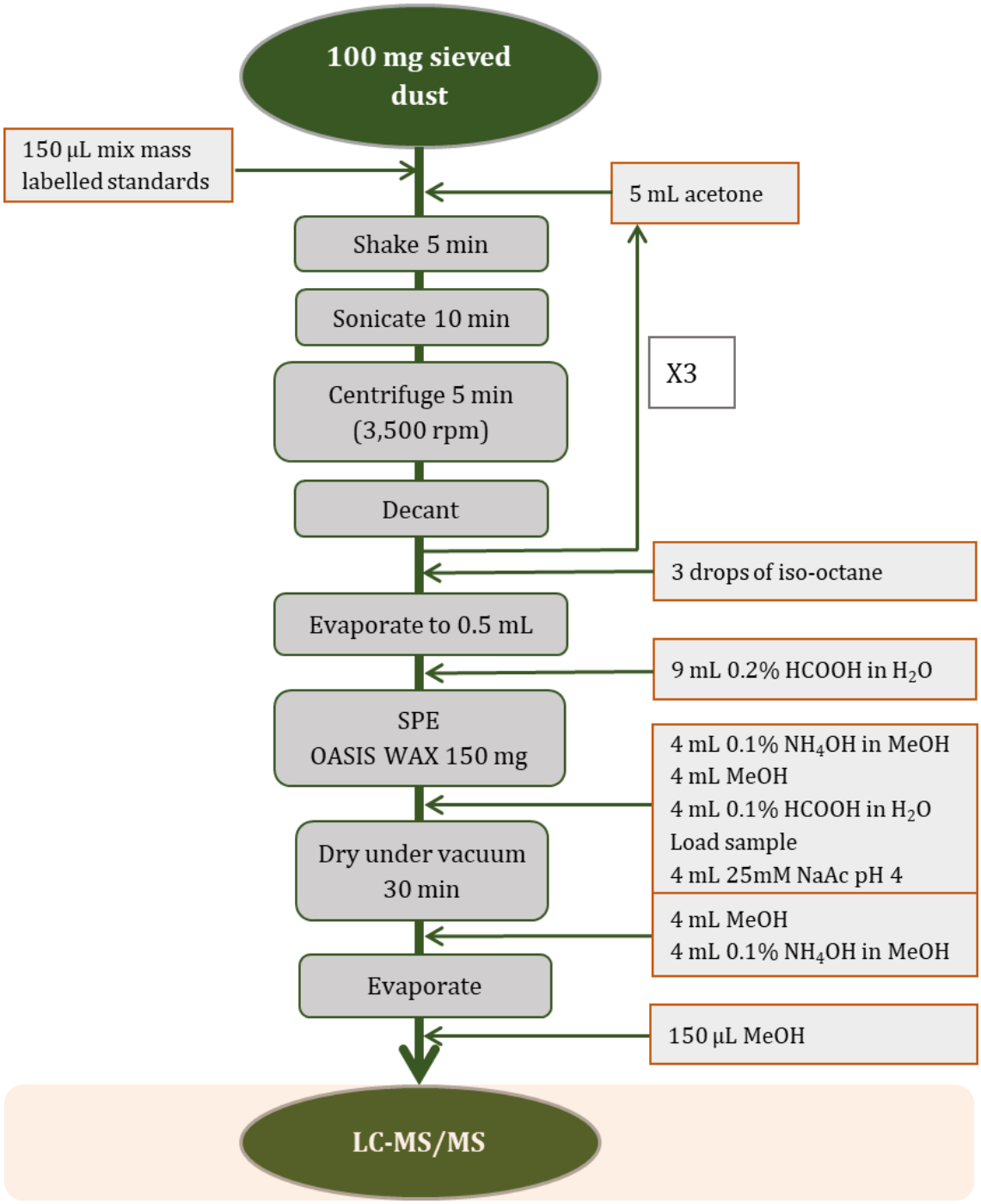


Figure 07. Schematic overview of the sample treatment employed for the analysis of dust samples. The shadowing indicates which parts of the sample preparation and acquisition methods have been improved and optimised from previously published methods, or developed for the first time

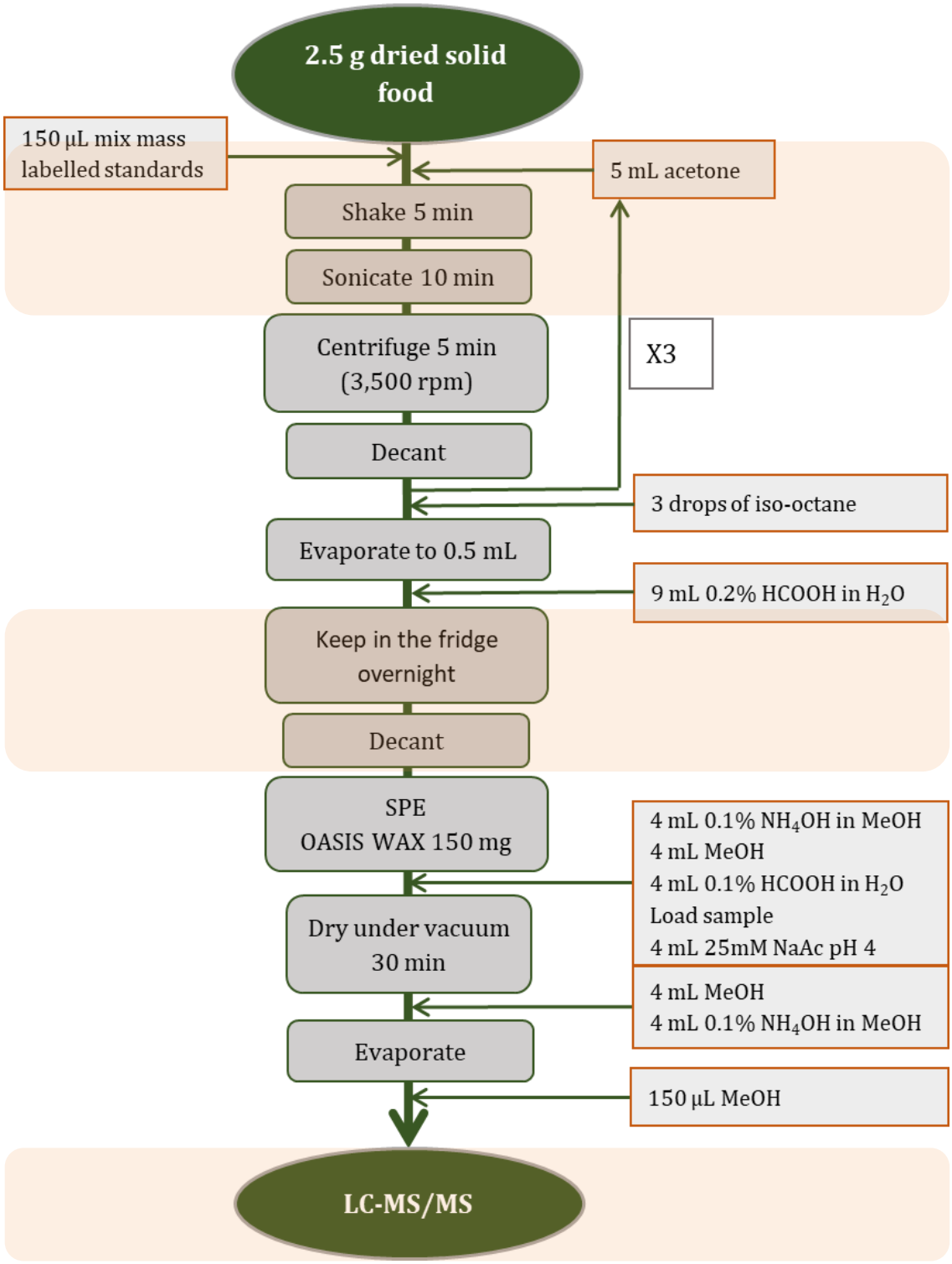


Figure 08. Schematic overview of the sample treatment employed for the analysis of solid food samples. The shadowing indicates which parts of the sample preparation and acquisition methods have been improved and optimised from previously published methods, or developed for the first time

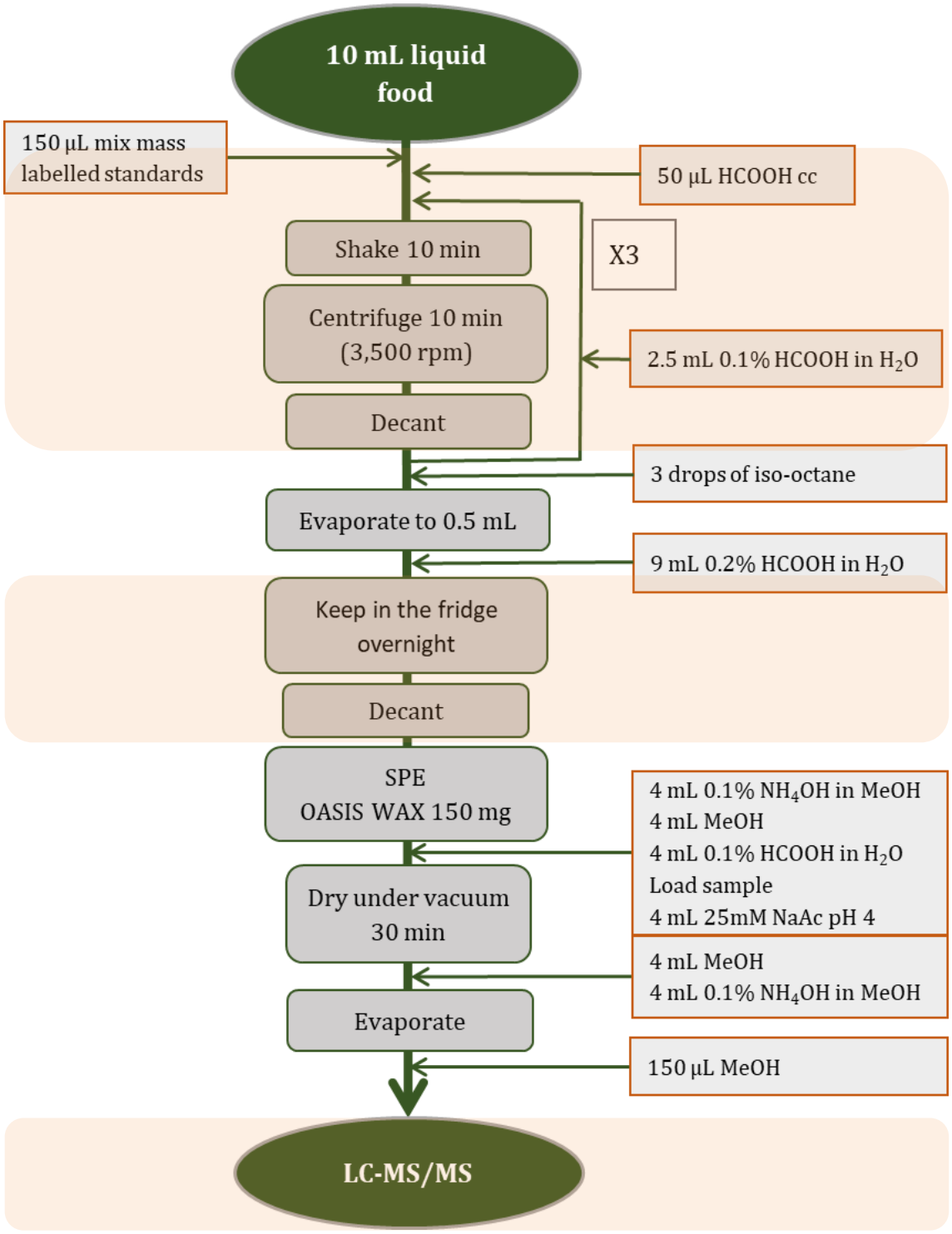


Figure 09. Schematic overview of the sample treatment employed for the analysis of liquid food samples. The shadowing indicates which parts of the sample preparation and acquisition methods have been improved and optimised from previously published methods, or developed for the first time

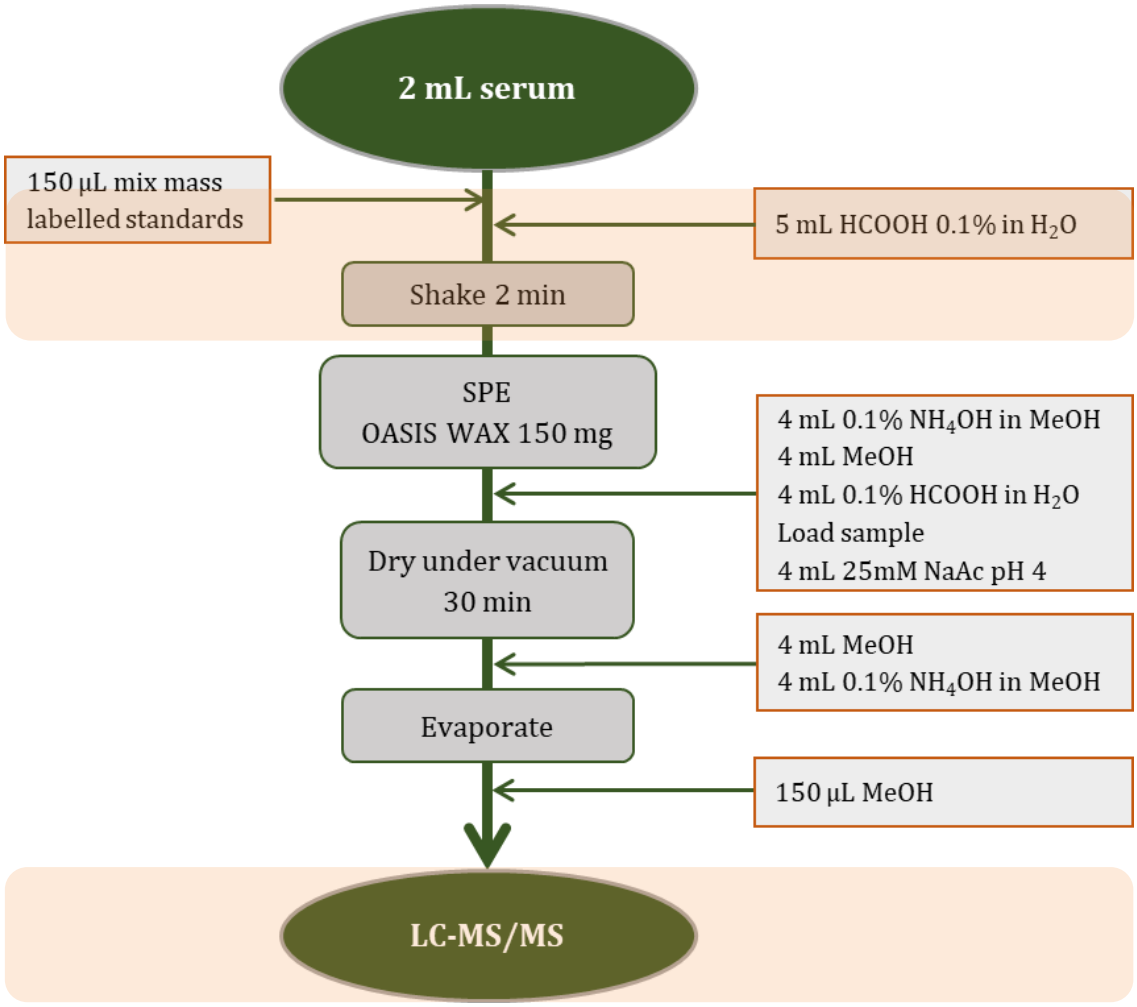


Figure 10. Schematic overview of the sample treatment employed for the analysis of serum samples. The shadowing indicates which parts of the sample preparation and acquisition methods have been improved and optimised from previously published methods, or developed for the first time

3.4.3 Liquid Food

For the liquid food sample preparation, a protein precipitation approach was proposed, similar to the commonly reported ones for blood samples, milk samples or *in vitro* studies (Mosch *et al.*, 2010; Luque *et al.*, 2012; Antignac *et al.*, 2013). Two alternatives were suggested: a) organic solvent precipitation and b) acidic precipitation. The sample treatment finally applied to the liquid food samples was as follows (also see *Figure 09*).

For the extraction, 10 mL (previously spiked with the mixture of internal standards, shaken and let rest for an hour) of the liquid sample was mixed with 50 µL of HCOOH in a 15 mL centrifuge tube. The mixture was shaken for 10 min, followed by 10 min centrifugation (3,500 rpm). The supernatant was collected in a new glass tube. 2.5 mL of 0.1 % HCOOH in H_20 were added to the remaining solid and the extraction process was repeated two more times, collecting all the supernatants together in a new glass tube (15 mL as final volume) which are subsequently passed though the SPE cartridges.

The SPE clean-up, evaporation and reconstitution remained as explained above for dust samples.

3.4.4 Serum

Serum samples are a relatively clean sample compared to those previously described. It is for this reason that quite simple extraction steps or even no extraction steps have been reported (Kuklenyik *et al.*, 2004; Poothong *et al.*, 2017). After checking the clarity of the serum extracts, it was decided that no extraction

step was required. 2 mL of serum sample (previously spiked with the mixture of internal standards, shaken and let rest for an hour) were added to a centrifuge tube containing 5 mL of 0.1 % HCOOH in H_2O. The mixture was vortex mixed and was ready to be introduced into the SPE cartridges.

SPE elution, evaporation and reconstitution remained the same as for the rest of the matrices.

The entire sample treatment is described in *Figure 10.*

3.5 ANALYSIS BY HPLC-MS/MS

Three different analytical methods were developed and optimised in Birmingham for the analysis of: a) total PFOS and PFOS precursors – including three FOSAs and two FOSEs – for the analysis of dust samples; b) total PFOS and PFOS precursors – including three FOSAs, two FOSEs, and three FOSAAs – for the analysis food and serum samples; and c) PFOS branched isomers for the analysis of selected extracts.

All such methods were run on a Shimadzu HPLC system, coupled to an SCIEX API 2000 triple quadrupole mass spectrometer (MS/MS(QqQ)). The MS/MS(QqQ) operated in negative ion mode, using electrospray ionisation (ESI).

3.5.1. Mass Spectrometry Optimisation

In order to enhance to the maximum the sensitivity of the methods, different parameters from the instrument were manually adjusted or optimised by design of

experiments (DoE) as Plackett-Burman and Central Composite Design (CCD), by the use of Minitab statistical software:

- Declustering potential, focusing potential, entrance potential and collision cell entrance potential were adjusted individually for every parent compound or precursor in a given working range of the instrument. Collision energy and collision cell exit potential were adjusted for every chosen transition. All these parameters were manually adjusted by direct injection of individual solutions of standards at a concentration of 2,000 ng/mL and tuning the signals to their maximum intensity. Two transitions were selected for every target compound, except for MeFOSE and EtFOSE, for which just one quantitative transition was found. One transition was selected for every internal standard.
- Ion source gas pressures were set initially at an intermediate value recommended by the engineers. These parameters were discarded for an optimisation by CCD after a preliminary Plackett-Burman experimental design.
- Curtain gas pressure, ion spray voltage, heater temperature and collision gas pressure were optimised by the use of a CCD experiment, a response surface designed experiment where central and axial values of the four parameters were defined (according to the user manual) and different experiments (n = 31) were carried out in a specific running order. All signals were tuned to their maximum intensity, and a compromise on sensitivity – optimal conditions for each analyte was achieved, in order to optimise the MS/MS method.

For the PFOS branched isomers the CCD experiment where the maximum sensitivity was achieved for PFOS transitions was selected.

Table 14. Liquid chromatography parameters for the analysis of PFOS, FOSAs and FOSEs in dust samples

Parameter	**Value**		
Mobile phase A (MPA)	2 mM NH_4Ac in H_2O		
Mobile phase B (MPB)	MeOH		
Injection volume	20 µL		
Guard column	Agilent C18, 4 x 3.0 mm		
Column	Agilent C18 Metasil Basic, 5 µm Si, 10 cm x 2.1 mm, 100 Å		
Gradient			
Time (min)	*MPA (%)*	*MPB (%)*	*Flow (mL/min)*
0.0	70	30	0.4
2.0	0	100	0.4
15.0	0	100	0.4
17.0	70	30	0.4
20.0	70	30	0.4

Table 15. Generic mass spectrometry parameters for the analysis of PFOS and PFOS precursors

Parameter	**Units**	**Value**
Ion source gas 1	Psi	45
Ion source gas 2	Psi	30
Curtain gas pressure	Psi	23.9
Ion spray voltage	V	-3,000
Heater temperature	°C	450
Collision gas pressure	Psi	7

Table 16. Specific mass spectrometry parameters for the analysis of PFOS and PFOS precursors

Native standards	**Units**	**PFOS**	**FOSA**	**EtFOSA**	**MeFOSA**	**EtFOSE**	**MeFOSE**	**FOSAA**	**EtFOSAA**	**MeFOSAA**
Parent compound (M-)	amu	499.0	497.8	525.9	511.8	629.9	615.8	555.9	583.9	569.9
Declustering potential	V	-110	-60	-60	-60	-20	-40	-50	-10	-20
Focusing potential	V	-300	-350	-350	-350	-370	-350	-300	-350	-380
Entrance potential	V	-10	-10	-10	-8	-6	-9	-9	-10	-8
Collision cell entrance potential	V	-30	-30	-30	-30	-40	-25	-26	-27	-27
Quantitation transition (Q)	amu	79.9	77.8	168.9	168.9	59.0	59.0	498.0	418.9	419.0
Collision energy	V	-90	-40	-37	-35	-50	-50	-35	-28	-25
Collision cell exit potential	V	-7	-7	-15	-10	-7	-5	-45	-35	-35
Confirmation transition (q) (amu)	amu	98.9	63.8	64.9	64.9	629.9	615.8	419.0	526.0	482.9
Collision energy	V	-65	-120	-70	-70	-5	-5	-35	-20	-20
Collision cell exit potential	V	-8	-8	-6	-5	-55	-55	-35	-40	-40

Mass labelled standards	**Units**	**PFOS (IS)**	**FOSA (IS)**	**MeFOSA (IS)**	**MeFOSE (IS)**	**FOSAA (IS)**
Parent compound (M-)	amu	502.9	505.9	514.9	625.8	572.9
Declustering potential	V	-100	-81	-65	-10	-10
Focusing potential	V	-360	-300	-310	-365	-350
Entrance potential	V	-9	-11	-8	-8	-10
Collision cell entrance potential	V	-40	-30	-25	-35	-26.5
Quantitation transition (Q)	amu	98.9	78.0	168.9	61.9	419.0
Collision energy	V	-70	-75	-35	-40	-28
Collision cell exit potential	V	-7	-6	-10	-7	-30

Table 17. Liquid chromatography parameters for the analysis of PFOS, FOSAs, FOSEs and FOSAAs in food and blood samples

Parameter	**Value**		
Mobile phase A (MPA)	0.1°% HN_4OH in H_2O		
Mobile phase B (MPB)	0.1°% HN_4OH in MeOH		
Injection volume	20 µL		
Guard column	Agilent C18, 4 x 3.0 mm		
Column	Agilent C18 Metasil Basic, 5 µm Si, 10 cm x 2.1 mm, 100 Å		
Gradient			
Time (min)	*MPA (%)*	*MPB (%)*	*Flow (mL/min)*
0.0	70	30	0.4
9.0	0	100	0.4
15.0	0	100	0.4
17.0	70	30	0.4
20.0	70	30	0.4

Table 18. Liquid chromatography parameters for the analysis of PFOS branched isomers

Parameter	**Value**		
Mobile phase A (MPA)	10 mM NH_4COOH in H_2O adjusted to pH 4		
Mobile phase B (MPB)	10 mM NH_4COOH in MeOH adjusted to pH 4		
Injection volume	20 µL		
Guard column	Agilent C18, 4 x 3.0 mm		
Column	ES Industries FluoroSep-RP Octyl, 5µm, 15 cm x 2.1 mm, 60 Å		
Gradient			
Time (min)	*MPA (%)*	*MPB (%)*	*Flow (mL/min)*
0	50	50	0.18
3.9	30	70	0.18
35	22	78	0.18
40	15	85	0.18
41	0	100	0.18
49	0	100	0.18
50	50	50	0.18
55	50	50	0.18

Table 19. Generic mass spectrometry parameters for the analysis of PFOS branched isomers

Parameter	Units	Value
Ion source gas 1	Psi	45
Ion source gas 2	Psi	30
Curtain gas pressure	Psi	32.5
Ion spray voltage	V	-2,000
Heater temperature	°C	412
Collision gas pressure	Psi	9

3.5.2. LC-MS/MS Analysis of PFOS and PFOS Precursors in Dust Samples: PFOS, FOSAs and FOSEs

The mobile phases for the HPLC separation were composed of 2 mM NH_4Ac in H_2O as aqueous phase and 2 mM NH_4Ac in MeOH as organic phase. The column used for the analysis was a C18 Metasil Basic, 5 µm Si, 100 x 2.1 mm (Agilent Technologies), 100 Å, attached to an Agilent C18 4 x 3 mm guard column. The flow was set to 0.4 mL/min. The gradient is described in *Table 14*.

Details regarding the MS/MS conditions are detailed in *Table 15* and *Table 16*.

3.5.3. LC-MS/MS Analysis of PFOS and PFOS Precursors: PFOS, FOSAs FOSEs and FOSAAs in Food and Serum Samples

A second chromatographic method was developed for the analysis of PFOS precursors in food and serum samples. On it, the mobile phases were modified from the previous method in order to achieve good separation and sensitivity for FOSAAs, due to their poor elution with the previously used modifiers. In this case, the mobile

phases were composed of 0.1 % NH_4OH in H_2O and 0.1 % NH_4OH in MeOH. The column was a C18 Metasil Basic, 5 µm Si, 100 x 2.1 mm, 100 Å (Agilent Technologies), attached to an Agilent C18 4 x 3 mm guard column. The gradient used can be found in *Table 17*.

Details regarding the MS/MS conditions are the ones previously detailed in *Table 15 and Table 16*.

3.5.4. LC-MS/MS Analysis of PFOS Branched Isomers

The mobile phases for the HPLC separation were composed of 10 mM NH_4COOH in H_2O as aqueous phase and 10 mM NH_4COOH in MeOH as organic phase, both adjusted to pH 4. The column used for the analysis was a FluoroSep-RP Octyl, 5 µm, 15 cm x 2.1 mm, 60 Å (ES Industries), attached to an Agilent C18 4 x 3 mm guard column. The flow was set to 0.18 mL/min. The gradient used can be found in *Table 18*.

Details regarding the MS/MS conditions are detailed in *Table 19*.

3.6 QUALITY ASSURANCE / QUALITY CONTROL

Reliability of analytical data is mandatory in order to ensure that all the reported data is accurate and reproducible. All the presented methods were carefully validated before being applied to the reported samples, and quality control requirements were checked every sequence to ensure the validity of the data.

Within this section, all quality assurance / quality control (QA/QC) procedures that have been applied to the entire batch of samples and procedures will be explained in detail.

3.6.1. Method Validation and Quality Control Criteria

All the analytical methods, as well as the sample treatments were validated before being applied to the real samples. This means the reported methods had to satisfy certain pre-established criteria in terms of precision, accuracy, sensitivity and relative response factors. All the methods were validated at, at least, two levels of the calibration standards. Absolute and relative recoveries, precision and accuracy were evaluated. Linear intervals and limits of detection and quantification were established according to these results. *Table 20* to *Table 23* summarise the main validated parameters.

Relative Response Factors

Six calibration points were used with concentrations ranging from 10 to 1,000 ng/mL (concentration in samples can be found in *Table 20* to *Table 23*). The response of each calibration was checked for all the native standards by the calculation of the relative response factor (RRF), defined as the instrument response for a unit amount of target pollutant relative to the instrument response obtained for the same amount of the mass labelled standard. It can be calculated as shown in *Equation 02*, where A_{NAT} is the area of the native standard, A_{IS} is the area of the mass labelled standard, C_{NAT} is the concentration of the native standard and C_{IS} is the

concentration of the mass labelled standard. Calculation of RRFs for each of the standards comprising the multi-point calibration should reveal them to be essentially identical for each concentration level. The relative standard deviation (% RSD) of RRFs for a given target compound should not exceed 10 %.

However, the use of a single internal standard for the quantification of more than one native standard (MeFOSA-IS for MeFOSA and EtFOSA, MeFOSE-IS for MeFOSE and EtFOSE, and MeFOSAA-IS for FOSAA, MeFOSAA and EtFOSAA) resulted in RRF values exceeding this 10 % RSD. The difference in the masses is responsible for the difference in the analytical response. Despite this variation between the native and the mass labelled standard, the results from the calibrations were consistent during the study and remained within 25 % of the original calibration RRFs, so these values were still accepted.

Equation 02 $$RRF = \frac{ANAT}{AIS} x \frac{CIS}{CNAT}$$

Precision and Accuracy

Blank and spiked samples were used in all validation exercises, together with previously reported samples or standard reference materials (SRM) when available. For dust samples, cleaned sodium sulphate (for blanks), SRM 2585 developed by the National Institute of Standards and Technology (NIST) and dust samples from the UK and Australia were used. For liquid food samples, methanol (for blanks) and an in-house spiked mixture of water:milk:juice (80:10:10) were used. For solid food samples, diatomaceous earth (for blanks), different brands of spiked dry cat food

and inter laboratory fish samples were used. For blood samples, methanol (for blanks) and spiked calf serum were used.

Each sample was prepared in triplicate (as a minimum) and on two different days. Percent recoveries (% Rec) (*Equation 03* for solid samples, and *Equation 04* for liquid samples) and relative standard deviation (% RSD) (*Equation 05*) both intra and inter day were calculated in order to evaluate accuracy – defined as closeness to the true value – and precision – defined as statistical variability related to reproducibility and repeatability- of the validated methods. In the equations below, A_{IS} is the area of mass labelled standard in every sample; A_{NAT} is the peak area of target pollutant in every sample, RRF is the relative response factor for the target pollutant (see *Equation 02*), M_{IS} is mass of mass labelled standard added to sample (pg), SS is the sample size (g), V_V is the final sample extract volume in the vial (mL), V_{LS} is the initial volume of liquid sample (mL) and σ_{n-1} is the standard deviation of the analysed batch of samples.

Equation 03 $$Conc\ (ng/g) = \frac{ANAT}{AIS} x \frac{1}{RRF}\ x \frac{MIS}{SS}$$

Equation 04 $$Conc\ (ng/mL) = \frac{ANAT}{AIS} x \frac{1}{RRF}\ x\ CIS\ x\ \frac{VV}{VLS}$$

Equation 05 $$\%\ RSD = \frac{\sigma\ n-1}{Average} x\ 100$$

Table 20. Dust samples main validation parameters

Compound	Range (ng/mL) (vial)	Range (ng/g) (sample)	R^2	Accuracy (%)	Precision (% RSD)	IS recovery (%)	LOD (ng/mL) instrument	LOD (pg/inj) instrument	LOQ (ng/g) sample	RRT (% RSD)
PFOS	10 - 1000	15-1500	> 0.999	107.02 114.41	1.31 1.06	94.24	0.06	1.2	0.10	0.197
FOSA	10 - 1000	15-1500	> 0.999	95.34 83.99	1.31 1.72	85.01	0.03	0.6	0.05	0.180
MeFOSA	10 - 1000	15-1500	> 0.999	84.23 77.41	6.78 17.65	44.55	0.02	0.4	0.07	0.487
EtFOSA	10 - 1000	15-1500	> 0.999	70.29 127.79	3.46 27.30	44.55	0.30	6.4	1.08	0.109
MeFOSE	10 - 1000	15-1500	> 0.999	100.38 61.00	6.85 39.65	54.31	0.90	17.8	2.46	0.118
EtFOSE	10 - 1000	15-1500	> 0.999	70.86 73.49	11.10 12.72	54.31	0.40	9.0	1.24	0.095

LOD = Limit of detection. LOQ = Limit of quantification. RRT = Relative retention time. IS = Mass labelled standard. RSD = Relative standard deviation

Table 21. Solid food samples main validation parameters

Compound	Range (ng/mL) (vial)	Range (ng/g) (sample)	R^2	Accuracy (%)	Precision (% RSD)	IS recovery (%)	LOD (ng/mL) instrument	LOD (pg/inj) instrument	LOQ (ng/kg) sample	RRT (% RSD)
PFOS	10 - 1000	0.6 - 60	> 0.999	104.41 97.95	5.03 5.32	77.2	0.063	1.200	0.0047	0.063
FOSA	10 - 1000	0.6 - 60	> 0.999	98.78 104.26	6.44 4.60	68.1	0.030	0.600	0.0026	0.061
MeFOSA	10 - 1000	0.6 - 60	> 0.998	98.51 106.69	8.06 17.40	34.9	0.020	0.400	0.0034	0.042
EtFOSA	10 - 1000	0.6 - 60	> 0.998	107.20 106.25	18.18 18.96	34.9	0.320	6.400	0.0551	3.099
MeFOSE	10 - 1000	0.6 - 60	> 0.998	84.92 100.71	29.96 22.73	57.5	0.890	17.800	0.0929	0.332
EtFOSE	10 - 1000	0.6 - 60	> 0.998	69.66 90.22	30.65 25.72	57.5	0.450	9.000	0.0470	0.302
FOSAA	10 - 1000	0.6 - 60	> 0.997	91.68 88.55	11.25 15.49	61.6	0.320	6.400	0.0312	4.861
MeFOSAA	10 - 1000	0.6 - 60	> 0.999	107.72 101.38	13.83 6.85	61.6	0.060	1.200	0.0058	0.138
EtFOSAA	10 - 1000	0.6 - 60	> 0.997	83.41 105.13	10.78 6.55	61.6	0.090	1.800	0.0088	8.517

LOD = Limit of detection. LOQ = Limit of quantification. RRT = Relative retention time. IS = Mass labelled standard. RSD = Relative standard deviation

Table 22. Liquid food samples main validation parameters

Compound	Range (ng/mL) (vial)	Range (ng/mL) (sample)	R^2	Accuracy (%)	Precision (% RSD)	IS recovery (%)	LOD (ng/mL) instrument	LOD (pg/inj) instrument	LOQ (ng/L) sample	RRT (% RSD)
PFOS	10 - 1000	0.15 - 15	> 0.999	104.68 110.22	8.70 6.69	77.2	0.063	1.200	0.0012	0.063
FOSA	10 - 1000	0.15 - 15	> 0.999	101.94 109.65	5.73 5.97	68.1	0.030	0.600	0.0007	0.061
MeFOSA	10 - 1000	0.15 - 15	> 0.998	82.65 118.79	23.12 5.67	34.9	0.020	0.400	0.0009	0.042
EtFOSA	10 - 1000	0.15 - 15	> 0.998	84.23 60.73	23.84 19.83	34.9	0.320	6.400	0.0138	3.099
MeFOSE	10 - 1000	0.15 - 15	> 0.998	99.08 88.69	29.41 24.19	57.5	0.890	17.800	0.0232	0.332
EtFOSE	10 - 1000	0.15 - 15	> 0.998	69.05 87.76	30.31 23.17	57.5	0.450	9.000	0.0117	0.302
FOSAA	10 - 1000	0.15 - 15	> 0.997	47.79 82.03	25.25 28.54	61.6	0.320	6.400	0.0078	4.861
MeFOSAA	10 - 1000	0.15 - 15	> 0.999	93.60 92.20	17.70 7.25	61.6	0.060	1.200	0.0015	0.138
EtFOSAA	10 - 1000	0.15 - 15	> 0.997	90.01 114.66	29.75 18.40	61.6	0.090	1.800	0.0022	8.517

LOD = Limit of detection. LOQ = Limit of quantification. RRT = Relative retention time. IS = Mass labelled standard. RSD = Relative standard deviation

Table 23. Serum samples main validation parameters

Compound	Range (ng/mL) (vial)	Range (ng/g) (sample)	R^2	Accuracy (%)	Precision (% RSD)	IS recovery (%)	LOD (ng/mL) instrument	LOD (pg/inj) instrument	LOQ (ng/g) sample	RRT (% RSD)
PFOS	10 - 1000	0.75 - 75	> 0.999	84.06 91.14	18.68 18.13	79.2	0.060	1.200	0.0057	0.204
FOSA	10 - 1000	0.75 - 75	> 0.999	98.07 87.07	8.27 8.61	88.0	0.030	0.600	0.0026	0.206
MeFOSA	10 - 1000	0.75 - 75	> 0.993	78.82 89.03	10.91 9.38	58.8	0.020	0.400	0.0025	0.106
EtFOSA	10 - 1000	0.75 - 75	> 0.998	82.73 88.66	22.10 18.84	58.8	0.320	6.400	0.0408	1.348
MeFOSE	10 - 1000	0.75 - 75	> 0.997	23.95 56.82	24.77 28.90	57.5	0.890	17.800	0.1160	0.271
EtFOSE	10 - 1000	0.75 - 75	> 0.997	60.05 78.45	56.26 32.49	78.7	0.450	9.000	0.0587	0.477
FOSAA	10 - 1000	0.75 - 75	> 0.999	91.26 82.39	41.65 28.59	78.7	0.320	6.400	0.0305	1.553
MeFOSAA	10 - 1000	0.75 - 75	> 0.998	82.18 90.61	14.18 8.08	78.7	0.060	1.200	0.0057	1.077
EtFOSAA	10 - 1000	0.75 - 75	> 0.999	46.85 59.13	9.83 10.09	78.7	0.090	1.800	0.0086	0.155

LOD = Limit of detection. LOQ = Limit of quantification. RRT = Relative retention time. IS = Mass labelled standard. RSD = Relative standard deviation

Limits of Detection and Quantification

The instrumental detection limit (LOD) was defined as the quantity of the analyte providing a signal to noise ratio of 3:1 and it was calculated by extrapolation of the lowest concentration standards (10 mg/mL) in the calibration standards injected during the validation process for each analyte. In the majority of cases, the blank concentrations were not expected to exceed 30 % above the LOD, but in cases where the blanks contained concentrations above this level, the blank concentration would be used as the LOD. The sample detection limit or limit of quantification (LOQ) was determined as the lowest measurable concentration in the extracted sample, with respect to the LOD, final extract volume (V_{FE}), volume of final extract injected (V_{FEI}), sample size (SS) and percentage of mass labelled standard recovery (% IS Rec), and is calculated as shown in *Equation 06*.

$$\textbf{\textit{Equation 06}} \quad LOQ = \frac{LoD \times VFE}{VFEI \times SS} x \frac{100}{IS\ Recovery\ (\%)}$$

Mass Labelled Standards

The mass labelled standards (IS) were added to every sample in a specified amount of 200 ng/mL in vial to all the native standards, blanks and samples. They are commonly used in analytical chemistry to avoid recovery corrections when calculating concentrations in samples. Recovery values between 30 and 150 % were accepted. The acceptance of this wide interval is mainly due to matrix effect reasons, which could enhance or suppress heavily the intensity of the target analytes. Their signal to noise ratio was also measured and a minimum value of 20:1 was required.

Blanks

Two types of blank samples were defined: field blanks and matrix blanks. Field blanks were used to evaluate the lack of cross contamination during the sampling, storage and sample manipulation steps and they were usually solvents or inorganic reagents. Meanwhile, matrix blanks were used (when possible) to better mimic the real sample composition, as well as to evaluate the lack of cross contamination during the sample treatment steps.

Field blank and matrix blanks were prepared and run five consecutive times each at the beginning of every validation sequence. Internal standards were also added to them at the beginning of the sample treatment. Values below 30 % of the LOD were accepted for field blanks. For matrix blanks, concentrations below 30 % of the LOQ were accepted if the field blanks prepared together with them met their previously mentioned criteria and the RSD was below 30 %. Matrix blanks were later used as spiked samples during the analysis of real samples when a suitable SRM was not available.

3.6.2. Monitoring of Method Performance

After every method validation, real samples were analysed and reported, following all the QA/QC parameters defined and established on that matrix. These were applied to all the analysed samples and in the event of the failure of one QA/QC parameter, the rejection and repetition of the sample.

Relative Response Factors

The six point calibration curve (see *Table 20* to *Table 23*) was injected at least once at the beginning of every sequence, and RRF values were calculated for every analyte. An RSD value below 10 % was accepted when the analytes matched the mass labelled standard, and 25 % when they did not, provided the obtained values were repeatable.

Precision and Accuracy

As an on-going measure of accuracy and precision, Standard Reference Materials (for dust samples) or spiked matrix blank samples (for the rest of the matrices) – as previously reported during the validation stage – were prepared every sequence and run together with the samples. Two of these well-characterised samples were injected every 20 samples. Accuracy should range between 60-120 % and precision should not exceed 30 %. These numerical values were stored for an on-going monitoring of reproducibility and long term repeatability.

SRM 2585 used for dust samples has been previously analysed and reported together with dust samples for some PFASs (Björklund, Thuresson and De Wit, 2009). In 2013, Reiner et al. (Reiner *et al.*, 2013) published an inter-laboratory study where PFASs in different abiotic reference materials were analysed. Five laboratories were involved in the analysis of SRM 2585, and 23 PFASs were included. PFOS was analysed in all of them with concentrations ranging from 2,000 to 2,410 ng/g, while FOSA was just analysed in two of them with concentrations

ranging from 7.78 to 11.6 ng/g. Finally, MeFOSAA and EtFOSAA were analysed in just one of the laboratories, with values of 150 and 675 ng/g respectively.

Blanks

Field blanks were prepared every sequence and run every five samples. Values not exceeding 30 % above the LOD were accepted. Any blank exceeding that established value required re-injection of the sample. If a second injection showed a decrease to the acceptance criteria, contamination from the HPLC system had to be checked, and the entire batch re-analysed. If a second injection still showed an unacceptable value, the entire batch was rejected and repeated.

Matrix blanks were injected at the beginning of every sequence. Their values had to meet the criteria previously defined in the validation stage. Where they did not, the entire batch was rejected and repeated.

During sample analysis, no blank samples (both, field and matrix) exceeded QA/QC parameters and therefore no transformations or rejections were required.

Mass Labelled Standards

A specific amount of a mix of mass labelled standards according to a final concentration of 200 ng/mL in vial was added to all the samples, blanks and controls. Mass labelled standard recovery values between 30 and 150 % with a minimum signal to noise ratio of 20 were accepted. Any sample identified to have a

mass labelled standard recovery outside the acceptable range or with a signal to noise ratio lower than 20:1 was discarded and repeated.

Limits of Detection and Quantification

Samples in which concentrations were below the LOD were reported as "ND" or not detected. For descriptive statistics purposes *Equation 07* was later used for all these non-detected samples.

Samples for which concentrations were between LOD and LOQ values, were reported as "<LOQ". For descriptive statistic purposes, *Equation 08* will be used for the samples with these values between LOD and LOQ.

Equation 07 $$Conc\ (< LOD) = \frac{1}{\sqrt{LoD}}$$

Equation 08 $$Conc\ (LOD < X < LOQ) = \frac{1}{\sqrt{LoQ}}$$

Relative Retention Time

The relative retention time (RRT) of all peaks was required to remain within 0.2 % of the average calibration RRT calculated from the calibration standards run at the beginning of that batch of samples. If this parameter was not satisfied, the quantification was not valid as a positive sample and was removed from the reported list.

3.7. SOFTWARES

All software used for the analysis of the samples included in this study are:

- ✓ Microsoft EXCEL 2010 and 2013 for sample quantification and descriptive statistics.
- ✓ IBM SPSS Statistics 22 and 24 for Windows for post-acquisition statistical analysis.
- ✓ Analyst 1.4.2 and Mass Spectrometry toolkit v3.3 for data acquisition.
- ✓ Minitab 16 for Plackett-Burman and Central Composite Design for the MS/MS optimisation.

4. PFOS AND PFOS PRECURSORS IN INDOOR DUST

The main objectives of this chapter are a) to report concentrations of PFOS and PFOS precursors, and to check how a specific indoor microenvironment – homes – contributes significantly to direct exposure to PFOS and PFOS precursors. b) to estimate daily intakes for the participants and for toddlers and children, and c) to compare current levels of the selected pollutants in house dust with those previously reported for Norwegian (Haug, Huber, Schlabach, *et al.*, 2011) and English (Goosey and Harrad, 2011) populations.

4.1 INTRODUCTION

According to previously reported papers, dust has been highlighted as one of the major pathways of external exposure to some persistent environmental chemicals (Jones-Otazo *et al.*, 2005; Harrad *et al.*, 2010; Ross Wilson *et al.*, 2013; von Lindern *et al.*, 2016). PFOS tends to be present in indoor environments as a combination of different sources such as a) fibres from textiles and fabrics from indoor furniture, b) abrasion from water and stain-proofed textiles, and c) abrasion from diverse coatings as food packaging materials. All of them then deposit to dust, as some publications have reported for diverse families of organic pollutants (Webster *et al.*, 2009; Beesoon *et al.*, 2012; Fraser *et al.*, 2013; Tian *et al.*, 2016). Many studies have been published – reviewed in the introduction chapter and summarised in *Table 03* – reporting levels and detection frequencies of indoor PFOS in several countries around the world (Kubwabo *et al.*, 2005; Shoeib *et al.*, 2005, 2011, 2016; Strynar and Lindstrom, 2008; Kato, Calafat and Needham, 2009; Goosey and Harrad, 2011; Haug,

Huber, Schlabach, *et al.*, 2011; Ericson Jogsten *et al.*, 2012; Fraser *et al.*, 2013; Tian *et al.*, 2016; Karásková *et al.*, 2016). Moreover, it is also known that PFOS precursors as FOSAs and FOSEs included on this study tend to be present as impurities, secondary and/or intermediate products from the synthesis of POSF related products, such as PFOS and some of their derivatives, so their origins in indoor dust are closely linked to those of PFOS. They have not been so widely reported as PFOS itself, but the currently available levels have been described and reviewed in the introduction chapter of this thesis. These facts suggest they are a major contributor to PFOS internal exposure when levels in blood, breast milk, or other non-invasive matrices have been detected.

In a more specific way when reviewing PFAs as an overall, both direct (Trudel *et al.*, 2008; D'Eon J and Mabury, 2011; Ericson Jogsten *et al.*, 2012) and indirect (Gebbink, Berger and Cousins, 2015) exposure to PFOS are influenced by dust ingestion and is frequently underestimated. Dust ingestion rates have been previously reported (Jones-Otazo *et al.*, 2005; R. Wilson *et al.*, 2013) and established, whereby adults ingest an average of 4.15 mg of dust per day in mean scenario, while this rate increases up to 100 mg per day for kids and toddlers in mean scenario (USEPA, 2011). The US EPA report on Child Specific Exposure Factors (USEPA, 2008) elaborates how children are more exposed to certain organic pollutants due to hand-mouth behaviour, suggesting their exposure patterns to differ from those for adults (Calafat, Wong, *et al.*, 2007).

As an average people spend around 90 % of their time indoors (Shoeib *et al.*, 2011), with this time mostly divided between working hours and home. This factor is especially relevant when considering this study was carried out in Norway during

the cold period from November 2013 to April 2014, when the average time spent outdoors is much lower than during the summer season. According to the indoor questionnaires related to this study, just one participant reported spending less than 10 hours per day at home (4 hours), while the rest spent ten or more hours (up to 21 for one participant) at home, with a mean value for the 61 participants of 13.2 hours (55 %) at home per day.

This chapter will present indoor vacuum cleaner bag dust data collected from 57 Norwegian homes, all of them belonging to the participants of the A-TEAM cohort. Six PFASs – PFOS, FOSA, EtFOSA, MeFOSA, EtFOSE and MeFOSE – are quantified. The differences within the participants from this cohort and among the similar studies cited above (Norway and UK) will be reported here for the first time.

4.2 INDOOR AND DEMOGRAPHIC QUESTIONNAIRES DESCRIPTION

An indoor environment questionnaire was given to all the participants during the first of the two agreed sampling appointments, and it was collected during the second visit. The data extracted from the questionnaires was provided by the NIPH. Among all the questions, a rough screening of the relevant ones to evaluate PFASs indoor exposure was done. From the 61 participants, just 57 vacuum cleaner bags were collected and shipped to Birmingham, so all the data presented onwards will be relative to these 57 participants, including the information extracted from the questionnaires provided along this chapter.

In terms of age, the participants ranged from 20 to 66 years old (average = 41.7 years), with75.5 % female and the remaining 24.5 % male. The average distance

from residence to the institute, was 12 km (range 0.3 – 77.3 km). Houses were also quite diverse; they ranged from small apartments of 30 m^2 surface to family houses of more than 200 m^2 surface area. The age they were built ranged from 1892 (the oldest one built) to newly built ones (2013 was the newest). Moreover, 13 participants gave a positive answer when they were asked about living close to busy traffic roads, and four of them declared they were living close to an industrial area. This overview gives a general idea about the broad range of differences in terms of environment, sources of exposure and daily routines the participants described here have.

For the indoor exposure itself, house facilities and cleaning habits varied as much as the environmental ones or the location of the residences. 44 % of the participants have separate kitchen and living rooms in their houses, while the other 56 % were living in houses with open kitchens, facilitating the cross contamination between sources coming from both rooms. The number of people living in the same residence was variable: some people lived alone while in the highest occupied house three adults and three children were living in. The overall number of children living in the sampled residences ranged from 0 to 3 children per house (51 kids in total from the included 57 participants). On the other hand, 14 participants owned pets, a fact which could enhance the dust transport around the house as well as in and out from the residence, besides significant alteration of the dust composition. Regarding to ventilation habits, 39 % of the participants ventilated their living rooms by opening the windows during the period of the sampling campaign, while 56 % of the participants ventilated their bedrooms over the same sampling period. More than the 50 % of the participants said they refurbished their living rooms (51 %),

kitchens (59 %) or bedrooms (58 %) recently. The average number of times the participants vacuum cleaned their living rooms was 4.5 times per month, with a wide range starting from 1 up to 15 times per month. The average value for bedrooms was slightly lower (3.6 times per month), while minimum and maximum values nearly remained the same. The participants were also asked about Gore-Tex® and Gore-Tex®-like clothing and shoes, with 48 "yes" for clothing and 51 for shoes.

A detailed graphical description of the main parameters discussed are available in *Figure 11* and *Figure 12.* These values were grouped in three groups of consumption for ANOVA test analysis: low, intermediate and high.

4.3 CONCENTRATIONS IN INDOOR DUST

Indoor dust samples from 57 Norwegian homes were collected and processed as explained in the methodology chapter.

In this section, PFOS – linear and branched isomers – and PFOS precursors – FOSAs and FOSEs – concentrations will be reported and compared with the information collected from the participants and extracted from the indoor environment questionnaires. Daily average intakes will be calculated. Significant differences and correlations will be presented too.

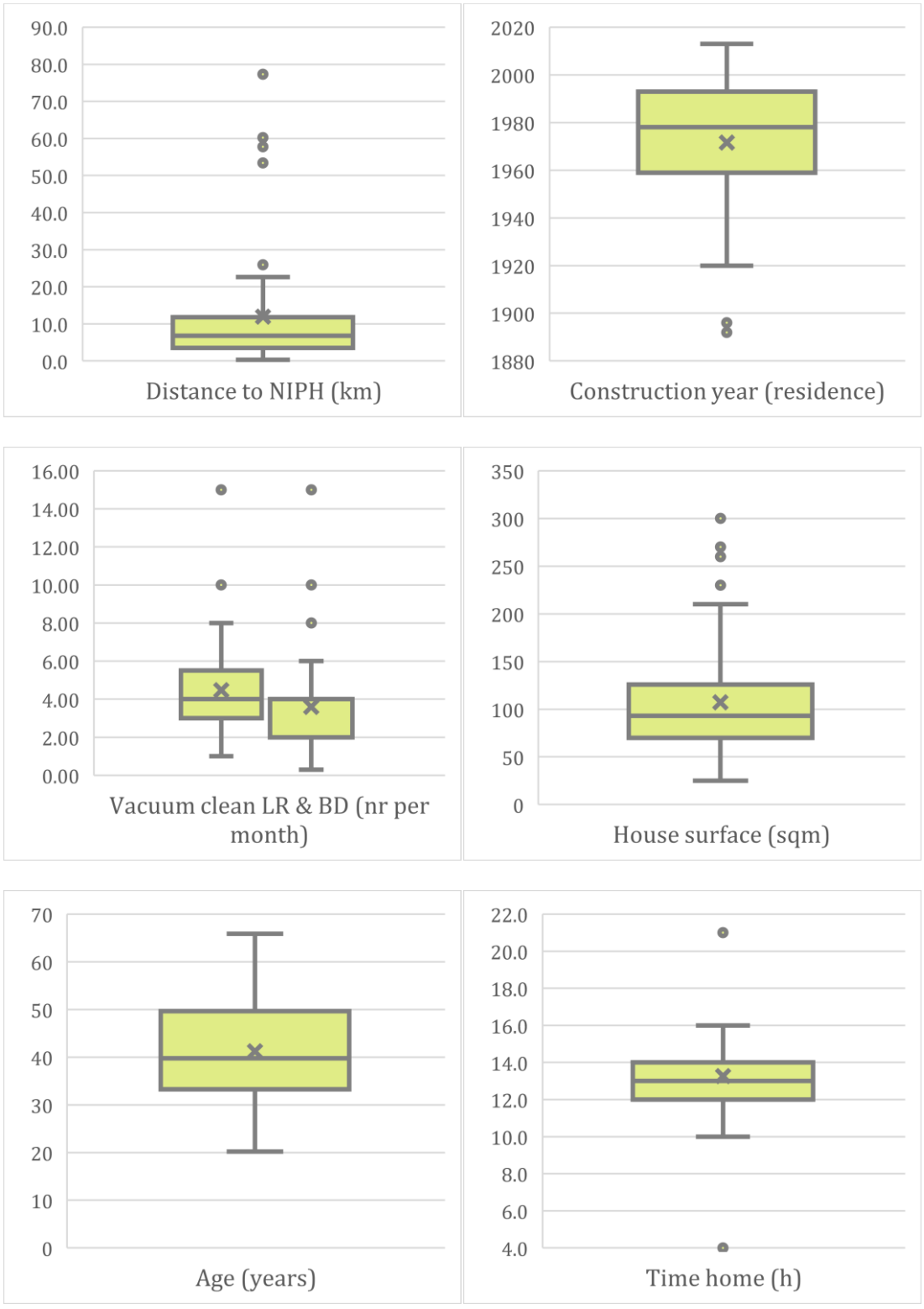


Figure 11. Box plots relative to the answers given by the participants for some of the indoor environment questionnaire questions

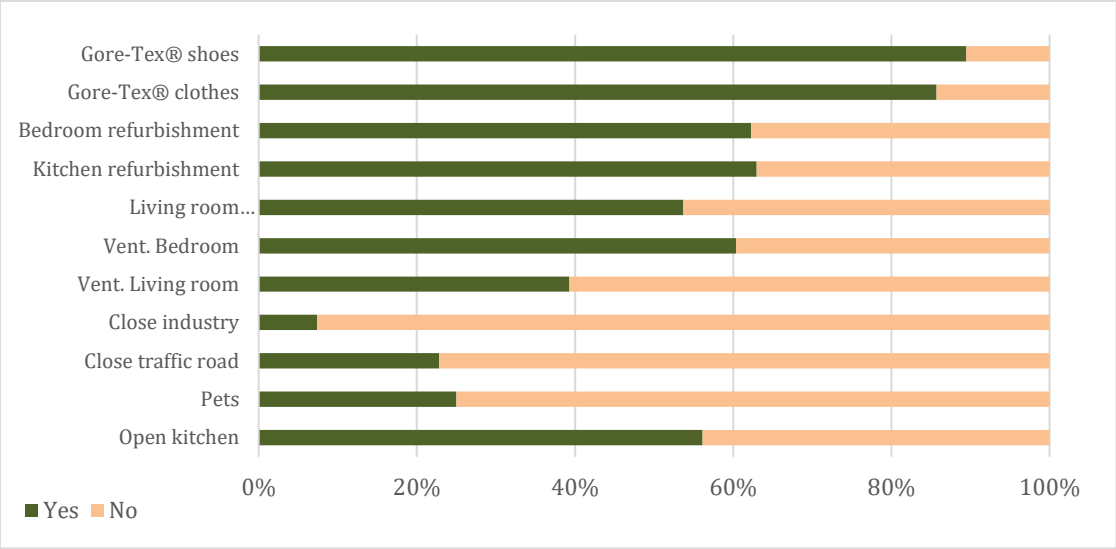


Figure 12. Percentages (yes/no) for participant answers related to some of the indoor environment questionnaire questions

4.3.1 Total PFOS and PFOS Precursors

Descriptive statistics from the vacuum cleaner dust samples where PFOS and PFOS precursors were analysed are shown in *Table 24*. Individual sample results are detailed in *Table SM01*.

PFOS was detected at measurable levels in the 100 % (n = 57) of the dust samples analysed (average = 8.95 ng/g), with concentrations in a range between 0.41 and 70.7 ng/g. FOSA and MeFOSA were also identified in very high detection frequencies – 96 % for both of them – in average concentrations of 0.43 (<LOD – 2.5 ng/g) and 30.94 ng/g (<LOD – 142.8 ng/g) respectively. Both FOSEs were detected in a range between 30 – 35 % of the dust samples, with an average concentration of 24.46 ng/g (<LOD – 839.1 ng/g) for MeFOSE, and 11.98 ng/g (<LOD – 231.7 ng/g) for EtFOSE. Finally, MeFOSA was the pollutant reported the lower number of samples – 9

positive samples, 16 % of the total– , with a reported average concentration of 0.41 ng/g (<LOD – 5.7 ng/g).

The pattern for dust exposure is clearly dominated by EtFOSA, followed by both FOSEs, even though these last two compounds are detected in around 1/3 of the analysed samples. On the other side PFOS, which is present in all the analysed samples, would contribute around a 10 % to the overall exposure to the presented PFASs. Same for FOSA, highly detected in percentage of samples, which would contribute to the overall exposure to PFASs less than 1 %.

Table 24. Concentrations (ng/g) of PFOS and PFOS precursors in vacuum cleaner bags (n = 57)

	PFOS	**FOSA**	**MeFOSA**	**EtFOSA**	**MeFOSE**	**EtFOSE**	**ΣPFAS**
Average	8.95	0.43	0.41	30.94	24.46	11.98	77.17
Mean	5.07	0.34	0.08	21.72	3.75	1.95	47.83
Median	4.45	0.39	0.05	26.06	1.74	0.88	50.90
Min	0.41	< LOD	< LOD	< LOD	< LOD	< LOD	4.04
Max	70.7	2.5	5.7	142.8	839.1	231.7	1122.4
Q1	3.27	0.22	0.05	12.23	1.74	0.88	28.37
Q3	8.53	0.52	0.05	39.70	4.39	2.87	73.50
5^{th}	0.90	0.12	0.05	4.31	1.74	0.88	15.19
95^{th}	31.26	0.88	3.13	70.58	70.50	84.20	148.86
SD	12.5	0.3	1.2	24.7	111.7	37.4	147.3
% RSD	140	80	298	80	457	312	191
n > LOQ	57	55	9	55	18	20	57
DF (%)	100	96	16	96	32	35	100

n = number of dust samples included in the statistics. DF = Detection frequency. Q1 = 25^{th} percentile. Q3 = 75^{th} percentile. SD = Standard deviation. RSD = Relative standard deviation

Table 25. Statistical differences in ANOVA and t-test for the analysis of PFOS and PFOS precursors in dust samples (p = 0.05)

Parameter	Test	Variable	PFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	ΣPFAS
Age of the participants	ANOVA [1]	Below 30 30-49 Over 50	✓	×	×	×	✓	×	✓
Industries less than 500 m	T-test [2]	Yes/No	×	×	×	✓	×	✓	×
Renovation kitchen	T-test [3]	Yes/No	×	×	×	✓	×	×	✓

1)With Scheffe post-hoc test. 2) Equal variances not assumed for PFOS and EtFOSE (p < 0.05 for Levene test) 3) Equal variances not assumed for EtFOSA and MeFOSE (p < 0.05 for Levene test)

For statistical analysis of the indoor dust samples from this study, a cut-off value of 50 % positive samples was ideally required. In this case, just PFOS, FOSA and EtFOSA met the criteria, so the percentage was reduced to a minimum of 30 % positive samples for a full statistical analysis – so, MeFOSE and EtFOSE could be also included – and to minimum of 10 % for t-test and ANOVA when they were used for mean comparison with preceding studies.

The Shapiro-Wilk test for normality of data distribution was firstly applied to the dataset, showing skewed data. A comparison between non-parametric statistics and parametric statistics with logarithmic transformed data was conducted, showing no significant differences on the data analysis. As a consequence of it, logarithmic transformed data was selected and used for data normalisation and subsequent statistical analysis of the dust samples. All the ANOVA tests were run with Scheffe post-hoc test with a significance level 95 % (p = 0.05). All the t-tests were also run

with a significance level of 95 % (p = 0.05). In all the correlations the significance level is specified (p = 0.01 or p = 0.05).

The age of the participants and their gender, the number of hours spent home, the type of residence, their location (distance) from the NIPH, from industrial areas and from busy traffic roads, the age, size and distribution of the residence, the presence of pets and smokers, the ventilation frequency, the recent renovation of the different rooms in the residence, the frequency the residence was vacuum cleaned, and the use of Gore-Tex® clothing of the participants (shoes and clothes) were considered. A summary of the statistical significances identified among the evaluated indoor parameters and the detected concentrations of the analytes is shown in *Table 25* and they will be detailed one by one along this section.

The age of the participants seemed to play a role in the detected levels of PFOS, MeFOSE and ΣPFAS when ANOVA test was conducted, as *Figure 13* and *Figure SM01* show. In case of PFOS, significant higher concentrations in houses of the participants over 50 years old were determined (mean log PFOS = 1.01) when compared with those in the range of 30-49 years old (mean log PFOS = 0.59). For ΣPFAS, the trend pattern was similar (mean concentrations for 30-49 < Below 30 < Over 50), but showing significant differences among the three groups of ages, while for MeFOSE the pattern was different, showing a significant differences for participants aged below 30 (see *Figure SM01*). Interestingly, other detected PFASs, such as FOSA – with high detection frequencies, low concentrations and no statistical differences – showed same pattern as PFOS and ΣPFAS (see *Figure SM02*). These statistical differences could be caused by a combination of differences related to trends in lifestyle associated to age, such as the cleaning and ventilation frequencies, the age

of the Gore-Tex® or Teflon® utensils in the residence, the number of hours spent indoors, the age of the house and/or refurbishments, etc., even though they did not show statistical significances as individual parameters.

The proximity (less than 500 metres) of the main residence of the participants to industrial areas also showed statistical differences in detected concentrations of EtFOSA and EtFOSE (see *Figure 14* and *Figure SM03*). Nevertheless, the overall sum of PFASs showed no statistical differences. Especially relevant is the fact that extreme values for EtFOSE concentrations were found in residences presumably not exposed to PFASs by industrial areas nearby. For EtFOSE, the mean concentration was lower (log EtFOSE = 0.895) for those reporting living close to an industrial area than those who answered they did not (log EtFOSA = 1.368). These facts suggest the statistical difference identified for this parameter occurs by chance.

The third indoor parameter – recent renovation of the kitchen shown in *Figure 15* – showing significant differences for some of the compounds of study here, showed higher levels of ΣPFAS (mean log ΣPFAS = 1.8660) for not recently renovated kitchens than for the renovated ones (mean log ΣPFAS = 1.5729). This trend was also observed for EtFOSA concentrations, with mean (logarithmic scale) values of 1.5165 for the not renovated ones in comparison to the renovated ones, with values of 1.25.

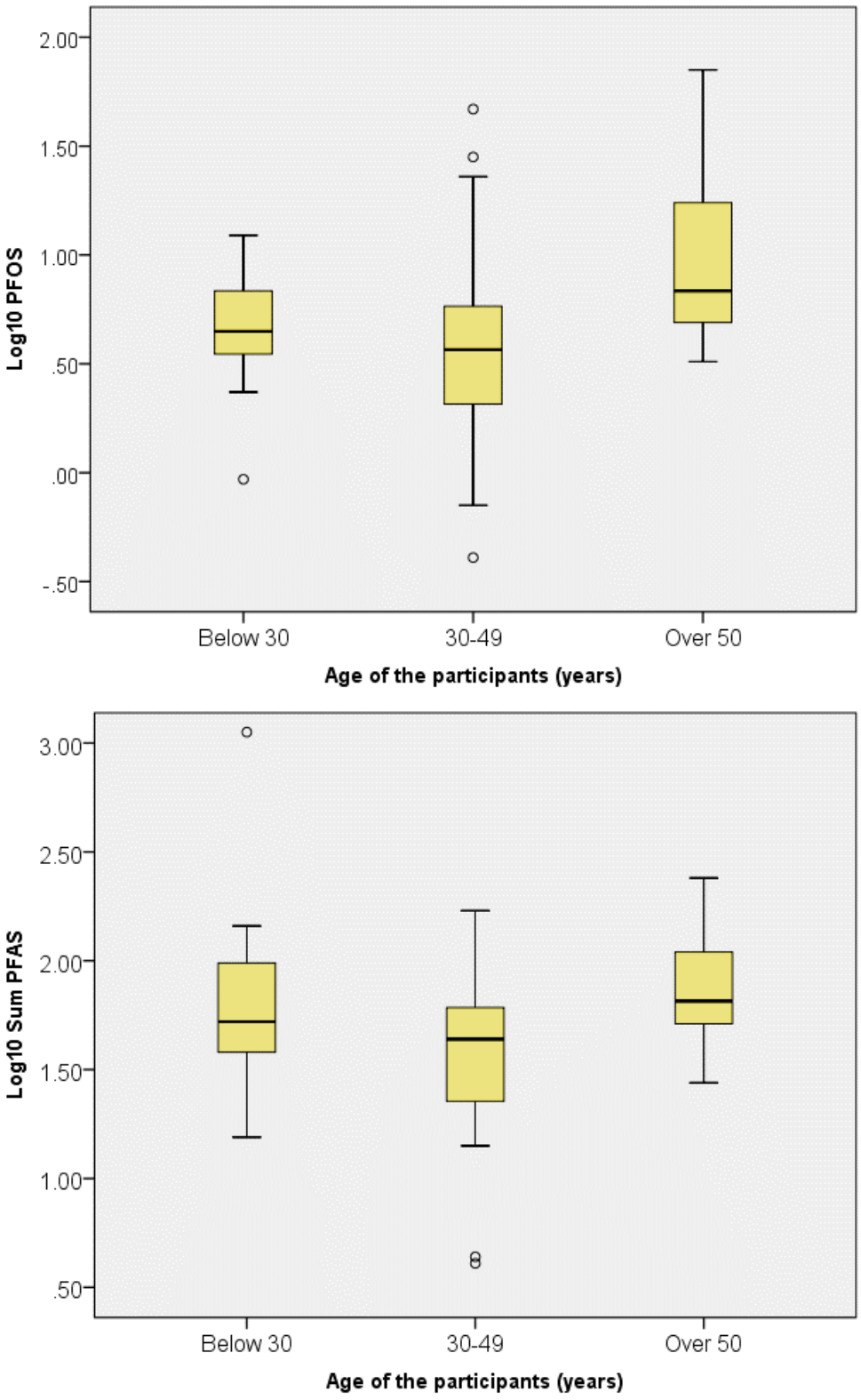


Figure 13. Box plots showing significant differences ($p < 0.01$) found among the three groups of ages when the concentrations of individual PFOS and the sum of PFOS precursors were considered

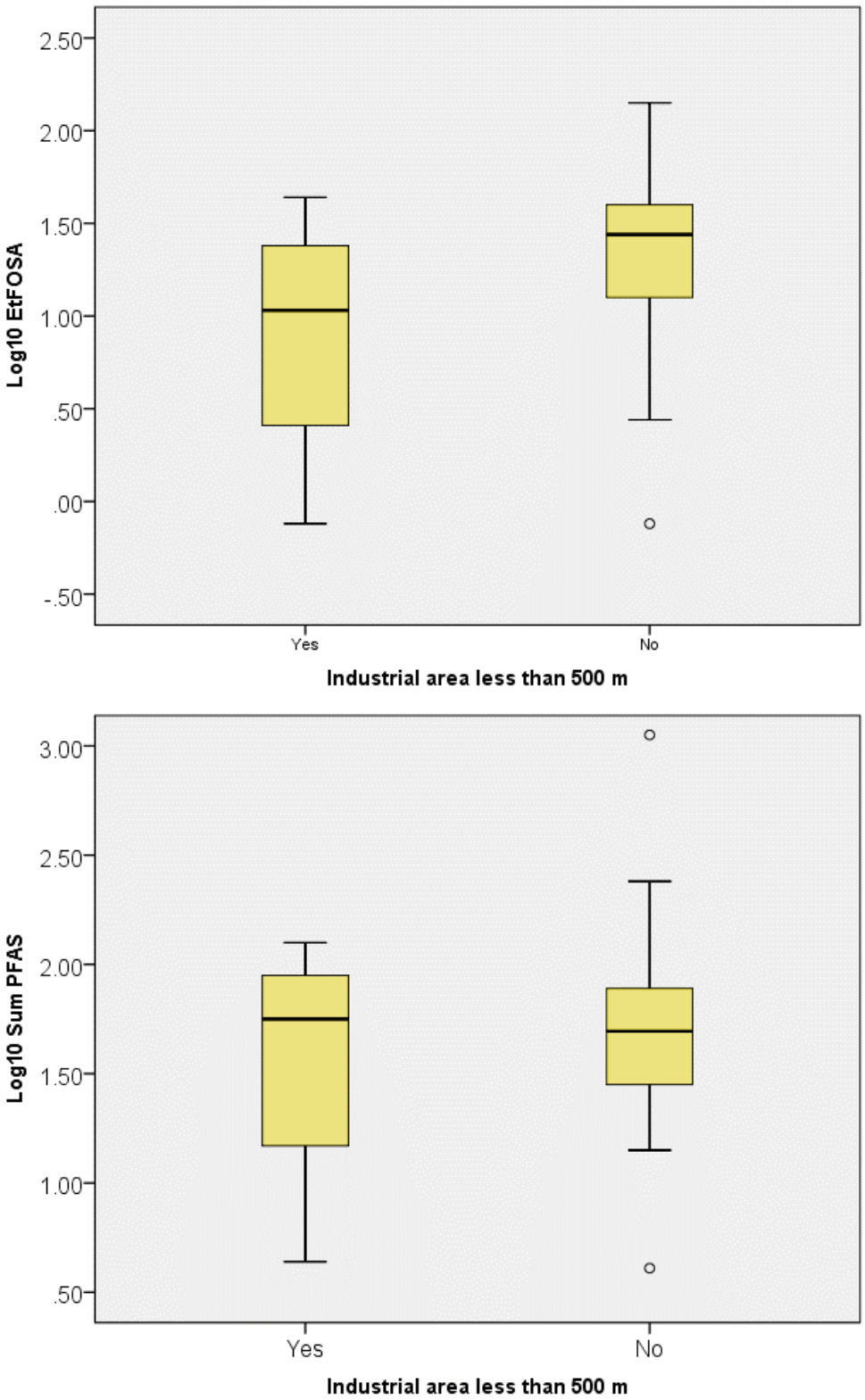
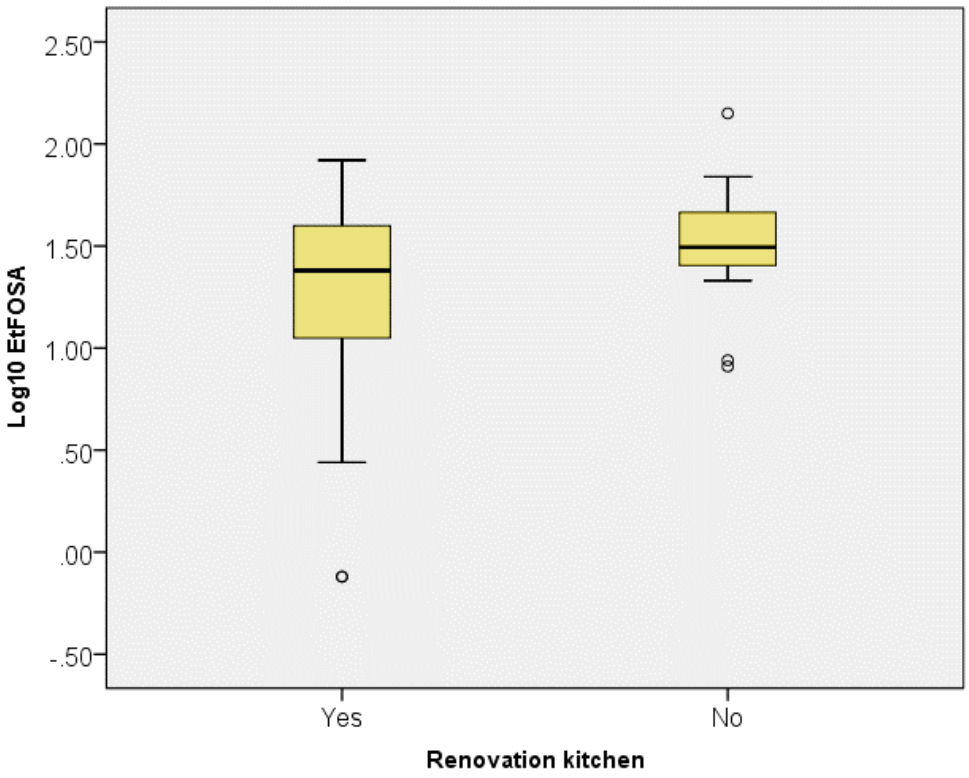


Figure 14. Box plots showing significant differences ($p < 0.01$) between the participants whose residences were close to industrial areas the ones not when the concentrations of individual EtFOSA and the sum of PFOS precursors were considered



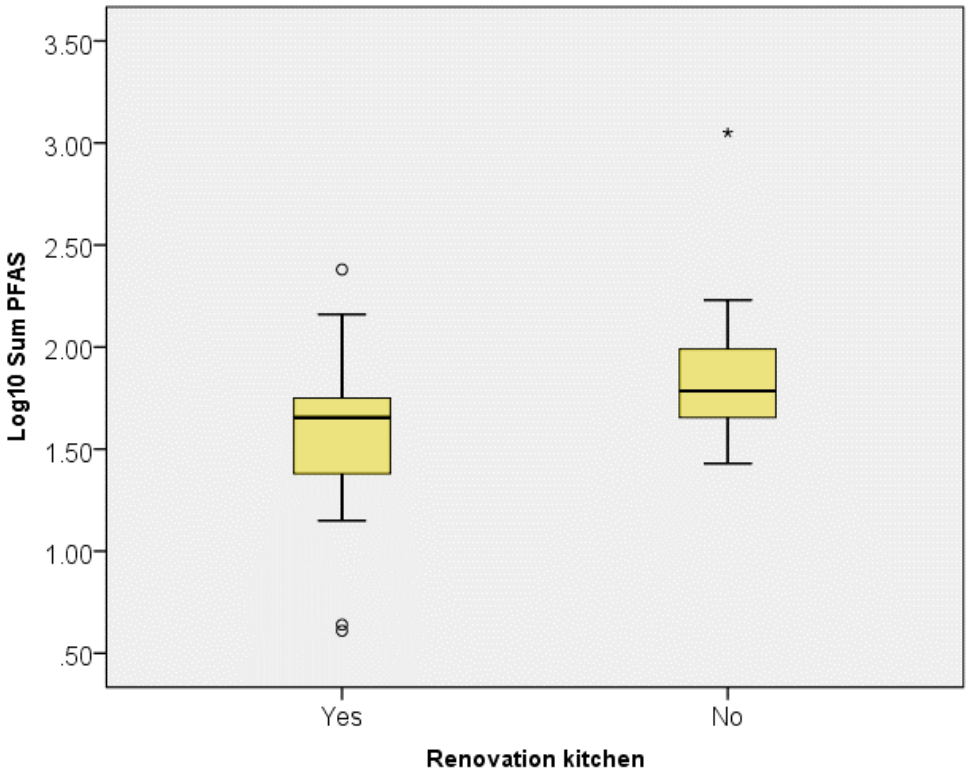
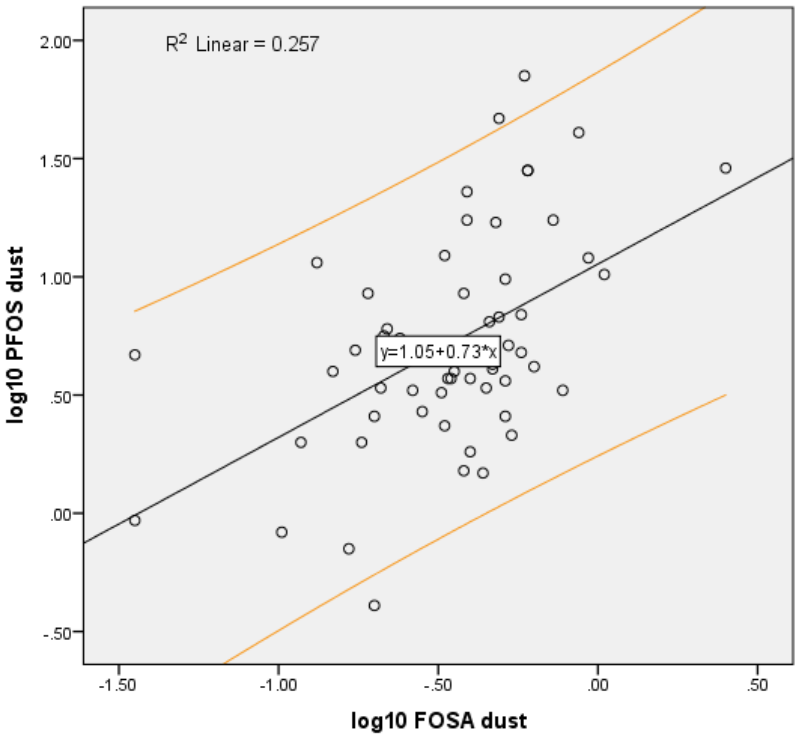


Figure 15. Box plots showing significant differences ($p < 0.01$) between the participants whose kitchens were recently renovated and not, when the concentrations of individual and the sum of PFOS precursors were considered



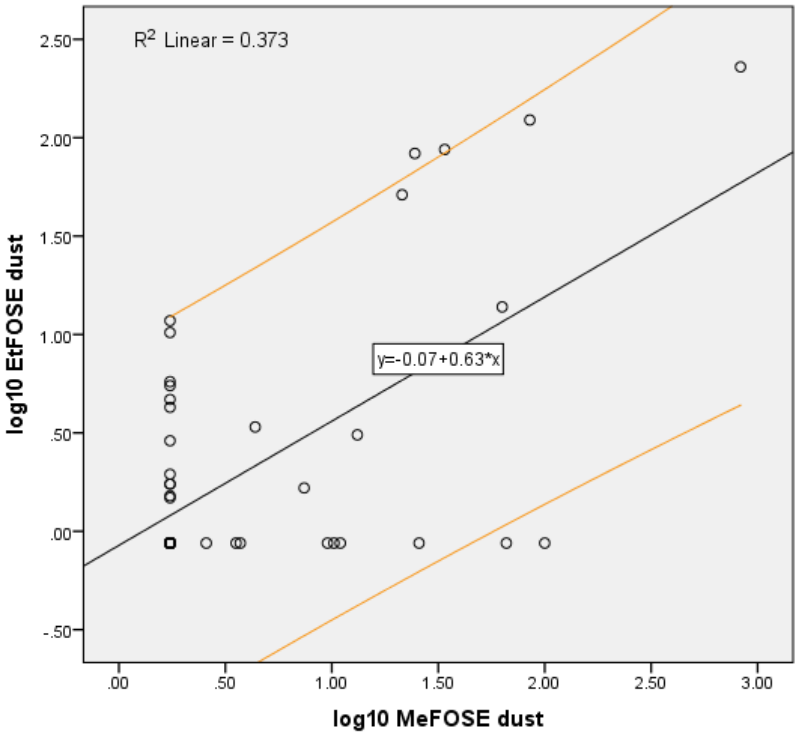


Figure 16. Scatter plots with significant positive correlations ($p < 0.01$) between indoor dust levels of the pairs PFOS and FOSA, and EtFOSE and MeFOSE

Correlations for PFOS, FOSA, EtFOSA, MeFOSE and EtFOSE and MeFOSE were also analysed. Significant positive correlations were found for the pairs PFOS and FOSA ($p < 0.01$) with a regression coefficient of 0.26, between EtFOSE and MeFOSE ($p < 0.01$) with a regression coefficient of 0.37 and between EtFOSA and PFOS ($p < 0.05$). *Figure 16* shows graphical representations of the two positive correlations at the level of 0.01.

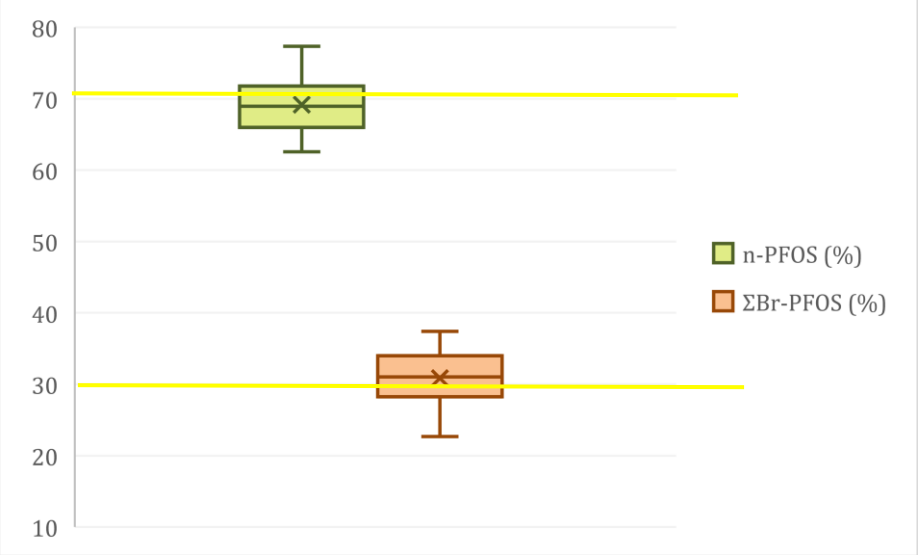


Figure 17. Percentages of linear and branched PFOS isomers identified in the positive vacuum cleaner dust samples from the A-TEAM cohort, where the yellow lines represent the theoretical percentage of the commercial PFOS mixture (70 % linear and 30 % branched isomers)

Besides the generic evaluation of the indoor and demographic parameters of the entire dataset, some abnormally high concentrations were found for two samples (at least one of the precursors exceeded 100 ng/g), so they were individually checked for further evaluation. The first sample corresponded to a participant who was a non-smoker living alone, around 20 km from the NIPH in a wooden semi-

detached house, not close to busy traffic roads or industrial areas. The house was 70 m^2 surface, recently renovated entirely and it was not ventilated during the sampling period. The participant owned Gore-Tex® clothing and shoes, but not waterproof sprays. On the other hand, the second one corresponded to a non-smoker living with another adult, 5 km from the NIPH in a brick apartment built within the last ten years, far from busy roads and industrialised areas. The apartment was 60 m^2 surface and the participant ventilated the bedroom during the sampling campaign period. The participant used Gore-Tex® shoes, clothing and sprays, and cooked with Teflon pans and utensils.

4.3.2 PFOS Branched Isomers

The expected ratio between linear:branched isomers for external exposure to PFOS is 70:30 (%), as in the manufactured product (Houde *et al.*, 2008; Buck *et al.*, 2011; Beesoon and Martin, 2015a). In order to verify or discard isomer specific degradation of the monitored PFOS precursors to PFOS in the environment, all the positive samples for total PFOS analysis, were re-analysed using the second of the methods detailed in the methodology chapter. As not all the individual isomers could be elucidated and identified from the standard mixture, the sum of the branched isomers (ΣBr-PFOS) versus the linear one (n-PFOS) was used. These ratios were used to check if the initial premise was confirmed.

Descriptive statistics from the vacuum cleaner dust samples where PFOS and PFOS precursors were analysed are shown in *Table 26* and represented in *Figure 17*. Individual sample results are detailed in *Table SM02.* As expected, mean value of the

experimental ratio (expressed as n-PFOS / ΣBr-PFOS) was 2.26, which corresponded to 69 % n-PFOS and 31 % ΣBr-PFOS, as expected from the technical mixture.

Table 26. Linear (n) and branched (Br) PFOS isomers (%) in vacuum cleaner bags (n = 57)

	n-PFOS / ΣBr-PFOS	**n-PFOS (%)**	**ΣBr-PFOS (%)**
Average	2.29	69	31
Mean	2.26	69	31
Median	2.22	69	31
Min	1.67	63	23
Max	3.41	77	37
Q1 (25th percentile)	1.96	66	28
Q3 (75th percentile)	2.51	72	34
SD	0.40	4	4
% RSD	17.67	5	12

SD = Standard deviation. RSD = Relative standard deviation. n = number of dust samples included in the statistics

4.4 DAILY INTAKES OF PFOS AND PFOS PRECURSORS VIA DUST INGESTION

Vacuum cleaner dust concentrations were used to estimate the daily exposure of the participants to the selected organic pollutants. Mean and high intake rate scenarios were selected. Estimated daily intakes (EDI) were calculated according to *Equation 09*:

Equation 09 $$EDI = C\ x \frac{Rdust}{W}\ x\ F$$

Where C is the concentration of the specified pollutant (ng/g), R is the daily dust ingestion rate (mg/day) set as 4.16 (mean scenario) and 55 (high scenario) for adults (USEPA, 2011), W is the body weight (kg) reported by the participants, and F is the uptake fraction. This last parameter was assumed to be F = 1 (100 % absorbed) as for other exposure studies (Fromme *et al.*, 2009; Goosey and Harrad, 2011; Xu *et al.*, 2013; Tian *et al.*, 2016), and as representation of the highest uptake scenario. No dermal absorption was considered.

Descriptive statistics of the estimated daily intakes of PFOS and PFOS precursors according to mean and high scenarios, and normalized by body weight for the participants are shown in *Table 27* and *Table 28*, respectively, while individual results are detailed in *Table SM03* and *Table SM04*. Daily intake values in mean scenario, which would represent exposure for most of the population, showed mean values ranged from 0.03 pg/bw kg/day for FOSA and MeFOSA to 1.88 for EtFOSA (ΣPFAS = 2.33 pg/bw kg/day). Meanwhile, in the high scenario, these values ranged from 0.35 pg/bw kg/day for FOSA and 24.80 for EtFOSA (ΣPFAS = 30.8 pg/bw kg/day).

4.4.1. Daily Intakes in Adults

For PFOS, daily intakes were estimated to be 0.56 and 7.37 pg/bw kg/day for mean and high scenarios, respectively. These values are significantly lower than the tolerable daily intakes of 150 ng/kg bw/day promoted by the European Food Safety Authority (EFSA) (EFSA, 2008), and for the 100 ng/kg bw/day promulgated by the German Federal Institute for Risk Assessment (BfR) (Miralles-Marco and Harrad,

2015), both established for PFOS exposure. Still, when considering direct and indirect exposure to PFOS, assuming same uptake fraction for PFOS and for its precursors, and according to the concentrations presented in this paper, external exposure to PFOS via FOSAs and FOSEs direct exposure would contribute significantly to the overall exposure to PFOS. These findings are in line with previously reported models based on Scenario-Based Risk Assessment approaches in which indirect exposure to PFOS via indoor dust in high exposed scenarios was estimated to contribute up to 68 % according to Vestergren et al. (Vestergren *et al.*, 2008), and up to 11 % (low exposure scenario) and 33 % (high exposure scenario) according to Gebbink et al. (Gebbink, Berger and Cousins, 2015).

Table 27. Daily intakes of total PFOS and PFOS precursors (pg/kg bw/day) through dust ingestion for adults in mean scenario (4.15 mg dust/day) (n = 57)

	PFOS	**FOSA**	**MeFOSA**	**EtFOSA**	**MeFOSE**	**EtFOSE**	**ΣPFAS**
Average	0.52	0.03	0.03	1.93	1.30	0.71	4.51
Mean	0.30	0.02	< 0.01	1.31	0.23	0.12	2.88
Median	0.29	0.02	< 0.01	1.60	0.12	0.06	2.94
Min	0.03	< 0.01	< 0.01	0.05	0.08	0.04	0.26
Max	3.59	0.19	0.42	10.61	43.63	12.05	58.36
Q1	0.16	0.16	0.01	0.00	0.75	0.10	0.05
Q3	0.56	0.56	0.03	0.00	2.63	0.28	0.12
5th percentile	0.05	0.01	< 0.01	0.29	0.08	0.04	0.92
95th percentile	1.86	0.04	0.15	4.68	3.82	5.80	10.23
SD	0.67	0.03	0.08	1.71	5.81	2.15	7.78
RSD (%)	129.87	95.17	317.47	88.79	445.27	302.08	172.52

Q1: 25th percentile..Q3: 75th percentile. SD = Standard deviation. RSD = Relative standard deviation. n = number of dust samples included in the statistics

Table 28. Daily intakes of total PFOS and PFOS precursors (pg/kg bw/day) through dust ingestion for adults in high scenario (55 mg dust/day) (n = 57)

	PFOS	**FOSA**	**MeFOSA**	**EtFOSA**	**MeFOSE**	**EtFOSE**	**ΣPFAS**
Average	6.87	0.35	0.34	25.46	17.25	9.39	59.66
Mean	4.03	0.27	0.06	17.27	2.98	1.55	38.04
Median	3.82	0.30	0.04	21.16	1.59	0.80	38.89
Min	0.40	0.02	0.02	0.61	1.01	0.50	3.47
Max	47.43	2.55	5.59	140.24	576.88	159.28	771.65
Q1	2.12	2.12	0.19	0.04	9.92	1.37	0.69
Q3	7.44	7.44	0.40	0.05	34.81	3.76	1.54
5th percentile	0.72	0.09	0.03	3.86	1.08	0.54	12.17
95th percentile	24.64	0.56	2.01	61.83	50.52	76.67	135.26
SD	8.92	0.34	1.07	22.60	76.81	28.37	102.92
RSD (%)	129.87	95.17	317.47	88.79	445.27	302.08	172.52

Q1: 25th percentile..Q3: 75th percentile. SD = Standard deviation. RSD = Relative standard deviation. n = number of dust samples included in the statistics

4.4.2. Daily Intakes in Children

In a second approach, PFOS and PFOS precursors concentrations were employed to estimate EDIs for toddlers (1 to < 3 years old) and children (3 to < 6 years old). On this approach, mean (100 mg/day) and high (200 ng/day) intake rate scenarios were selected, as well as three concentration levels (5th percentile, 50th percentile, and 95th percentile) (Karásková *et al.*, 2016).

Table 29. Estimated daily intakes of total PFOS and PFOS precursors (ng/kg bw/day) through dust ingestion for toddlers and children

	PFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	ΣPFAS
1 to <2 years old							
Mean intake scenario							
5th percentile	0.008	0.001	<0.001	0.038	0.015	0.008	0.133
50th percentile	0.044	0.003	0.001	0.191	0.033	0.017	0.420
95th percentile	0.274	0.008	0.027	0.619	0.618	0.739	1.306
High intake scenario							
5th percentile	0.016	0.002	<0.001	0.076	0.031	0.015	0.267
50th percentile	0.089	0.006	0.001	0.381	0.066	0.034	0.839
95th percentile	0.548	0.015	0.055	1.238	1.237	1.477	2.612
2 to <3 years old							
Mean intake scenario							
5th percentile	0.007	0.001	<0.001	0.031	0.013	0.006	0.110
50th percentile	0.037	0.002	0.001	0.157	0.027	0.014	0.347
95th percentile	0.227	0.006	0.023	0.511	0.511	0.610	1.079
High intake scenario							
5th percentile	0.013	0.002	0.001	0.062	0.025	0.013	0.220
50th percentile	0.073	0.005	0.001	0.315	0.054	0.028	0.693
95th percentile	0.453	0.013	0.045	1.023	1.022	1.220	2.157
3 to < 6 years old							
Mean intake scenario							
5th percentile	0.005	0.001	<0.001	0.023	0.009	0.005	0.082
50th percentile	0.027	0.002	<0.001	0.117	0.020	0.010	0.257
95th percentile	0.168	0.005	0.017	0.379	0.379	0.453	0.800
High intake scenario							
5th percentile	0.010	0.001	<0.001	0.046	0.019	0.009	0.163
50th percentile	0.055	0.004	0.001	0.234	0.040	0.021	0.514
95th percentile	0.336	0.009	0.034	0.759	0.758	0.905	1.601

Mean intake scenario = 100 mg/day. High intake scenario = 200 mg/day

Weights: 1-2 years old = 11.4 kg. 2-3 years old = 13.8 kg. 3-6 years old = 18.6 kg

5th percentile, 50th percentile (mean) and 95th percentile concentrations (ng/g) as calculated in Table 27 and *Table 28 (n = 57)*

In the case toddlers and children, hand to mouth dust ingestion is more relevant than in adults (25 times higher exposure in mean exposed scenario, while four times higher for the higher exposed one). According to the personal questionnaires, 26 % of the participants reported the presence of at least one child up to 6 years old living in the sampled residences, and so, exposed to same concentrations of PFOS and PFOS precursors as the participants. For the calculation of the daily intakes, the data was also normalised to body weight according to the range of ages considered (1 to 6): 1 to <2 years old (11.4 kg), 2 to <3 years old (13.8 kg), and 3 to < 6 years old (18.6 kg) (USEPA, 2011). EDIs (ng/bw kg/day) for children in mean and high dust intake scenarios for three exposure levels are represented in *Table 29*. Daily intake values in mean intake scenario (50^{th} percentile) ranged from ΣPFAS = 0.26 ng/bw kg/day for 3 to 6 years old children to 0.42 for 1 to 2 years old toddlers, while same percentile (50^{th}) in high exposed scenario showed values from 0.51 to 0.84 ng/bw kg/day, respectively. In case of worst case scenario (high intake, 95^{th} percentile), values ranged from 1.6 to 2.6 ng/bw kg/day for ΣPFAS, and from 0.34 to 0.55 ng/bw kg/day when just considering PFOS, being both values two orders of magnitude higher than the previously estimated in the high exposed scenario for adult population. This fact evidence how lower body weights – younger children –, besides the higher ingestion of dust due to hand to mouth contact, contribute to higher daily intake of dust, and so, to the associated risk assessment concerns. As for adult population, these values are significantly lower than tolerable daily intakes, even when considering ΣPFAS instead of just PFOS. However, even knowing dust is not the only source of internal levels of PFOS but a relevant contributor, dust exposure

has to be carefully considered for the estimation of the overall body burdens of PFOS, especially for children.

4.5 COMPARISON WITH PREVIOUS STUDIES

Two previously reported datasets were used for statistical comparison with the data reported in this paper. The Norwegian study conducted by Haug et al. (Haug, Huber, Schlabach, *et al.*, 2011) analysed the same PFOS precursors as those selected in this study for another Norwegian population, while the British study conducted by Goosey and Harrad (Goosey and Harrad, 2011) employed the same instrumentation and laboratory presented here, and in both cases, there was available raw data for the comparison. The importance of this comparison relies in two different aspects:

- Trends in PFOS and PFOS precursors tend to decline
- Settled dust is usually less contaminated with PFAS than floor dust

The cohort from this study included 57 dust samples of vacuum cleaner bags collected as described along the methodology chapter. The study by Goosey and Harrad included 45 floor dust samples collected from homes in the UK, collected by the procedure described in the mentioned paper. The study by Haug et al. included 41 settled dust samples collected and analysed as specified in the referenced paper.

In a first general overview, *Figure 18* shows the individual percentage contributions of individual target PFASs to ΣPFAS – study by study – for PFOS and PFOS precursors. One of the charts (Haug, Huber, Becher, *et al.*, 2011) differs from the other two because EtFOSE was not measured in that study, but the other two studies

also differ substantially in the PFASs profile. A second comparison is shown in *Figure 19,* where relative percentages are shown next to each other, and EtFOSE was excluded. In there, the different profiles are clearly visible. The study presented here (A-TEAM) is clearly dominated by the presence of EtFOSA (as percentage of amount of individual PFASs versus ΣPFAS), and followed by PFOS and MeFOSE, while for Goosey & Harrad the exposure was clearly dominated by PFOS followed by MeFOSE, and in case of Haug et al., it was equally dominated by PFOS and MeFOSE. In all cases, the lowest contributors to dust exposure are FOSA and MeFOSA.

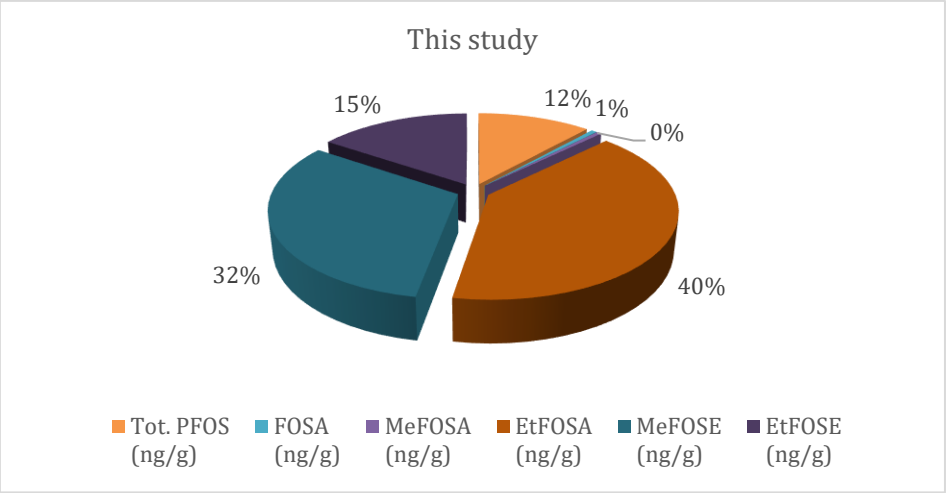
In terms of percentage of positive samples, the three studies revealed 100 % of positive samples for PFOS, followed by FOSA as another compound detected in a high percentage of samples (76 % for Goosey & Harrad, 24 % for Haug et al. and 96 % for this study). MeFOSA was in all three cases the compound detected in the lowest percentage of samples (29 % for Goosey & Harrad, 2 % for Haug et al. and 16 % for this study). On the other hand, larger diversity was found for EtFOSA and MeFOSE, with much higher detection frequencies for Goosey & Harrad (89 % for both of them) when they were compared to this study (96 and 35 % respectively) and to Haug et al. (15 and 10 % respectively).

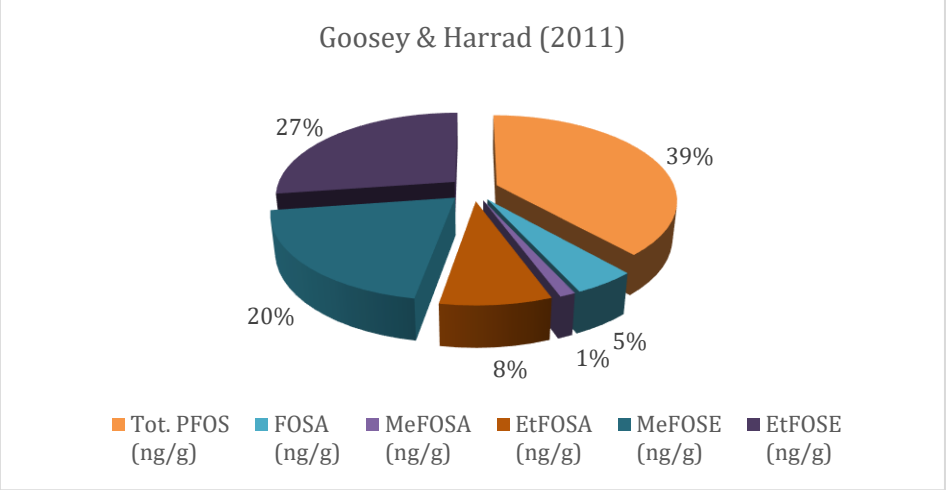
Figure 20 gives an individual and more detailed overview of the different exposure patterns beyond just percentages or detection frequencies. The figure shows the box plots relative to the t-test - significance level of 95 % (p = 0.05) – in case of EtFOSE (not included in Haug et al.) and MeFOSA (detection frequency lower than 10°% in Haug et al.), and the ANOVA tests – Scheffe post-hoc test with a significance level 95 % (p = 0.05) – for PFOS, FOSA, EtFOSA and MeFOSE. Significant differences for

all the studied pollutants were found when comparing the determined concentrations from the three cohorts.

For both compounds, PFOS and for FOSA, significant higher concentrations were identified in the UK study of Goosey & Harrad, while statistically comparable values were detected in Haug et al. when compared to this study. For EtFOSA, significant differences were found among the three studies, with statistically higher concentrations for Goosey & Harrad, followed by Haug et al. with this study displaying the lowest concentrations. A similar trend was identified for MeFOSE, with significant higher concentrations in Goosey & Harrad, but no significant difference between Haug et al. and this study, even though higher concentrations were detected for the Norwegian one. Data for MeFOSA and EtFOSE was just compared to the UK cohort, and the two compounds showed two different patterns. MeFOSA concentrations were significantly higher, while EtFOSE concentrations were significantly lower in this study when both were compared to the British data.

In addition to the differences due to the nature of the cohort itself, there are other factors which could lead to differences in exposure patterns related to the concentrations of PFOS precursors (especially EtFOSE and MeFOSE, the most volatile ones):





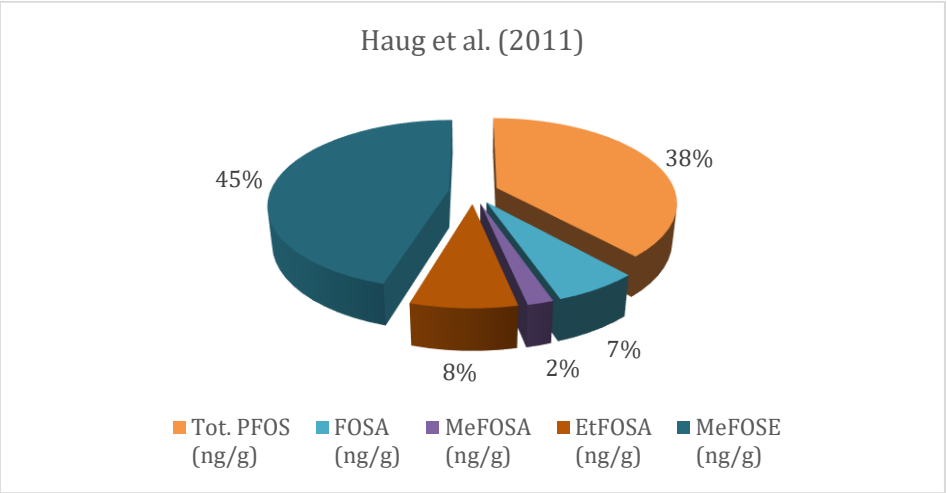


Figure 18. Different exposure profiles, expressed as percentage of ΣPFAS, for PFOS, FOSAs and FOSEs , where n = 45 (Goosey & Harrad), n = 41 (Haug et al.) and n = 57 (this study). The relative abundances differ substantially among the three studies

- Different populations and locations: especially when considering two of the studies were carried out in Norway and the third one in England.
- Different sampling methods: Haug et al. collected settled dust samples from living rooms, Goosey & Harrad collected floor dust from a specific area (1 m^2) of the houses, while this study collected vacuum cleaner bag dust samples representatives from the entire house.
- Seasonal variations: Goosey & Harrad carried out the sample campaign for two entire years, Haug et al. between February and May, and this study between November and April.

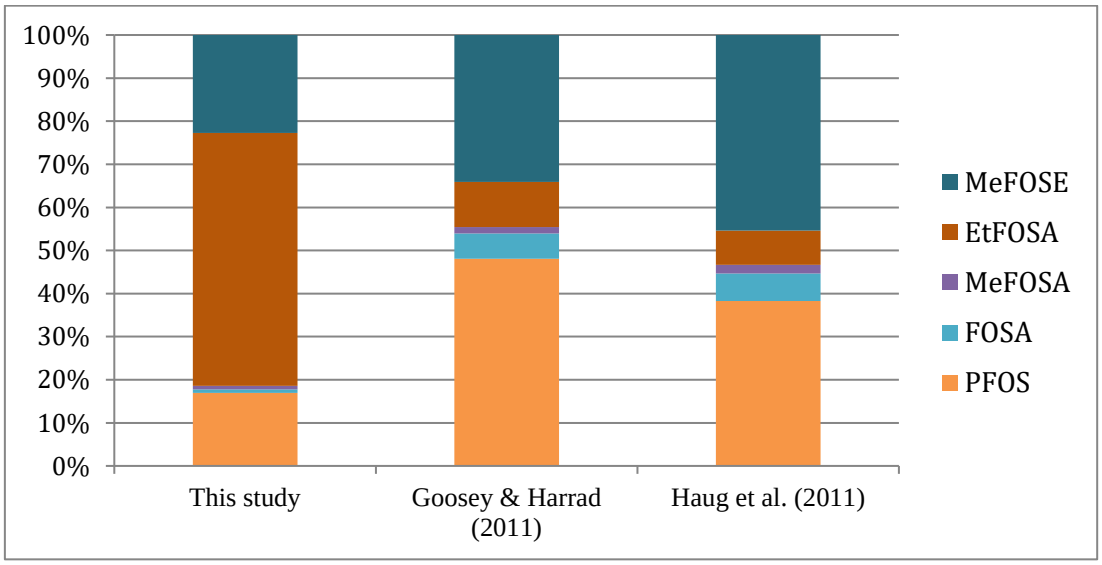


Figure 19. Different exposure profiles, expressed as percentage of ΣPFAS, for PFOS, FOSAs, and FOSEs from this study (n = 57) UK (n = 45), and Norway (n = 41) studies. (EtFOSE omitted as not measured by Haug et al. (2011))

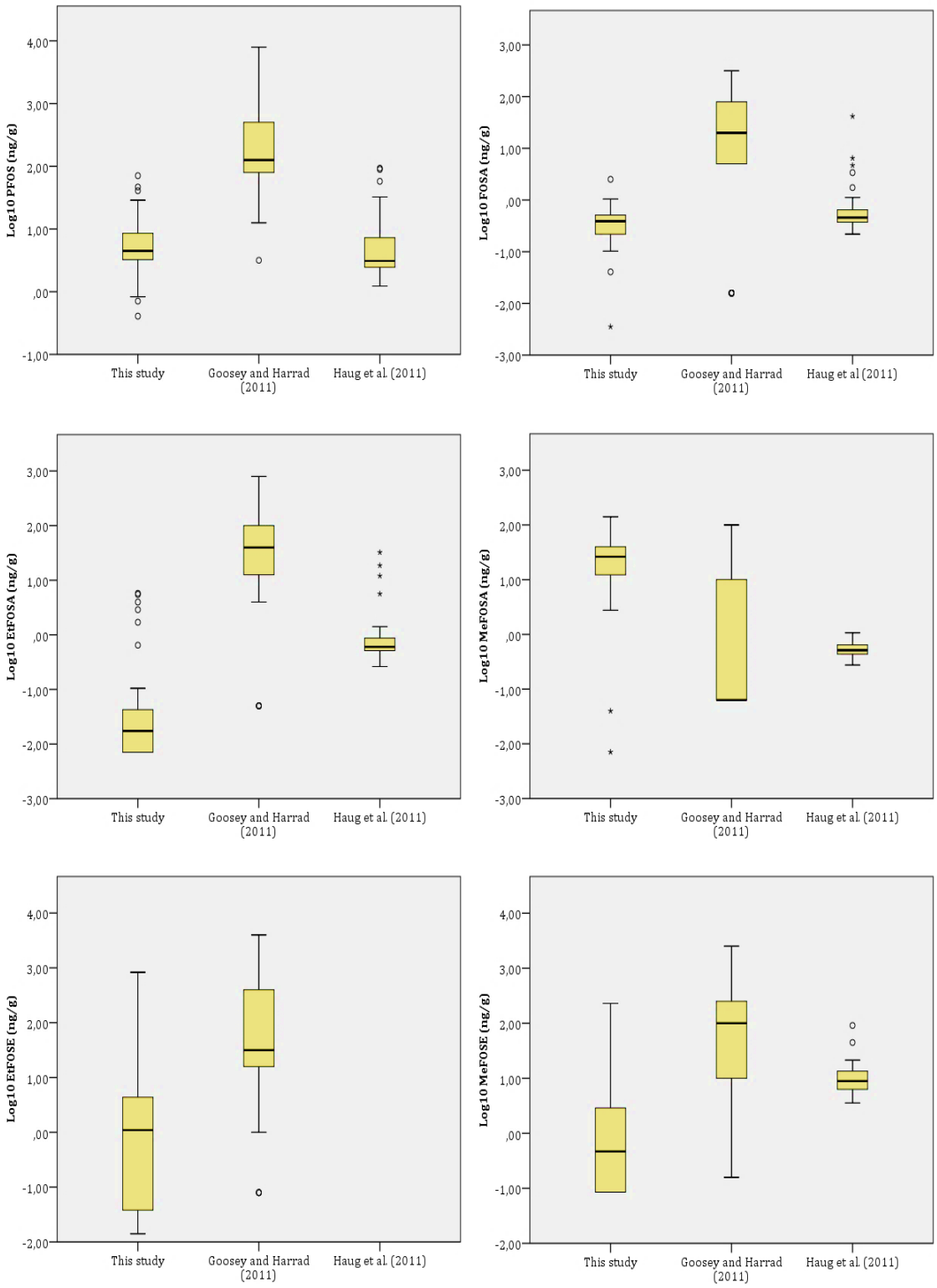


Figure 20. Comparison of three different studies where PFOS, FOSAs and FOSEs have been analysed. Significant differences (p = 0.05) were observed between the concentrations of the six analysed pollutants in the three studies

4.6 SUMMARY AND CONCLUSIONS

This study has shown that PFOS and PFOS precursors are still detectable in a high percentage of samples from indoor dust samples in Norwegian population (100 % positive samples for PFOS) , even though their use was restricted in 2002 and PFOS was included as a POP under the Stockholm convention in 2009 (UNEP, 2010). After more than 15 years of restriction, they remain present at measurable concentrations in indoor dust samples (average concentration ΣPFAS reported in this study of 77.2 ng/g). This fact indicates that indoor environments are still a source, or a reservoir, for PFASs.

Since the early 2000s, PFOS has been measured in indoor dust samples by many authors (Moriwaki, Takata and Arakawa, 2003; Björklund, Thuresson and De Wit, 2009; Kato, Calafat and Needham, 2009; Ericson Jogsten *et al.*, 2012; Fraser *et al.*, 2013; Xu *et al.*, 2013; Shoeib *et al.*, 2016) in different indoor microenvironments like homes, offices and cars. Since then, trends in average values have been decreasing from the average value of 443.7 ng/g detected by Kubwabo et al. (Kubwabo *et al.*, 2005) for Canadian home dust, 640.7 ng/g by Goosey & Harrad (Goosey and Harrad, 2011) for English classrooms, or 175.2 ng/g by Bjorklund et al. (Björklund, Thuresson and De Wit, 2009) for Swedish homes, but all of them reporting different orders of magnitude data depending on the location of the study (see *Table 03*). More recent studies reported lower concentrations for these indoor micro environments, as Tian et al. (Tian *et al.*, 2016) who reported average values of 13.7 ng/g for Korean houses or Shoeib et al. (Shoeib *et al.*, 2016) who reported median values of 0.29 ng/g for Egyptian homes, but still relevant in terms of both, concentrations and detection frequencies. Average values found in this study for

PFOS (average = 8.95 ng/g), are in line with the decreasing trends, much lower than the reported by Goosey and Harrad (Goosey and Harrad, 2011) for the UK (average = 144.7 ng/g), and slightly lower than the value reported by Haug et al. (Haug, Huber, Schlabach, *et al.*, 2011) for the Norwegian population (average = 10.9 ng/g), even though this last ones corresponded to settled dust (sampled in 2010) – usually presenting lower concentrations of PFASs – versus the analysed vacuum cleaner bags dust (sampled in 2014) reported in this thesis.

Interestingly, as *Figure 18* and *Figure 19* revealed, the overall indoor exposure to PFOS and PFOS precursors in this study is not dominated by PFOS, although this is the most frequently determined PFASs from those included in this study. Instead, the profile was: EtFOSA (40 %), followed by MeFOSE (32 %), EtFOSE (15 %) and PFOS (12 %). It is well known that perfluorinated substances like EtFOSA – among other FOSAs – were employed in grease and water repellent coatings (Tittlemier, Pepper and Edwards, 2006). As a consequence of it, its presence in indoor microenvironments is feasible due to its release from coatings and later deposition in dust particles. On the other hand, FOSA was present in most of the samples (DF = 96 %) but in much lower concentrations, contributing around 1 % to the overall PFASs contribution. These findings are in agreement with recently reported papers where the underestimation of PFOS precursors was suggested when evaluating the overall exposure to PFOS, and its consequent body burdens (Vestergren *et al.*, 2008; D'Eon J and Mabury, 2011; Gebbink, Berger and Cousins, 2015). Moreover, statistical regression analysis showed significant positive correlations between PFOS and FOSA, EtFOSE and MeFOSE, as well as EtFOSA and PFOS, suggesting common sources of indoor contamination with these PFASs, in

agreement with previous published studies (Strynar and Lindstrom, 2008; Björklund, Thuresson and De Wit, 2009; Shoeib *et al.*, 2016). This information is also in agreement with the need to further investigate the sources of contamination by PFOS and its precursors.

Concentrations for FOSAs and FOSEs have been reported by Goosey & Harrad (Goosey and Harrad, 2011), Haug et al. (Haug, Huber, Schlabach, *et al.*, 2011), Shoeib et al. (Shoeib *et al.*, 2016) and Fraser et al. (Fraser *et al.*, 2013). Different patterns of exposure were identified when comparing this study with the English and Norwegian cohorts, which could be partially attributed to differences between the participants, seasonal differences leading to air/dust distribution changes and ventilation habits, and the origin of the collected dust. Still, all of the studies revealed appreciable concentrations of EtFOSA, MeFOSA, and MeFOSE.

The sources of PFOS and PFOS precursors could not be determined from correlations with personal data, indoor questionnaires and room contents, besides the age of the participants, the proximity to industrial areas and the renovation of the house. Some other parameters like age and origin of the furniture, carpets or waterproof clothing were not included in the indoor questionnaires, some of which could contribute to a deeper knowledge of indoor exposure origin.

Average estimate daily intake of ΣPFAS for the investigated cohort was estimated to be 4.51 pg/kg bw/day in mean scenario, and 59.7 pg/kg bw/day for the highest one. In case of children population, worst case scenario (high intake scenario, 95^{th} percentile) showed an estimated daily intake of 2.6 ng/kg bw/day, two orders of magnitude higher than the values reported for adult population. Still, these values

are significantly lower than the tolerable daily intakes of 150 ng/kg bw/day (EFSA, 2008), and 100 ng/kg bw/day (BfR) promulgated by European food authorities.

Moreover, indirect exposure pathways and their certain contribution to the overall body burdens of PFOS remain partially unknown. Metabolic pathways and conversion ratios, discussed later in this thesis, need further research to better model, and understand the link between external and internal exposure to PFOS precursors, which nowadays are present in Norwegian indoor environments in higher levels (88 %) than the historically reported PFOS (12 %).

5. PFOS AND PFOS PRECURSORS IN FOOD SAMPLES

The main hypothesis of this chapter is that the diet contributes to: a) direct exposure to PFOS and b) direct exposure to PFOS precursors which might contribute to indirect exposure to PFOS via subsequent metabolism of FOSAs and FOSEs – discussed briefly in the conclusions from this chapter, and in the later chapter focused on *in vitro* metabolism – for the Norwegian A-TEAM cohort. A secondary aim is to find correlations between different groups of food items and food packaging with the presence or absence of PFASs and identify those contributing substantially to human body burdens.

5.1 INTRODUCTION

As mentioned in the introduction chapter, diet – as solid food and drinking water - has been suggested by some authors as the main, or one of the most important routes of external exposure to PFOS (Tittlemier, Pepper and Edwards, 2006; D'Eon J and Mabury, 2011; Gebbink, Berger and Cousins, 2015; Miralles-Marco and Harrad, 2015), with its presence extensively reported in food items and water for the last years (Martin *et al.*, 2004; Ericson, Nadal, *et al.*, 2008; Kärrman *et al.*, 2009; Haug *et al.*, 2010; Noorlander *et al.*, 2011; van Asselt *et al.*, 2011; Domingo, 2012; Klenow *et al.*, 2013; Rahman, Peldszus and Anderson, 2014; Gebbink *et al.*, 2015). In a previously reported study from Norway (Haug *et al.*, 2010), total dietary intake for the 16 perfluorinated substances was measured, reporting estimated intakes of 100 ng/day for the sum of the 16 compounds, with around 50 % of that contribution due to PFOS and PFOA combined. PFOS was detected in all food matrices except in tea.

Other European authors, such as Fromme et al. (Fromme, Midasch, *et al.*, 2007) reported in 2007 median values for PFOS intakes in German population of 1.4 ng/kg/day, while in 2010, Schuetze et al. (Schuetze *et al.*, 2010) estimated – also for German samples - concentrations up to 225 µg/kg ww in wild fish from populated regions. In Sweden, Berger et al. (Berger *et al.*, 2009) also identified fish from polluted water systems as a potential source of PFOS exposure. Moreover, in Denmark, Halldorsson et al. (Halldorsson *et al.*, 2008) highlighted meats and snacks as predictors of blood levels of PFOS.

While direct exposure has traditionally been suggested as the main route of exposure humans can be exposed to PFOS – and to PFASs in general – directly or indirectly, with indirect exposure a consequence of external exposure to PFOS precursors and the subsequent metabolism. In recent years, the growing concern about PFOS precursors, the number of papers also including data on some of these PFOS precursors have increased in number, as mentioned in the introduction and dust chapters. Still, the number of papers reporting levels of PFOS precursors in food items remains rather limited (Tittlemier, Pepper and Edwards, 2006; Ericson, Nadal, *et al.*, 2008; Ullah *et al.*, 2014; Krafft and Riess, 2015).

The presence of PFOS (and potentially of PFOS precursors) in food items is a combination of a mixture of two different pathways: a) bio-concentration though the food chain and b) contact with contaminated food packaging materials. The first one is observed when moving up within the food chain and trophic levels (Krafft and Riess, 2015), this is especially relevant for long chain PFASs – as PFOS and its precursors – due to their low elimination rate and high persistence in the body (Houde *et al.*, 2008; Loi *et al.*, 2011). The second one likely arises from the presence

of PFOS precursors in food items, where such precursors incorporated within coatings of paper products migrate to the food items they are in contact with (Skutlarek, Exner and Färber, 2006; Tittlemier, Pepper and Edwards, 2006; Ericson, Martí-Cid, *et al.*, 2008; Jogsten *et al.*, 2009; Shoeib *et al.*, 2016).

Among the broad spectrum of food items included in common human diets, fish, meat, egg, and dairy products have been considered as the most important food items constituting sources of PFASs by bioaccumulation (Tittlemier, Pepper and Edwards, 2006; Ericson *et al.*, 2009; Haug, Huber, Becher, *et al.*, 2011; Vestergren *et al.*, 2012), while pre-packed food and the number of paper and plastic wrapping items was added to the list of variables of interest when considering migration from food packaging as a source of PFOS related contamination.

5.2 FOOD DIARIES DESCRIPTION AND COMPOSITION OF THE FOOD SAMPLES

An empty food diary, a food frequency questionnaire, and a kitchen scale were given to every participant when the food collection bottles were given to them. Instructions on how to fill the questionnaires were also attached. In total, 113 solid food samples and 121 liquid food samples were received and analysed in Birmingham from the 61 participants.

A brief introduction describing the main nutritional parameters extracted from both types of questionnaires – FD and FFQ – will be introduced in this section, with the purpose to evidence differences in the food composition and the extracted nutritional data when the two types of questionnaires were compared.

5.2.1. Food Diaries Versus Food Frequency Questionnaires

The average weight of solid and liquid food composites collected and reported in the FDs (≈ 2,500 g/day) was lower than the one calculated from the information extracted from the FFQs (≈ 2,800 g/day). Same pattern was observed when the energy intakes (kcal/day) were compared, as *Figure 21* shows.

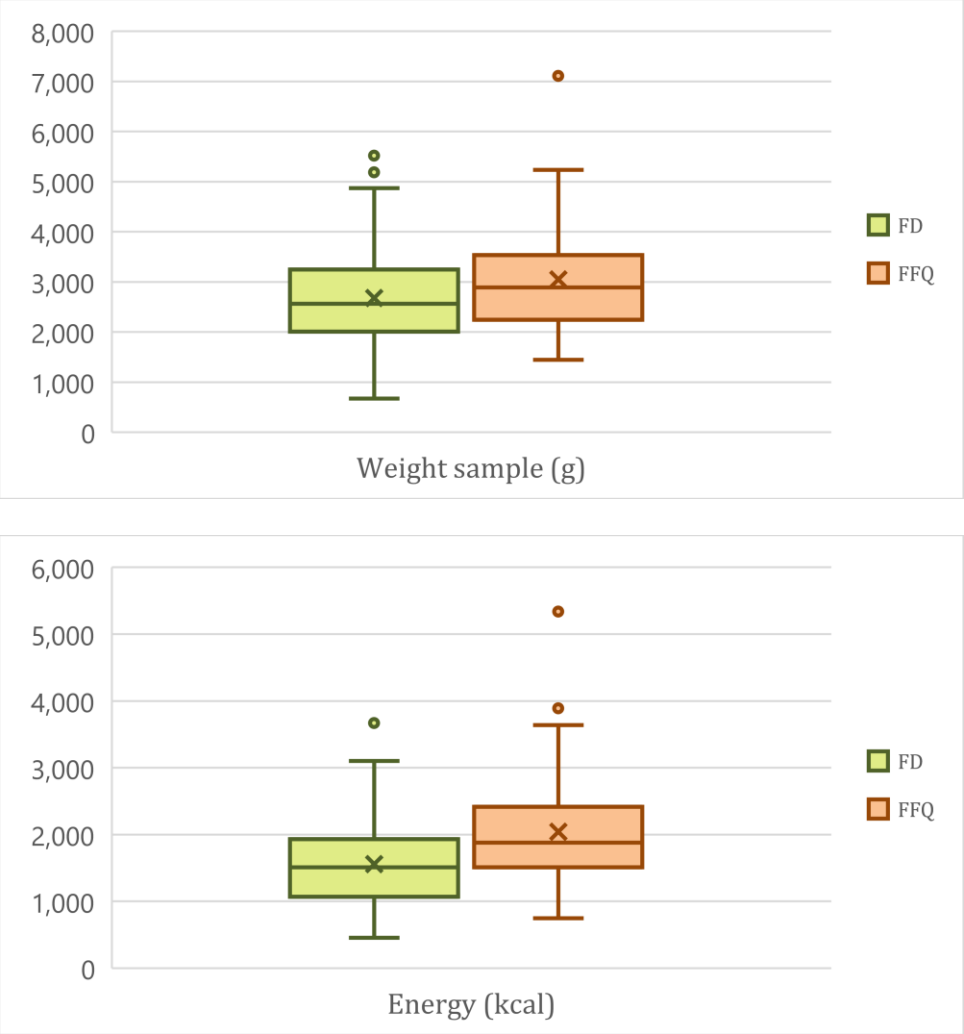


Figure 21. Box plots showing weights of food and amount of energy (kcal) – calculated per day – reported and extracted from the food diaries (FD) and from the food frequency questionnaires (FFQ)

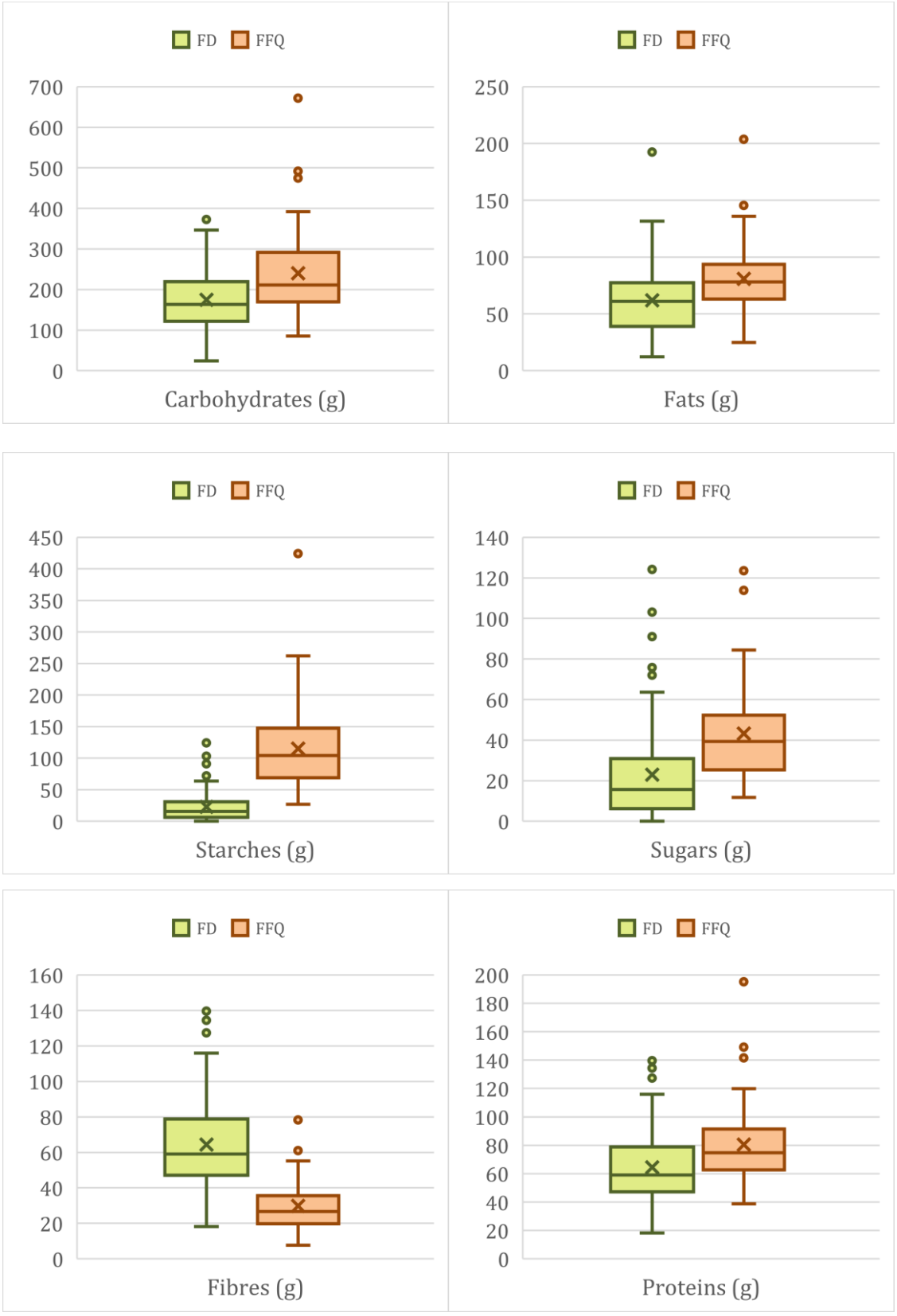


Figure 22. Box plots showing carbohydrates, fats, starch, sugars, fibre and proteins – calculated per day – reported and extracted from the food diaries (FD) and from the food frequency questionnaires (FFQ)

Main nutritional groups (carbohydrates, fats, starch, sugars, fibres and proteins) were compared, as shown in *Figure 22*. Five of six of the parameters evaluated presented lower values in the food diaries, while fibre was the only one for which the participants reported a much lower values in the long term exposure questionnaires (≈ 22 g/day) than in the food diaries linked to the food composites (≈ 60 g/day). Besides fibre, starch consumption varied from an average of ≈ 20 g/day in the food diaries to an average of ≈ 100 g/day for the food frequency questionnaires, same as for sugars, with averages of ≈ 15 and ≈40 g/day, respectively.

As a consequence of these differences, just the parameters from the food diaries were included in the statistical analysis of the food samples, while the food frequency questionnaires – linked to long term exposure – will be employed for statistical purposes in a later chapter of this thesis.

5.2.2. Food Diaries

Main descriptive statistics derived from the food diaries provided by the NIPH according to the information obtained from the participants included in the study, are showed in *Table 30.*

As a general overview, solid food composites were a mixture ranging from 4 to 47 (average = 17) food items combined and blended, while the liquid food samples were a mixture of 3 to 27 (average = 9) different liquids combined in the same bottle. When combining solid food and liquid food samples, the average weight of a single day (24 hours) total food sample was 2,627 g, being the minimum amount collected

670 g and the maximum 5,665 g. An average of 86 % of it was water, while the remaining 14 % was a mixture of nutrients. For the nutritional study, the average for daily food energy intake was 1,502 kcal (within a range of 418 – 3,670 kcal), while the averages for fat, carbohydrates, starch, sugar, fibre and protein were 60, 169, 101, 21.1, 20.6 and 62 g respectively. Some of these parameters are represented in *Figure 23.*

The participants also had to include some information about cooking and consumption of the food items, as well as the material of all cookware and storage containers.

5.3 CONCENTRATIONS IN FOOD COMPOSITES

113 duplicate diet solid food samples and 121 liquid ones from the Norwegian participants were collected and processed as explained in the methodology chapter. In this section, PFOS – total – and PFOS precursors – FOSAs, FOSEs and FOSAAs – concentrations are given.

Descriptive statistics from the solid and liquid food samples where PFOS and PFOS precursors were analysed are shown in *Table 31* and *Table 32* respectively. Individual sample results are detailed in *Table SM05* and *Table SM06*.

PFOS was detected in 7 solid food samples (average = 0.005 ng/kg) in a range between <LOQ and 0.054 ng/kg, while FOSA was detected in 18 samples (average = 0.004 ng/kg) in a range between <LOQ and 0.051 ng/kg, MeFOSE in 9 samples (average = 1.437 ng/kg) in a range between <LOQ and 42.302 ng/kg, EtFOSE in 19 samples (average = 3.683 ng/kg) in a range between <LOQ and 53.125

ng/kg and MeFOSAA in 3 samples (average = 0.005) in a range between <LOQ and 0.045 ng/kg. MeFOSA, EtFOSA and EtFOSAA were not detected in any sample.

For the liquid food samples, PFOS was detected in 4 samples (average = 0.029 ng/L) in a range between <LOQ and 3.195 ng/L, while FOSA was detected in 3 samples (average = 0.031 ng/L) in a range between <LOQ and 3.67 ng/L, same number as for MeFOSA (average = 0.044 ng/L) in a range between <LOQ and 0.131 ng/L. EtFOSA was detected in one sample with a value of 0.011 ng/L, and another positive sample was identified for MeFOSE, with a value of 0.080 ng/L. FOSAA and MeFOSAA were both detected in two samples, with values of 0.0217 and 2.77 ng/L for FOSAA and 0.0147 and 0.0038 ng/L for MeFOSAA. No positive samples were found for EtFOSE and EtFOSAA.

As with dust samples, the Shapiro-Wilk test for normality of data distribution was applied, showing skewed data. A comparison between non-parametric statistics and parametric statistics with logarithmic transformed data was conducted, showing no significant differences on the data analysis. As a consequence of it, logarithmic transformed data was selected and used for data normalisation and subsequent statistical analysis of the food composite samples. For further statistical analysis of the samples from this study, a cut-off value of 50 % positive samples was ideally required. None of the target compounds were detected in such high frequency, and even the cut-off value of 30 % positive samples – applied for dust samples – was not enough for a full statistical analysis of individual analytes, regardless their importance in dust and serum exposure.

Table 30. Descriptive statistics for the food samples as a composite of solid and liquid sample for 24 hours

	Weight (g)	Water (g)	Energy (kcal)	Fat (g)	Carbohydrates (g)	Starch (g)	Sugar (g)	Fibre (g)	Protein (g)
Average	2,627	2,282	1,502	59.5	168.6	101.0	21.7	20.6	62.4
Mean	2,435	2,099	1,376	51.8	150.1	87.5	6.6	18.1	57.1
Median	2,534	2,169	1,434	58.3	156.7	99.1	14.3	19.8	58.3
Min	670	579	418	12.3	24.4	9.7	0.0	3.0	17.5
Max	5,665	5,192	3,670	192.4	372.7	255.9	124.2	50.1	141.1
Q1	1,950	1,697	1,024	37.1	115.3	61.6	5.5	14.8	44.7
Q3	3,208	2,818	1,872	73.6	213.2	130.4	28.9	25.7	76.7
SD	980.9	891.7	609.1	30.7	75.7	50.7	23.5	9.8	26.4
%RSD	37.3	39.1	40.5	51.5	44.9	50.2	108.4	47.7	42.3

Q1: 25th percentile. Q3: 75th percentile. SD = Standard deviation. RSD = Relative standard deviation

Table 31. Concentrations of PFOS and PFOS precursors (ng/kg wet weight) in solid food samples (n = 113)

	PFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA	ΣPFAS
Average	0.005	0.004	< LOD	< LOD	1.902	3.746	< LOD	0.005	< LOD	5.203
Mean	0.004	0.003	< LOD	< LOD	0.105	0.099	< LOD	0.004	< LOD	0.429
Median	0.003	0.002	< LOD	< LOD	0.066	0.033	< LOD	0.004	< LOD	0.178
Min	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Max	0.054	0.051	< LOD	< LOD	42.302	53.125	< LOD	0.045	< LOD	73.750
Q1	0.003	0.002	< LOD	< LOD	0.066	0.033	< LOD	0.004	< LOD	0.178
Q3	0.003	0.002	< LOD	< LOD	0.066	0.033	< LOD	0.004	< LOD	0.192
5th	0.003	0.002	0.002	0.039	0.066	0.033	0.022	0.004	0.006	0.178
95th	0.012	0.020	0.002	0.039	9.106	21.908	0.022	0.004	0.006	40.179
SD	0.006	0.010	< LOD	< LOD	5.723	9.819	< LOD	0.005	< LOD	13.497
% RSD	129.43	176.03	< LOD	< LOD	398.33	266.63	< LOD	105.99	< LOD	259.42
n > LOQ	7	18	0	0	9	19	0	3	0	39
% Positive	6.2	15.9	0.0	0.0	8.0	16.8	0.0	2.7	0.0	34.5

n = number of samples included in the statistics. DF: Detection frequency. Q1: 25th percentile. Q3: 75th percentile. SD = Standard deviation. RSD = Relative standard deviation

Table 32. Concentrations of PFOS and PFOS precursors (ng/L) in liquid food samples (n = 121)

	PFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA	ΣPFAS
Average	0.029	0.031	0.044	NR	NR	< LOD	0.029	0.001	< LOD	0.23
Mean	0.001	0.001	0.001	NR	NR	< LOD	0.006	0.001	< LOD	0.05
Median	0.001	0.000	0.001	NR	NR	< LOD	0.006	0.001	< LOD	0.04
Min	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Max	3.195	3.677	2.763	0.131	7.743	< LOD	2.771	0.015	< LOD	19.57
Q1	0.001	0.000	0.001	0.010	0.016	< LOD	0.006	0.001	< LOD	0.04
Q3	0.001	0.000	0.001	0.010	0.016	< LOD	0.006	0.001	< LOD	0.04
5th	0.001	0.000	0.001	0.010	0.016	0.008	0.006	0.001	0.002	0.045
95th	0.001	0.000	0.001	0.010	0.016	0.008	0.006	0.001	0.002	0.061
SD	0.291	0.334	0.319	0.011	0.702	< LOD	0.251	0.001	< LOD	1.79
% RSD	1,008	1,080	719	103	875	< LOD	882	106	< LOD	763
n >LOQ	4	3	3	1	1	0	2	2	0	11.0
% Positive	3.3	2.5	2.5	0.8	0.8	0.0	1.7	1.7	0.0	9.1

n = number of samples included in the statistics. DF: Detection frequency. Q1: 25th percentile. Q3: 75th percentile. LOD = Limit of detection.

NR = Not reported. SD = Standard deviation. RSD = Relative standard deviation

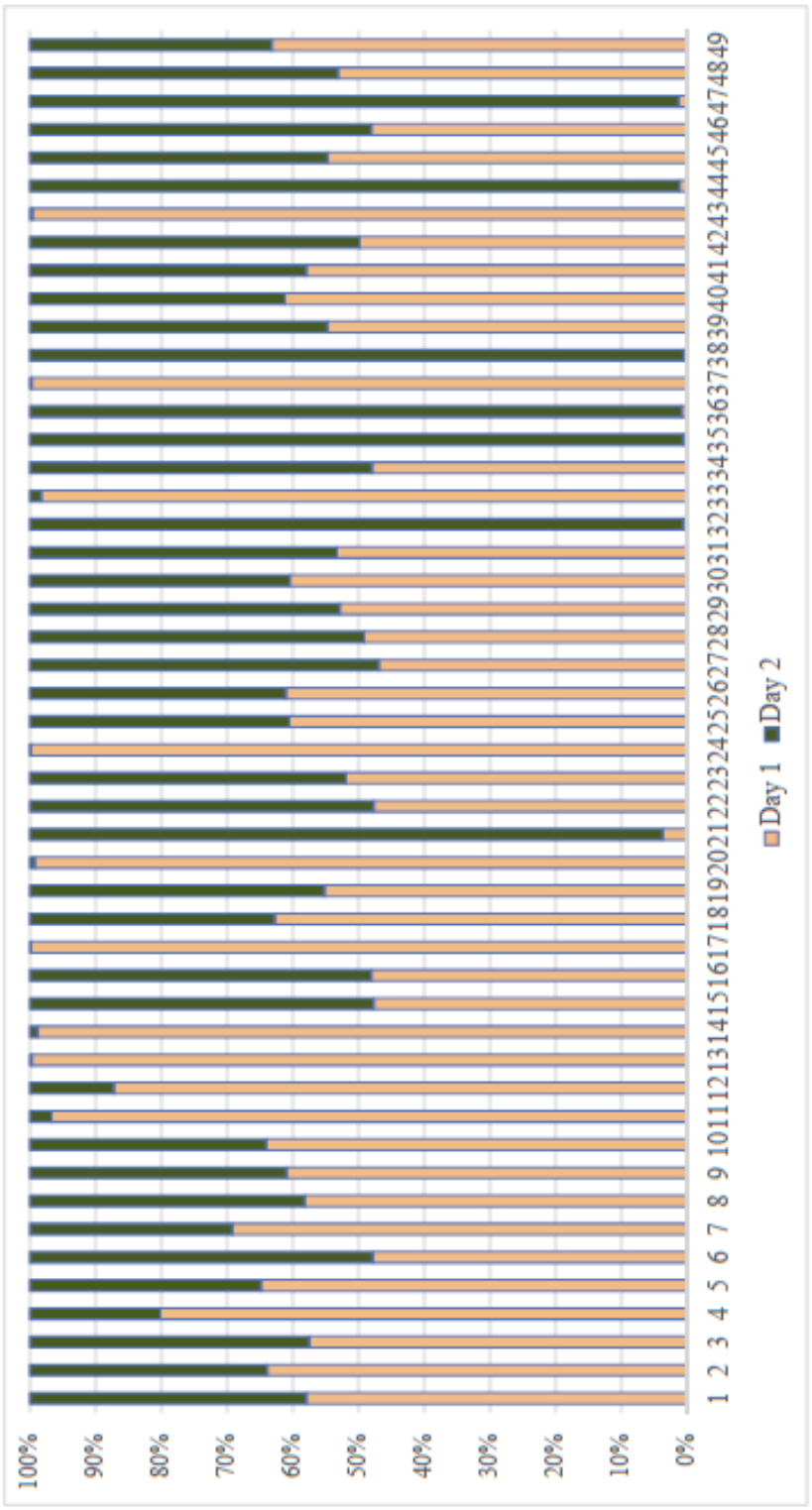


Figure 23. Stacked column chart showing individual solid food samples and how the distribution between ΣPFAS daily exposure (as percentage when the daily intakes were body weight corrected calculated) from day 1 and 2 are distributed

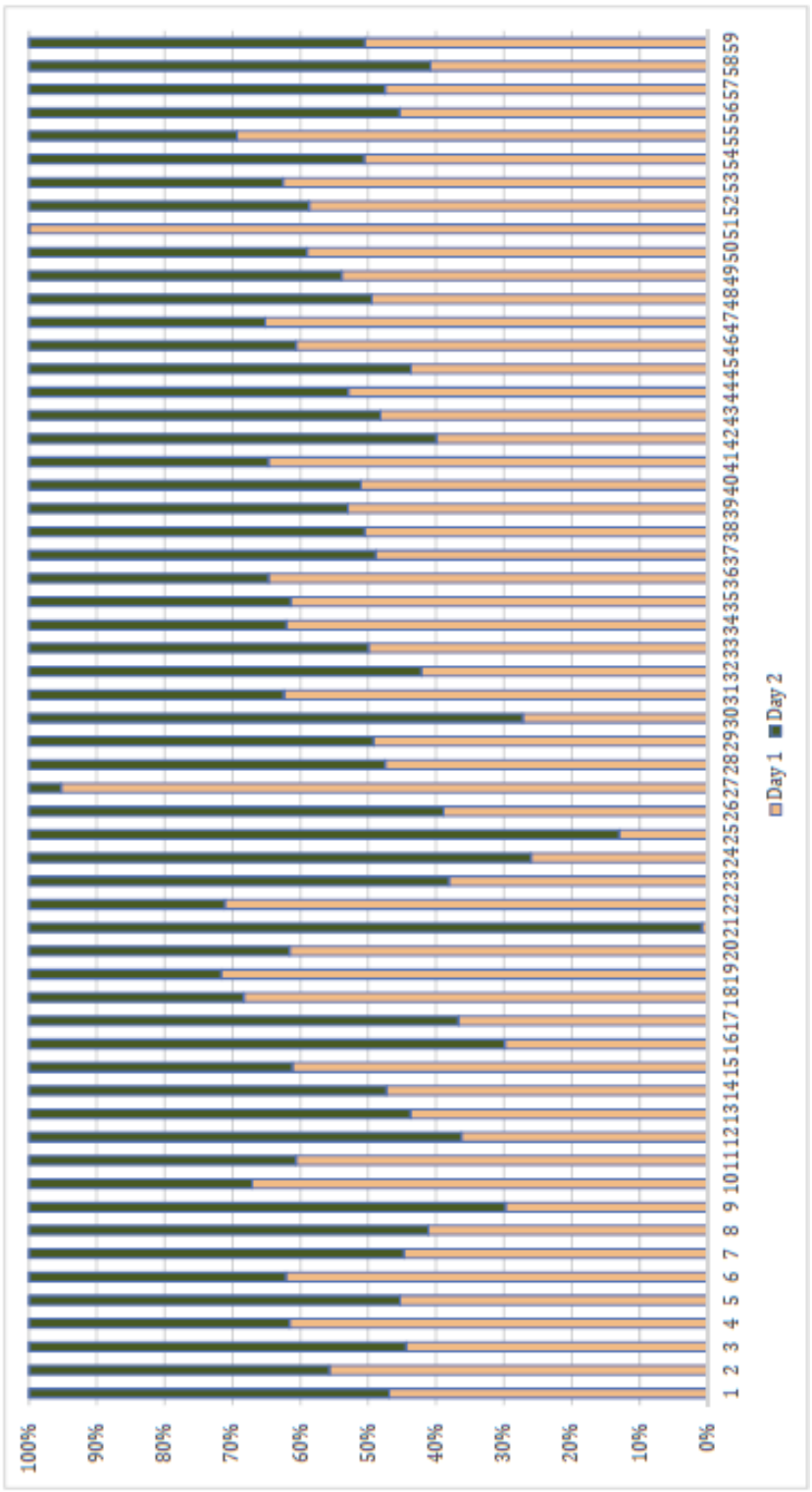


Figure 24. Stacked column chart showing individual liquid food samples and how the distribution between ΣPFAS daily exposure (as percentage when the daily intakes were body weight corrected calculated) from day 1 and 2 are distributed

In case of the solid food samples, just ΣPFAS (36 % of the samples were positive for at least one of the PFASs included in the study) was analysed by SPSS, while no statistical analysis could be conducted for the liquid samples. ANOVA tests for ΣPFAS in solid food samples were run with Scheffe post-hoc test with a significance level 95 % ($p = 0.05$). All the t-tests were also run with a significance level of 95 % ($p = 0.05$).

No statistical differences were identified when ΣPFAS were grouped by age and gender. For correlations, just the positive samples (i.e. concentration > detection limit) were considered and the significance level was specified ($p = 0.01$ or $p = 0.05$) later on in the specific analysis. No positive correlations were found for group of nutrients (energy, water, proteins, fats, carbohydrates, starches, sugars and fibres), so individual food items were selected for the correlations. Butter, margarine, different types of cheese, low and high fat fish, shellfish, low and high fat meat (chicken, beef, pork, bacon, lamb, turkey, game and organs), eggs, mayonnaise, jam, honey, nuts, cereals, bread, yogurt, chocolate, sweets, potatoes, mustard, olive oil, beans, green salads, vegetables (raw and cooked), mushrooms, corn, berries, common and exotic fruits, onion and leak, tomatoes and cucumbers, spirits, rice, flour, and pasta were tested as individual products. Sauces and gravies, ice creams, waffles, cakes, puddings, pizzas, casseroles, salty snacks, cookies, sweets, tomato based sauces, soups, prepared meals and food complements were also included in the statistical analysis as processed products. Positive correlations were found for ΣPFAS and the consumption of fish with high content in fat ($p < 0.01$), raw vegetables ($p < 0.01$), tomatoes and cucumbers ($p < 0.05$). On the other side, no significant correlations were found for food packaging (plastics, paper, etc.), cooking

(Teflon® pans, plastic or wooden utensils, etc.) and storage (plastic, glass, etc.) of the solid food items.

Milk, juices, soya drinks, yogurt drinks, beer, wine, coffee and spirits were excluded from the list of food items, as they were part of the liquid food composites, not the solid ones, and no statistical analysis was conducted due to the low detection frequencies.

5.4 DAILY INTAKES OF PFOS AND PFOS PRECURSORS VIA FOOD INGESTION

Daily intakes of ΣPFAS for solid and for liquid composites were calculated for all the participants (except for one liquid sample, excluded due to the lack of complete information on the total weight of the food composite), per day and body weight corrected, for lower and medium bounds (see *Table SM07* and *Table SM08*). For the lower bound approach, a value of zero was assigned for the samples which concentrations were below the LOQ, while in the medium bound the LOQ of each compound divided by the square root of two was employed (Tennant *et al.*, 2017). No calculations were conducted for the higher bound, as they are strongly dependent on the instrumental LOD and LOQ of the instruments, so less representative to real intakes (Kettler *et al.*, 2015).

Estimated daily intakes were calculated according to *Equation 10*:

Equation 10 $$EDI = C\ x \frac{1}{W}\ x\ WF$$

Where C is the concentration of the specified pollutant (ng/kg for solid composites and ng/L for the liquid ones), W is the body weight (kg), and WF is the weight of ingested food per day (kg). The uptake fraction was assumed to be 100 % absorbed as representation of the worst uptake scenario.

Descriptive statistics of the estimated daily intakes of ΣPFAS are shown in *Table 33* for solid food samples, and in *Table 34* for the liquid ones.

An average intake of ΣPFAS of 16.7 ng/day (0.25 ng/kg bw/day) was obtained for the lower bound in solid food composites. For the medium bound, an average intake of ΣPFAS of 17.2 ng/day (0.23 ng/kg bw/day) was obtained, with a median value of 0.53 ng/day (0.007 ng/kg bw/day) and an extreme value of 417 ng/day (6.96 ng/kg bw/day) for one of the participants. *Figure 23* and *Figure 24* show relative contributions (%) in terms of exposure by participant for the medium bound, when the two sampled solid food and liquid food composites were compared.

An average intake of ΣPFAS of 0.46 ng/day (5.73 pg/kg bw/day) was calculated for the medium bound for the liquid food composites, with a median value of 0.08 ng/day (1.10 pg/kg bw/day). The maximum intake calculated for the entire dataset of liquid samples was 41.09 ng/day (483.4 pg/kg bw/day). *Figure 24* shows relative contributions (%) in terms of exposure by participant, when the two sampled solid food composites were compared. For the lower bound, an average value of 0.39 ng/day (5 pg/kg bw/day) was calculated.

Table 33. Calculated daily intakes ΣPFAS for lower and medium bounds (ng/day and ng/kg bw/day) through solid food consumption (n = 113)

	Intake (ng/day) Lower bound	**Intake (ng/kg bw/day) Lower bound**	**Intake (ng/day) Median bound**	**Intake (ng/kg bw/day) Median bound**
Average	16.79	0.252	17.24	0.258
Mean	NR	NR	1.07	0.015
Median	<0.01	<0.001	0.53	0.007
Min	<0.01	<0.001	0.12	0.002
Max	417.35	6.956	417.79	6.963
5th percentile	<0.01	<0.001	0.24	0.003
25th percentile	<0.01	<0.001	0.38	0.006
75th percentile	0.04	0.001	0.70	0.010
95th percentile	110.89	1.47	111.60	1.479
SD	53.56	0.85	53.54	0.85
%RSD	NR	NR	310.63	328.82

NR = Not reported. SD = Standard deviation. RSD = Relative standard deviation. n = number of samples included in the statistics

Table 34. Calculated daily intakes ΣPFAS for lower and medium bounds (ng/day and ng/kg bw/day) through liquid food consumption (n = 120)

	Intake (ng/day) Lower bound	**Intake (ng/kg bw/day) Lower bound**	**Intake (ng/day) Median bound**	**Intake (ng/kg bw/day) Median bound**
Average	0.387	0.005	0.465	0.0057
Mean	NR	NR	0.082	0.0012
Median	<0.001	<0.001	0.079	0.0011
Min	<0.001	<0.001	0.021	0.0003
Max	41.044	0.483	41.087	0.4834
5th percentile	<0.001	<0.001	0.033	0.0005
25th percentile	<0.001	<0.001	0.057	0.0008
75th percentile	<0.001	<0.001	0.097	0.0014
95th percentile	0.039	0.001	0.180	0.0025
SD	3.761	0.044	3.757	0.0442
%RSD	NR	NR	808.14	771.0840

NR = Not reported. SD = Standard deviation. RSD = Relative standard deviation. n = number of samples included in the statistics

Table 35. Calculated daily intakes of ΣPFAS for lower and medium bounds (ng/day and ng/kg bw/day) through combined solid and food consumption (n = 94)

	Intake (ng/day) Lower bound	**Intake (ng/kg bw/day) Lower bound**	**Intake (ng/day) Median bound**	**Intake (ng/kg bw/day) Median bound**
Average	18.771	0.280	19.294	0.287
Mean	NR	NR	1.278	0.018
Median	<0.001	<0.001	0.599	0.008
Min	<0.001	<0.001	0.173	0.002
Max	417.347	6.956	417.974	6.966
5th percentile	<0.001	<0.001	0.291	0.005
25th percentile	<0.001	<0.001	0.460	0.007
75th percentile	0.055	0.001	0.886	0.012
95th percentile	139.532	1.797	140.246	1.806
SD	58.025	0.920	58.020	0.921
% RSD	NR	NR	300.72	320.78

NR = Not reported. SD = Standard deviation. RSD = Relative standard deviation. n = number of samples included in the statistics

Table 36. Calculated daily intakes of ΣPFAS for lower and medium bounds (ng/day and ng/kg bw/day) through combined solid and food consumption when the average concentrations of day 1 and day 2 were considered (n = 47)

	Intake (ng/day) Lower bound	**Intake (ng/kg bw/day) Lower bound**	**Intake (ng/day) Median bound**	**Intake (ng/kg bw/day) Median bound**
Average	15.181	0.232	15.652	0.239
Mean	NR	NR	2.083	0.030
Median	0.026	<0.001	0.607	0.008
Min	<0.001	<0.001	0.280	0.004
Max	139.358	1.991	140.013	2.000
5th percentile	<0.001	<0.001	0.318	0.004
25th percentile	0.002	<0.001	0.472	0.007
75th percentile	17.418	0.259	17.912	0.266
95th percentile	76.882	1.277	77.375	1.285
SD	29.773	0.456	29.820	0.457
% RSD	NR	NR	190.518	191.349

NR = Not reported. SD = Standard deviation. RSD = Relative standard deviation. n = number of samples included in the statistics

Table 34 and *Table 35* show two different ways of presenting the estimated daily intakes as a sum of solid and liquid food ingestion. In the first approach, represented in *Table 35*, concentrations of solid and liquid food composites were combined, and the calculation of the daily intakes was performed per sample/day (n = 94, two for each participant). On the other hand, the second approach represented in *Table 36*, reported concentrations of combined solid and liquid composites and averaged the data from day one and from day two, as well as the food weights corresponding to the specific samples. For both approaches, average and mean results for the estimated daily intakes were comparable, despite maximum and minimum ones differed substantially. In view of these results, the second approach was selected to be employed in later chapters of this thesis, due to its higher representability – more food items, so more realistic of real diet – when comparing both approaches to long term exposure data. Averaged individual daily intakes for the participants are detailed in *Table SM09*.

On the other hand, lower and medium bounds also showed comparable daily intakes of PFASs (see *Table 34* to *Table 36*), especially when they were body weight corrected. As a consequence of it, medium bound approach (substitution of the concentrations <LOQ for their LOQ value divided the square root of two) will be employed in later chapters for the calculation of the total estimated intakes for external exposure, and for the correlation with internal exposure levels.

5.5 SUMMARY AND CONCLUSIONS

This study shows that PFOS is present in only some food items (around 6 % positive samples and contributing less than 5 % to the overall exposure to ΣPFAS). It also shows that some PFOS precursors – i.e. FOSA (16 % positive samples) and FOSEs (both EtFOSE (8 % positive samples and MeFOSE (17 % positive samples)) are present in food items and constitute an additional potential source of indirect exposure to PFOS. Combined, PFOS and PFOS precursors are present in around 1/3 of the food composites (35 %) resulting an average concentration of 5.2 ng/g wet weight for solid food composites. In view of these results, it can be concluded that PFOS and PFOS precursors remain present in every day diet, indicating that diet is still a potential source for external – and mainly indirect – exposure to PFASs.

Even though the number of positive samples was not ideal for statistical analysis, three positive correlations were found and were described in previous sections of this chapter. The first of them was related to the overall consumption of fatty fish, widely consumed in Scandinavian countries. This finding is in agreement with the higher than average reported levels in fish samples by other authors (Berger *et al.*, 2009; Haug *et al.*, 2010; Hrádková *et al.*, 2010) when they were compared to other studies or to other food items within the same study, reporting them as high potential sources of PFOS dietary exposure. For Haug et al., meat, fish and seafood products, together with milk and dairy produce represented between 50 – 70 % of the overall daily dietary exposure The main suggested reason for fatty fish to be a source of PFOS and PFOS related compounds is bioaccumulation by the trophic chain.

The second of the relationships identified was related to the amount of raw vegetables ingested. Among the questions from the questionnaires, some of them were related to the habit of washing or not washing raw fruits and vegetables before consumption. No correlation was found for washed vegetables, while it was for unwashed vegetables. The third of the correlations was found for the daily ingestion of tomatoes and cucumbers, two food items usually ingested without cooking or peeling. This combination of factors suggest the contamination from these specific items is most likely to migration from their original envelopes or bags. The number of studies where packed and non-packed food items have been investigated together is not large in number (Tittlemier, Pepper and Edwards, 2006; Ericson, Martí-Cid, *et al.*, 2008; Halldorsson *et al.*, 2008; Jogsten *et al.*, 2009; Noorlander *et al.*, 2011; Herzke *et al.*, 2013) despite food packaging having been suggested as an important source of indirect exposure to PFOS by several papers.

For liquid food samples, no comparison could be conducted due to the reduced number of positive samples. This fact, could be attributed to the large volume of water necessary to carry out the analysis, suggesting that water exposure could be better addresses if sampled and analysed as an independent sample instead of as a liquid food composite.

Estimated daily intakes of 15.6 ng ΣPFAS/day (0.24 ng/kg bw/day) were obtained in medium bound approach when concentrations and food diaries data for the four samples collected from each participant were combined. This study was compared to the previous dietary report by the Norwegian Institute for Public Health in 2010 (Haug *et al.*, 2010), where total dietary intake for 16 perfluorinated substances was measured. When comparing the results obtained here with the ones obtained from

the Norwegian cohort, many differences are apparent. The main one is the nature of the food items, as the previously reported in 2010, was not carried out by the duplicate diet method of sampling, but on an individual food item basis. Although in this study, no statistical differences were identified between age groups and gender, Haug et al. found significant differences in daily ingestion by age. Haug et al. estimated average daily exposure around 100 ng/day for all the measured compounds, with around 50 % of that due to PFOS and PFOA combined. In this study, the average daily intake reported for PFOS, FOSAs, FOSEs and FOSAAs was 17.3 ng/day. Here it is necessary to point out the smaller number of PFASs included in this study when compared to Haug et al, while FOSAs, FOSEs and FOSAAs were not included for identification by Haug et al. In terms of comparison, PFOS daily intake from Haug et al. should be equated to the ΣPFAS presented here, as all of the pollutants included in this thesis are susceptible to be metabolised or degraded to PFOS. That way, Haug et al. reported an intake of 18 ng/day for PFOS, while this study reports 15.6 ng/day for ΣPFAS.

A second relevant comparison has been done with duplicate diet studies for the Japanese population by Kärrman et al. (Kärrman *et al.*, 2009) and for the German population by Fromme et al. (Fromme, Schlummer, *et al.*, 2007). In Fromme et al., 214 food composites were collected from 30 different participants, reporting a mean value for PFOS intake of 123.4 ng/day. Clearly, present reported levels in Norwegian food composites are significantly lower than the ones presented ten years ago for the German population. The Japanese study reported by Kärrman et al. also presented higher exposures to total PFOS in food composites (average values of 85.5 and 88 ng/day for Osaka and Miyagi respectively), and identified in 100 %

of the food composites. Decreasing trends in direct exposure to PFOS, as well as in overall exposure to long chain PFASs, can be identified from the reported values from both dietary papers. But as mentioned for Haug et al., patterns of exposure have changed drastically since the 3M Company phase out of PFOS, fitting much better with current indirect exposure estimations (Vestergren *et al.*, 2008; Gebbink, Glynn and Berger, 2015): Total PFOS direct exposure – i.e. as PFOS itself – has decreased significantly relative to levels of FOSAs, FOSEs and FOSAAs. This does not mean the exposure to these precursors is increasing, but that their contribution to ΣPFAS has increased due to the decline in PFOS.

When the reported values are compared to the tolerable daily intakes (TDI) for PFOS established by the European Food Safety Authority (EFSA, 2004, 2008), it shows – as an overall – values much lower than the TDI of 150 ng/kg bw/day. This is not a fixed value per participant, so when average daily intake (calculated as ng/day) from this study is converted to ng/kg bw by the assumption of an average weight of 75 kg, the TDI is 11.25 µg/day. Even for extreme values presented in this study (daily intake = 418 ng/day), the daily ingested amount of PFOS and PFOS precursors for the participant represents just 4.6 % of the TDI (9 µg/day for 60 kg weight).

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6. *IN VITRO* METABOLISM OF PFOS PRECURSORS

The main objectives of this chapter are: a) to elucidate if the PFOS precursors MeFOSA, MeFOSE and MeFOSAA are *in vitro* metabolised by human liver microsomes (HLM) to PFOS or to other intermediate products susceptible to further metabolism leading to PFOS as an end-product, b) to suggest *in vitro* metabolism pathways for MeFOSA, MeFOSE and MeFOSAA leading to PFOS, and c) to evaluate the significance of indirect exposure to PFOS via exposure to FOSAs, FOSEs and FOSAAs.

6.1 INTRODUCTION

Metabolism occurs in different organs and tissues, with liver as the most important one. It is well known that liver is rich in heme-containing enzymes (CYP 450), which play a major role in phase I oxidation reactions. *In vitro* assays with human liver-derived experimental systems have constituted the most effective approach to estimate the human metabolic fates *in vivo* (Li, 2004). Within the available models, hepatocytes, S9 fractions, liver slices and human liver microsomes have been employed. Specifically, human liver microsomes have been the most popular model in the study of drugs metabolism and environmental pollutants due to their low cost, simplicity in use, easy storage, and rich concentration in many drug-metabolizing enzymes like cytochrome P450s, flavin monooxygenases, carboxyl esterases and epoxide hydrolase, and UDP glucuronyl transferases (Asha and Vidyavathi, 2010).

FOSAs, FOSEs and FOSAAs are listed among the organic pollutants suggested as PFOS precursors. Recently, papers reporting *in vitro* studies of potential PFOS precursors including FOSA (Benskin, Holt and Martin, 2009; Ross, Wong and Martin, 2012; Chen *et al.*, 2015), EtFOSA (Tomy *et al.*, 2004; Fu *et al.*, 2015) and EtFOSE (Xu *et al.*, 2004; Zhao *et al.*, 2016; Chang *et al.*, 2017) have been published. Moreover, a few *in vivo* studies and results have been reported showing different conversion rates for these same compounds (Xie *et al.*, 2009; Chen *et al.*, 2015; Chang *et al.*, 2017). Nevertheless, some other substances listed in *Table 01* still require further study to confirm them as sources of human exposure to PFOS. For that reason, in this study MeFOSA, MeFOSE and MeFOSAA were chosen for a qualitative study of phase I & II metabolism and plausible conversion to PFOS; and also to see if any other intermediate metabolites were detected by the use of UHPLC-HRMS(QTOF) analysis. These experiments were conducted by the author in collaboration with Luisa Lucattini at the Institute for Environmental Studies (IVM) of the Vrije Universiteit Amsterdam (VU) in the Netherlands during the months of September and October 2015.

6.2 *IN SILICO* PREDICTION

In silico approaches for the theoretical study of metabolism pathways are becoming a conventional tool to be used together with experimental observations and measurements. Nowadays, specific software such as MetaSite (Molecular Discovery Ltd Middlesex, UK), MetaDrug (Thomson Reuters, NY, USA), or Meteor (Lhasa Limited, Leeds, UK) are commercially available and frequently used as theoretical support when metabolism studies are carried out. The advantages conferred by use

of such software, the increasing number of database sets available, together with the availability of HRMS techniques and methodologies allow the identification of: a) predicted metabolites generated by the *in silico* software by a targeted quantification or targeted screening approaches, and b) "unpredicted" metabolites by non targeted screening and even retrospective screening (Ballesteros-Gómez *et al.*, 2015; Negreira *et al.*, 2016).

The growing *in silico* approaches together with the current uncertainties related to the metabolism of the selected perfluoroalkyl compounds provide evidence about the need to further study of these PFOS precursors substances. However, to date only a few papers examining *in vitro* and *in vivo* metabolism pathways of FOSAs and FOSEs leading to PFOS have been published. On this paper, qualitative evaluation of phase I and phase II metabolism of MeFOSA, MeFOSA and MeFOSAA incubated with human liver microsomes is presented (see *Figure 25*).

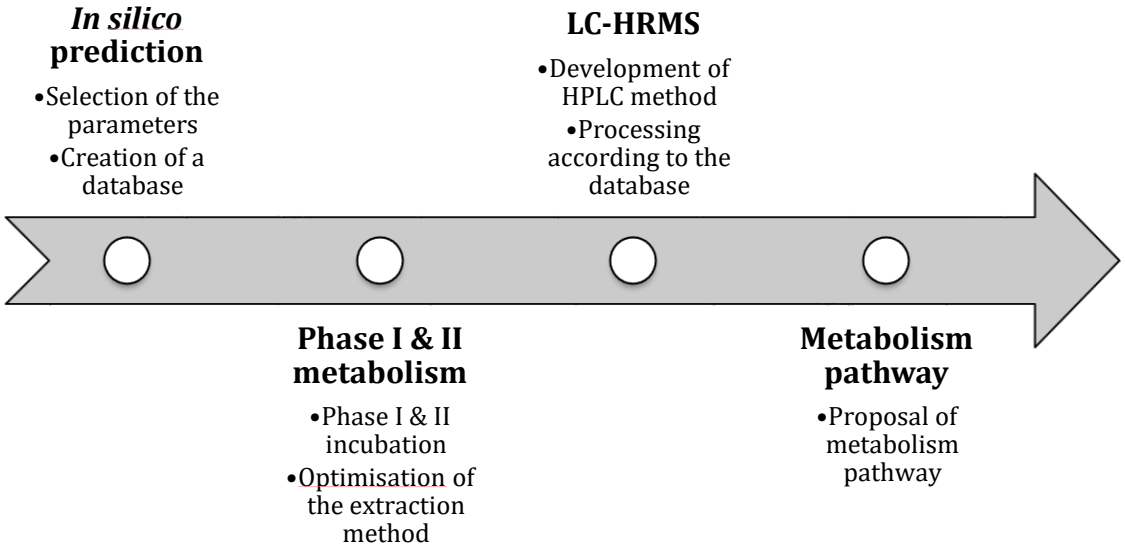


Figure 25. General workflow used for the study of phase I & II metabolism for MeFOSA, MeFOSE and MeFOSAA incubated with human liver microsomes

In this study, the Meteor Nexus (Lhasa Limited) software – whose use was possible thanks to the University of Antwerp – was employed for the *in silico* predictions. It enables the fast prediction of accurate metabolism of chemicals based on their molecular structure and the Lhasa metabolism database (see *Table 37*). In it, phase I and phase II metabolism were selected for the three analytes of interest, and the conditions of the *in vitro* experiments specified: the use of human liver microsomes (HLM) for phase I; and the use of human liver microsomes (HLM) and human liver cytosols (HLCyt) for phase II metabolism, including glucuronidation and sulfonation products as the expected phase II metabolites. With these premises, the software predicted:

- 60 theoretical possible metabolites for MeFOSA
- 59 theoretical possible metabolites for MeFOSAA
- 68 theoretical possible metabolites for MeFOSE

An extended description of them is provided in *Table SM10*, *Table SM11* and *Table SM12*, while an abstract of them is shown in *Table 38*. The software discriminated among analytes being probable, plausible or equivocal, according to the specified conditions selected during its design. Data obtained by the software was used afterwards to generate three complete databases – one per parent compound – with all the molecular structures and accurate masses from the suspected intermediates and metabolites generated according to the selected enzymes and cofactors, together with their capability to catalyse these reactions in the human body.

These databases, including all the generated metabolites and end products, were imported to the processing methods for proper identification – target and suspect screening analysis – after the analytical acquisition.

Table 37. Selected PFOS precursors for the study of *in vitro* metabolism, their molecular formula and their monoisotopic masses

Compound	Name	Molecular formula	Monoisotopic mass	Average mass	log Kow
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	N-MeFOSE	$C_{11}H_8F_{17}NO_3S$	556.99536	557.2240	7.29 *
N-methylperfluoro-1-octanesulfonamide	N-MeFOSA	$C_9H_4F_{17}NO_2S$	512.96912	513.1710	8.05 *
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	$C_{11}H_6F_{17}NO_4S$	570.97461	571.2075	NF

Table 38. Abstract from the Meteor predictions relatives to the three analytes of interest

Compound	Total metabolites	Stage	Number metabolites	Probable	Plausible	Equivocal
N-MeFOSE	68	*Phase I*	37	13	9	46
		Phase II	31			
N-MeFOSA	60	*Phase I*	23	11	14	35
		Phase II	37			
N-MeFOSAA	59	*Phase I*	26	13	5	41
		Phase II	33			

6.3 ANALYTICAL METHOD BY UHPLC-HRMS

All the *in vitro* assays were carried out at the Institute for Environmental Studies – IVM (University of Amsterdam, The Netherlands), where no chromatographic method was already set up for the analytes of interest. As a consequence of it, a new

analytical method including MeFOSA, MeFOSE and MeFOSAA as well as their corresponding metabolites needed to be rapidly developed. Different modifiers, pHs, and combinations of both of them were tested in order to optimise the mobile phases: HCOOH (0.01 – 0.1%), HH_4Ac (5 – 10 mM), NH_4COOH (5 – 10 mM). The column was selected based in previously published research from IVM staff, where other PFASs were analysed (Ballesteros-Gomez, Rubio and van Leeuwen, 2010). The gradient, the flow and the oven temperature were optimised based on the separation of the available standards, together with the consideration of the detection of early elution of polar metabolites generated during the incubation stages.

For the analytical separation, a UHPLC system (Nexera, Shimadzu, Den Bosch, the Netherlands) was employed. The mobile phases were composed of 5 mM NH_4COOH in H_2O and methanol as aqueous and organic phase respectively, both adjusted to pH 4. The column used for the analysis was a FluoroSep-RP Octyl, 5 µm, 15 cm x 2.1 mm, 60 Å (ES Industries). The flow rate was set to 0.4 mL/min, the oven set to 35 °C and the injection volume was 5 µL. The elution gradient started at 10 % MeOH set isocratically for 3 minutes, followed by a linear gradient up to 89 % MeOH at minute 25 to allow the elution of the predicted metabolites. Then, the flow was kept isocratic at 87 % MeOH for another 5 minutes. For cleaning purposes, the gradient was raised to 100 % MeOH at minute 33 and kept isocratic at 100 % for a further 7 minutes.

For the HRMS acquisition, a high-resolution time of flight (QTOF) instrument (maXis 4G, Bruker Daltonics, Bremen, Germany) attached to an electrospray ionization (ESI)-ion booster source operating in negative mode were employed. The

acquisition was set to full scan mode in m/z scan range from 0 to 1,000 Da. The source parameters were selected as from previous methods from that same instrument: capillary 1 kV; end plate offset, 400 V; charging voltage, 500 V; nebulizer gas, 4.1 bar; dry gas, 3.0 L/min; dry temperature, 200 °C; and vaporizer temperature, 320 °C (Ballesteros-Gómez *et al.*, 2016).

6.4 PHASE I & II *IN VITRO* METABOLISM ASSAYS

Out of all the possible metabolism reactions, just the most likely to happen in human liver microsomes and commonly studied were selected. For that reason, nicotinamide adenine dinucleotide phosphate (NADPH) was added to the HLM as co-enzyme for the phase I metabolism. For the phase II, uridine 5'-diphospho-glucuronic acid (UDPGA) – together with the addition of alamatechin (Alam) as membrane permeabilizing – was selected as co-enzyme for the uridine 5'-diphospho-glucuronosyltransferase (UGT) in the study of glucuronidation with HLM, and 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as chosen co-enzyme for the sulfotransferases (SULTs) in the study of sulfonation with HLCyt.

6.4.1 Reagents and Solutions

All the solutions and reagents needed to be prepared carefully and stored following the instructions given below to ensure the reagents are active and the metabolic reactions were not inhibited or stopped.

- Buffer TRIS 50 mM as reaction medium with adjusted pH to 7.73 at 25 °C (with 0.1 M HCl or NaOH) and stored in the fridge at 4 °C. The pH was checked prior to every experiment to be 7.4 at the incubation temperature (37 °C).
- HLM 10 mg/mL in buffer used in phase I and phase II metabolism stages and freshly prepared for every batch of experiments. HLM solutions were just agitated by hand, not vortex mixed.
- NADPH 100 mM in buffer as phase I metabolism co-enzyme solution freshly prepared for every batch of experiments and stored in the fridge during them.
- HLCyt 10 mg/mL in buffer used in phase II metabolism stages and freshly prepared for every batch of experiments. HLCyt solutions were just agitated by hand, not vortex mixed.
- UDPGA 100 mM in buffer as phase II glucuronidation co-enzyme solution freshly prepared for every batch of experiments and stored in the fridge during them.
- Alamethicin 1 mg/mL in DMSO as membrane permeabilizing in glucuronidation tests and freshly prepared every day.
- PAPs 10 mM in buffer as phase II sulfonation co-enzyme solution freshly prepared for every batch of experiments and stored in the freezer (- 20 °C) for the day.

6.4.2 Incubation Stage: Experimental Design and Preparation

As previously established during the *in silico* approach stage, the number of experiments was limited to three analytes (MeFOSA, MeFOSE and MeFOSAA) and to HLM and HLCyt with specific co-enzymes. As a consequence of the limited time available to prepare and run the experiments, just a selection of the most important controls were selected and tested, and they included the following:

- Uridine glucuronic acid transferase (UGT) and sulfotransferase (SULT) enzymes substrate negative controls, where all the reagents but the substrates were added.
- UGT and SULT enzyme negative controls, where nothing but buffer and substrates were added.
- UGT and SULT positive controls where all the reagents and substrates were added.

Prior to phase I &II metabolism assays, just phase I assays were carried out to ensure the process (including the later extraction stage) was reliable and the recoveries were sufficient to avoid quantitation or identification problems during the injection of the samples in the UHPLC-HRMS system. These experiments were carried out in triplicate and the incubation time was set as two hours.

Table 39. Schematic overview of the phase I and phase II glucoronidation experiments

Tube	Content	Buffer	HLM	HLM#2	Subs (50ppm)	Alam	NADPH	NADPH#2	UDPGA	UDPGA#2	Start	Stop
1	UGT substrate neg cont a	930	50	50		10	10	10	10	10	0	plus 4h
2	UGT substrate neg cont b	930	50	50		10	10	10	10	10	+ 10 sec	plus 4h
3	UGT substrate neg cont c	930	50	50		10	10	10	10	10	+ 20 sec	plus 4h
4	Enz neg cont: MeFOSE a	990			10						+ 240 sec	plus 4h
5	Enz neg cont: MeFOSE b	990			10						+ 250 sec	plus 4h
6	Enz neg cont: MeFOSE c	990			10						+ 260 sec	plus 4h
7	Enz neg cont: MeFOSA a	990			10						+ 270 sec	plus 4h
8	Enz neg cont: MeFOSA b	990			10						+ 280 sec	plus 4h
9	Enz neg cont: MeFOSA c	990			10						+ 290 sec	plus 4h
10	Enz neg cont: MeFOSAA a	990			10						+ 300 sec	plus 4h
11	Enz neg cont: MeFOSAA b	990			10						+ 310 sec	plus 4h
12	Enz neg cont: MeFOSAA c	990			10						+ 320 sec	plus 4h
13	UGT: MeFOSE a	920	50	50	10	10	10	10	10	10	+ 30 sec	plus 4h
14	UGT: MeFOSE b	920	50	50	10	10	10	10	10	10	+ 40 sec	plus 4h
15	UGT: MeFOSE c	920	50	50	10	10	10	10	10	10	+ 50 sec	plus 4h
16	UGT: MeFOSA a	920	50	50	10	10	10	10	10	10	+ 60 sec	plus 4h
17	UGT: MeFOSA b	920	50	50	10	10	10	10	10	10	+ 70 sec	plus 4h
18	UGT: MeFOSA c	920	50	50	10	10	10	10	10	10	+ 80 sec	plus 4h
19	UGT: MeFOSAA a	920	50	50	10	10	10	10	10	10	+ 90 sec	plus 4h
20	UGT: MeFOSAA b	920	50	50	10	10	10	10	10	10	+ 100 sec	plus 4h
21	UGT: MeFOSAA c	920	50	50	10	10	10	10	10	10	+ 110 sec	plus 4h
	Addition time (min)	**0**	**0**	**120**	**0**	**120**	**0**	**60**	**120**	**180**		

Table 40. Schematic overview of the phase I and phase II sulfonation experiments

Tube	Content	Buffer	HLM	HLCyt	Subs (50ppm)	NADPH#	NADPH#2	PAPS	PAPS#2	Start	Stop
22	SULT substrate neg cont a	930	50	50		10	10	10	10	+ 120 sec	plus 4h
23	SULT substrate neg cont b	930	50	50		10	10	10	10	+ 130 sec	plus 4h
24	SULT substrate neg cont c	930	50	50		10	10	10	10	+ 140 sec	plus 4h
25	SULT: MeFOSE a	920	50	50	10	10	10	10	10	+ 150 sec	plus 4h
26	SULT: MeFOSE b	920	50	50	10	10	10	10	10	+ 160 sec	plus 4h
27	SULT: MeFOSE c	920	50	50	10	10	10	10	10	+ 170 sec	plus 4h
28	SULT: MeFOSA a	920	50	50	10	10	10	10	10	+ 180 sec	plus 4h
29	SULT: MeFOSA b	920	50	50	10	10	10	10	10	+ 190 sec	plus 4h
30	SULT: MeFOSA c	920	50	50	10	10	10	10	10	+ 200 sec	plus 4h
31	SULT: MeFOSAA a	920	50	50	10	10	10	10	10	+ 210 sec	plus 4h
32	SULT: MeFOSAA b	920	50	50	10	10	10	10	10	+ 220 sec	plus 4h
33	SULT: MeFOSAA c	920	50	50	10	10	10	10	10	+ 230 sec	plus 4h
	Addition time (min)	**0**	**0**	**120**	**0**	**0**	**60**	**120**	**180**		

Subs: Substrate

Table 39 and *Table 40* show schematic abstracts of how the experiment was designed for both phases after the optimisation attempts. In them, test tubes 1 to 3 are UGT substrate negative controls, while tubes 22 to 24 are the SULT ones. These controls were set as background chromatograms for the correct identification of peaks coming from the analytes and not from residual matrix from the microsomes. They could be also used for subtracted chromatograms and the identification of non-target compounds Test tubes 4 to 12 are enzyme negative controls, where just buffer and standards were added. These controls were included to identify and discard as metabolism products, hydrolysis products coming from reactions due to the medium (buffer) itself. Finally, test tubes 13 to 21 and 25 to 33 are the positive controls for both glucuronidation and sulfonation processes. Every experiment was prepared in triplicate, and the total incubation time was two hours for phase I metabolism, followed by another two for phase II.

A covered water bath with mild agitation was used for the incubation step. The water was held at a constant 37 °C, as well as the tris buffer solution before the beginning of every experiment. The 2 mL Eppendorf vials were numbered as indicated above and the specified amount (see *Table 39* and *Table 40)* of buffer was added to all of them, followed by 10 µL of substrate and vortex mixed for 10 sec (when proceeded). Then, 50 µL of HLM were added to the specified vials and lightly hand mixed. At this point the timer was started when finally 10 µL of NADPH were added to the first vial containing HLM, with an interval of 10 sec. between one addition and the next one. During that 10 sec. slot, the vials were sealed, hand mixed and placed in the incubation bath. At minute 60, a second addition of 10 µL of NADPH was carried out in the same order and with the same interval of 10 seconds for

opening, adding, hand mixing and closing the vial. A third addition was made at minute 120, in this case of 50 µL of HLM, and 10 µL of alamethicin and UDPGA solutions for the glucuronidation vials; and of 50 µL of HLCyt and 10 µL of PAPs solutions for the sulfonation ones. A fourth addition was made at minute 180 of 10 µL of UDPGA or PAPs solutions. Finally, at minute 240 the reactions were quenched by the addition of 200 µL of cold (4 °C) MeOH followed by 15 µL of cold HCOOH and vortex mixed for 10 sec.

6.4.3 Sample Preparation

A new extraction method was developed, so, different approaches were tested. All of them included a liquid-liquid extraction to transfer all the analytes and metabolites from the aqueous phase – where the incubation was carried out – to the organic solvent and concentrated before LC-MS analysis.

In this case, three different extraction solvents were tested: acetonitrile (ACN), ethyl acetate (EtAc) and dichloromethane (DCM). The addition of ammonium acetate to enhance the salting-out process was also tested, as well as the removal or not – via precipitation – of the denatured proteins from the incubation step before the extraction stage.

The first and major difference among all the different tests, was the higher recoveries obtained when the precipitant proteins were not removed by centrifugation, a step commonly carried out for many other organic pollutants after the incubation (Van den Eede *et al.*, 2015). PFOS – and so PFOS precursors or another intermediate substances – tend to bind to proteins (Beesoon and Martin,

2015b), leading to poor extraction recoveries (even not detected when analysed) as target compounds were not present in the aqueous phase, they were attached to the solid fraction. Among the three solvents tested, DCM showed similar recoveries to EtAc for the precursor compounds (MeFOSA, MeFOSE and MeFOSAA) as well as for PFOS, but DCM was discarded due to its higher density compared to water and the added complication it posed to the extraction. Acetonitrile recoveries were lower than those calculated for the other two solvents, so EtAc was finally selected as extraction solvent. Finally, the salting-out process slightly enhanced recoveries for all the tested solvents, but led to lower signal to noise ratios due to ion suppression caused by the remaining salts from the extraction in the final extract.

The selected extraction to carry out after the reaction was stopped was as follows:

- 700 µL of EtAc were added to the Eppendorf tubes. They were vortex-mixed for 1 min. and ultra-centrifuged (8,000 rpm) for 30 sec.
- After the phase-separation by salting-out with ammonium acetate, the ethyl acetate layer was separated and transferred into a new glass tube.
- After the phase-separation, the upper layer (organic phase) was separated and transferred into new glass tubes. The liquid-liquid extraction was repeated for another three times by adding new aliquots of 0.5 mL of EtAc.
- The supernatants were combined and evaporated to dryness under a mild nitrogen stream (30 °C).
- The extracts were reconstituted in 100 µL of methanol.

6.5 EXPERIMENTAL RESULTS

In this section, the specific qualitative results coming from the *in vitro* experiments detailed above will be presented. As previously explained in this chapter, the established workflow for the entire procedure, is described in *Figure 25*.

For the proper identification of suspected intermediate metabolites, three databases with the accurate mass of all the compounds generated by the theoretical software, together with data reported in previous papers including common intermediates (Benskin, Holt and Martin, 2009; Ross, Wong and Martin, 2012; Chen *et al.*, 2015), were created – one for each analyte investigated – and uploaded to the processing methods. During the processing stage, two different approaches were considered:

- "Target screening" (TS), for the metabolites predicted by the software and/or previously identified in the literature, and for which standards were available.
- "Suspect screening" (SS) for any additional compound experimentally identified and suspected to be a PFOS precursor or intermediate metabolite not predicted by the *in silico* approach and/or not identified previously by the literature.

Besides, three different levels of identification were defined:

- "Confirmation" for these compounds for which standards were available and their relative retention times (RRT) were set as a confirmation parameter when injected together with the samples within an m/z tolerance of 5 ppm.

- "Identification" for these compounds predicted by the software or previously identified in the literature for which no standards were available within an m/z tolerance of 5 ppm.
- "Suspect" for any additional compound experimentally identified and suspected to be a PFOS precursor or intermediate metabolite not predicted by the *in silico* approach and not identified previously by the literature within an m/z tolerance of 5 ppm from the monoisotopic accurate mass.

Moreover, extracted chromatograms were processed in the same way as the original sequence to verify the occurrence of the suspected peaks was not due to matrix interferences or organic solvent impurities employed during the process.

Out of all the possible metabolism reactions, just the most likely to happen and commonly studied for HLM were selected. With these premises, the software predicted 60 theoretical possible metabolites for MeFOSA, 59 for MeFOSAA, and 68 for MeFOSE, as shown in *Table 38* (see *Tables S109, Table SM11* and *Table SM12* for detailed databases). The software discriminated among analytes being probable, plausible or equivocal, according to the specified conditions selected during its design. Data obtained by the software was used to generate a complete database with all the molecular structures and accurate masses from all the suspected intermediates and metabolites generated according to the selected enzymes and cofactors, together with their capability to catalyse these reactions in the human body.

Table 41. Predicted origin for the theoretically detected MeFOSE intermediate products and the reactions leading to them

Formula	Nominal Mass (Da)	Name	Parent	Biotransformation Name	Phase	Enzyme
C11H8F17NO3S	557	A 90507 (Query)				
C8H2F17NO2S	499	M9	M32	Oxidative N-Dealkylation	Phase I	CYP450
C8H2F17NO2S	499	M9	M6	Oxidative N-Dealkylation	Phase I	CYP450
C8H2F17NO2S	499	M9	M2	Oxidative N-Demethylation	Phase I	CYP450
C9H4F17NO2S	513	M2	M32	Decarboxylation of alpha-Amino, Aromatic and beta-Keto Carboxylic Acids	Phase I	Decarboxylase/ AAAD
C9H4F17NO2S	513	M2	M25	Oxidative N-Demethylation	Phase I	CYP450
C9H4F17NO2S	513	M2	M5	Oxidative N-Dealkylation	Phase I	CYP450
C9H4F17NO2S	513	M2	90507 (Query)	Oxidative N-Dealkylation	Phase I	CYP450
C10H6F17NO3S	543	M6	90507 (Query)	Oxidative N-Demethylation	Phase I	CYP450
C11H6F17NO4S	571	M5	90507 (Query)	Oxidation of Primary Alcohols	Phase I	ADH

Table 42. Predicted origin for the theoretically detected MeFOSA intermediate products and the reactions leading to them

Formula	Nominal Mass (Da)	Name	Parent	Biotransformation Name	Phase	Enzyme
C9H4F17NO2S	513	A 3034468 (Query)				
C8H2F17NO2S	499	M1	3034468 (Query)	Oxidative N-Demethylation	Phase I	CYP450
C14H8F17NO8S	673	M29	M3	Oxidation of Secondary (Alicyclic) Alcohols	Phase I	ADH
C14H8F17NO8S	673	M29	M10	Oxidative N-Demethylation	Phase I	CYP450
C14H10F17NO8S	675	M3	M1	N-Glucuronidation of Amides and Related Compounds	Phase I	UGT
C14H10F17NO8S	675	M3	M2	Oxidative N-Demethylation	Phase I	CYP450

Table 43. Predicted origin for the theoretically detected MeFOSAA intermediate products and the reactions leading to them

Formula	Nominal Mass (Da)	Name	Parent	Biotransformation Name	Phase	Enzyme
C11H6F17NO4S	571	A 22286931 (Query)				
C10H4F17NO4S	557	M11	22286931 (Query)	Oxidative N-Demethylation	Phase I	CYP450
C8H12O9	252	M23	M6	Glucuronidation of Carboxylic Acids	Phase II	UGT

For MeFOSE, three compounds were identified from the entire predicted metabolite list, while four were identified for MeFOSA and two for MeFOSAA. Besides these, PFOS was also identified for the three of them. The metabolite names assigned by the software, molecular formulae and names of the reactions leading to them are described in *Table 41* for MeFOSE*, Table 42* for MeFOSA and *Table 43* for MeFOSAA.

For MeFOSA and MeFOSE mechanisms could be suggested, while for MeFOSAA, the possible pathways could not be identified when combining the *in silico* predictions and the experimental results obtained after the analysis by HPLC-HRMS. As consequence of it, along the next sub sections discuss only those results related to MeFOSA and MeFOSE.

6.5.1 MeFOSE

According to the processed Phase I and Phase II sequences for MeFOSE, four compounds were identified by their RRT and/or exact mass. Three of them were predicted by the software (see *Table 41*), while a fourth one was not present in the theoretical approach. Despite this fact, the fourth compound was easily identified as

PFOS, widely reported as the stable end product for other FOSAs and FOSEs. Even though the main database of predicted MeFOSE metabolites was originally created by the Meteor software, some other compounds previously identified or suggested in literature as intermediates from FOSA were added to the processing list.

Three of the four standards were available and they were included in the sequence together with the reported experiments. All of them satisfied the accurate mass (5 ppm) and RRT criteria. For the remaining compounds, no standard could be injected, so just the exact mass identification criteria could be applied.

Figure 26 shows the overlapped extracted ion chromatogram (XIC) of these compounds, named as MeFOSE M5 (or MeFOSAA), M6, M9 (or FOSA) according to the Meteor software nomenclature, all of them formed during the phase I incubation step. MeFOSE metabolite M5 ($C_{11}H_6F_{17}NO_4S$) was predicted to be formed by the alcoholic oxidation of the parent compound ($C_{11}H_8F_{17}NO_3S$ – MeFOSE). MeFOSE M6 metabolite ($C_{10}H_6F_{17}NO_3S$) by its oxidative demethylation and metabolite MeFOSE M9 ($C_8H_2F_{17}NO_2S$) by three different oxidative reactions, all of them coming from other intermediate metabolites.

Figure 27 shows a schematic abstract of the three hypothetical routes of PFOS formation from MeFOSE according to the experimental results. In the first one, MeFOSE M5 – detected – would be directly formed by the oxidation of the precursor to be metabolised afterwards to MeFOSE M9 – detected – via another intermediate (MeFOSE M2 – $C_9H_4F_{17}NO_2S$) which could not be detected chromatographically. A second route could lead as well to MeFOSE M9 intermediate via MeFOSE M2 metabolite, but metabolised in this case straight from the parent compound (MeFOSE). A third proposed metabolic pathway suggested the formation of MeFOSE

M9 intermediate by an oxidative dealkylation of MeFOSE M6 intermediate – detected – formed as a consequence of a previous demethylation of the parent compound.

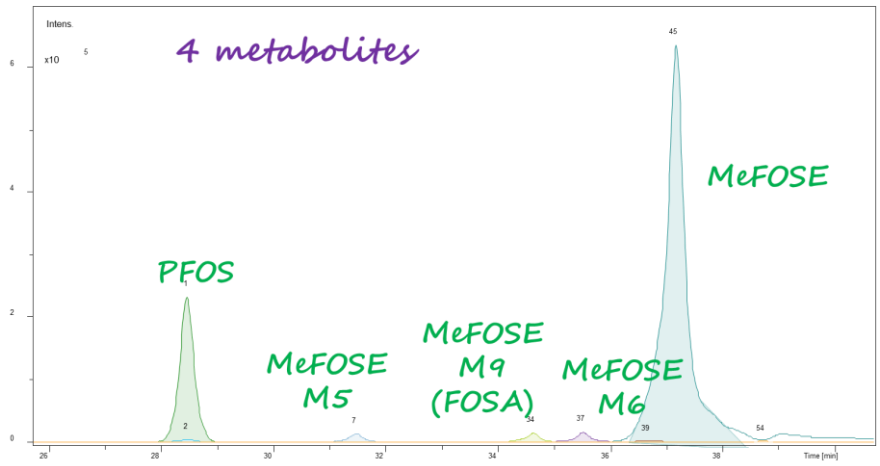


Figure 26. Overlapped XIC chromatograms of the main metabolites detected for MeFOSE

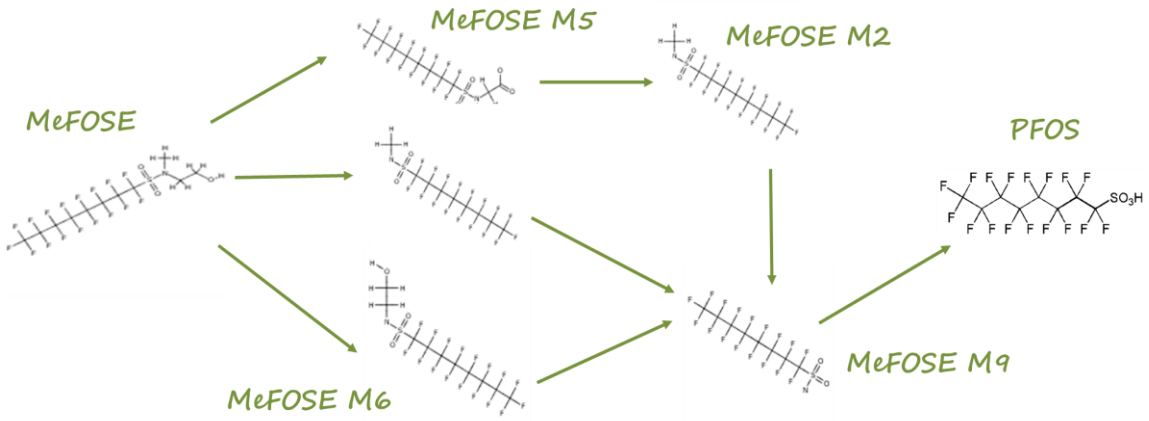


Figure 27. Suggested possibilities for the observed metabolism of MeFOSE

Dealkylation reactions during the incubation process have been previously reported for EtFOSE in rat and human cytochromes by Xu et al. (Xu *et al.*, 2004) and *in vivo* experiments with rats (Xie *et al.*, 2009), supporting the mechanisms presented here, which are expected to be similar but with a methylated group instead of the ethyl reported. Once MeFOSE M9 (FOSA) is formed, different published papers (Tomy *et al.*, 2004; Xu *et al.*, 2004; Riddell *et al.*, 2009; Ross, Wong and Martin, 2012; Chang *et al.*, 2017) support its transformation to PFOS.

6.5.2 MeFOSA

Five peaks were identified when processing the data relative to MeFOSA incubation. Four of them were predicted by the software (see *Table 42*) while the fifth one was identified as PFOS. As per MeFOSE, two available standards lead to the confirmation of MeFOSA M1 – or FOSA – and PFOS, but no standards were available for the rest of the intermediate identified, being just characterised by their accurate mass.

Figure 28 shows the overlapped XIC for the identified peaks predicted by Meteor software, named MeFOSA M1, M3 and M29. Metabolite MeFOSA M1 ($C_8H_2F_{17}NO_2S$, – FOSA) was formed directly by the oxidative dealkylation of the parent compound ($C_9H_4F_{17}NO_2S$ – MeFOSA), while M3 (C_{14} $H_{10}F_{17}NO_8S$) could be present due to an oxidative demethylation of an unidentified metabolite or a glucuronidation. MeFOSA 29 ($C_{14}H_8F_{17}NO_8S$) was formed by oxidation of secondary alcohols, but even identified, was present in much lower proportion than the rest of metabolites, and no further information about later metabolism could be found. The fifth

identified compound did not seem to have relation with the other discovered metabolites, so was discarded.

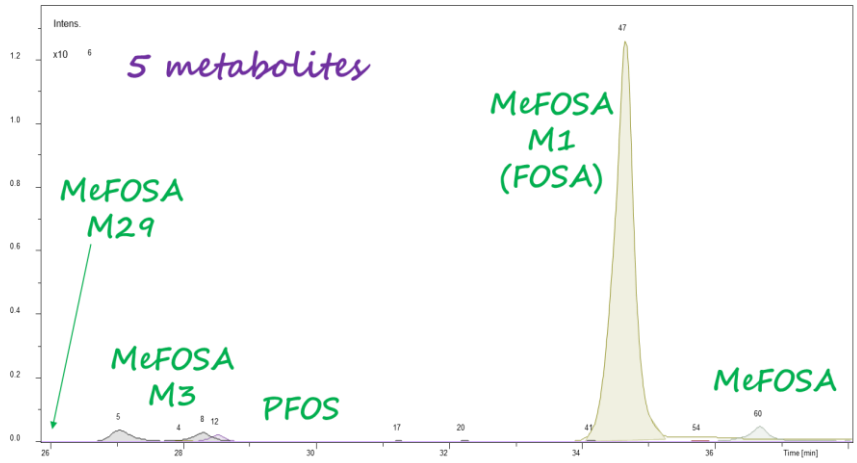


Figure 28 Overlapped XIC chromatograms of the main metabolites detected for MeFOSA

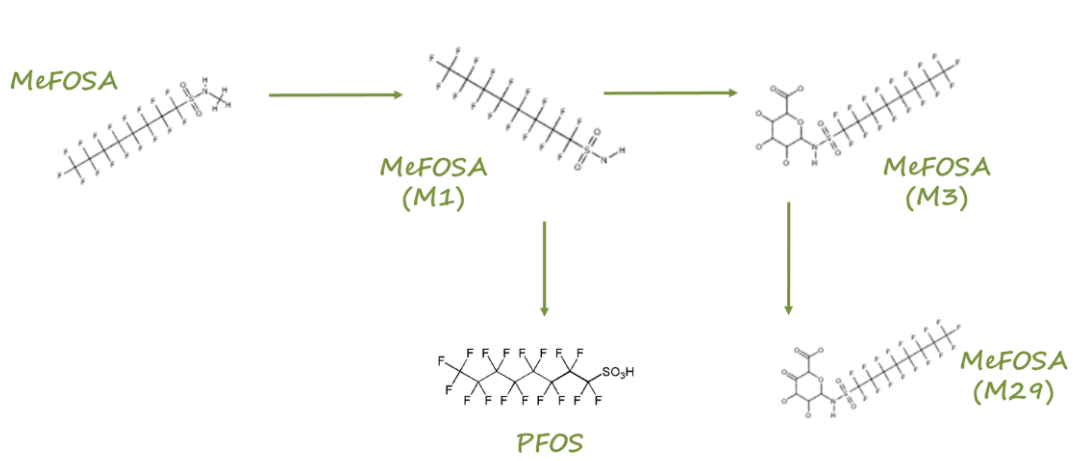


Figure 29. Suggested possibilities for the observed metabolism of MeFOSA

Figure 29 shows the scheme of the two suggested routes for MeFOSA metabolism, one of them leading to PFOS as end-product, and the second one leading to a second product M29, without possibility to track its possible conversion to PFOS. For the

first route ending in the formation of PFOS, the suggested pathway seems easier to define and characterise. MeFOSA M1 was directly formed from the parent compound and it is metabolised, same way as for MeFOSE, to PFOS. This mechanism would be in agreement with the study carried out in 2015 by Fu et al. (Fu *et al.*, 2015)., in which an *in silico* approximation was performed to investigate the metabolism of certain PFOS precursors catalysed by cytochrome P45 enzymes. Moreover, Tomy et al. (Tomy *et al.*, 2004) already reported dealkylation and deamination reactions for the proposed metabolism of EtFOSA in fish liver microsomes.

6.6 SUMMARY AND CONCLUSIONS

According to the results of these experiments – from the qualitative point of view – the data reported here is in line with previous published data from *in vitro* and *in vivo* studies for compounds from the same family (Tomy *et al.*, 2004; Xu *et al.*, 2004; Riddell *et al.*, 2009; Fu *et al.*, 2015). In this case, kinetics was not deeply studied as the experiments were just carried out for a short time period of two hours thus permitting only a qualitative perspective.

Notwithstanding this, it is evident that there is rapid biotransformation of our target PFOS precursors to PFOS, via the stable and detectable intermediate FOSA. The mechanisms leading to the formation of PFOS do not occur in the same way for MeFOSA and MeFOSE. For MeFOSA, the demethylation to FOSA seems to be fast, with the conversion from FOSA to PFOS the limiting step to the final conversion to PFOS, while for MeFOSE the conversion to FOSA seems to be the limiting step, with

a faster conversion of FOSA to PFOS, both of them taking into account the relative abundance of the involved and detected compounds. This can be established just as a rough approximation due to the lack of knowledge of the real kinetics of the proposed mechanisms and the relatively short incubation times.

On the other hand, FOSAA metabolites seem also to play an important role in the metabolic formation of PFOS when MeFOSE was studied, being detected and identified as another stable intermediate before FOSA formation, though the experiments carried out spiking FOSAA itself did not provide a clear pathway for a better understanding of the entire process.

Overall, these experiments make an important contribution to testing the initial hypothesis of this thesis, by demonstrating that *in vivo* metabolism of precursors like FOSAs, FOSEs and FOSAAs to yield PFOS is viable.

7. PFOS AND PFOS PRECURSORS IN HUMAN SERUM SAMPLES

The main objective of this chapter is to determine concentrations of PFOS and PFOS precursors in human serum samples to evaluate the relative contribution of the external exposure to PFOS itself, as well as the indirect exposure to PFOS via FOSAs, FOSEs and FOSAAs via dust and food ingestion, already reported in previous chapters of this thesis.

7.1 INTRODUCTION

Perfluoroalkyl substances in blood samples have been studied and reported for years. Since fluorine was firstly identified by Taves (Taves, 1968a, 1968b), many papers have been reporting levels of PFOS in blood. Total PFOS – as a sum of linear and branched isomers and later years also individually – has been one of the most common PFAS analysed and detected together with PFOA, even after its restrictions in 2002 – by 3M Company phase out – and in 2009 – under the Stockholm Convention – (Zhou *et al.*, 2014; Gebbink, Berger and Cousins, 2015; Gebbink, Glynn and Berger, 2015; Liu *et al.*, 2015; Miralles-Marco and Harrad, 2015; Shan *et al.*, 2016). PFOS was a POP co be concerned about before 2009 (Ehresman *et al.*, 2007; Ericson *et al.*, 2007; Jin *et al.*, 2007; Olsen, Burris, *et al.*, 2007; Wilhelm *et al.*, 2009), and it still is due to its ubiquity and persistence in human blood samples.

Isomer patterns of PFOS started to be reported for environmental, external and internal exposure to PFOS in representative matrices. For environmental samples,

the percentage of branched isomers relative to linear PFOS is usually comparable to the ECF product isomer pattern, containing a mixture of linear and branched isomers around (70:30) (Butt *et al.*, 2010; Beesoon *et al.*, 2011; Esparza *et al.*, 2011; Kärrman *et al.*, 2011; Rahman, Peldszus and Anderson, 2014). Conversely, in human serum samples the ECF product composition of 70 % linear isomers is not true: the isomeric pattern tends to vary among individuals even from the same study, and most of them show an enrichment up to 50 % in PFOS branched isomers (Kärrman, Langlois, *et al.*, 2007; Wang *et al.*, 2011; Zhang *et al.*, 2013; Gebbink, Glynn and Berger, 2015),while some other studies show the opposite tendency (see *Table 10*). The lack of an explanation for this fact evidences that the mechanisms causing this enrichment of branched isomers in blood have not been fully elucidated yet. On the other hand, differences in the isomeric profiles have been observed between humans and animals (Loveless *et al.*, 2006; Benskin *et al.*, 2009; Ross, Wong and Martin, 2012; Chen *et al.*, 2015).

Moreover, PFOS precursors such as FOSAs, FOSEs and FOSAAs have been reported in blood and serum samples, albeit in far fewer publications, as just direct exposure to environmental and external PFOS was considered (Fraser *et al.*, 2013; Gebbink, Berger and Cousins, 2015; Poothong *et al.*, 2017). The interest in these organic pollutants increased substantially after their identification in environmental samples, indoor microenvironments and food samples (Jahnke *et al.*, 2007; Ahrens *et al.*, 2011; Buck *et al.*, 2011; Noorlander *et al.*, 2011; Shoeib *et al.*, 2011, 2016; Ericson Jogsten *et al.*, 2012; Kim *et al.*, 2012; Gebbink *et al.*, 2015), their previously underestimated transfer from furniture, clothing and food packaging materials to air, dust or food items (Tittlemier, Pepper and Edwards, 2006; Ericson, Martí-Cid, *et*

al., 2008; Jogsten *et al.*, 2009; Domingo, 2012), and the growing volume of studies suggesting routes of environmental degradation or metabolism from the precursors to PFOS (Xu *et al.*, 2004; Wang *et al.*, 2009; Xie *et al.*, 2009; Asher *et al.*, 2012; Peng *et al.*, 2014; Chen *et al.*, 2015).

This chapter will present results relative to the human serum samples collected from the participants of the A-TEAM cohort. Nine PFASs – PFOS, FOSA, EtFOSA, MeFOSA, EtFOSE, MeFOSE, FOSAA, EtFOSAA and MeFOSAA – have been quantified and they will be presented here for the first time.

7.2 LONG TERM EXPOSURE QUESTIONNAIRES

Together with the indoor questionnaires and the food diaries, food frequency questionnaires (FFQ) were given to all the participants before the first of the two agreed sampling appointments, and they were collected during the second visit. The main difference between a FD and a FFQ is that the latter records data relating to long term exposure. The food diaries just collect accurate information about the food items consumed during the 48 hours of the sampling period – so are related to the food analysis directly linked to it – while the FFQ reflects eating habits over a longer period of time, covering a wider range of food items which are more representative for the long term food consumption exposure linked to internal exposure to persistent organic pollutants. Using this information, a rough screening of the relevant food items likely contaminated by PFASs and leading to higher levels in blood serum was carried out.

Dietary exposure to PFOS and PFOS precursors was estimated by the use of data generated by this study and the available food intake data. With this, generic parameters – as for the food results chapter – were considered as a first approach. The selected information extracted from food frequency questionnaires was: average weight of food and contents of carbohydrates, fats, sugar, proteins, fibres and energy consumed per participant. Descriptive statistics from these parameters are shown in *Table 44*, and represented in *Figure 21* and *Figure 22* from the food chapter, where both types of questionnaires were compared. The information from the food frequency questionnaires was divided in three groups of consumption for ANOVA test analysis: low, intermediate and high.

The average weight of food consumed by a participant per day according to the FFQ, was 3,050 g, with the lowest consumption being 1,447 and the maximum 7,113 g. In terms of energy, the minimum consumption was 746 kcal, the maximum 5,338 kcal, and the average value 2,040 kcal. On the other side, the average fat consumption was 80.9 g (24.8 – 203.7 g), 240.4 g (84.9 – 671.6 g) for carbohydrates, 43.3 g for sugars (11.7 – 123.5 g), 29.8 g (7.7 – 78.3 g) for fibre and 80.4 g (38.7 – 195.3 g) for proteins.

No information about the food packaging or the cooking utensils was available for the food frequency questionnaires. So, out of the large number of food items listed in the questionnaires (same groups as for the food diaries), just the animal-origin protein ones were considered as more relevant for the exposure of PFASs. Hence cheese, eggs, milk, mixed meat, shellfish and mixed fish will be further discussed together with concentrations of PFASs.

Table 44. Descriptive statistics extracted from the food frequency questionnaires

	Weight (g)	**Energy (kcal)**	**Fat (g)**	**Carbohydrates (g)**	**Sugar (g)**	**Fibre (g)**	**Protein (g)**
Average	3,051	2,040	80.9	240.4	43.3	29.8	80.4
Mean	2,898	1,928	76.5	221.3	38.0	27.4	77.0
Median	2,891	1,880	78.1	210.9	39.4	26.7	74.7
Min	1,447	746	24.8	84.9	11.7	7.7	38.7
Max	7,113	5,338	203.7	671.6	123.5	78.3	195.3
Q1	2,315	1,516	63.6	174.5	25.7	19.7	62.8
Q3	3,522	2,413	93.5	291.2	52.2	35.4	91.2
SD	1035.4	754.4	28.4	106.7	23.3	13.1	26.3
% RSD	33.9	37.0	35.1	44.4	53.9	43.8	32.7

Q1: 25th percentile. Q3: 75th percentile. SD = Standard deviation. RSD = Relative standard deviation

Besides the FFQ, indoor questionnaires are also important when evaluating long term exposure to organic pollutants, and these already described and also mentioned when evaluating indoor dust exposure (see *Figure 11* and *Figure 12*), will be included in this chapter as well.

Finally, age and gender play a role in terms of accumulation and elimination of organic pollutants, so they are both important parameters to consider. As mentioned in the dust chapter, the participants ranged from 20 to 66 years old (average = 41.7 years), being 75.5 % of them female and the remaining 24.5 % male. Ages were divided in three groups for ANOVA evaluation: less than 30 years, between 30 and 50, and more than 50 years old.

7.3 CONCENTRATIONS IN SERUM SAMPLES

From the 61 participants, 60 tubes containing human blood samples were collected, the serum extracted and shipped to Birmingham, so all the data presented onwards will relate to these 60 participants, including the information extracted from the questionnaires provided along this chapter.

In this section, PFOS – linear and branched isomers – and PFOS precursors – FOSAs, FOSEs and FOSAAs – concentrations will be given and related to the different indoor and food questionnaires parameters mentioned previously.

7.3.1 Total PFOS and PFOS Precursors

Descriptive statistics from the serum samples where PFOS and PFOS precursors were analysed are shown in *Table 45*. Individual sample results are detailed in *Table SM13.*

Statistical requirements for the analysis of the serum samples are the same as established for the other two matrices previously reported: minimum value of 30 % positive samples for a full statistical analysis, and a minimum of 10 % for t-test and ANOVA in scenarios when they were used for comparison of means with preceding studies.

For the present set of samples, just ΣPFOS (86.7 % positive samples) met the criteria, with a mean concentration of 5.29 ng/mL (<LOQ – 16.52). The second most detected compound was MeFOSAA (23 % positive samples) with a mean concentration of 0.04 ng/mL (<LOD – 0.38). For the rest of the analytes, three samples were positive for EtFOSE (1.11, 3.73 and 0.61 ng/mL), two for MeFOSA

(0.013 and 0.005 ng/mL) and EtFOSAA (0.02 and 14.72 ng/mL), one for FOSA (0.003 ng/mL) and MeFOSE (1.17 ng/mL), and no positive samples were found for EtFOSA and for FOSAA. Even though FOSAs and FOSEs were main contributors to dust and food exposure pathways, no statistical analysis could be conducted in serum samples as a consequence of their low detection frequencies.

As with dust and food samples, the Shapiro-Wilk test for normality of data distribution was applied, showing skewed data. A comparison between non-parametric statistics and parametric statistics with logarithmic transformed data was conducted, showing no significant differences on the data analysis. As a consequence of it, the dataset was normalised by the use of logarithmic transformed values for the statistical analysis. T-tests were run with a significance level of 95 % ($p = 0.05$), ANOVA tests with Scheffe post-hoc test with a significance level 95 % ($p = 0.05$), while for the correlations they were specified case by case ($p = 0.01$ or $p = 0.05$).

Age and gender did not show significant differences either for ΣPFOS when ANOVA and t-test were applied, but mean values were higher for participants aged over 50 years old, followed by these under 30 and lower concentrations for the participants aged between 30 and 50 years old (see *Figure 30*). Similarly, mean concentration values for males were higher than for women, as *Figure 31* shows.

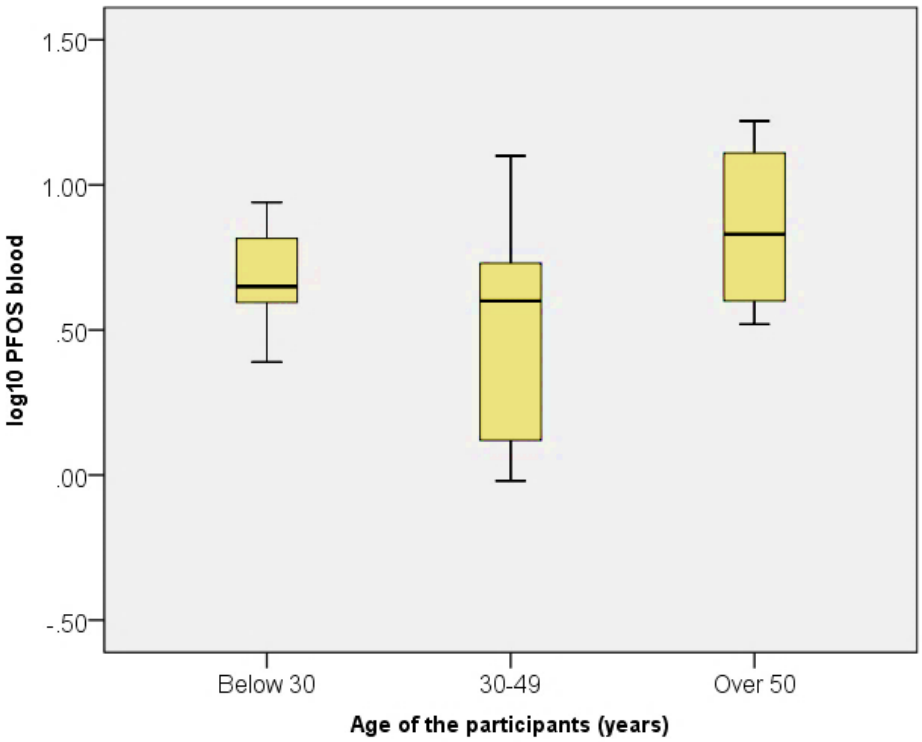


Figure 30. Box plots showing different concentrations of ΣPFOS in serum samples according to the age of the participants. No significant differences were found (p = 0.05)

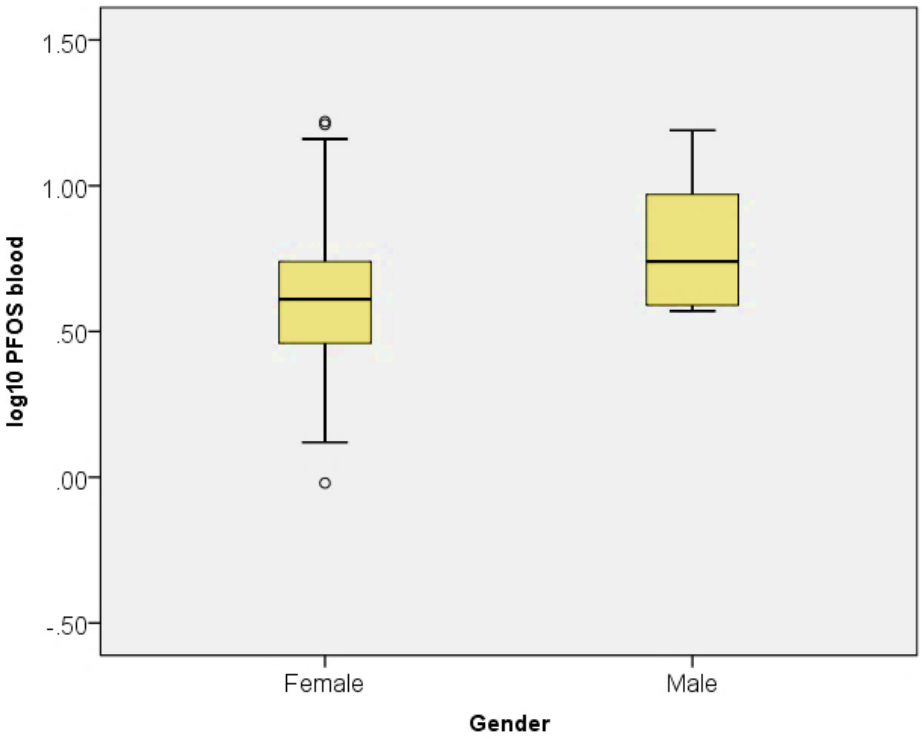
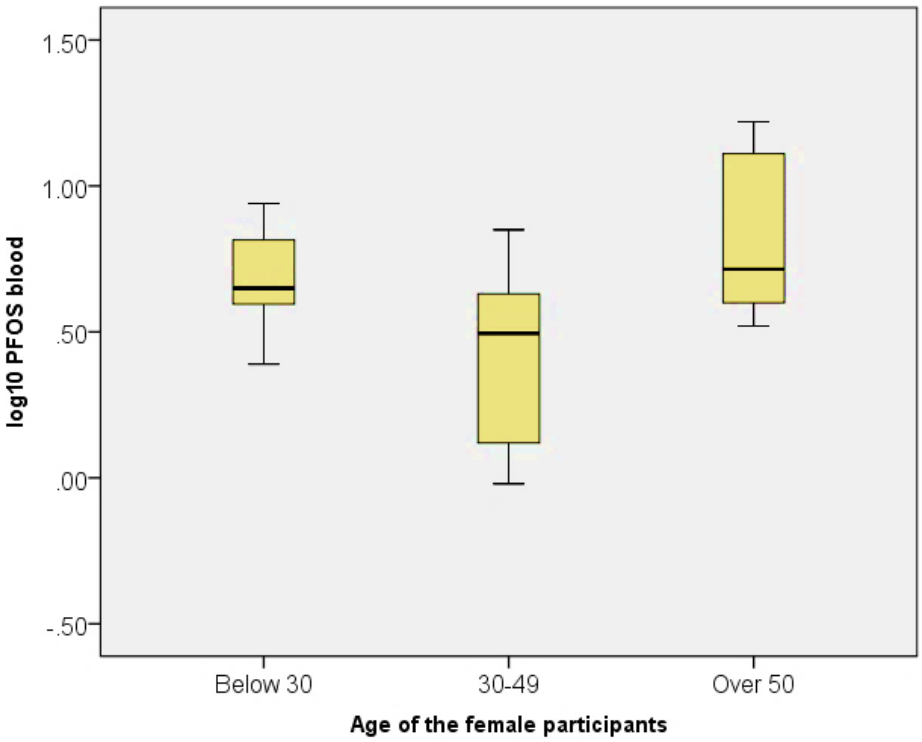


Figure 31. Box plots showing different concentrations of ΣPFOS in serum samples according to the gender of the participants. No significant differences were found (p = 0.05)



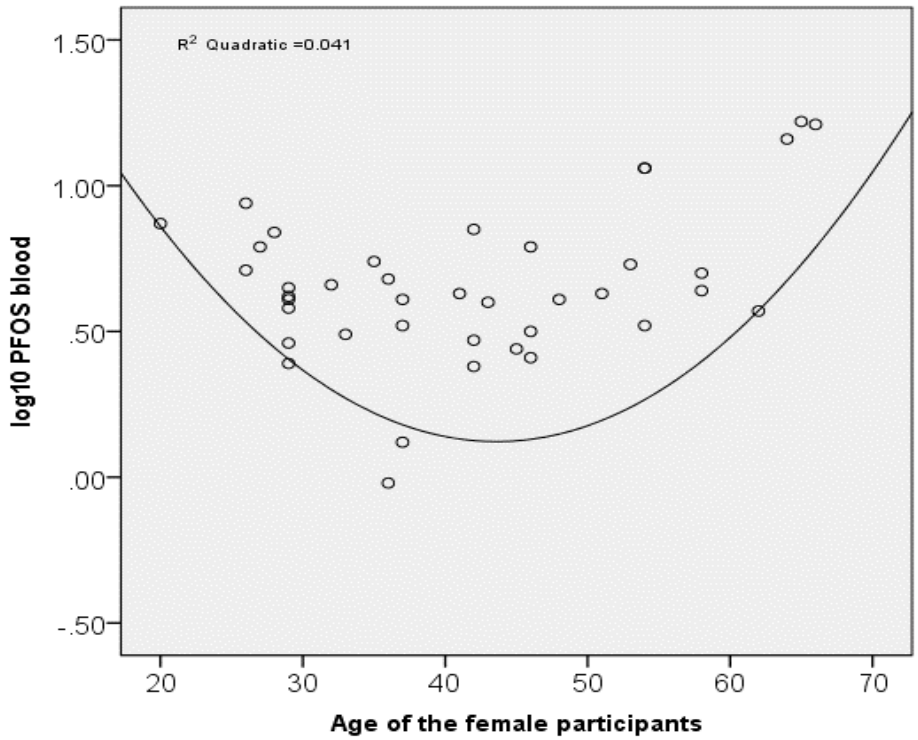
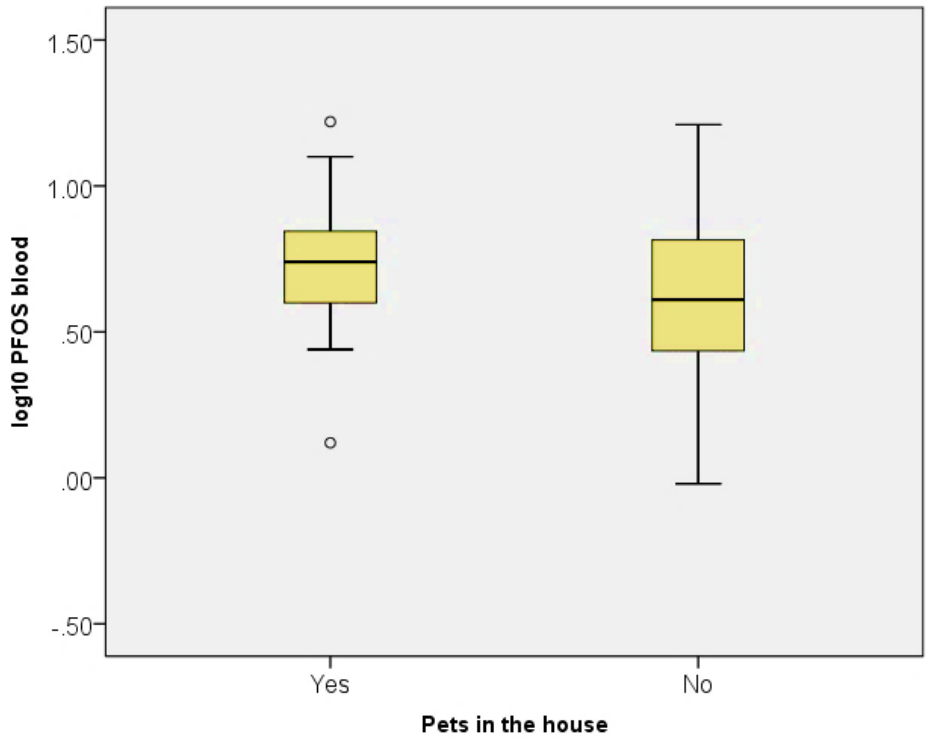


Figure 32. Box plots showing different concentrations of ΣPFOS in serum samples according to the gender of the female participants, followed by a scatter plot with trends of levels of PFOS in serum for female participants of the cohort according to age

Table 45. Concentrations of PFOS and PFOS precursors (ng/mL) in serum samples (n = 60)

	PFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA	ΣPFAS
Average	5.292	NR	0.002	< LOD	NR	0.131	< LOD	0.036	0.251	5.86
Mean	1.973	NR	0.002	< LOD	NR	0.049	< LOD	0.009	0.007	3.79
Median	4.301	NR	0.002	< LOD	NR	0.042	< LOD	0.004	0.006	4.54
Min	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.19
Max	16.525	0.0028	0.013	< LOD	1.167	3.739	< LOD	0.379	14.717	17.78
5th percentile	0.004	0.002	0.002	0.029	0.082	0.042	0.022	0.004	0.006	0.19
Q1	2.909	0.0018	0.002	< LOD	0.082	0.042	< LOD	0.004	0.006	3.25
Q3	6.715	0.0018	0.002	< LOD	0.082	0.042	< LOD	0.004	0.006	7.34
95th percentile	14.376	0.002	0.002	0.029	0.082	0.070	0.022	0.180	0.006	14.95
SD	4.164	0.000	0.001	< LOD	0.140	0.499	< LOD	0.072	1.899	4.36
% RSD	78.68	7.01	72.08	< LOD	139.95	382.05	< LOD	198.15	755.32	74
% Positive	86.7	1.7	3.3	0.0	1.7	5.0	0.0	23.3	3.3	90.0

n = number of dust samples included in the statistics. DF: Detection frequency. Q1: 25th percentile. Q3: 75th percentile. NR = Not reported. SD = Standard deviation. RSD = Relative standard deviation. LOD = limit of detection



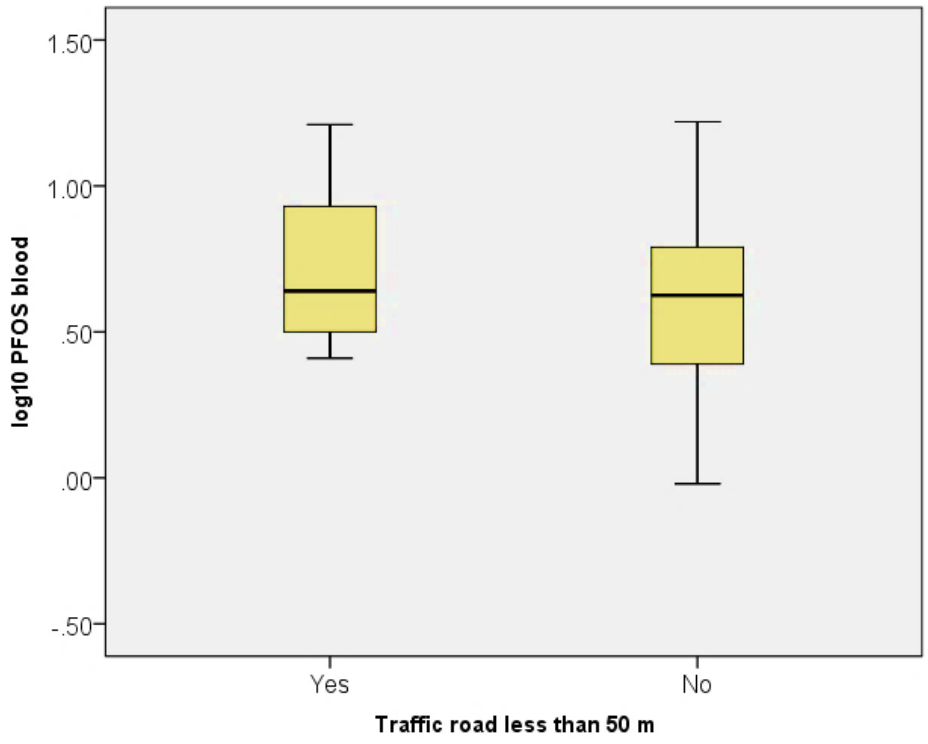


Figure 33. Box plots showing significant differences (t-test, $p < 0.05$) for concentrations of ΣPFOS in serum samples according to the location –distance from busy traffic roads – and the presence/absence of pets in the house

The female subgroup of samples was selected (75.5 %) for further evaluation of the levels of ΣPFOS according to the three groups of ages, showing same pattern as when all the participants were selected. The highest levels were for oldest participants, followed by the youngest ones, with the lowest levels for those aged between 30 and 50 years old (*Figure 32*) even though no significant differences were found for ANOVA and Pearson correlation tests.

These findings strongly suggest – even with the lack of significant differences in the date – that age and gender do play a role when estimating internal exposure to PFOS (and by extension, to PFASs), further discussed in the conclusions section of this chapter.

The same indoor parameters evaluated for the dust samples – age of the participants and their gender, the number of hours spent home, the type of residence, their location (distance) from the NIPH, from industrial areas and from busy traffic roads, the age, size and distribution of the residence, the presence of pets and smokers, the ventilation frequency, the recent renovation of the different rooms in the residence, the frequency the residence was vacuum cleaned, and the use of Gore-Tex® clothing of the participants (shoes and clothes) – were evaluated for the serum data. Just two significant differences were found: proximity to busy traffic roads, and the presence/absence of pets in the house, as represented in *Figure 33*. Mean values of ΣPFOS in serum were significantly higher for these participants living less than 50 meters from a road with traffic (log ΣPFOS = 0.7207) when compared with those participants who were not (log ΣPFOS = 0.1661). Similarly, mean values of ΣPFOS were higher in participants living with one – or more animals – in the house (log ΣPFOS = 0.7321) compared to the ones who were not (log ΣPFOS = 0.2075).

The serum concentrations were also evaluated versus the parameters extracted from the food frequency questionnaires. Energy, fat, carbohydrates, sugar, fibre and protein consumption were the first parameters selected for evaluation. No significant differences were found when the ANOVA tests were performed, and just fat showed a positive correlation with ΣPFOS concentrations at $p = 0.05$, as represented in *Figure SM04*. In a second approach, specific food items, as fish (shell fish, lean fish, cooked fish, oily fish and all kind of fish combined), meat (chicken meat, read meat, and all kinds of meat combined), eggs, butter and milk were selected for significant differences among three different scenarios (low, intermediate and high consumption) and correlations. Egg consumption was the only parameter which showed a positive correlation with ΣPFOS concentrations at $p = 0.05$, but no statistical differences were identified among the three scenarios.

7.3.2 PFOS Branched Isomers

The expected ratio between linear and branched isomers for external exposure to PFOS via dust exposure is 70:30 = 2.33 as previously reported for dust samples. In contrast, for internal exposure the percentage of the linear isomer has been reported to be lower according to most of the papers (Kärrman *et al*., 2007b; Zhang *et al.*, 2013; Beesoon *et al.*, 2011; Liu *et al.,* 2015) due to the different absorption and elimination rates for the multiple PFOS isomers (Benskin, Holt and Martin, 2009; De Silva *et al.*, 2009; Peng *et al.*, 2014), but also higher according to other authors (Ross, Wong and Martin, 2012).

In this section, some of the positive serum samples for total PFOS were re-analysed using the second of the methods detailed in the methodology chapter, and the levels of the samples sensitive enough for a proper quantification of the branched isomers are reported. As not all of the individual isomers could be elucidated and identified from the standard mixture, the sum of the branched isomers (ΣBr-PFOS) versus the linear one (n-PFOS) was selected for identification and quantitation.

Table 46. Linear and branched PFOS isomers (%) in serum samples (n = 40)

	n-PFOS / ΣBr-PFOS	**n-PFOS (%)**	**ΣBr-PFOS (%)**
Average	2.46	69	31
Mean	2.30	69	30
Median	2.37	70	30
Min	1.06	51	18
Max	4.60	82	49
5th percentile	1.208	55	18
Q1 (25th percentile)	1.99	67	27
Q3 (75th percentile)	2.70	73	33
95th percentile	4.498	82	45
SD	0.93	8.02	8.02
% RSD	38.67	11.61	25.97

SD = Standard deviation. RSD = Relative standard deviation

Descriptive statistics from the serum samples where linear PFOS and branched PFOS isomers were analysed (n = 40) are shown in *Table 46* and represented in *Figure 34*. Individual sample results are detailed in *Table SM14*.

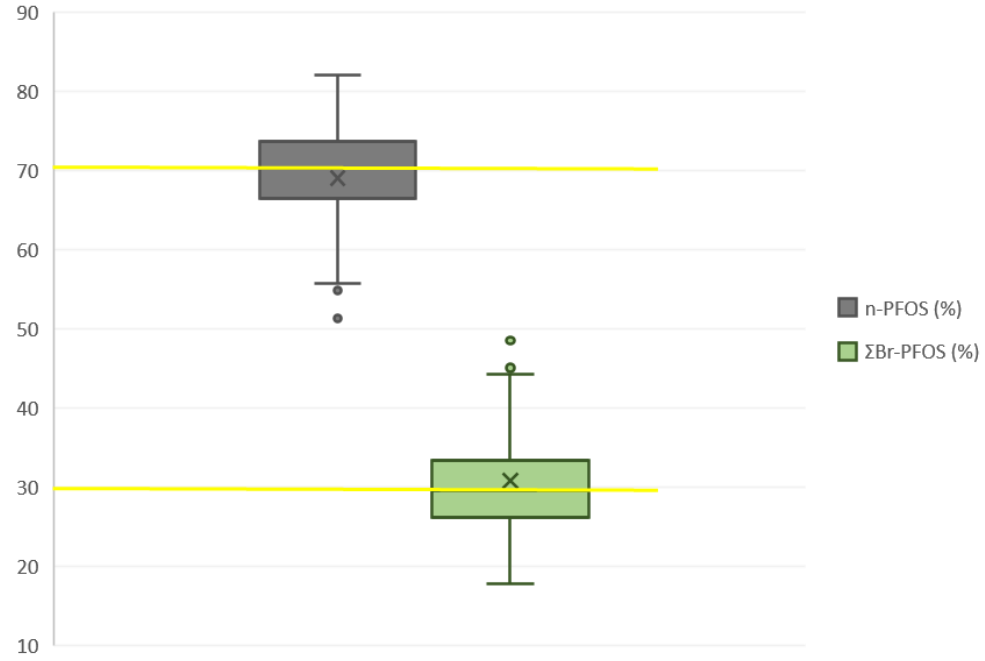


Figure 34. Box plots representing the percentages of linear and branched PFOS isomers identified in the serum samples (n = 40), where the yellow lines represent the theoretical percentage of the commercial PFOS mixture (70 % linear and 30 % branched isomers)

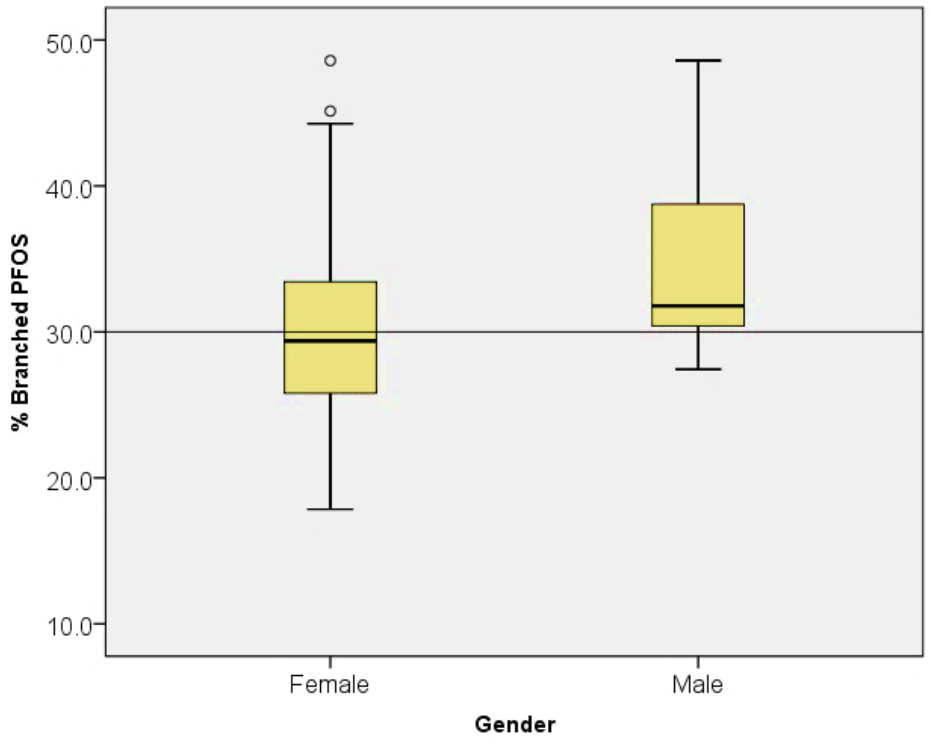


Figure 35. Box plots showing the percentages of branched PFOS isomers for male and female participants identified in the serum samples

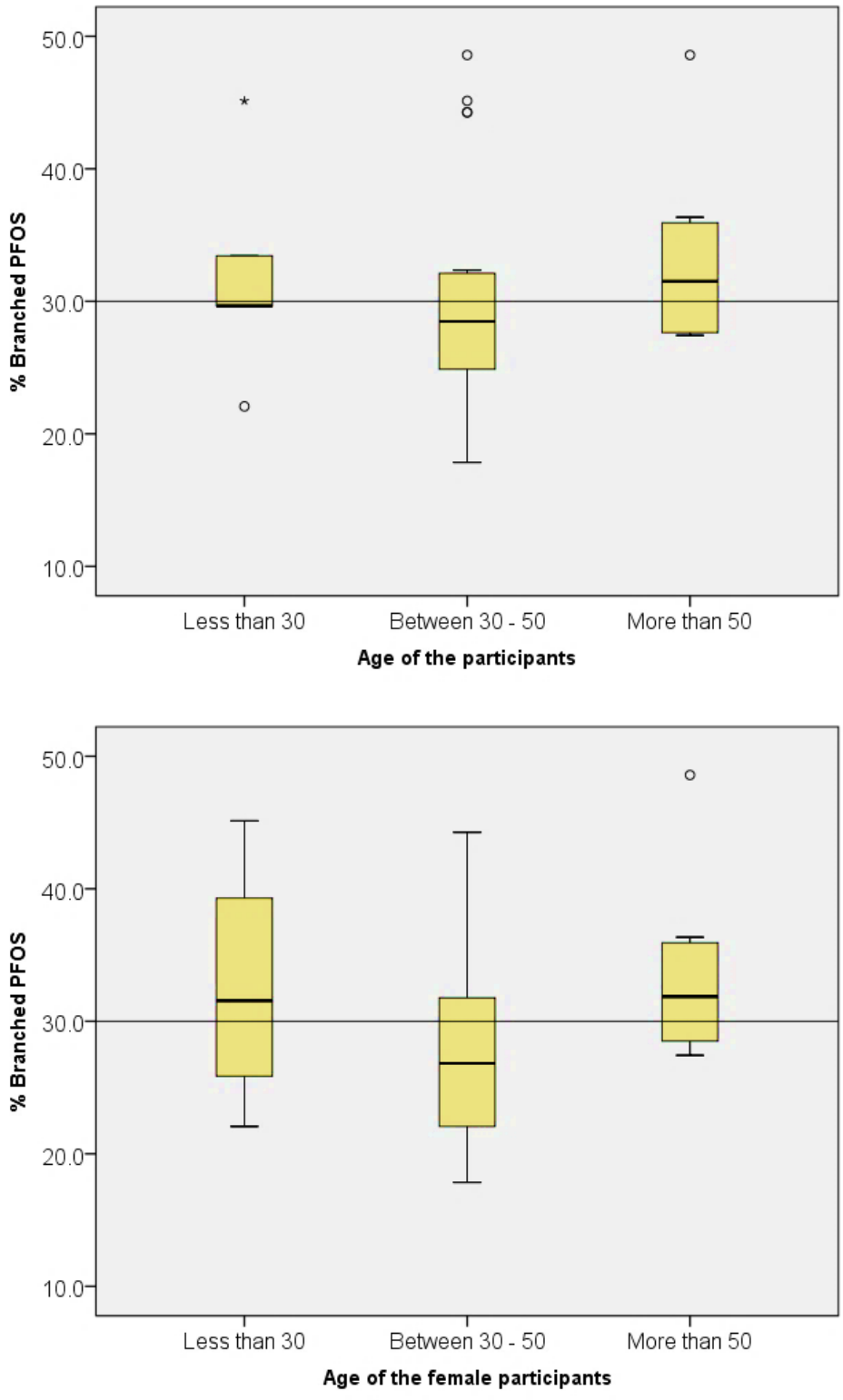


Figure 36. Box plots relatives to the percentages of branched PFOS isomers identified in the serum samples from the entire set of participants (upper figure) and for the female participants (lower figure)according to age when ANOVA test (p = 0.05) were conducted. No significant differences were identified

According to what has been established in the introduction of this chapter, the most commonly reported in literature are samples showing increased proportions of the branched isomers, while still a small percentage showed the opposite tendency. The samples presented in this work presented linear:branched quotients ranging from 1.06 to 4.60 (equivalent linear:branched ratios of 51:49 and 82:18, respectively), when compared to the ECF value of 2.33 (equivalent to linear:branched ratio of 70:30). This results are consistent with initial assumptions and suggests that there is substantial inter-individual variation in both exposure to different PFOS isomers and (likely more importantly) their metabolism.

In order to try to elucidate and discriminate among the participants according to their PFOS isomer ratios, age and gender were evaluated.

No significant differences were identified when concentrations of ΣBr-PFOS and the gender of the participants was evaluated (t test, $p = 0.05$). Nevertheless, the representative box blots of the two subsets of participants showed some differences: mean value ΣBr-PFOS for males was 35.2 %, while the mean value for female participants was < 30 % (See *Figure 35*).

As for gender, no significant differences were found when the participants were grouped by ages for ANOVA test. Still, some trends could be identified, albeit not statistically significant: the percentage of branched isomers in participants ranged between 30 and 50 years old (mean = 29 %) were lower than those reported for participants under 30 years old (mean = 32 %) and above 50 years old (mean = 31 %), as *Figure 36* shows. The difference became slightly higher when just considering the female participants from the cohort (see *Figure 36*): the mean value of ΣBr-PFOS for female participants ranged from 30 to 50 years old was 27.7 %,

while they were 32.6 % and 33.1 % for female participants below 30 years old and for the ones older than 50 years old, respectively.

The concentrations of PFOS branched isomers were also compared with the same parameters as total PFOS from the food frequency questionnaires: by groups of nutrients in a first approach (energy, fat, carbohydrates, sugar, fibre and protein consumption), and by specific food items in a second approach (fish, meat, eggs, butter and milk).

None of the groups of nutrients showed significant differences, but different patterns were observed when the three scenarios (low, intermediate and high consumption) were compared. Participants reporting an average energy intake higher than 2,000 kcal/day showed higher percentage in ΣBr-PFOS (31.5 %) than the ones reporting lower caloric intakes (< 30 %), as *Figure37* shows. Same pattern was observed for fibre, carbohydrates and for proteins, represented in *Figure SM5*. On the other side, fat consumption seemed not to contribute to enhance or reduce the percentage of PFOS isomers in serum.

7.4 LINKING EXTERNAL AND INTERNAL EXPOSURE TO PFOS AND PFOS PRECURSORS

Estimated daily intakes for the sum of food and dust ingestion for the Norwegian participants of the A-TEAM cohort, in medium bound and mean scenario respectively, are reported in *Table SM15*. Descriptive statistics are shown in *Table 47*.

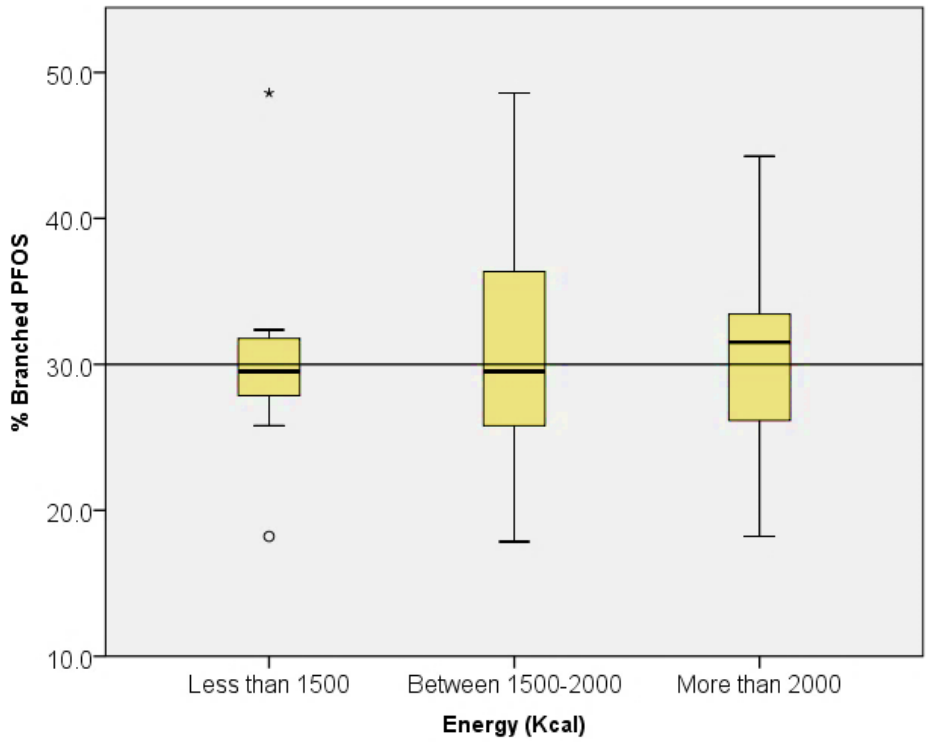


Figure 37. Box plots showing the percentages of branched PFOS isomers in the serum samples for different energy intakes reported by the participants. ECF ratio (30% branched isomers) is marked as reference

Correlations between concentrations of ΣPFOS in serum versus PFOS, FOSA, EtFOSA, MeFOSE and EtFOSE in dust samples, versus ΣPFAS in food samples, and versus estimated daily intakes were evaluated. Positive correlations were identified for PFOS and for ΣPFAS when they were compared with ΣPFOS concentrations in serum samples.

Table 47. Calculated daily intakes of ΣPFAS (ng/day and ng/kg bw/day) through dust and food ingestion (n = 46)

	Daily intake (mg/day)	**Daily intake (ng/kg bw/day)**
Average	16.314	0.265
Mean	2.882	0.042
Median	0.981	0.016
Min	0.468	0.006
Max	140.286	2.551
5th percentile	0.495	0.007
25th percentile	0.688	0.009
75th percentile	18.455	0.271
95th percentile	78.471	1.368
SD	30.028	0.533
%RSD	184.067	201.388

SD = Standard deviation. RSD = Relative standard deviation

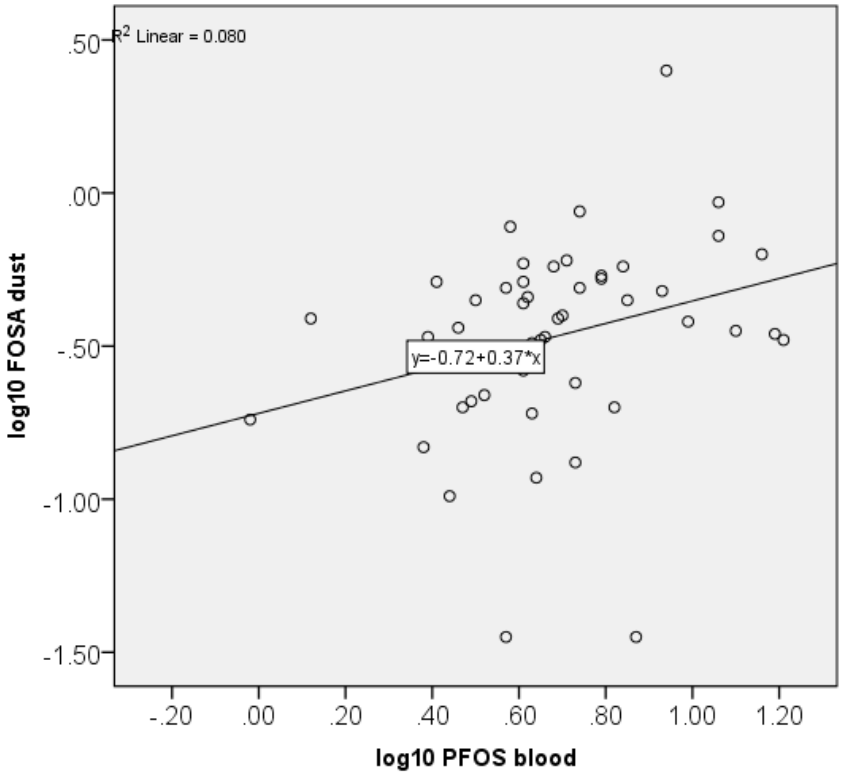


Figure 38. Scatter plot showing the positive correlation (0.348) at p = 0.05 between the concentrations of FOSA in dust samples and the ones in blood for PFOS

A positive correlation (regression coefficient of 0.35) was identified for the concentrations of ΣPFOS in blood and the concentrations of FOSA in dust samples, as *Figure 38* shows. A similar correlation (regression coefficient of 0.38) was identified for the estimated daily intakes of PFOS in dust, shown in *Figure 39*. Interestingly, no other positive correlation was identified for dust or food concentrations, neither for the sum of both of them, versus the reported levels of PFOS in serum.

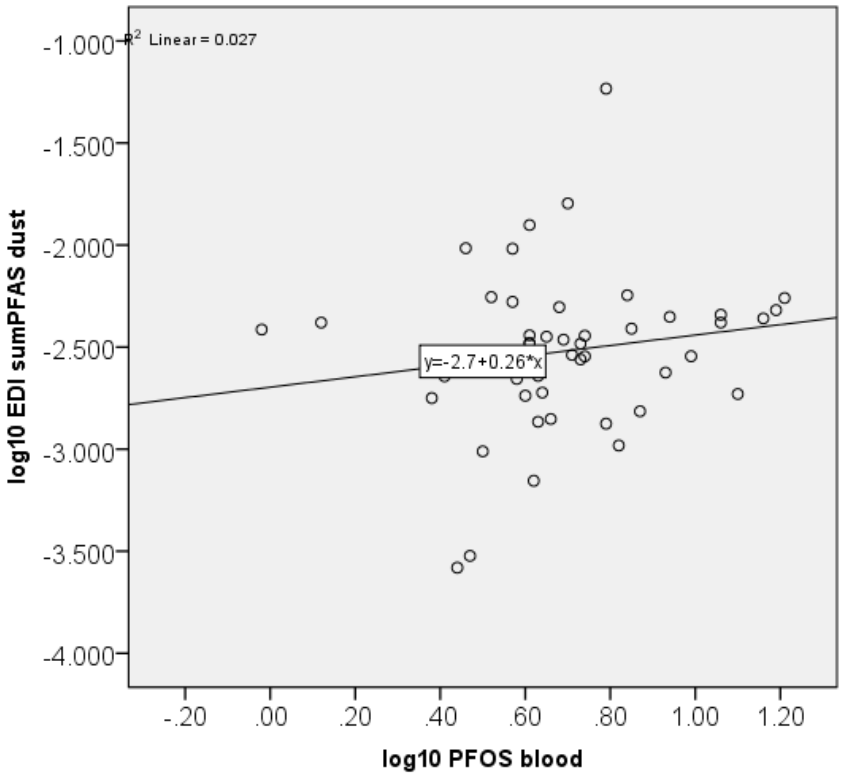


Figure 39. Scatter plot showing the positive correlation (0.38) at p = 0.05 between the estimated daily intakes of PFOS in mean scenario for dust ingestion and the concentrations in blood for PFOS

7.5 SUMMARY AND CONCLUSIONS

Since 2001, the ubiquity of PFOS in the environment and its high levels reported in blood samples has been a fact and a cause of concern (Hansen *et al.*, 2001). Now, decreasing patterns for internal exposure levels as a consequence of PFOS derivatives restriction are evident too (Kato *et al.*, 2011), but still, many authors are reporting measurable levels of PFOS in blood samples in the order of ng/mL (Ericson *et al.*, 2007; Fromme *et al.*, 2009; Haug, Huber, Becher, *et al.*, 2011).

This study has shown that ΣPFOS is detectable in a high percentage of serum samples (87 %) from Norwegian population, clearly dominating the internal exposure levels with an average concentration of 5.3 ng/mL. MeFOSAA was identified as the second most detected compound (23 %), with an average concentration of 0.036 ng/mL, while the rest of the PFOS precursor were detected in much lower ranges: FOSA and MeFOSE were detected in one sample (0.003 and 1.167 ng/mL respectively), MeFOSA was identified in two samples (0.013 and 0.05 ng/mL), same as EtFOSAA (0.016 and 14. 71 ng/mL), while three samples were positive for EtFOSE (average = 0.131 ng/mL). EtFOSA and FOSAA were not detected in any of the serum samples.

This study is not the first one reporting levels of FOSAAs in blood or serum samples. In 2015, Gebbink et al. (Gebbink, Glynn and Berger, 2015) reported FOSAAs concentrations in human serum samples from Swedish population collected between 1991 and 2012. On it, they showed how trends in FOSA and FOSAAs internal exposure decreased as for PFOS through the years, in a range of concentrations of 0.015 to 0.5 ng/g when considering individual concentrations of FOSAA, EtFOSAA and MeFOSAA. Other authors also reported levels in human serum

samples in the same order of magnitude for FOSAAs (Olsen *et al.*, 2004; Lee and Mabury, 2011), for FOSAs (Haug, Thomsen and Becher, 2009a), and for FOSEs (Fraser *et al.*, 2013), being and all of them 1-2 orders of magnitude lower than PFOS.

In terms of exposure, Ullah et al. (Ullah *et al.*, 2014) attributed internal levels of FOSAAs to direct exposure through food or dust ingestion, while other authors as Shoeib et al. (Shoeib *et al.*, 2011) suggested indirect exposure to FOSEs via dust or food ingestion as the main source of FOSAAs. On the other side, detected levels of FOSAs in blood samples have been attributed to both, direct and indirect – via MeFOSA or EtFOSA – exposure to air, dust or food (Peng *et al.*, 2014).

Age and gender of the participants seemed to play a role in internal concentrations of ΣPFOS in serum, even when no statistical differences were identified. Participants aged over 50 years old showed higher concentrations than the rest of them, followed by the participants below 30 years old. Same pattern was detected for the female subset of participants. These differences could be attributed to different accumulation and elimination rates as consequence of menstruation, birth giving or breastfeeding of the female participants.

For PFOS isomers, a slight enrichment for the sum of branched isomers was observed for the overall cohort, but remaining unclear if it is due to different patterns of exposure or – as most suggested – bioaccumulation. When male and female participants were compared, female participants showed a different pattern than the male ones, showing a percentage of branched isomers higher than 30 % for men, while it was lower than 30 % for women. Interestingly, when the sum of branched isomers was evaluated by age for the female participants, these between 30 and 50 years old presented were the only ones showing percentages below 30 %,

while the other two groups of age showed mean values higher than 30 %. This finding suggest than the different uptakes and elimination rates of linear and the different branched PFOS isomers could be even more relevant when considering the factors of menstruation, maternity and breastfeeding mentioned above.

The study presented in this thesis could not find strong correlations between levels of external exposure to PFOS precursors or PFOS – via dust and food ingestion when they were combined –and the internal ones, even when there are evidences supporting dust and food as the main sources of exposure. Still, two correlations – for FOSA and for ΣPFAS – were identified for dust exposure and intake, even when dust constituted around 2 % to the overall exposure presented in this thesis (average intake of 0.24 ng/kg bw/day for food in median bound versus 0.0045 ng/kg bw/day for dust in medium scenario).

Current findings point out the need to further investigate the link between their external and internal exposure patterns by the use of long term monitoring to properly evaluate trends and correlations.

8. CONCLUSIONS AND SUMMARY

Major exposure to PFOS and PFOS related compounds is an every-day exposure for most of the population. According to the literature review (Vestergren *et al.*, 2008; D'Eon J and Mabury, 2011; Gebbink, Berger and Cousins, 2015; Miralles-Marco and Harrad, 2015), main routes of exposure to these pollutants suggest diet as the first contributor (combination of food and drinking water), followed by dust ingestion as the second one, in all cases remarking the unavoidable daily exposure to these environmental pollutants.

Direct exposure to PFOS has been long studied and widely reported. Recent years indirect exposure to PFOS precursors – such as FOSAs, FOSEs and FOSAAs – has been pointed out as the most feasible source of current internal levels of PFOS in human biomonitoring studies, especially after the 3M Company phase out (2002) and the later inclusion of PFOS as a POP under the Stockholm Convention (2009). Consequently, legacy routes of exposure to PFOS related compounds – clearly dominated by direct exposure to PFOS – changed, and new studies further investigating exposure and metabolism of the so-called PFOS precursors and their relative contribution to the overall body burdens of PFOS, started growing in number.

This thesis was addressed to the idea of partially contribute to the understanding of the remaining unknowns related to real exposure and body burdens of PFOS – indirect sources of exposure inclusive – by the study of a reduced number of selected

PFASs suspected to be PFOS precursors in a small population group (n = 61), set as the Norwegian A-TEAM cohort. Concentrations, intakes, exposure patterns, correlations, and novel findings will be described and remarked along this conclusive chapter.

8.1. RESULTS SYNOPSIS

Diet, as a combination of food and drinking water, has been extensively suggested to constitute the most important external source of PFASs exposure. Due to these statements, food exposure – duplicate diet method– was studied and evaluated for the participants of the A-TEAM cohort. In agreement with what has been previously reported by some authors (Tittlemier, Pepper and Edwards, 2006; Vestergren *et al.*, 2008; D'Eon J and Mabury, 2011; Gebbink, Berger and Cousins, 2015), this study pointed diet as the main source of exposure to PFOS and PFOS precursors. Average daily intake for the sum of PFOS and PFOS precursors was estimated to be 0.23 ng/kg bw/day (15.6 ng/day) for the combined exposure to both, solid and liquid food. Still, in this study just seven solid food composites showed detectable concentrations of PFOS, with an average value of 0.005 ng/g. FOSA (18 positive samples, 0.004 ng/kg), MeFOSE (9 positive samples, 1.902 ng/kg) and EtFOSE (19 positive samples, 3.746 ng/kg) were identified in a larger number of samples and in higher concentrations (maximum amount of 53.1 ng/kg for EtFOSE for one of the reported samples) than PFOS, revealing PFOS precursors as higher contributors to solid food exposure than PFOS itself. Lower detection frequencies were reported for the liquid food composites, albeit water has been established as a source of PFASs (Loos *et al.*, 2007; Ericson, Nadal, *et al.*, 2008): 4 samples were positive for PFOS

(0.029 ng/L), three for FOSA (0.031 ng/L) and for MeFOSA (0.044 ng/L), two samples for FOSAA, MeFOSAA, one for EtFOSAA and for MeFOSEE, and none for EtFOSE and EtFOSAA. The underpinning reason behind the lack of positive samples derived from the liquid food analysis, could be attributed to the reduced volume of sample employed in the analysis (10 mL) when it is compared to the large volume of sample (up to one litre) of water employed in the papers reporting PFOS in high detection frequencies, introduced in *Table 02*.

Home – as the most relevant indoor environment due to the number of hours spent in there – is commonly considered an important source of PFOS and PFOS related products, and dust, a representative matrix of indoor exposure for semi-volatile and non-volatile organic pollutants. Daily intakes reported in this thesis estimated average daily intakes of 4.5 and 59.7 pg/kg bw/day in mean and high scenarios respectively for the sum of the six PFASs investigated. The relative contribution to dust exposure was dominated by EtFOSA, with an average concentration of 30.9 ng/g (average EDI in mean scenario of 1.9 pg/kg bw/day, DF = 96 %). EtFOSA constituted around 40 % of the overall dust exposure (see *Figure 18*), while PFOS was contributing around 12 %, with a reported average concentration of 8.9 ng/g (average EDI in mean scenario of 0.5 pg/kg bw/day, DF = 96 %). MeFOSE and EtFOSE were also identified as significant contributors to the overall PFASs, constituting the sum of them approximately the 50 % of the dust exposure to ΣPFAS reported in this thesis. They showed average concentrations of 24.4 (intake) and 12.0 ng/g (EDI) for MeFOSE and EtFOSE respectively.

In view of the results obtained for the two selected matrices to evaluate external exposure to PFOS and PFOS related substances, can be concluded that both, dust and food are potential sources of PFOS and of PFOS precursors suspected to contribute to indirect exposure to PFOS, even more than to direct exposure to PFOS itself. As an overall, both routes of exposure combined represent an estimated daily intake of 0.25 ng/kg bw/day (15.3 ng/day) of ΣPFAS, with a much larger contribution of diet (around 98 %) to this value.

Finally, internal levels of PFOS in serum samples were evaluated. Serum samples showed expected patterns of exposure to PFOS and PFOS precursors when they were compared to literature (Ericson *et al.*, 2007; Holzer *et al.*, 2008; Kato *et al.*, 2011): PFOS was the main contributor to internal exposure to PFOS and PFOS related products. While it was detected in 87 % of the analysed samples showing an average value of 5.3 ng/mL, all the PFOS precursors but MeFOSAA (which showed 23 % positive samples) were just detected in a few samples, either in none of them.

PFOS branched isomeric ratio was also evaluated in these samples for which ΣPFOS was quantified. In dust samples, linear:branched ratio showed the expected pattern of 70 % of linear and 30 % of branched PFOS isomers. In serum ones, the calculated ratio revealed different composition (in percentage) than the one estimated in dust, and showing an important inter-individual variation, especially when age and gender were considered.

And at this point, is were external and internal exposure merge and explanations to the different exposure profiles can be further understood: as reported in the *in vitro* chapter, two of the selected PFOS precursors (MeFOSA and MeFOSE) were fast metabolised (two hours) to the end up product – PFOS – , to a stable and well

characterised intermediate (FOSA), or to some other intermediates which could or not lead to the final formation of PFOS, same way as for their previously reported ethylated analogues (Ericson *et al.*, 2009; Ross, Wong and Martin, 2012; Chang *et al.*, 2017). This fast conversion, supported by previously reported data on *in vitro* and *in vivo* studies, would prevent reporting high levels of PFOS precursors in human biomonitoring studies – as presented here –, even when population is still externally exposed to them.

8.2. CONCLUSIONS BY HYPOTHESES

8.2.1. Indoor home dust is a significant source of exposure to long chain PFASs such as PFOS, FOSAs and FOSEs

PFOS has shown to be still present at detectable levels – 12 years after its phase-out and 5 years after its inclusion as a POP – in indoor environments, as home dust concentrations (ranging from 0.41 to 70.7 ng/g,) presented in this thesis revealed. Still, when comparing concentrations in dust with previous studies, PFOS values reported here (average = 8.9 ng/g) for the Norwegian cohort are lower than the 49 ng/g reported in 2009 by Bjorklund et al. (Björklund, Thuresson and De Wit, 2009) in Sweden, the 27 ng/g by Fraser et al. (Fraser *et al.*, 2013) for the Czech Republic in 2013, or the 144 ng/g by Goosey and Harrad (Goosey and Harrad, 2011) for the UK in 2011. Nowadays, levels of PFOS for Norwegian indoor floor dust are comparable to the ones reported in previous studies by Haug et al. (Haug, Huber, Becher, *et al.*, 2011) (average = 10.9 ng/g), even considering the one conducted in 2011 corresponded to settled dust – usually cleaner and presenting lower concentrations

of environmental organic pollutants – and the present one, to vacuum cleaner floor dust samples. These findings are in line with recent studies stating that (direct) external exposure to PFOS through dust ingestion tend to decline, even though its ubiquity and prevalence in indoor dust reveal its exposure should still be considered and monitored.

Contrary to reported information where just "traditionally characterised" perfluoroalkyl acids and sulfonates are analysed, dust exposure to the PFASs included in this thesis project showed a strong dominance of two PFOS precursors instead of PFOS: EtFOSA (40 %) and MeFOSE (32 %), while PFOS appeared to contribute around 12 % to the overall exposure (see *Figure 18*). Even though these precursors have been much less reported in indoor environments, concentrations up to 75,000 ng/g for EtFOSE (with detection frequencies of 100 % for EtFOSE and MeFOSE) by Shoeib et al. (Shoeib *et al.*, 2005) in Canada, and up to 3,000 ng/g in Boston by Fraser et al. (Fraser *et al.*, 2013) can be found in the literature. These extreme values represent 0.05 and 1.3 % respectively when compared to the approximated concentration of 36 ng/g ΣFOSE reported here.

The nature of the vacuumed dust, its particle size, the longevity of the vacuum cleaner bag containing the dust together with the bag itself, and its storage location are also factors to consider: Unlike the presumable exposure to settled dust is via ingestion, it won't likely be the equivalent for long term vacuum cleaned dust. Vacuum cleaned dust can give a better estimation of the potential exposure of all the residence or all the cleaned rooms, but it will not reflect the overall time spent indoors, neither accuracy in sampling time lines (Harrad *et al.*, 2010), fact which could lead to an under or overestimations in daily dust ingestion rates estimated for

this study. Besides, factors as ventilation, location of the residence and daily routines would affect and interfere in the temporal/seasonal concentration of PFOS and PFOS precursors in indoor environments, fact that might completely change the distribution pattern of the pollutants, especially for the more volatile ones (FOSEs).

Significant differences and correlations between – individual and combined – concentrations of PFOS and PFOS precursors were evaluated versus the variables extracted from the personal, demographic and indoor questionnaires. Just a reduced number of significant differences were identified:

- Age of the participants versus concentrations of PFOS ($p < 0.01$), MeFOSE ($p < 0.05$)and ΣPFAS ($p < 0.01$).
- Location of the residences according to industrial areas versus concentrations of EtFOSA ($p < 0.01$)and EtFOSE ($p < 0.05$).
- Recent renovation of the kitchen and concentrations of EtFOSA ($p < 0.01$) and ΣPFAS ($p < 0.01$).

These statistical differences could be consequence of a combination of differences related to trends in lifestyle related to age, such as the cleaning and ventilation frequencies, the age of the Gore-Tex® or Teflon® utensils in the residence, the number of hours spent indoors, the age of the house and/or refurbishments, etc., even though they did not show statistical significances as individual parameters and no principal component analysis (PCA) could be conducted due to the low score obtained when the Kaiser-Meyer-Olkin (KMO) test for sampling adequacy was performed.

Surprisingly, some of the indoor environment related questions for which direct and strong significant differences and/or correlations would have been expected, such as the ventilation frequency (especially for FOSEs), the use of Gore-Tex® clothing and water proof sprays, or the use of Teflon® kitchen utensils, showed no statistical difference, neither correlation. On the other hand, some other parameters like age (pre or post phase-out) and origin (Europe/America or Asia) of the furniture, floor, carpets or the waterproof clothing were not included in the indoor questionnaires, some of which could contribute to a deeper knowledge of indoor exposure origin.

Still, and even several uncertainties remain unclear about the sources of PFOS and PFOS precursors, this research revealed that their presence in indoor dust is ubiquitous, even years after the restriction of POSF related products. Moreover, the presence of positive correlations among the reported concentrations (pairs PFOS – FOSA and EtFOSE – MeFOSE), suggested common sources of indoor contamination with PFASs, even though they could not be fully elucidated.

8.2.2. FOSAs and FOSEs contribute to the overall external exposure to PFASs via indoor dust ingestion

According to Vestergren et al. (Vestergren *et al.*, 2008), relative contributions of direct and indirect exposure are dependent on the level of exposure: the relative contribution of high exposure scenario can be dominated by indirect exposure via dust ingestion (40 – 60 %), while for Gebbink et al. (Gebbink, Glynn and Berger, 2015), in a high scenario exposure indirect exposure to PFOS would contribute around 33 %.

Estimated daily intakes (body weight corrected) for adults and children were calculated for all the individual analytes, and for the combination of all of them in both, median and high scenarios. For PFOS in adult population, daily intakes were estimated to be 0.56 and 7.37 pg/bw kg/day for mean and high scenarios, while for the overall exposure to PFASs they were 4.51 and 59.7 pg/bw kg/day. As already described in the previous hypothesis, PFASs concentrations – and subsequently, exposure – was dominated by precursors, but PFOS. For children, estimated daily intakes of PFOS ranged from 0.005 ng/kg bw/day (mean scenario, 5th percentile, from 3 to 6 years old) to 0.548 ng/kg bw/day (high scenario, 95th percentile, from 1 to 2 years old), while for ΣPFAS they ranged from 0.082 to 2.612 ng/kg bw/day for the same scenarios, percentiles and ages, respectively. Estimated daily intakes for children were reported to be two orders of magnitude higher than the estimated in the high exposed scenario for adult population.

The estimations reported in this thesis clearly evidence how lower body weights – younger children –, besides the higher ingestion of dust due to hand to mouth contact, contribute to higher daily intake of dust, and so, to the associated risk assessment concerns of PFOS and PFOS related compounds, such as FOSAs and FOSEs present in indoor dust. In addition to it, this research project also evidenced that expected patterns of indoor dust exposure for "commonly analysed" PFASs might not be valid if precursors are not included in the exposure models, because as shown for PFOS precursors, they contribute in a much higher proportion (88 %) to the external exposure to the PFASs selected for this study than PFOS itself, so even direct exposure to PFASs might be significantly underestimated.

8.2.3. Diet is a significant source of exposure to long chain PFASs such as PFOS, FOSAs, FOSAAs and FOSEs

PFOS has shown to be present at detectable levels in food composites, even though nowadays the exposure pattern is dominated by PFOS precursors, as the data reported in this thesis revealed. PFOS was detected in seven solid food samples in a range between <LOQ and 0.054 ng/kg, while FOSA was detected in 18 samples in a range between <LOQ and 0.051 ng/kg, MeFOSE in 9 samples between <LOQ and 42.302 ng/kg, EtFOSE between <LOQ and 53.125 ng/kg and MeFOSAA between <LOQ and 0.045 ng/kg. Detection frequencies for liquid food samples were too low to be included in the statistical analysis, as mentioned along the thesis. For the sum of all the investigated PFASs, an average concentration of 5.2 ng/kg was calculated and employed for the statistical analysis.

Unlike in dust samples, age and gender did not seem to play a role in the reported concentrations of ΣPFAS in solid food composites. Moreover, no significant differences and/or positive correlations were identified when the samples were compared to energy, water, proteins, fats, carbohydrates, starches, sugars and fibres. Because of it, individual food items derived from the food diaries were evaluated, showing positive correlations for three items:

- ΣPFAS versus the consumption of fish with high content in fat ($p < 0.01$).
- ΣPFAS versus raw vegetables consumption ($p < 0.01$)
- ΣPFAS versus tomatoes and cucumbers consumption ($p < 0.05$).

The suspicious sources of contamination for oily fish, vegetables, cucumbers and tomatoes were evaluated, and two main explanations were proposed: food packaging and bioaccumulation. The second one would be more likely to occur in big animals, carnivores and high content in protein food, as reported by Haug et al. (Haug *et al.*, 2010). Otherwise, the first one would be linked to the sample manipulation, transference from cooking utensils or food packaging, more than to the composition of the food item itself (Tittlemier, Pepper and Edwards, 2006; Noorlander *et al.*, 2011). In view of these results, fatty fish consumption was expected to show positive correlations with the concentrations of ΣPFAS, especially when these types of fish are widely consumed in Scandinavian countries. Moreover, this finding was in agreement with the higher than average reported levels in fish samples by other authors (Berger *et al.*, 2009; Haug *et al.*, 2010; Hrádková *et al.*, 2010), reporting them as high potential sources of PFOS dietary exposure. On the other hand, no significant correlations were found for food packaging (plastics, paper, etc.), cooking (Teflon® pans, plastic or wooden utensils, etc.) and storage (plastic, glass, etc.) which could be considered as a source to FOSAs and FOSEs. Still, the positive correlation could be a consequence of an earlier contamination from the production stages, derived from the fact of not washing or peeling the food items before consumption, of from a combination of these with the ones not reporting significant differences, even when they were not showing significant differences as individual parameters. Unfortunately, no PCA could be conducted to further investigate these sources, due to the low score obtained when the KMO test for sampling adequacy was evaluated.

Some other parameters such as protein content in general, or individual food items rich in proteins (e.g. meats, eggs, or other types of fish) were expected to show strong significant differences or correlations, known the fact that PFASs tend to bind proteins. On the other hand, dairies (e.g. milk or liquid yogurts) and water would have been expected to show also strong correlations for the liquid food samples too, but they could not be evaluated due to the dilution of all the liquids as a composite sample, together with the incapability of pre-concentration of the samples as much as usually conducted for water samples.

8.2.4. FOSAs, FOSEs and FOSAAs contribute to the overall external exposure to PFASs via food consumption

Food consumption is well known to be an important source of direct exposure to PFOS and PFOS precursors, according to reported values in different food items and composites (Martin *et al.*, 2004; Ericson, Nadal, *et al.*, 2008; Kärrman *et al.*, 2009; Haug *et al.*, 2010; Noorlander *et al.*, 2011; van Asselt *et al.*, 2011; Domingo, 2012; Klenow *et al.*, 2013; Rahman, Peldszus and Anderson, 2014; Gebbink *et al.*, 2015).

The estimation of the food daily intakes was evaluated for lower and medium bounds, with reported comparable daily intakes for both of them. For the solid food composites (n = 113) average daily intake in medium bound was 0.26 ng/kg bw/day (17.24 ng/day), while for the combination of solid and food composites (n = 94), its average was 0.29 ng/kg bw/day (19.30 ng/day). Average daily intake when concentrations from both days were averaged, was 0.24 ng/kg bw/day (15.6 ng/day) in medium bound approach. These reported values are two orders of

magnitude higher than the previously ones described for dust samples (which were 4.5 and 60.0 pg/kg bw/day for mean and high scenario approaches).These findings revealed important information about the overall understanding of PFASs exposure:

- PFOS contributed less than 5 % to the overall exposure to ΣPFAS reported in this thesis.
- Food contributeed around 98 % to the total external exposure to ΣPFAS included in this study.

Moreover – as predicted – food could be considered the main source of external exposure to PFOS and especially, to PFOS precursors.

8.2.5. PFOS is ubiquitous in serum

PFOS has been extensively reported in human blood and serum samples for the last ten years, with reported detection frequencies up to 100 % in some studies, as *Table 07* from the Introduction chapter reflected. Nowadays, trends show decreasing concentrations in general population – as for all the environmental and biological matrices were PFOS is analysed–, but still and after more than 10 years after its restriction, PFOS concentrations reported in high percentages of the studied populations.

For the present set of serum samples, ΣPFOS was detected in the 87 % of the analysed samples in an average concentration of 5.29 ng/mL (<LOQ – 16.52). The second most detected compound was MeFOSAA (23 % positive samples) with a mean concentration of 0.04 ng/mL (<LOD – 0.38). None of the other compounds was identified in detected frequencies higher than 5 %.

As for dust and food samples, statistical analysis for PFOS concentrations was conducted to further evaluate significant differences and correlations, in this case, by comparison with personal, demographic, indoor and food frequency questionnaires. Age and gender of the participants did not show significant differences, albeit clear trends could be identified: mean values were higher for participants aged over 50 years old, followed by these under 30, while lower concentrations were identified for the participants aged between 30 and 50 years old (see *Figure 30*). Similarly, mean concentration values for males were higher than for female participants. Such differences were attributed to different accumulation and elimination patterns for the female participants between 30 to 50 years old, as consequence of menstruation, birth-giving or breastfeeding.

Significant differences and correlations between concentrations of ΣPFOS versus the variables extracted from the personal, demographic and indoor questionnaires were evaluated, and just a reduced number of significant differences were identified:

- Significantly higher concentrations for participants living less than 50 metres from busy traffic roads ($p < 0.05$).
- Significantly higher concentrations for participants with one or more animals in the house ($p < 0.05$).

Besides these two demographic and personal differences, just egg consumption showed a positive correlation versus ΣPFOS concentrations ($p = 0.05$) when individual food items extracted from the food frequency questionnaires were evaluated. Such correlation was attributed to the nature of the food item itself, rich in proteins, which tends to retain PFASs. Similar correlations and differences were

expected to be evidenced for other food items such as meat, fish, cheese or dairies, all of them content rich in animal origin protein. Conversely, no statistical differences or correlations were identified. Food packaging (plastics, paper, etc.), cooking utensils (Teflon® pans, plastic or wooden utensils, etc.) and food storage containers (plastic, glass, etc.) were not included in the food frequency questionnaires, so they could not be evaluated.

Besides PFOS, MeFOSAA was the second most detected analyte in serum samples. Its levels in human serum samples reported here are comparable with other studies reporting FOSAAs (Olsen *et al.*, 2004; Lee and Mabury, 2011).

The exposure pattern identified for serum samples from the A-TEAM cohort – higher detection frequencies of PFOS in serum when compared with these reported for indoor dust and diet composites, and lower for PFOS precursors than the ones reported for external exposure – leaded to the hypothesis which suggested that PFOS precursors were not detected in blood samples due to their previous metabolism to PFOS in the human body.

8.2.6. PFOS branched isomer ratios in serum differ from the reported for environmental external exposure

The expected ratio between linear:branched isomers for external exposure to PFOS is 70:30 (%), as in the manufactured product (Houde *et al.*, 2008; Buck *et al.*, 2011; Beesoon and Martin, 2015a). In order to verify or discard isomer specific degradation of the monitored PFOS precursors to PFOS in the environment, PFOS linear and branched isomers were reported for dust samples, and showing average

percentages of 69 % linear PFOS versus 31 % branched PFOS, as would correspond to the absence of environmental degradation. When the same analysis was conducted for serum samples, a few differences were observed: while average value for the estimated percentage of linear isomers remained 69 %, the range of percentages observed for the entire set of samples varied from 51 to 82 %, showing much more variability than the ones reported for dust samples. Moreover, trends (but no significant differences) were identified according to age and gender of the participants: male participants reported higher percentages of linear PFOS than females, showing these last ones different patterns according to age and lower percentage of branched isomers for women aged between 30 and 50 years old. This finding could suggest that different uptakes and elimination rates of linear and the different branched PFOS isomers could be even more relevant when considering the factors of menstruation, maternity and breastfeeding mentioned above.

In conclusion, the information extracted from the evaluation of PFOS branched isomers in serum samples showed large interpersonal variability, even though still remained unclear if they are due to different patterns of exposure or – as most suggested – bioaccumulation. Moreover, this variability could be strongly influenced in case of females by factors as maternity or menstruation.

8.2.7. FOSAs and FOSEs are rapidly metabolised to PFOS

During the last 15 years, papers reporting in vitro studies of potential PFOS precursors including FOSA (Benskin, Holt and Martin, 2009; Ross, Wong and Martin, 2012; Chen *et al.*, 2015), EtFOSA (Tomy *et al.*, 2004; Fu et al., 2015) and EtFOSE (Xu

et al., 2004; Zhao *et al*., 2016; Chang *et al.*, 2017) have been published. Moreover, a few in vivo studies and results have been reported showing different conversion rates for these same compounds (Xie *et al.*, 2009; Chen *et al.*, 2015; Chang *et al.*, 2017). Nevertheless, some other substances still require further study to confirm them as sources of human exposure to PFOS. For that reason, MeFOSA and MeFOSE were chosen for a qualitative study of phase I & II metabolism and plausible conversion to FOSA.

According to the results of these experiments – from the qualitative point of view – the data reported here was in line with previous published data from *in vitro* and *in vivo* studies for compounds as EtFOSA, EtFOSE and FOSA. In this case, kinetic was not studied as the experiments were just carried out for a short time period of two hours thus permitting only a qualitative perspective.

Notwithstanding this, it is evident that there was rapid biotransformation of the two selected target PFOS precursors to PFOS, via the stable and detectable intermediate FOSA. The mechanisms leading to the formation of PFOS did not seem to occur in the same way for MeFOSA and MeFOSE. For MeFOSA, the demethylation to FOSA seemed to be fast, with the conversion from FOSA to PFOS the limiting step to the final conversion to PFOS, while for MeFOSE the conversion to FOSA seemed to be the limiting step, with a faster conversion of FOSA to PFOS, both of them taking into account the relative abundance of the involved and detected compounds. This could be established just as a rough approximation due to the lack of knowledge of the real kinetics of the proposed mechanisms and the relatively short incubation times.

Overall, these experiments would make a contribution to testing that *in vivo* metabolism of precursors like FOSAs, FOSEs and FOSAAs to yield PFOS is viable, and

further studies are needed to better understanding their metabolism pathways and the overall internal exposure to PFOS.

8.3. SUMMARY AND FINAL REMARKS

In summary, findings from this thesis can be briefly listed as:

- Food ingestion can be considered the primary source of direct exposure to PFOS precursors, via bioaccumulation or migration from food packaging, with an estimated daily intakes of 0.24 pg/kg bw/day for Norwegian adult population.
- Dust can be considered as well a significant source of direct exposure to PFOS precursors and to PFOS itself, with estimated daily intakes for the studied population ranging from 4.5 to 60.0 pg/kg bw/day for mean and high scenarios, from which just 12 % is due to PFOS contamination.
- Methylated PFOS precursors, as MeFOSA and MeFOSE are *in vitro* metabolised to PFOS, both via FOSA, a stable and well characterised intermediate during the metabolism to PFOS.
- PFOS is the main contributor to the internal exposure to PFOS, with a reported average concentration for Norwegian population of 5.3 ng/mL in serum.
- As a consequence of PFOS precursors metabolism and their significant presence in dust and food, these two matrices can be considered a significant source of indirect exposure to PFOS, with a relative contribution of 98 % from diet and 2 % from dust ingestion.

- PFOS branched isomers need further study. Different patterns in accumulation and elimination rates of the different isomers can lead to enrichment of sum of branched isomers in the human body.
- Daily intake of PFOS and PFOS precursors for Norwegians has been estimated to be around 0.265 ng/kg bw/day for adult population when dust and food ingestion pathways were combined. Even though several uncertainties remain, these values can be considered safe according to the TDI of 150 ng/kg bw/day established by EFSA.

8.4. RECOMMENDATIONS AND FUTURE WORK

The elucidation of direct and indirect sources of exposure to PFOS, the understanding of the substances called PFOS precursors, together with the evaluation of the metabolism pathways leading to a better knowledge of PFOS body burdens are not an easy task. Several uncertainties remain unclear despite the growing number of available research moving towards their better understanding.

According to the experience earned through the project development, some recommendations for future research projects within the same context this thesis was addressed would be:

- The selection of a more representative cohort. The A-TEAM cohort was entirely constituted by workers from the NIPH, a research institute focused on health safety, and so, with poor representation of the general population in terms of educational level, concern and knowledge about exposure to

environmental pollutants, and socio-economical level. These parameters could lead to biased results and conclusions, being just representative of a specific part of the overall population.

- To sample drinking water in an independent way than (liquid) food. Water is considered a very clean matrix and thus, easy to pre-concentrate in larger factors than any other liquid food item, such as juice or milk, which would require entirely different sample treatments.
- The specific and independent analysis – besides the generic food composites – of relevant food items such as meat, fish, eggs, dairies and alternative sources of protein, to a better evaluation of PFOS precursors and PFOS isomers exposure in such matrices.
- Food frequency questionnaires should include information relative to food packaging and cooking ware, to allow a better estimation of suspected migration from them to the food items.
- Information about the age and the origin of specific clothes items, furniture, and other relevant materials could help to better understand suspected migration from them to dust.

Consequently, further research should be addressed towards:

- The analysis of current levels of FOSAs, FOSEs and FOSAAs in environmental matrices, indoor environments, food and human samples. They would provide a better estimation of real external exposure to pollutants which could potentially be metabolised to PFOS.

- The analysis of individual isomers of PFOS and PFOS precursors in representative matrices for internal exposure to PFOS. It would conduct to a better knowledge of their specific accumulation and elimination rates and so, to a better understanding of the behaviour of the individual PFOS isomers.
- The instrumental development and the analysis of chiral signatures of PFOS isomers in both, external and internal exposure representative matrices.
- The specific study of contamination derived from the migration of PFASs from food packing materials and cooking utensils, as well as the effect of washing, peeling and other cooking related processes, and the mechanisms involved.
- The specific study of contamination derived from abrasion processes and aging of clothing, furniture and materials commonly present in indoor environments, their migration, and the mechanism involved.
- The further study of the *in vitro* and *in vivo* metabolism of PFOS precursors (FOSAs and FOSEs) with the support of *in silico* approaches, which would allow a better understanding of the real kinetics and of the intermediate metabolites involved in the metabolic pathways leading to PFOS.
- The better comprehension of the relative human metabolism and toxicity of individual PFOS isomers.
- The evaluation of alternative sources of both, external and internal exposure to PFOS, e.g. the study of non-invasive internal exposure matrices.
- The evaluation of the impact derived from the restricted use of long chain PFASs pro the short chain ones, such as perfluoroether carboxylic acids (PFECAs) and perfluoroether sulfonic acids (PFESAs).

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLES

Table SM01. Individual concentrations (ng/g) of PFASs in vacuum cleaner bag samples (n = 57)

Sample	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE
01	2.347	0.331	<LOQ	45.939	2.574	<LOQ
02	2.582	0.200	<LOQ	11.339	<LOQ	<LOQ
03	2.699	0.284	<LOQ	21.148	<LOQ	<LOQ
04	0.410	0.198	<LOQ	<LOQ	<LOQ	<LOQ
05	4.653	<LOQ	0.077	69.143	<LOQ	2.869
06	3.267	0.327	<LOQ	21.586	<LOQ	<LOQ
07	3.316	0.263	<LOQ	44.137	<LOQ	<LOQ
08	5.974	0.221	<LOQ	83.522	<LOQ	4.688
09	17.535	0.385	<LOQ	38.602	3.689	<LOQ
10	3.359	0.447	<LOQ	73.070	<LOQ	<LOQ
11	3.958	0.149	5.528	11.538	<LOQ	1.465
12	3.686	0.336	<LOQ	8.050	7.492	1.652
13	0.823	0.103	<LOQ	<LOQ	<LOQ	<LOQ
14	3.591	0.517	5.695	142.788	13.053	3.076
15	28.482	0.597	1.682	25.511	<LOQ	1.738
16	11.375	0.131	<LOQ	33.144	<LOQ	<LOQ
17	1.977	0.183	<LOQ	46.599	<LOQ	<LOQ
18	3.393	0.208	<LOQ	56.777	<LOQ	<LOQ
19	4.270	0.468	<LOQ	33.113	<LOQ	5.441
20	1.988	0.119	<LOQ	12.584	<LOQ	<LOQ
21	4.865	0.175	2.917	26.118	<LOQ	1.966
22	0.714	0.165	<LOQ	69.960	<LOQ	<LOQ
23	5.683	0.212	0.106	39.703	<LOQ	<LOQ
24	1.522	0.381	<LOQ	24.183	<LOQ	<LOQ
25	17.537	0.726	<LOQ	39.431	<LOQ	<LOQ
26	70.716	0.585	<LOQ	15.807	<LOQ	<LOQ
27	4.054	0.467	<LOQ	30.647	9.630	<LOQ
28	1.816	0.397	<LOQ	10.123	11.003	<LOQ
29	2.552	0.516	<LOQ	19.762	<LOQ	<LOQ

Sample	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE
30	10.204	1.049	<LOQ	23.835	<LOQ	<LOQ
31	4.765	0.580	<LOQ	10.500	<LOQ	<LOQ
32	9.775	0.511	<LOQ	49.340	<LOQ	4.265
33	11.998	0.929	<LOQ	35.312	<LOQ	<LOQ
34	4.154	0.632	<LOQ	62.702	<LOQ	<LOQ
35	28.963	2.501	<LOQ	11.636	<LOQ	11.836
36	12.188	0.330	<LOQ	8.122	21.605	51.790
37	17.015	0.479	<LOQ	8.772	99.795	<LOQ
38	6.522	0.452	<LOQ	32.425	<LOQ	10.154
39	3.676	0.344	<LOQ	8.791	<LOQ	<LOQ
40	40.454	0.863	4.005	61.700	<LOQ	<LOQ
41	8.521	0.191	<LOQ	31.784	<LOQ	<LOQ
42	1.469	0.438	<LOQ	30.776	10.311	<LOQ
43	23.062	0.391	<LOQ	29.062	<LOQ	1.531
44	3.978	0.358	0.077	34.153	3.555	<LOQ
45	3.311	0.779	<LOQ	42.303	4.386	3.383
46	3.758	0.398	<LOQ	23.998	<LOQ	<LOQ
47	6.863	0.574	<LOQ	26.057	86.011	122.808
48	5.121	0.526	<LOQ	29.228	66.626	<LOQ
49	5.453	0.240	<LOQ	45.873	839.095	231.684
50	6.723	0.487	<LOQ	12.231	25.951	<LOQ
51	5.180	0.447	<LOQ	24.973	24.360	83.423
52	2.116	0.535	<LOQ	8.751	<LOQ	<LOQ
53	5.024	0.360	0.639	4.687	<LOQ	5.817
54	4.449	0.341	<LOQ	18.156	33.594	87.307
55	0.925	<LOQ	<LOQ	2.780	63.479	13.676
56	8.531	0.376	<LOQ	16.793	<LOQ	<LOQ
57	46.785	0.485	<LOQ	13.166	<LOQ	<LOQ

Table SM2. Individual ratios of linear:branched PFOS isomers in vacuum cleaner bag samples (n = 57)

Sample	n-PFOS / ΣBr-PFOS	n-PFOS (%)	ΣBr-PFOS (%)
01	2.16	68.33	31.67
02	2.35	70.18	29.82
03	2.88	74.24	25.76
04	1.99	66.54	33.46
05	2.39	70.48	29.52
06	2.11	67.79	32.21
07	2.68	72.81	27.19
08	2.25	69.27	30.73
09	2.15	68.28	31.72
10	1.99	66.59	33.41
11	2.03	66.98	33.02
12	2.29	69.60	30.40
13	1.91	65.59	34.41
14	2.30	69.67	30.33
15	1.89	65.37	34.63
16	1.88	65.31	34.69
17	1.89	65.37	34.63
18	1.71	63.06	36.94
19	2.22	68.92	31.08
20	2.05	67.17	32.83
21	1.77	63.94	36.06
22	2.25	69.27	30.73
23	2.51	71.51	28.49
24	2.77	73.49	26.51
25	2.22	68.95	31.05
26	2.64	72.55	27.45
27	2.14	68.13	31.87
28	2.31	69.81	30.19
29	2.95	74.67	25.33
30	3.41	77.33	22.67
31	2.17	68.45	31.55
32	2.50	71.43	28.57
33	2.57	72.00	28.00
34	1.82	64.59	35.41
35	2.87	74.19	25.81

Sample	n-PFOS / ΣBr-PFOS	n-PFOS (%)	ΣBr-PFOS (%)
36	2.42	70.75	29.25
37	1.75	63.63	36.37
38	2.50	71.43	28.57
39	1.84	64.74	35.26
40	1.96	66.27	33.73
41	1.88	65.28	34.72
42	2.51	71.53	28.47
43	1.92	65.70	34.30
44	2.46	71.12	28.88
45	3.05	75.33	24.67
46	3.12	75.71	24.29
47	2.58	72.07	27.93
48	2.21	68.82	31.18
49	2.63	72.46	27.54
50	1.67	62.58	37.42
51	1.98	66.44	33.56
52	2.59	72.12	27.88
53	3.13	75.77	24.23
54	1.89	65.42	34.58
55	1.84	64.75	35.25
56	2.39	70.49	29.51
57	2.12	67.94	32.06

Table SM03. Individual estimated daily intakes of total PFOS and PFOS precursors (ng/kg bw/day) through dust ingestion for adults in mean scenario (4.15 mg dust/day) (n = 57)

Sample	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	∑PFAS
01	1.60E-04	2.26E-05	3.38E-06	3.13E-03	1.76E-04	5.98E-05	3.55E-03
02	1.60E-04	1.24E-05	3.07E-06	7.04E-04	1.08E-04	5.44E-05	1.04E-03
03	3.05E-05	1.47E-05	3.68E-06	5.67E-05	1.29E-04	6.51E-05	3.00E-04
04	3.12E-04	2.37E-06	5.18E-06	4.64E-03	1.17E-04	1.92E-04	5.27E-03
05	1.60E-04	1.60E-05	2.42E-06	1.06E-03	8.51E-05	4.29E-05	1.36E-03
06	3.45E-04	1.28E-05	2.86E-06	4.83E-03	1.01E-04	2.71E-04	5.56E-03
07	1.20E-03	2.63E-05	3.38E-06	2.63E-03	2.52E-04	5.98E-05	4.17E-03
08	2.89E-04	1.09E-05	4.03E-04	8.42E-04	1.27E-04	1.07E-04	1.78E-03
09	2.43E-04	2.22E-05	3.27E-06	5.32E-04	4.95E-04	1.09E-04	1.40E-03
10	4.96E-05	6.23E-06	2.98E-06	4.60E-05	1.05E-04	5.29E-05	2.63E-04
11	2.67E-04	3.84E-05	4.23E-04	1.06E-02	9.70E-04	2.29E-04	1.25E-02
12	1.72E-03	3.60E-05	1.01E-04	1.54E-03	1.05E-04	1.05E-04	3.60E-03
13	6.96E-04	7.99E-06	3.03E-06	2.03E-03	1.06E-04	5.36E-05	2.89E-03
14	1.27E-04	1.17E-05	3.17E-06	2.98E-03	1.11E-04	5.61E-05	3.29E-03
15	2.08E-04	1.28E-05	3.03E-06	3.47E-03	1.06E-04	5.36E-05	3.86E-03
16	1.18E-04	7.05E-06	2.94E-06	7.48E-04	1.03E-04	5.21E-05	1.03E-03
17	2.44E-04	8.75E-06	1.46E-04	1.31E-03	8.72E-05	9.86E-05	1.89E-03
18	5.60E-05	1.29E-05	3.89E-06	5.49E-03	1.37E-04	6.88E-05	5.77E-03
19	4.30E-04	1.60E-05	7.98E-06	3.00E-03	1.32E-04	6.63E-05	3.65E-03
20	8.29E-04	3.43E-05	2.34E-06	1.86E-03	8.22E-05	4.14E-05	2.85E-03
21	2.91E-04	3.35E-05	3.55E-06	2.20E-03	6.91E-04	6.29E-05	3.28E-03
22	1.08E-04	2.36E-05	2.94E-06	6.02E-04	6.54E-04	5.21E-05	1.44E-03
23	1.45E-04	2.94E-05	2.82E-06	1.13E-03	9.91E-05	5.00E-05	1.45E-03
24	7.39E-04	3.87E-05	3.74E-06	3.73E-03	1.32E-04	3.23E-04	4.97E-03
25	6.93E-04	5.37E-05	2.86E-06	2.04E-03	1.01E-04	5.07E-05	2.94E-03
26	2.23E-03	1.93E-04	3.81E-06	8.96E-04	1.34E-04	9.12E-04	4.37E-03
27	3.01E-04	2.09E-05	2.29E-06	1.50E-03	8.04E-05	4.69E-04	2.37E-03
28	1.66E-04	1.55E-05	2.24E-06	3.98E-04	7.87E-05	3.96E-05	7.00E-04
29	3.18E-04	2.86E-05	6.13E-06	2.73E-03	2.84E-04	7.01E-05	3.44E-03
30	1.14E-04	2.68E-05	1.70E-06	1.45E-03	1.51E-04	1.16E-04	1.86E-03
31	2.70E-04	2.85E-05	3.55E-06	1.72E-03	1.25E-04	6.29E-05	2.21E-03
32	1.47E-04	3.71E-05	3.43E-06	6.07E-04	1.21E-04	6.08E-05	9.75E-04
33	3.67E-04	2.63E-05	4.67E-05	3.42E-04	1.27E-04	4.25E-04	1.33E-03

Sample	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	∑PFAS
34	1.84E-04	1.94E-05	3.38E-06	1.44E-03	1.19E-04	5.98E-05	1.83E-03
35	2.38E-04	1.88E-05	3.55E-06	3.17E-03	1.25E-04	6.29E-05	3.61E-03
36	2.73E-04	2.99E-05	3.17E-06	2.12E-03	1.11E-04	3.48E-04	2.89E-03
37	1.17E-04	2.94E-05	3.81E-06	1.86E-03	1.34E-04	6.75E-05	2.21E-03
38	3.59E-03	2.97E-05	2.51E-06	8.02E-04	8.82E-05	4.45E-05	4.55E-03
39	6.15E-04	6.33E-05	2.98E-06	1.44E-03	1.05E-04	5.29E-05	2.28E-03
40	1.64E-04	2.19E-05	2.42E-06	3.58E-03	8.51E-05	4.29E-05	3.89E-03
41	3.25E-04	3.96E-05	3.38E-06	7.16E-04	1.19E-04	5.98E-05	1.26E-03
42	2.47E-04	3.76E-05	2.94E-06	3.73E-03	1.03E-04	5.21E-05	4.17E-03
43	5.76E-04	1.56E-05	2.34E-06	3.84E-04	1.02E-03	2.45E-03	4.45E-03
44	7.37E-04	2.08E-05	2.14E-06	3.80E-04	4.32E-03	3.80E-05	5.50E-03
45	1.77E-03	3.78E-05	1.75E-04	2.70E-03	7.62E-05	3.84E-05	4.80E-03
46	5.63E-04	1.26E-05	3.27E-06	2.10E-03	1.15E-04	5.79E-05	2.85E-03
47	7.64E-05	2.28E-05	2.57E-06	1.60E-03	5.36E-04	4.56E-05	2.28E-03
48	1.37E-03	2.32E-05	2.94E-06	1.73E-03	1.03E-04	9.10E-05	3.32E-03
49	4.53E-04	3.79E-05	3.27E-06	1.72E-03	5.68E-03	8.11E-03	1.60E-02
50	2.84E-04	2.92E-05	2.75E-06	1.62E-03	3.70E-03	4.86E-05	5.68E-03
51	2.84E-04	1.25E-05	2.57E-06	2.39E-03	4.36E-02	1.20E-02	5.84E-02
52	4.00E-04	2.89E-05	2.94E-06	7.27E-04	1.54E-03	5.21E-05	2.75E-03
53	3.59E-04	3.10E-05	3.43E-06	1.73E-03	1.69E-03	5.78E-03	9.60E-03
54	2.98E-04	2.29E-05	3.32E-06	1.22E-03	2.25E-03	5.86E-03	9.65E-03
55	3.08E-05	1.18E-06	1.65E-06	9.25E-05	2.11E-03	4.55E-04	2.69E-03
56	4.61E-04	2.03E-05	2.67E-06	9.07E-04	9.40E-05	4.74E-05	1.53E-03
57	2.67E-03	2.76E-05	2.82E-06	7.50E-04	9.91E-05	5.00E-05	3.60E-03

Table SM04. Individual estimated daily intakes of total PFOS and PFOS precursors (ng/kg bw/day) through dust ingestion for adults in high scenario (55 mg dust/day) (n = 57)

Sample	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	∑PFAS
01	2.12E-03	2.98E-04	4.46E-05	4.14E-02	2.32E-03	7.91E-04	4.70E-02
02	2.12E-03	1.64E-04	4.06E-05	9.31E-03	1.43E-03	7.20E-04	1.38E-02
03	4.03E-04	1.94E-04	4.86E-05	7.50E-04	1.71E-03	8.61E-04	3.97E-03
04	4.13E-03	3.14E-05	6.85E-05	6.13E-02	1.54E-03	2.54E-03	6.97E-02
05	2.11E-03	2.12E-04	3.20E-05	1.40E-02	1.13E-03	5.67E-04	1.80E-02
06	4.56E-03	1.69E-04	3.78E-05	6.38E-02	1.33E-03	3.58E-03	7.35E-02
07	1.58E-02	3.47E-04	4.46E-05	3.48E-02	3.33E-03	7.91E-04	5.51E-02
08	3.82E-03	1.44E-04	5.33E-03	1.11E-02	1.68E-03	1.41E-03	2.35E-02
09	3.22E-03	2.94E-04	4.32E-05	7.03E-03	6.54E-03	1.44E-03	1.86E-02
10	6.56E-04	8.24E-05	3.95E-05	6.09E-04	1.39E-03	6.99E-04	3.47E-03
11	3.53E-03	5.07E-04	5.59E-03	1.40E-01	1.28E-02	3.02E-03	1.66E-01
12	2.27E-02	4.76E-04	1.34E-03	2.03E-02	1.39E-03	1.39E-03	4.76E-02
13	9.20E-03	1.06E-04	4.00E-05	2.68E-02	1.41E-03	7.09E-04	3.83E-02
14	1.67E-03	1.55E-04	4.19E-05	3.94E-02	1.47E-03	7.42E-04	4.35E-02
15	2.74E-03	1.69E-04	4.00E-05	4.59E-02	1.41E-03	7.09E-04	5.10E-02
16	1.56E-03	9.32E-05	3.89E-05	9.89E-03	1.37E-03	6.89E-04	1.36E-02
17	3.22E-03	1.16E-04	1.93E-03	1.73E-02	1.15E-03	1.30E-03	2.50E-02
18	7.41E-04	1.71E-04	5.14E-05	7.26E-02	1.81E-03	9.10E-04	7.63E-02
19	5.68E-03	2.12E-04	1.06E-04	3.97E-02	1.74E-03	8.77E-04	4.83E-02
20	1.10E-02	4.54E-04	3.09E-05	2.46E-02	1.09E-03	5.48E-04	3.77E-02
21	3.84E-03	4.43E-04	4.69E-05	2.91E-02	9.13E-03	8.31E-04	4.34E-02
22	1.43E-03	3.12E-04	3.89E-05	7.95E-03	8.64E-03	6.89E-04	1.91E-02
23	1.92E-03	3.89E-04	3.73E-05	1.49E-02	1.31E-03	6.61E-04	1.92E-02
24	9.78E-03	5.11E-04	4.95E-05	4.93E-02	1.74E-03	4.26E-03	6.57E-02
25	9.17E-03	7.09E-04	3.78E-05	2.70E-02	1.33E-03	6.70E-04	3.89E-02
26	2.95E-02	2.55E-03	5.04E-05	1.19E-02	1.77E-03	1.21E-02	5.78E-02
27	3.99E-03	2.76E-04	3.02E-05	1.98E-02	1.06E-03	6.21E-03	3.14E-02
28	2.20E-03	2.06E-04	2.96E-05	5.26E-03	1.04E-03	5.24E-04	9.25E-03
29	4.21E-03	3.79E-04	8.11E-05	3.61E-02	3.76E-03	9.27E-04	4.55E-02
30	1.50E-03	3.54E-04	2.25E-05	1.92E-02	1.99E-03	1.54E-03	2.46E-02
31	3.56E-03	3.77E-04	4.69E-05	2.28E-02	1.65E-03	8.31E-04	2.92E-02
32	1.94E-03	4.91E-04	4.54E-05	8.02E-03	1.59E-03	8.04E-04	1.29E-02
33	4.85E-03	3.47E-04	6.17E-04	4.52E-03	1.68E-03	5.61E-03	1.76E-02

Sample	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	∑PFAS
34	2.43E-03	2.56E-04	4.46E-05	1.91E-02	1.57E-03	7.91E-04	2.42E-02
35	3.14E-03	2.49E-04	4.69E-05	4.19E-02	1.65E-03	8.31E-04	4.78E-02
36	3.61E-03	3.96E-04	4.19E-05	2.80E-02	1.47E-03	4.60E-03	3.81E-02
37	1.55E-03	3.89E-04	5.04E-05	2.46E-02	1.77E-03	8.93E-04	2.93E-02
38	4.74E-02	3.92E-04	3.32E-05	1.06E-02	1.17E-03	5.88E-04	6.02E-02
39	8.13E-03	8.37E-04	3.95E-05	1.90E-02	1.39E-03	6.99E-04	3.01E-02
40	2.17E-03	2.89E-04	3.20E-05	4.73E-02	1.13E-03	5.67E-04	5.15E-02
41	4.30E-03	5.23E-04	4.46E-05	9.47E-03	1.57E-03	7.91E-04	1.67E-02
42	3.26E-03	4.97E-04	3.89E-05	4.93E-02	1.37E-03	6.89E-04	5.51E-02
43	7.62E-03	2.06E-04	3.09E-05	5.08E-03	1.35E-02	3.24E-02	5.88E-02
44	9.75E-03	2.75E-04	2.84E-05	5.03E-03	5.72E-02	5.02E-04	7.28E-02
45	2.34E-02	5.00E-04	2.32E-03	3.57E-02	1.01E-03	5.08E-04	6.35E-02
46	7.44E-03	1.67E-04	4.32E-05	2.77E-02	1.52E-03	7.65E-04	3.77E-02
47	1.01E-03	3.01E-04	3.40E-05	2.12E-02	7.09E-03	6.03E-04	3.02E-02
48	1.81E-02	3.07E-04	3.89E-05	2.28E-02	1.37E-03	1.20E-03	4.39E-02
49	5.99E-03	5.01E-04	4.32E-05	2.27E-02	7.51E-02	1.07E-01	2.12E-01
50	3.76E-03	3.86E-04	3.63E-05	2.14E-02	4.89E-02	6.43E-04	7.51E-02
51	3.75E-03	1.65E-04	3.40E-05	3.15E-02	5.77E-01	1.59E-01	7.72E-01
52	5.28E-03	3.82E-04	3.89E-05	9.61E-03	2.04E-02	6.89E-04	3.64E-02
53	4.75E-03	4.10E-04	4.54E-05	2.29E-02	2.23E-02	7.65E-02	1.27E-01
54	3.95E-03	3.02E-04	4.39E-05	1.61E-02	2.98E-02	7.75E-02	1.28E-01
55	4.07E-04	1.56E-05	2.18E-05	1.22E-03	2.79E-02	6.02E-03	3.56E-02
56	6.09E-03	2.69E-04	3.54E-05	1.20E-02	1.24E-03	6.26E-04	2.03E-02
57	3.52E-02	3.65E-04	3.73E-05	9.92E-03	1.31E-03	6.61E-04	4.75E-02

Table SM05. Individual concentrations of PFOS and PFOS precursors (ng/kg wet weight) in solid food samples (n = 113)

Sample	Day	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
01	1	<LOQ	0.022	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
01	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
02	1	<LOQ	0.016	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
02	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
03	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
03	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
03	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	14.611	<LOQ	<LOQ	<LOQ
04	1	<LOQ	0.009	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
04	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
05	1	<LOQ	0.016	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
05	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
06	1	<LOQ	0.013	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
07	1	<LOQ	0.033	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.040	<LOQ
08	1	0.054	0.019	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
08	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
09	1	<LOQ	0.027	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
11	1	<LOQ	0.003	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
11	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
12	1	<LOQ	0.014	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Sample	Day	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
12	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
13	1	<LOQ	0.009	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
13	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
14	1	<LOQ	0.005	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
14	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
15	1	0.021	0.028	<LOQ	<LOQ	<LOQ	1.964	<LOQ	<LOQ	<LOQ
15	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
16	1	<LOQ	0.070	<LOQ	<LOQ	56.295	<LOQ	<LOQ	<LOQ	<LOQ
16	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
17	1	0.025	0.051	<LOQ	<LOQ	0.903	<LOQ	<LOQ	<LOQ	<LOQ
17	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
18	1	<LOQ	0.014	<LOQ	<LOQ	<LOQ	20.346	<LOQ	0.015	<LOQ
19	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
20	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	14.577	<LOQ	<LOQ	<LOQ
21	1	<LOQ	<LOQ	<LOQ	<LOQ	24.839	21.256	<LOQ	<LOQ	<LOQ
21	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
22	1	<LOQ	0.032	<LOQ	<LOQ	<LOQ	16.530	<LOQ	<LOQ	<LOQ
22	2	0.013	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
23	1	<LOQ	0.005	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
23	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
24	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
24	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Sample	Day	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
25	1	<LOQ	<LOQ	<LOQ	<LOQ	42.302	15.615	<LOQ	0.045	<LOQ
25	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
26	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
26	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
26	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
27	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
27	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
28	1	<LOQ	<LOQ	<LOQ	<LOQ	9.692	14.377	<LOQ	<LOQ	<LOQ
28	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
29	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
29	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.559	<LOQ	<LOQ	<LOQ
30	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
31	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
32	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
32	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
33	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
33	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
34	1	<LOQ	<LOQ	<LOQ	<LOQ	20.546	53.125	<LOQ	<LOQ	<LOQ
34	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
35	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
35	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
36	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Sample	Day	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
36	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
37	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
37	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
38	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
38	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	14.678	<LOQ	<LOQ	<LOQ
39	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
39	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
40	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
40	2	0.012	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
41	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
41	2	0.021	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
42	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
42	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
43	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
43	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	29.765	<LOQ	<LOQ	<LOQ
44	1	<LOQ	<LOQ	<LOQ	<LOQ	8.439	<LOQ	<LOQ	<LOQ	<LOQ
44	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
45	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
45	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
46	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
46	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	48.490	<LOQ	<LOQ	<LOQ
47	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Sample	Day	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
47	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	42.720	<LOQ	<LOQ	<LOQ
48	1	<LOQ	<LOQ	<LOQ	<LOQ	23.726	28.667	<LOQ	<LOQ	<LOQ
48	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
49	1	<LOQ	0.010	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
49	2	<LOQ	<LOQ	<LOQ	<LOQ	16.352	21.958	<LOQ	<LOQ	<LOQ
50	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
50	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
51	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
51	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
52	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
52	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
53	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	21.875	<LOQ	<LOQ	<LOQ
54	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
54	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
55	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	18.665	<LOQ	<LOQ	<LOQ
55	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
56	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
56	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12.543	<LOQ	<LOQ	<LOQ
58	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
58	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
59	1	0.023	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
59	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Sample	Day	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
60	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
60	2	<LOQ	<LOQ	<LOQ	<LOQ	8.715	10.363	<LOQ	<LOQ	<LOQ
61	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
61	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
62	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
62	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Table SM06. Individual concentrations of PFOS and PFOS precursors (ng/L) in liquid food samples (n = 121)

Sample	Day	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
01	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.022	<LOQ	<LOQ
01	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
02	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
02	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
03	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
03	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
04	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
04	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
05	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
05	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
06	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
06	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
07	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
07	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
08	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
08	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
09	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
09	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
11	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Sample	Day	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
11	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
12	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
12	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
13	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
13	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
14	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
14	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
15	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
15	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
16	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.015	<LOQ
16	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
17	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
17	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
18	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
18	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
19	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
19	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
20	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
20	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
21	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
21	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
22	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Sample	Day	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
22	2	<LOQ	0.006	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
23	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
23	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
24	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
24	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
25	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
25	2	<LOQ	0.003	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
26	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
26	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
26	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
27	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
27	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
28	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
28	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
29	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
29	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
30	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
30	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
31	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
31	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
32	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
32	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Sample	Day	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
33	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
33	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
34	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
34	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
35	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
35	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
36	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
36	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
37	1	<LOQ	<LOQ	<LOQ	0.131	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
37	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
38	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
38	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
39	1	0.032	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
39	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
40	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
40	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
41	1	0.015	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
41	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
42	1	0.147	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
42	2	3.195	3.677	2.160	<LOQ	7.743	<LOQ	2.771	<LOQ	<LOQ
43	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
43	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Sample	Day	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
44	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
44	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
45	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
45	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
46	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
46	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
47	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
47	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
48	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
48	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
49	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
49	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
50	1	<LOQ	<LOQ	0.365	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
50	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
51	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
51	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
52	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
52	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
53	1	<LOQ	<LOQ	2.763	<LOQ	<LOQ	<LOQ	<LOQ	0.004	<LOQ
53	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
54	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
54	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Sample	Day	ΣPFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
55	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
55	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
56	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
56	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
58	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
58	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
59	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
59	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
60	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
60	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
61	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
62	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Table SM07. Individual daily intakes of ΣPFAS for lower and medium bounds (ng/day and ng/kg bw/day) through solid food consumption (n = 113)

Sample	Day	Daily intake (ng/day) lower bound	Daily intake (ng/kg bw/day) lower bound	∑PFAS (ng/kg) lower	Daily intake (ng/day) medium bound	Daily intake (ng/kg bw/day) medium bound	∑PFAS (ng/kg) medium
01FS15	1	0.038	0.0006	0.022	0.338	0.0055	0.198
01FS25	2	0.000	0.0000	0.000	0.245	0.0040	0.178
02FS15	1	0.045	0.0007	0.016	0.551	0.0082	0.192
02FS25	2	0.000	0.0000	0.000	0.312	0.0047	0.178
03FS15	1	0.000	0.0000	0.000	0.450	0.0080	0.178
03FS25	2	0.000	0.0000	0.000	0.577	0.0103	0.178
03FS25	2	47.398	0.8464	14.611	47.867	0.8548	14.756
04FS15	1	0.026	0.0004	0.009	0.554	0.0089	0.185
04FS25	2	0.000	0.0000	0.000	0.410	0.0066	0.178
05FS15	1	0.053	0.0006	0.016	0.628	0.0074	0.192
05FS25	2	0.000	0.0000	0.000	0.156	0.0018	0.178
06FS15	1	0.037	0.0005	0.013	0.527	0.0073	0.189
07FS15	1	0.228	0.0037	0.073	0.766	0.0126	0.245
08FS15	1	0.205	0.0036	0.072	0.693	0.0122	0.245
08FS25	2	0.000	0.0000	0.000	0.376	0.0066	0.178
09FS15	1	0.092	0.0015	0.027	0.701	0.0111	0.203
10FS15	1	0.000	0.0000	0.000	0.487	0.0071	0.178
10FS25	2	0.000	0.0000	0.000	0.532	0.0077	0.178
11FS15	1	0.012	0.0002	0.003	0.601	0.0107	0.179
11FS25	2	0.000	0.0000	0.000	0.268	0.0048	0.178
12FS15	1	0.035	0.0005	0.014	0.468	0.0068	0.190
12FS25	2	0.000	0.0000	0.000	0.336	0.0049	0.178
13FS15	1	0.028	0.0004	0.009	0.591	0.0087	0.185
13FS25	2	0.000	0.0000	0.000	0.379	0.0056	0.178
14FS15	1	0.018	0.0003	0.005	0.619	0.0095	0.181
14FS25	2	0.000	0.0000	0.000	0.347	0.0053	0.178
15FS15	1	3.271	0.0454	2.013	3.498	0.0486	2.153
15FS25	2	0.000	0.0000	0.000	0.119	0.0017	0.178
16FS25	2	0.000	0.0000	0.000	0.242	0.0030	0.178
17FS15	1	1.787	0.0263	0.979	1.983	0.0292	1.086
17FS25	2	0.000	0.0000	0.000	0.291	0.0043	0.178
18FS15	1	65.402	0.9343	20.374	65.847	0.9407	20.513
19FS15	1	0.000	0.0000	0.000	0.776	0.0094	0.178

Sample	Day	Daily intake (ng/day) lower bound	Daily intake (ng/kg bw/day) lower bound	∑PFAS (ng/kg) lower	Daily intake (ng/day) medium bound	Daily intake (ng/kg bw/day) medium bound	∑PFAS (ng/kg) medium
20FS25	2	23.848	0.4500	14.577	24.084	0.4544	14.722
21FS15	1	157.046	2.8554	46.095	157.314	2.8603	46.174
21FS25	2	0.000	0.0000	0.000	0.453	0.0082	0.178
22FS15	1	41.007	0.4660	16.562	41.360	0.4700	16.704
22FS25	2	0.033	0.0004	0.013	0.487	0.0055	0.187
23FS15	1	0.008	0.0001	0.005	0.309	0.0053	0.181
23FS25	2	0.000	0.0000	0.000	0.338	0.0058	0.178
24FS15	1	0.000	0.0000	0.000	0.565	0.0081	0.178
24FS25	2	0.000	0.0000	0.000	0.612	0.0087	0.178
25FS15	1	174.814	2.3947	57.962	175.039	2.3978	58.037
25FS25	2	0.000	0.0000	0.000	0.407	0.0056	0.178
26FS05	2	0.000	0.0000	0.000	0.259	0.0047	0.178
26FS15	1	0.000	0.0000	0.000	0.436	0.0079	0.178
26FS25	2	0.000	0.0000	0.000	0.259	0.0036	0.178
27FS15	1	0.000	0.0000	0.000	0.508	0.0071	0.178
27FS25	2	0.000	0.0000	0.000	0.412	0.0076	0.178
28FS15	1	61.038	1.1303	24.069	61.238	1.1340	24.147
28FS25	2	0.000	0.0000	0.000	0.444	0.0049	0.178
29FS15	1	0.000	0.0000	0.000	0.710	0.0079	0.178
29FS25	2	18.051	0.1492	5.559	18.521	0.1531	5.704
30FS15	1	0.000	0.0000	0.000	0.681	0.0074	0.178
31FS15	1	0.000	0.0000	0.000	0.256	0.0049	0.178
32FS15	1	0.000	0.0000	0.000	0.790	0.0065	0.178
32FS25	2	0.000	0.0000	0.000	0.866	0.0149	0.178
33FS15	1	0.000	0.0000	0.000	0.481	0.0083	0.178
33FS25	2	0.000	0.0000	0.000	0.446	0.0074	0.178
34FS15	1	417.347	6.9558	73.671	417.794	6.9632	73.750
34FS25	2	0.000	0.0000	0.000	0.529	0.0093	0.178
35FS15	1	0.000	0.0000	0.000	0.554	0.0097	0.178
35FS25	2	0.000	0.0000	0.000	0.361	0.0059	0.178
36FS15	1	0.000	0.0000	0.000	0.381	0.0062	0.178
36FS25	2	0.000	0.0000	0.000	0.243	0.0042	0.178
37FS15	1	0.000	0.0000	0.000	0.364	0.0063	0.178
37FS25	2	0.000	0.0000	0.000	0.412	0.0063	0.178
38FS15	1	0.000	0.0000	0.000	0.665	0.0102	0.178

Sample	Day	Daily intake (ng/day) lower bound	Daily intake (ng/kg bw/day) lower bound	∑PFAS (ng/kg) lower	Daily intake (ng/day) medium bound	Daily intake (ng/kg bw/day) medium bound	∑PFAS (ng/kg) medium
39FS15	1	0.000	0.0000	0.000	0.295	0.0055	0.178
39FS25	2	0.000	0.0000	0.000	0.306	0.0057	0.178
40FS15	1	0.000	0.0000	0.000	0.492	0.0060	0.178
40FS25	2	0.027	0.0004	0.012	0.439	0.0064	0.186
41FS15	1	0.000	0.0000	0.000	0.690	0.0100	0.178
41FS25	2	0.049	0.0006	0.021	0.452	0.0053	0.196
42FS15	1	0.000	0.0000	0.000	0.615	0.0072	0.178
42FS25	2	0.000	0.0000	0.000	0.539	0.0088	0.178
43FS15	1	0.000	0.0000	0.000	0.834	0.0137	0.178
43FS25	2	131.949	2.1631	29.765	132.590	2.1736	29.910
44FS15	1	22.220	0.3174	8.439	22.515	0.3216	8.551
44FS25	2	0.000	0.0000	0.000	0.397	0.0057	0.178
45FS15	1	0.000	0.0000	0.000	0.456	0.0052	0.178
45FS25	2	0.000	0.0000	0.000	0.495	0.0056	0.178
46FS15	1	0.000	0.0000	0.000	0.981	0.0102	0.178
46FS25	2	153.615	1.6002	48.490	154.073	1.6049	48.634
47FS15	1	0.000	0.0000	0.000	0.686	0.0072	0.178
47FS25	2	85.696	0.9021	42.720	85.986	0.9051	42.865
48FS15	1	199.095	3.1602	52.393	199.395	3.1650	52.472
48FS25	2	0.000	0.0000	0.000	0.546	0.0087	0.178
49FS15	1	0.029	0.0004	0.010	0.520	0.0065	0.186
49FS25	2	96.272	1.2034	38.309	96.470	1.2059	38.388
50FS15	1	0.000	0.0000	0.000	0.562	0.0080	0.178
50FS25	2	0.000	0.0000	0.000	0.465	0.0066	0.178
51FS15	1	0.000	0.0000	0.000	0.435	0.0069	0.178
51FS25	2	0.000	0.0000	0.000	0.276	0.0044	0.178
52FS15	1	0.000	0.0000	0.000	0.596	0.0080	0.178
52FS25	2	0.000	0.0000	0.000	0.433	0.0058	0.178
53FS15	1	38.893	0.4986	21.875	39.150	0.5019	22.019
54FS15	1	0.000	0.0000	0.000	0.640	0.0080	0.178
54FS25	2	0.000	0.0000	0.000	0.645	0.0081	0.178
55FS15	1	96.852	1.3836	18.665	97.602	1.3943	18.809
55FS25	2	0.000	0.0000	0.000	0.392	0.0056	0.178
56FS15	1	0.000	0.0000	0.000	0.503	0.0084	0.178
56FS25	2	44.692	0.7449	12.543	45.207	0.7534	12.688

Sample	Day	Daily intake (ng/day) lower bound	Daily intake (ng/kg bw/day) lower bound	∑PFAS (ng/kg) lower	Daily intake (ng/day) medium bound	Daily intake (ng/kg bw/day) medium bound	∑PFAS (ng/kg) medium
58FS15	1	0.000	0.0000	0.000	0.410	0.0066	0.178
58FS25	2	0.000	0.0000	0.000	0.339	0.0055	0.178
59FS15	1	0.065	0.0005	0.023	0.563	0.0045	0.197
59FS25	2	0.000	0.0000	0.000	0.609	0.0049	0.178
60FS15	1	0.000	0.0000	0.000	0.224	0.0029	0.178
60FS25	2	16.502	0.2143	19.078	16.571	0.2152	19.157
61FS15	1	0.000	0.0000	0.000	0.168	0.0021	0.178
61FS25	2	0.000	0.0000	0.000	0.148	0.0018	0.178
62FS15	1	0.000	0.0000	0.000	0.595	0.0081	0.178
62FS25	2	0.000	0.0000	0.000	0.347	0.0047	0.178

Table SM08. Individual daily intakes of ΣPFAS for lower and medium bounds (ng/day and ng/kg bw/day) through liquid food consumption (n = 120)

Sample	Day	Daily intake (ng day) lower bound	Daily intake (ng/kg bw/day) lower bound	∑PFAS (ng/L) lower	Daily intake (ng day) medium bound	Daily intake (ng/kg bw/day) medium bound	∑PFAS (ng/L) medium
01FL15	1	0.018	0.0003	0.022	0.050	0.0008	0.061
01FL25	2	0.000	0.0000	0.000	0.034	0.0006	0.045
02FL15	1	0.000	0.0000	0.000	0.062	0.0009	0.045
02FL25	2	0.000	0.0000	0.000	0.041	0.0006	0.045
03FL15	1	0.000	0.0000	0.000	0.077	0.0014	0.045
03FL25	2	0.000	0.0000	0.000	0.083	0.0015	0.045
04FL15	1	0.000	0.0000	0.000	0.079	0.0013	0.045
04FL25	2	0.000	0.0000	0.000	0.062	0.0010	0.045
05FL15	1	0.000	0.0000	0.000	0.087	0.0010	0.045
05FL25	2	0.000	0.0000	0.000	0.038	0.0004	0.045
06FL15	1	0.000	0.0000	0.000	0.072	0.0010	0.045
06FL25	2	0.000	0.0000	0.000	0.060	0.0008	0.045
07FL15	1	0.000	0.0000	0.000	0.107	0.0018	0.045
07FL25	2	0.000	0.0000	0.000	0.084	0.0014	0.045
08FL15	1	0.000	0.0000	0.000	0.066	0.0011	0.045
08FL25	2	0.000	0.0000	0.000	0.046	0.0008	0.045
09FL15	1	0.000	0.0000	0.000	0.080	0.0013	0.045
09FL25	2	0.000	0.0000	0.000	0.060	0.0010	0.045
10FL15	1	0.000	0.0000	0.000	0.092	0.0013	0.045
10FL25	2	0.000	0.0000	0.000	0.092	0.0013	0.045
11FL15	1	0.000	0.0000	0.000	0.122	0.0022	0.045
11FL25	2	0.000	0.0000	0.000	0.050	0.0009	0.045
12FL15	1	0.000	0.0000	0.000	0.078	0.0011	0.045
12FL25	2	0.000	0.0000	0.000	0.052	0.0007	0.045
13FL15	1	0.000	0.0000	0.000	0.085	0.0012	0.045
13FL25	2	0.000	0.0000	0.000	0.069	0.0010	0.045
14FL15	1	0.000	0.0000	0.000	0.107	0.0016	0.045
14FL25	2	0.000	0.0000	0.000	0.074	0.0011	0.045
15FL15	1	0.000	0.0000	0.000	0.033	0.0005	0.045
15FL25	2	0.000	0.0000	0.000	0.053	0.0007	0.045
16FL15	1	0.021	0.0003	0.015	0.083	0.0010	0.058
16FL25	2	0.000	0.0000	0.000	0.056	0.0007	0.045
17FL15	1	0.000	0.0000	0.000	0.055	0.0008	0.045
17FL25	2	0.000	0.0000	0.000	0.035	0.0005	0.045
18FL15	1	0.000	0.0000	0.000	0.079	0.0011	0.045

Sample	Day	Daily intake (ng day) lower bound	Daily intake (ng/kg bw/day) lower bound	∑PFAS (ng/L) lower	Daily intake (ng day) medium bound	Daily intake (ng/kg bw/day) medium bound	∑PFAS (ng/L) medium
18FL25	2	0.000	0.0000	0.000	0.140	0.0020	0.045
19FL15	1	0.000	0.0000	0.000	0.152	0.0018	0.045
19FL25	2	0.000	0.0000	0.000	0.142	0.0017	0.045
20FL15	1	0.000	0.0000	0.000	0.069	0.0013	0.045
20FL25	2	0.000	0.0000	0.000	0.027	0.0005	0.045
21FL15	1	0.000	0.0000	0.000	0.102	0.0019	0.045
21FL25	2	0.000	0.0000	0.000	0.071	0.0013	0.045
22FL15	1	0.000	0.0000	0.000	0.085	0.0010	0.045
22FL25	2	0.012	0.0001	0.006	0.096	0.0011	0.051
23FL16	1	0.000	0.0000	0.000	0.036	0.0006	0.045
23FL25	2	0.000	0.0000	0.000	0.056	0.0010	0.045
24FL15	1	0.000	0.0000	0.000	0.099	0.0014	0.045
24FL25	2	0.000	0.0000	0.000	0.096	0.0014	0.045
25FL15	1	0.000	0.0000	0.000	0.083	0.0011	0.045
25FL25	2	0.004	0.0001	0.003	0.076	0.0010	0.047
26FL15	1	0.000	0.0000	0.000	0.098	0.0018	0.045
26FL25	2	0.000	0.0000	0.000	0.057	0.0010	0.045
27FL15	1	0.000	0.0000	0.000	0.079	0.0011	0.045
27FL25	2	0.000	0.0000	0.000	0.080	0.0011	0.045
28FL15	1	0.000	0.0000	0.000	0.063	0.0012	0.045
28FL25	2	0.000	0.0000	0.000	0.070	0.0013	0.045
29FL15	1	0.000	0.0000	0.000	0.116	0.0013	0.045
29FL25	2	0.000	0.0000	0.000	0.093	0.0010	0.045
30FL15	1	0.000	0.0000	0.000	0.059	0.0006	0.045
30FL25	2	0.000	0.0000	0.000	0.076	0.0008	0.045
31FL15	1	0.000	0.0000	0.000	0.037	0.0007	0.045
31FL25	2	0.000	0.0000	0.000	0.023	0.0004	0.045
32FL15	1	0.000	0.0000	0.000	0.138	0.0011	0.045
32FL25	2	0.000	0.0000	0.000	0.190	0.0016	0.045
33FL15	1	0.000	0.0000	0.000	0.081	0.0014	0.045
33FL25	2	0.000	0.0000	0.000	0.081	0.0014	0.045
34FL15	1	0.000	0.0000	0.000	0.180	0.0030	0.045
34FL25	2	0.000	0.0000	0.000	0.110	0.0018	0.045
35FL15	1	0.000	0.0000	0.000	0.089	0.0016	0.045
35FL25	2	0.000	0.0000	0.000	0.056	0.0010	0.045
36FL15	1	0.000	0.0000	0.000	0.047	0.0008	0.045
36FL25	2	0.000	0.0000	0.000	0.025	0.0004	0.045

Sample	Day	Daily intake (ng day) lower bound	Daily intake (ng/kg bw/day) lower bound	∑PFAS (ng/L) lower	Daily intake (ng day) medium bound	Daily intake (ng/kg bw/day) medium bound	∑PFAS (ng/L) medium
37FL15	1	0.198	0.0034	0.131	0.251	0.0043	0.166
37FL25	2	0.000	0.0000	0.000	0.070	0.0012	0.045
38FL15	1	0.000	0.0000	0.000	0.073	0.0011	0.045
38FL25	2	0.000	0.0000	0.000	0.071	0.0011	0.045
39FL15	1	0.038	0.0007	0.032	0.090	0.0017	0.076
39FL25	2	0.000	0.0000	0.000	0.047	0.0009	0.045
40FL15	1	0.000	0.0000	0.000	0.065	0.0008	0.045
40FL25	2	0.000	0.0000	0.000	0.062	0.0008	0.045
41FL15	1	0.043	0.0006	0.015	0.170	0.0025	0.059
41FL25	2	0.000	0.0000	0.000	0.080	0.0012	0.045
42FL15	1	0.216	0.0025	0.147	0.280	0.0033	0.191
42FL25	2	41.044	0.4829	19.545	41.087	0.4834	19.565
43FL15	1	0.000	0.0000	0.000	0.138	0.0023	0.045
43FL25	2	0.000	0.0000	0.000	0.148	0.0024	0.045
44FL15	1	0.000	0.0000	0.000	0.071	0.0010	0.045
44FL25	2	0.000	0.0000	0.000	0.063	0.0009	0.045
45FL15	1	0.000	0.0000	0.000	0.072	0.0008	0.045
45FL25	2	0.000	0.0000	0.000	0.093	0.0011	0.045
46FL15	1	0.000	0.0000	0.000	0.177	0.0018	0.045
46FL25	2	0.000	0.0000	0.000	0.115	0.0012	0.045
47FL15	1	0.000	0.0000	0.000	0.088	0.0009	0.045
47FL25	2	0.000	0.0000	0.000	0.047	0.0005	0.045
48FL15	1	0.000	0.0000	0.000	0.126	0.0020	0.045
48FL25	2	0.000	0.0000	0.000	0.129	0.0020	0.045
49FL15	1	0.000	0.0000	0.000	0.091	0.0011	0.045
49FL25	2	0.000	0.0000	0.000	0.077	0.0010	0.045
50FL15	1	0.932	0.0133	0.365	1.044	0.0149	0.409
50FL25	2	0.000	0.0000	0.000	0.079	0.0011	0.045
51FL15	1	0.000	0.0000	0.000	0.082	0.0013	0.045
51FL25	2	0.000	0.0000	0.000	0.021	0.0003	0.045
52FL15	1	0.000	0.0000	0.000	0.113	0.0015	0.045
52FL25	2	0.000	0.0000	0.000	0.079	0.0011	0.045
53FL15	1	3.904	0.0500	2.767	3.964	0.0508	2.809
53FL25	2	0.000	0.0000	0.000	0.038	0.0005	0.045
54FL15	1	0.000	0.0000	0.000	0.083	0.0010	0.045
54FL25	2	0.000	0.0000	0.000	0.081	0.0010	0.045
55FL15	1	0.000	0.0000	0.000	0.129	0.0018	0.045

Sample	Day	Daily intake (ng day) lower bound	Daily intake (ng/kg bw/day) lower bound	∑PFAS (ng/L) lower	Daily intake (ng day) medium bound	Daily intake (ng/kg bw/day) medium bound	∑PFAS (ng/L) medium
55FL25	2	0.000	0.0000	0.000	0.057	0.0008	0.045
56FL15	1	0.000	0.0000	0.000	0.083	0.0014	0.045
56FL25	2	0.000	0.0000	0.000	0.100	0.0017	0.045
58FL15	1	0.000	0.0000	0.000	0.051	0.0008	0.045
58FL25	2	0.000	0.0000	0.000	0.057	0.0009	0.045
59FL15	1	0.000	0.0000	0.000	0.092	0.0007	0.045
59FL25	2	0.000	0.0000	0.000	0.133	0.0011	0.045
60FL15	1	0.000	0.0000	0.000	0.024	0.0003	0.045
60FL25	2	0.000	0.0000	0.000	0.024	0.0003	0.045
61FL25	2	0.000	0.0000	0.000	0.040	0.0005	0.045
62FL25	2	0.000	0.0000	0.000	0.055	0.0007	0.045

Table SM09. Individual daily intakes of ΣPFAS for lower and medium bounds (ng/day and ng/kg bw/day) through combined solid and food consumption when the average concentrations of day 1 and day 2 were considered (n = 47)

Sample	Daily intake (ng/day) lower bound	Daily intake (ng/kg bw/day) lower bound	∑PFAS (ng/kg) lower	Daily intake (ng/day) medium bound	Daily intake ng/kg bw/day medium bound	∑PFAS (ng/kg) medium
01FS15	0.0	0.000	0.022	0.3	0.005	0.241
02FS15	0.0	0.000	0.008	0.4	0.006	0.229
04FS15	0.0	0.000	0.004	0.5	0.009	0.226
05FS15	0.0	0.000	0.008	0.4	0.007	0.230
08FS15	0.1	0.001	0.036	0.5	0.006	0.256
10FS15	0.0	0.000	0.000	0.5	0.008	0.222
11FS15	0.0	0.000	0.002	0.5	0.008	0.223
12FS15	0.0	0.000	0.007	0.4	0.007	0.229
13FS15	0.0	0.000	0.004	0.5	0.008	0.226
14FS15	0.0	0.000	0.003	0.5	0.008	0.224
15FS15	1.1	0.019	1.007	1.3	0.023	1.210
17FS15	0.7	0.010	0.490	0.9	0.013	0.676
21FS15	56.8	0.835	23.047	57.2	0.841	23.220
22FS15	18.4	0.283	8.290	18.9	0.290	8.493
23FS15	0.0	0.000	0.002	0.3	0.004	0.224
24FS15	0.0	0.000	0.000	0.6	0.008	0.222
25FS15	63.7	0.937	28.982	64.1	0.942	29.153
26FS15	0.0	0.000	0.000	0.4	0.006	0.222
27FS15	0.0	0.000	0.000	0.5	0.006	0.222
28FS15	24.1	0.439	12.034	24.5	0.445	12.207
29FS15	8.3	0.151	2.780	8.9	0.162	2.985
32FS15	0.0	0.000	0.000	0.9	0.011	0.222
33FS15	0.0	0.000	0.000	0.5	0.008	0.222
34FS15	139.4	1.991	36.836	140.0	2.000	37.009
35FS15	0.0	0.000	0.000	0.5	0.006	0.222
36FS15	0.0	0.000	0.000	0.3	0.005	0.222
37FS15	0.1	0.002	0.066	0.5	0.007	0.283
39FS15	0.0	0.000	0.016	0.3	0.006	0.238
40FS15	0.0	0.000	0.006	0.5	0.005	0.227
41FS15	0.0	0.001	0.018	0.6	0.007	0.238
42FS15	24.8	0.476	9.846	25.3	0.486	10.056
43FS15	57.8	0.478	14.883	58.6	0.484	15.088
44FS15	8.3	0.143	4.219	8.7	0.149	4.409
45FS15	0.0	0.000	0.000	0.5	0.008	0.222
46FS15	92.4	1.621	24.245	93.2	1.635	24.451

Sample	Daily intake (ng/day) lower bound	Daily intake (ng/kg bw/day) lower bound	∑PFAS (ng/kg) lower	Daily intake (ng/day) medium bound	Daily intake ng/kg bw/day medium bound	∑PFAS (ng/kg) medium
47FS15	39.9	0.654	21.360	40.3	0.661	21.566
48FS15	82.5	1.423	26.197	83.1	1.432	26.370
49FS15	43.5	0.669	19.160	43.8	0.675	19.332
50FS15	0.5	0.009	0.183	1.0	0.019	0.405
51FS15	0.0	0.000	0.000	0.4	0.004	0.222
52FS15	0.0	0.000	0.007	0.6	0.009	0.229
54FS15	0.0	0.000	0.000	0.6	0.007	0.222
55FS15	27.0	0.443	9.332	27.6	0.453	9.538
56FS15	16.4	0.235	6.272	17.0	0.242	6.477
58FS15	0.0	0.000	0.000	0.4	0.004	0.222
59FS15	0.0	0.000	0.011	0.7	0.007	0.232
60FS15	7.6	0.080	9.539	7.8	0.082	9.712

Table SM10. Meteor predictions for MeFOSE

Name	Parent	Intermediates	Biotransformation Name	Formula	Exact Mass	Nominal Mass	LogP	Phase	Enzyme	Likelihood
90507 (Query)				C11H8F17NO3S	556.9953	557	3.34			
M5	90507 (Query)	I6a	Oxidation of Primary Alcohols	C11H6F17NO4S	570.9746	571	3.88	Phase I	ADH	PROBABLE
M32	M5	I37o	Oxidative N-Demethylation	C10H4F17NO4S	556.959	557	3.91	Phase I	CYP450	PROBABLE
M66	M32		Glucuronidation of Carboxylic Acids	C16H12F17NO10S	732.991	733	1.87	Phase II	UGT	PLAUSIBLE
M71	M32	I84b	Conjugation of Carboxylic Acids with Glutamine	C15H12F17N3O6S	685.0175	685	1.87	Phase II	ACS, AANAT	EQUIVOCAL
M2	M32		Decarboxylation of alpha-Amino, Aromatic and beta-Keto Carboxylic Acids	C9H4F17NO2S	512.9691	513	3.83	Phase I	Decarboxylase/AAAD	EQUIVOCAL
M3	M32	I72o, I73a	Oxidative N-Dealkylation	C2H4O3	76.016	76	-1.04	Phase I	CYP450	EQUIVOCAL
M9	M32	I69o	Oxidative N-Dealkylation	C8H2F17NO2S	498.9535	499	3.2	Phase I	CYP450	EQUIVOCAL
M15	M32	I70o, I71a	Oxidative N-Dealkylation	C2H2O4	89.9953	90	-1.75	Phase I	CYP450	EQUIVOCAL
M68	M32	I75a, I76a, I77a	Glucuronidation of Carboxylic Acids	C16H12F17NO10S	732.991	733	1.37	Phase II	UGT	EQUIVOCAL
M69	M32	I78a, I79a, I80a	Glucuronidation of Carboxylic Acids	C16H12F17NO10S	732.991	733	2.03	Phase II	UGT	EQUIVOCAL
M70	M32	I81a, I82a, I83a	Glucuronidation of Carboxylic Acids	C16H12F17NO10S	732.991	733	1.37	Phase II	UGT	EQUIVOCAL
M72	M32		N-Glucuronidation of Amides and Related Compounds	C16H12F17NO10S	732.991	733	2.39	Phase I	UGT	EQUIVOCAL
M27	M5		Glucuronidation of Carboxylic Acids	C17H14F17NO10S	747.0067	747	1.79	Phase II	UGT	PLAUSIBLE
M33	M5	I38b	Conjugation of Carboxylic Acids with Glutamine	C16H14F17N3O6S	699.0332	699	1.78	Phase II	ACS, AANAT	EQUIVOCAL
M25	M5		Decarboxylation of alpha-Amino, Aromatic and beta-Keto Carboxylic Acids	C10H6F17NO2S	526.9848	527	3.59	Phase I	Decarboxylase/AAAD	EQUIVOCAL
M2	M25	I68o	Oxidative N-Demethylation	C9H4F17NO2S	512.9691	513	3.83	Phase I	CYP450	PROBABLE
M2	M5	I22o	Oxidative N-Dealkylation	C9H4F17NO2S	512.9691	513	3.83	Phase I	CYP450	EQUIVOCAL
M3	M5	I25o, I26a	Oxidative N-Dealkylation	C2H4O3	76.016	76	-1.04	Phase I	CYP450	EQUIVOCAL
M15	M5	I23o, I24a	Oxidative N-Dealkylation	C2H2O4	89.9953	90	-1.75	Phase I	CYP450	EQUIVOCAL

Name	Parent	Intermediates	Biotransformation Name	Formula	Exact Mass	Nominal Mass	LogP	Phase	Enzyme	Likelihood
M29	M5	I28a, I29a, I30a	Glucuronidation of Carboxylic Acids	C17H14F17NO10S	747.0067	747	1.29	Phase II	UGT	EQUIVOCAL
M30	M5	I31a, I32a, I33a	Glucuronidation of Carboxylic Acids	C17H14F17NO10S	747.0067	747	1.94	Phase II	UGT	EQUIVOCAL
M31	M5	I34a, I35a, I36a	Glucuronidation of Carboxylic Acids	C17H14F17NO10S	747.0067	747	1.29	Phase II	UGT	EQUIVOCAL
M6	90507 (Query)	I7o	Oxidative N-Demethylation	C10H6F17NO3S	542.9797	543	3.45	Phase I	CYP450	PROBABLE
M32	M6	I44a	Oxidation of Primary Alcohols	C10H4F17NO4S	556.959	557	3.91	Phase I	ADH	PROBABLE
M35	M6		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C16H14F17NO9S	719.0118	719	2.06	Phase II	UGT	EQUIVOCAL
M3	M6	I40o, I41a	Oxidative N-Dealkylation	C2H4O3	76.016	76	-1.04	Phase I	CYP450	EQUIVOCAL
M4	M6	I42o, I43a	Oxidative N-Dealkylation	C2H6O2	62.0368	62	-1.37	Phase I	CYP450	EQUIVOCAL
M9	M6	I39o	Oxidative N-Dealkylation	C8H2F17NO2S	498.9535	499	3.2	Phase I	CYP450	EQUIVOCAL
M37	M6		N-Glucuronidation of Amides and Related Compounds	C16H14F17NO9S	719.0118	719	1.85	Phase I	UGT	EQUIVOCAL
M74	M37		Glucuronidation of Carboxylic Acids	C22H22F17NO15S	895.0439	895	0.07	Phase II	UGT	PLAUSIBLE
M80	M37		Oxidation of Secondary (Alicyclic) Alcohols	C16H12F17NO9S	716.9961	717	1.64	Phase I	ADH	PLAUSIBLE
M81	M37		Oxidation of Secondary (Alicyclic) Alcohols	C16H12F17NO9S	716.9961	717	1.76	Phase I	ADH	PLAUSIBLE
M82	M37		Oxidation of Secondary (Alicyclic) Alcohols	C16H12F17NO9S	716.9961	717	-0.07	Phase I	ADH	PLAUSIBLE
M72	M37	I91a	Oxidation of Primary Alcohols	C16H12F17NO10S	732.991	733	2.39	Phase I	ADH	PLAUSIBLE
M86	M37		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C22H22F17NO15S	895.0439	895	-0.16	Phase II	UGT	EQUIVOCAL
M87	M37		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C22H22F17NO15S	895.0439	895	0.5	Phase II	UGT	EQUIVOCAL
M88	M37		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C22H22F17NO15S	895.0439	895	-0.16	Phase II	UGT	EQUIVOCAL

Name	Parent	Intermediates	Biotransformation Name	Formula	Exact Mass	Nominal Mass	LogP	Phase	Enzyme	Likelihood
M89	M37		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C22H22F17NO15S	895.0439	895	0.46	Phase II	UGT	EQUIVOCAL
M94	M37	I101b	Conjugation of Carboxylic Acids with Glutamine	C21H22F17N3O11S	847.0704	847	0.23	Phase II	ACS, AANAT	EQUIVOCAL
M3	M37	I86o, I87a	Oxidative N-Dealkylation	C2H4O3	76.016	76	-1.04	Phase I	CYP450	EQUIVOCAL
M4	M37	I88o, I89a	Oxidative N-Dealkylation	C2H6O2	62.0368	62	-1.37	Phase I	CYP450	EQUIVOCAL
M38	M37	I85o	Oxidative N-Dealkylation	C14H10F17NO8S	674.9856	675	2.12	Phase I	CYP450	EQUIVOCAL
M83	M37	I92a, I93a, I94a	Glucuronidation of Carboxylic Acids	C22H22F17NO15S	895.0439	895	-0.42	Phase II	UGT	EQUIVOCAL
M84	M37	I95a, I96a, I97a	Glucuronidation of Carboxylic Acids	C22H22F17NO15S	895.0439	895	0.23	Phase II	UGT	EQUIVOCAL
M85	M37	I98a, I99a, I100a	Glucuronidation of Carboxylic Acids	C22H22F17NO15S	895.0439	895	-0.42	Phase II	UGT	EQUIVOCAL
M2	90507 (Query)	I1o	Oxidative N-Dealkylation	C9H4F17NO2S	512.9691	513	3.83	Phase I	CYP450	PROBABLE
M9	M2	I8o	Oxidative N-Demethylation	C8H2F17NO2S	498.9535	499	3.2	Phase I	CYP450	PROBABLE
M38	M9		N-Glucuronidation of Amides and Related Compounds	C14H10F17NO8S	674.9856	675	2.12	Phase I	UGT	EQUIVOCAL
M10	M2		N-Glucuronidation of Amides and Related Compounds	C15H12F17NO8S	689.0012	689	2.1	Phase I	UGT	EQUIVOCAL
M45	M10		Oxidation of Secondary (Alicyclic) Alcohols	C15H10F17NO8S	686.9856	687	1.89	Phase I	ADH	PROBABLE
M46	M10		Oxidation of Secondary (Alicyclic) Alcohols	C15H10F17NO8S	686.9856	687	2.01	Phase I	ADH	PROBABLE
M47	M10		Oxidation of Secondary (Alicyclic) Alcohols	C15H10F17NO8S	686.9856	687	0.18	Phase I	ADH	PROBABLE
M38	M10	I55o	Oxidative N-Demethylation	C14H10F17NO8S	674.9856	675	2.12	Phase I	CYP450	PROBABLE
M40	M10		Glucuronidation of Carboxylic Acids	C21H20F17NO14S	865.0333	865	0.32	Phase II	UGT	PLAUSIBLE
M51	M10		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H20F17NO14S	865.0333	865	0.1	Phase II	UGT	EQUIVOCAL
M52	M10		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H20F17NO14S	865.0333	865	0.75	Phase II	UGT	EQUIVOCAL

Name	Parent	Intermediates	Biotransformation Name	Formula	Exact Mass	Nominal Mass	LogP	Phase	Enzyme	Likelihood
M53	M10		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H20F17NO14S	865.0333	865	0.1	Phase II	UGT	EQUIVOCAL
M57	M10	I56b	Conjugation of Carboxylic Acids with Glutamine	C20H20F17N3O10S	817.0598	817	0.49	Phase II	ACS, AANAT	EQUIVOCAL
M48	M10	I46a, I47a, I48a	Glucuronidation of Carboxylic Acids	C21H20F17NO14S	865.0333	865	-0.17	Phase II	UGT	EQUIVOCAL
M49	M10	I49a, I50a, I51a	Glucuronidation of Carboxylic Acids	C21H20F17NO14S	865.0333	865	0.48	Phase II	UGT	EQUIVOCAL
M50	M10	I52a, I53a, I54a	Glucuronidation of Carboxylic Acids	C21H20F17NO14S	865.0333	865	-0.17	Phase II	UGT	EQUIVOCAL
M3	90507 (Query)	I2o, I3a	Oxidative N-Dealkylation	C2H4O3	76.016	76	-1.04	Phase I	CYP450	PROBABLE
M12	M3		Glucuronidation of Carboxylic Acids	C8H12O9	252.0481	252	-2.69	Phase II	UGT	EQUIVOCAL
M15	M3	I10a	Oxidation of Primary Alcohols	C2H2O4	89.9953	90	-1.75	Phase I	ADH	EQUIVOCAL
M59	M15		Glucuronidation of Carboxylic Acids	C8H10O10	266.0274	266	-2.92	Phase II	UGT	EQUIVOCAL
M4	90507 (Query)	I4o, I5a	Oxidative N-Dealkylation	C2H6O2	62.0368	62	-1.37	Phase I	CYP450	PROBABLE
M3	M4	I21a	Oxidation of Primary Alcohols	C2H4O3	76.016	76	-1.04	Phase I	ADH	EQUIVOCAL

Table SM11. Meteor predictions for MeFOSA

Name	Parent	Intermediates	Biotransformation Name	Formula	Exact Mass	Nominal Mass	LogP	Phase	Enzyme	Likelihood
A 3034468 (Query)				C9H4F17NO2S	512.9691	513	3.83			
M1	3034468 (Query)	I1o	Oxidative N-Demethylation	C8H2F17NO2S	498.9535	499	3.2	Phase I	CYP450	PROBABLE
M10	M2		Oxidation of Secondary (Alicyclic) Alcohols	C15H10F17NO8S	686.9856	687	1.89	Phase I	ADH	PROBABLE
M11	M2		Oxidation of Secondary (Alicyclic) Alcohols	C15H10F17NO8S	686.9856	687	2.01	Phase I	ADH	PROBABLE
M12	M2		Oxidation of Secondary (Alicyclic) Alcohols	C15H10F17NO8S	686.9856	687	0.18	Phase I	ADH	PROBABLE
M13	M2	I3a, I4a, I5a	Glucuronidation of Carboxylic Acids	C21H20F17NO14S	865.0333	865	-0.17	Phase II	UGT	EQUIVOCAL
M14	M2	I6a, I7a, I8a	Glucuronidation of Carboxylic Acids	C21H20F17NO14S	865.0333	865	0.48	Phase II	UGT	EQUIVOCAL
M15	M2	I9a, I10a, I11a	Glucuronidation of Carboxylic Acids	C21H20F17NO14S	865.0333	865	-0.17	Phase II	UGT	EQUIVOCAL
M16	M2		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H20F17NO14S	865.0333	865	0.1	Phase II	UGT	EQUIVOCAL
M17	M2		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H20F17NO14S	865.0333	865	0.75	Phase II	UGT	EQUIVOCAL
M18	M2		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H20F17NO14S	865.0333	865	0.1	Phase II	UGT	EQUIVOCAL
M2	3034468 (Query)		N-Glucuronidation of Amides and Related Compounds	C15H12F17NO8S	689.0012	689	2.1	Phase I	UGT	EQUIVOCAL
M2	M10		Reduction of Alicyclic Ketones	C15H12F17NO8S	689.0012	689	2.1	Phase I	ADH	PLAUSIBLE
M2	M11		Reduction of Alicyclic Ketones	C15H12F17NO8S	689.0012	689	2.1	Phase I	ADH	PLAUSIBLE
M2	M12		Reduction of Alicyclic Ketones	C15H12F17NO8S	689.0012	689	2.1	Phase I	ADH	PLAUSIBLE
M22	M2	I13b	Conjugation of Carboxylic Acids with Glutamine	C20H20F17N3O10S	817.0598	817	0.49	Phase II	ACS, AANAT	EQUIVOCAL
M24	M3		Glucuronidation of Carboxylic Acids	C20H18F17NO14S	851.0177	851	0.34	Phase II	UGT	PLAUSIBLE

Name	Parent	Intermediates	Biotransformation Name	Formula	Exact Mass	Nominal Mass	LogP	Phase	Enzyme	Likelihood
M29	M3		Oxidation of Secondary (Alicyclic) Alcohols	C14H8F17NO8S	672.9699	673	1.91	Phase I	ADH	PROBABLE
M29	M10	I35o	Oxidative N-Demethylation	C14H8F17NO8S	672.9699	673	1.91	Phase I	CYP450	PLAUSIBLE
M3	M1		N-Glucuronidation of Amides and Related Compounds	C14H10F17NO8S	674.9856	675	2.12	Phase I	UGT	EQUIVOCAL
M3	M2	I12o	Oxidative N-Demethylation	C14H10F17NO8S	674.9856	675	2.12	Phase I	CYP450	PROBABLE
M30	M3		Oxidation of Secondary (Alicyclic) Alcohols	C14H8F17NO8S	672.9699	673	1.93	Phase I	ADH	PROBABLE
M30	M11	I47o	Oxidative N-Demethylation	C14H8F17NO8S	672.9699	673	1.93	Phase I	CYP450	PROBABLE
M31	M3		Oxidation of Secondary (Alicyclic) Alcohols	C14H8F17NO8S	672.9699	673	0.26	Phase I	ADH	PROBABLE
M31	M12	I59o	Oxidative N-Demethylation	C14H8F17NO8S	672.9699	673	0.26	Phase I	CYP450	PLAUSIBLE
M32	M3	I15a, I16a, I17a	Glucuronidation of Carboxylic Acids	C20H18F17NO14S	851.0177	851	-0.15	Phase II	UGT	EQUIVOCAL
M33	M3	I18a, I19a, I20a	Glucuronidation of Carboxylic Acids	C20H18F17NO14S	851.0177	851	0.5	Phase II	UGT	EQUIVOCAL
M34	M3	I21a, I22a, I23a	Glucuronidation of Carboxylic Acids	C20H18F17NO14S	851.0177	851	-0.15	Phase II	UGT	EQUIVOCAL
M35	M3		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C20H18F17NO14S	851.0177	851	0.11	Phase II	UGT	EQUIVOCAL
M36	M3		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C20H18F17NO14S	851.0177	851	0.77	Phase II	UGT	EQUIVOCAL
M37	M3		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C20H18F17NO14S	851.0177	851	0.11	Phase II	UGT	EQUIVOCAL
M41	M3	I24b	Conjugation of Carboxylic Acids with Glutamine	C19H18F17N3O10S	803.0441	803	0.5	Phase II	ACS, AANAT	EQUIVOCAL
M42	M3		N-Glucuronidation of Amides and Related Compounds	C20H18F17NO14S	851.0177	851	0.62	Phase I	UGT	EQUIVOCAL
M44	M10		Glucuronidation of Carboxylic Acids	C21H18F17NO14S	863.0177	863	0.06	Phase II	UGT	PLAUSIBLE
M48	M10		Oxidation of Secondary (Alicyclic) Alcohols	C15H8F17NO8S	684.9699	685	2.51	Phase I	ADH	PLAUSIBLE
M48	M11		Oxidation of Secondary (Alicyclic) Alcohols	C15H8F17NO8S	684.9699	685	2.51	Phase I	ADH	PROBABLE

Name	Parent	Intermediates	Biotransformation Name	Formula	Exact Mass	Nominal Mass	LogP	Phase	Enzyme	Likelihood
M49	M10		Oxidation of Secondary (Alicyclic) Alcohols	C15H8F17NO8S	684.9699	685	1.61	Phase I	ADH	PLAUSIBLE
M49	M12		Oxidation of Secondary (Alicyclic) Alcohols	C15H8F17NO8S	684.9699	685	1.61	Phase I	ADH	PLAUSIBLE
M5	M2		Glucuronidation of Carboxylic Acids	C21H20F17NO14S	865.0333	865	0.32	Phase II	UGT	PLAUSIBLE
M50	M10	I26a, I27a, I28a	Glucuronidation of Carboxylic Acids	C21H18F17NO14S	863.0177	863	-0.44	Phase II	UGT	EQUIVOCAL
M51	M10	I29a, I30a, I31a	Glucuronidation of Carboxylic Acids	C21H18F17NO14S	863.0177	863	0.22	Phase II	UGT	EQUIVOCAL
M52	M10	I32a, I33a, I34a	Glucuronidation of Carboxylic Acids	C21H18F17NO14S	863.0177	863	-0.44	Phase II	UGT	EQUIVOCAL
M53	M10		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H18F17NO14S	863.0177	863	0.36	Phase II	UGT	EQUIVOCAL
M54	M10		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H18F17NO14S	863.0177	863	0.54	Phase II	UGT	EQUIVOCAL
M57	M10	I36b	Conjugation of Carboxylic Acids with Glutamine	C20H18F17N3O10S	815.0441	815	0.34	Phase II	ACS, AANAT	EQUIVOCAL
M59	M11		Glucuronidation of Carboxylic Acids	C21H18F17NO14S	863.0177	863	0.23	Phase II	UGT	PLAUSIBLE
M63	M11		Oxidation of Secondary (Alicyclic) Alcohols	C15H8F17NO8S	684.9699	685	1.92	Phase I	ADH	PROBABLE
M63	M12		Oxidation of Secondary (Alicyclic) Alcohols	C15H8F17NO8S	684.9699	685	1.92	Phase I	ADH	PLAUSIBLE
M64	M11	I38a, I39a, I40a	Glucuronidation of Carboxylic Acids	C21H18F17NO14S	863.0177	863	-0.27	Phase II	UGT	EQUIVOCAL
M65	M11	I41a, I42a, I43a	Glucuronidation of Carboxylic Acids	C21H18F17NO14S	863.0177	863	0.39	Phase II	UGT	EQUIVOCAL
M66	M11	I44a, I45a, I46a	Glucuronidation of Carboxylic Acids	C21H18F17NO14S	863.0177	863	-0.27	Phase II	UGT	EQUIVOCAL
M67	M11		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H18F17NO14S	863.0177	863	0.65	Phase II	UGT	EQUIVOCAL
M68	M11		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H18F17NO14S	863.0177	863	0.47	Phase II	UGT	EQUIVOCAL

Name	Parent	Intermediates	Biotransformation Name	Formula	Exact Mass	Nominal Mass	LogP	Phase	Enzyme	Likelihood
M71	M11	I48b	Conjugation of Carboxylic Acids with Glutamine	C20H18F17N3O10S	815.0441	815	0.39	Phase II	ACS, AANAT	EQUIVOCAL
M73	M12		Glucuronidation of Carboxylic Acids	C21H18F17NO14S	863.0177	863	-1.65	Phase II	UGT	PLAUSIBLE
M77	M12	I50a, I51a, I52a	Glucuronidation of Carboxylic Acids	C21H18F17NO14S	863.0177	863	-2.15	Phase II	UGT	EQUIVOCAL
M78	M12	I53a, I54a, I55a	Glucuronidation of Carboxylic Acids	C21H18F17NO14S	863.0177	863	-1.49	Phase II	UGT	EQUIVOCAL
M79	M12	I56a, I57a, I58a	Glucuronidation of Carboxylic Acids	C21H18F17NO14S	863.0177	863	-2.15	Phase II	UGT	EQUIVOCAL
M80	M12		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H18F17NO14S	863.0177	863	-1.38	Phase II	UGT	EQUIVOCAL
M81	M12		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H18F17NO14S	863.0177	863	-1.38	Phase II	UGT	EQUIVOCAL
M84	M12	I60b	Conjugation of Carboxylic Acids with Glutamine	C20H18F17N3O10S	815.0441	815	-1.65	Phase II	ACS, AANAT	EQUIVOCAL

Table SM12. Meteor predictions for MeFOSAA

Name	Parent	Intermediates	Biotransformation Name	Formula	Exact Mass	Nominal Mass	LogP	Phase	Enzyme	Likelihood
A 2228693 1 (Query)				C11H6F17NO4S	570.9746	571	3.88			
M1	2228693 1 (Query)		Decarboxylation of alpha-Amino, Aromatic and beta-Keto Carboxylic Acids	C10H6F17NO2S	526.9848	527	3.59	Phase I	Decarboxylase / AAAD	EQUIVOCAL
M10	2228693 1 (Query)	I13a, I14a, I15a	Glucuronidation of Carboxylic Acids	C17H14F17NO10S	747.0067	747	1.29	Phase II	UGT	EQUIVOCAL
M11	2228693 1 (Query)	I16o	Oxidative N-Demethylation	C10H4F17NO4S	556.959	557	3.91	Phase I	CYP450	PROBABLE
M12	2228693 1 (Query)	I17b	Conjugation of Carboxylic Acids with Glutamine	C16H14F17N3O6S	699.0332	699	1.78	Phase II	ACS, AANAT	EQUIVOCAL
M13	M11	I43o	Oxidative N-Dealkylation	C8H2F17NO2S	498.9535	499	3.2	Phase I	CYP450	EQUIVOCAL
M13	M4	I19o	Oxidative N-Demethylation	C8H2F17NO2S	498.9535	499	3.2	Phase I	CYP450	PROBABLE
M14	M39		Decarboxylation of alpha-Amino, Aromatic and beta-Keto Carboxylic Acids	C15H12F17NO8S	689.0012	689	2.1	Phase I	Decarboxylase /AAAD	EQUIVOCAL
M14	M4		N-Glucuronidation of Amides and Related Compounds	C15H12F17NO8S	689.0012	689	2.1	Phase I	UGT	EQUIVOCAL
M16	M5		Glucuronidation of Carboxylic Acids	C8H10O10	266.0274	266	-2.92	Phase II	UGT	EQUIVOCAL
M23	M6		Glucuronidation of Carboxylic Acids	C8H12O9	252.0481	252	-2.69	Phase II	UGT	EQUIVOCAL
M3	2228693 1 (Query)		Glucuronidation of Carboxylic Acids	C17H14F17NO10S	747.0067	747	1.79	Phase II	UGT	PLAUSIBLE
M33	M11		Glucuronidation of Carboxylic Acids	C16H12F17NO10S	732.991	733	1.87	Phase II	UGT	PLAUSIBLE
M35	M11	I49a, I50a, I51a	Glucuronidation of Carboxylic Acids	C16H12F17NO10S	732.991	733	1.37	Phase II	UGT	EQUIVOCAL
M36	M11	I52a, I53a, I54a	Glucuronidation of Carboxylic Acids	C16H12F17NO10S	732.991	733	2.03	Phase II	UGT	EQUIVOCAL
M37	M11	I55a, I56a, I57a	Glucuronidation of Carboxylic Acids	C16H12F17NO10S	732.991	733	1.37	Phase II	UGT	EQUIVOCAL
M38	M11	I58b	Conjugation of Carboxylic Acids with Glutamine	C15H12F17N3O6S	685.0175	685	1.87	Phase II	ACS, AANAT	EQUIVOCAL

Name	Parent	Intermediates	Biotransformation Name	Formula	Exact Mass	Nominal Mass	LogP	Phase	Enzyme	Likelihood
M39	M11		N-Glucuronidation of Amides and Related Compounds	C16H12F17NO10S	732.991	733	2.39	Phase I	UGT	EQUIVOCAL
M4	M11		Decarboxylation of alpha-Amino, Aromatic and beta-Keto Carboxylic Acids	C9H4F17NO2S	512.9691	513	3.83	Phase I	Decarboxylase /AAAD	EQUIVOCAL
M4	2228693 1 (Query)	I1o	Oxidative N-Dealkylation	C9H4F17NO2S	512.9691	513	3.83	Phase I	CYP450	PROBABLE
M4	M1	I18o	Oxidative N-Demethylation	C9H4F17NO2S	512.9691	513	3.83	Phase I	CYP450	PROBABLE
M40	M39	I71o	Oxidative N-Dealkylation	C14H10F17NO8S	674.9856	675	2.12	Phase I	CYP450	EQUIVOCAL
M40	M13		N-Glucuronidation of Amides and Related Compounds	C14H10F17NO8S	674.9856	675	2.12	Phase I	UGT	EQUIVOCAL
M40	M14	I69o	Oxidative N-Demethylation	C14H10F17NO8S	674.9856	675	2.12	Phase I	CYP450	PROBABLE
M42	M14		Glucuronidation of Carboxylic Acids	C21H20F17NO14S	865.0333	865	0.32	Phase II	UGT	PLAUSIBLE
M47	M14		Oxidation of Secondary (Alicyclic) Alcohols	C15H10F17NO8S	686.9856	687	1.89	Phase I	ADH	PROBABLE
M48	M14		Oxidation of Secondary (Alicyclic) Alcohols	C15H10F17NO8S	686.9856	687	2.01	Phase I	ADH	PROBABLE
M49	M14		Oxidation of Secondary (Alicyclic) Alcohols	C15H10F17NO8S	686.9856	687	0.18	Phase I	ADH	PROBABLE
M5	M11	I44o, I45a	Oxidative N-Dealkylation	C2H2O4	89.9953	90	-1.75	Phase I	CYP450	EQUIVOCAL
M5	M39	I72o, I73a	Oxidative N-Dealkylation	C2H2O4	89.9953	90	-1.75	Phase I	CYP450	EQUIVOCAL
M5	2228693 1 (Query)	I2o, I3a	Oxidative N-Dealkylation	C2H2O4	89.9953	90	-1.75	Phase I	CYP450	PROBABLE
M5	M6	I32a	Oxidation of Primary Alcohols	C2H2O4	89.9953	90	-1.75	Phase I	ADH	EQUIVOCAL
M50	M14	I60a, I61a, I62a	Glucuronidation of Carboxylic Acids	C21H20F17NO14S	865.0333	865	-0.17	Phase II	UGT	EQUIVOCAL
M51	M14	I63a, I64a, I65a	Glucuronidation of Carboxylic Acids	C21H20F17NO14S	865.0333	865	0.48	Phase II	UGT	EQUIVOCAL
M52	M14	I66a, I67a, I68a	Glucuronidation of Carboxylic Acids	C21H20F17NO14S	865.0333	865	-0.17	Phase II	UGT	EQUIVOCAL
M53	M14		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H20F17NO14S	865.0333	865	0.1	Phase II	UGT	EQUIVOCAL
M54	M14		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H20F17NO14S	865.0333	865	0.75	Phase II	UGT	EQUIVOCAL

Name	Parent	Intermediates	Biotransformation Name	Formula	Exact Mass	Nominal Mass	LogP	Phase	Enzyme	Likelihood
M55	M14		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C21H20F17NO14S	865.0333	865	0.1	Phase II	UGT	EQUIVOCAL
M59	M14	I70b	Conjugation of Carboxylic Acids with Glutamine	C20H20F17N3O10S	817.0598	817	0.49	Phase II	ACS, AANAT	EQUIVOCAL
M6	M11	I46o, I47a	Oxidative N-Dealkylation	C2H4O3	76.016	76	-1.04	Phase I	CYP450	EQUIVOCAL
M6	M39	I74o, I75a	Oxidative N-Dealkylation	C2H4O3	76.016	76	-1.04	Phase I	CYP450	EQUIVOCAL
M6	2228693 1 (Query)	I4o, I5a	Oxidative N-Dealkylation	C2H4O3	76.016	76	-1.04	Phase I	CYP450	PROBABLE
M62	M39		Glucuronidation of Carboxylic Acids	C22H20F17NO16S	909.0231	909	0.62	Phase II	UGT	PLAUSIBLE
M63	M39		Glucuronidation of Carboxylic Acids	C22H20F17NO16S	909.0231	909	0.3	Phase II	UGT	PLAUSIBLE
M69	M39		Oxidation of Secondary (Alicyclic) Alcohols	C16H10F17NO10S	730.9754	731	2.18	Phase I	ADH	PROBABLE
M70	M39		Oxidation of Secondary (Alicyclic) Alcohols	C16H10F17NO10S	730.9754	731	2.3	Phase I	ADH	PROBABLE
M71	M39		Oxidation of Secondary (Alicyclic) Alcohols	C16H10F17NO10S	730.9754	731	0.47	Phase I	ADH	PROBABLE
M72	M39	I78a, I79a, I80a	Glucuronidation of Carboxylic Acids	C22H20F17NO16S	909.0231	909	0.12	Phase II	UGT	EQUIVOCAL
M73	M39	I81a, I82a, I83a	Glucuronidation of Carboxylic Acids	C22H20F17NO16S	909.0231	909	-0.2	Phase II	UGT	EQUIVOCAL
M74	M39	I84a, I85a, I86a	Glucuronidation of Carboxylic Acids	C22H20F17NO16S	909.0231	909	0.77	Phase II	UGT	EQUIVOCAL
M75	M39	I87a, I88a, I89a	Glucuronidation of Carboxylic Acids	C22H20F17NO16S	909.0231	909	0.46	Phase II	UGT	EQUIVOCAL
M76	M39	I90a, I91a, I92a	Glucuronidation of Carboxylic Acids	C22H20F17NO16S	909.0231	909	0.12	Phase II	UGT	EQUIVOCAL
M77	M39	I93a, I94a, I95a	Glucuronidation of Carboxylic Acids	C22H20F17NO16S	909.0231	909	-0.2	Phase II	UGT	EQUIVOCAL
M78	M39		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C22H20F17NO16S	909.0231	909	0.39	Phase II	UGT	EQUIVOCAL
M79	M39		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C22H20F17NO16S	909.0231	909	1.05	Phase II	UGT	EQUIVOCAL

Name	Parent	Intermediates	Biotransformation Name	Formula	Exact Mass	Nominal Mass	LogP	Phase	Enzyme	Likelihood
M8	2228693 1 (Query)	I7a, I8a, I9a	Glucuronidation of Carboxylic Acids	C17H14F17NO10S	747.0067	747	1.29	Phase II	UGT	EQUIVOCAL
M80	M39		Glucuronidation of Primary and Secondary Aliphatic and Benzylic Alcohols	C22H20F17NO16S	909.0231	909	0.39	Phase II	UGT	EQUIVOCAL
M84	M39	I96b	Conjugation of Carboxylic Acids with Glutamine	C21H20F17N3O12S	861.0496	861	0.3	Phase II	ACS, AANAT	EQUIVOCAL
M85	M39	I97b	Conjugation of Carboxylic Acids with Glutamine	C21H20F17N3O12S	861.0496	861	0.78	Phase II	ACS, AANAT	EQUIVOCAL
M9	2228693 1 (Query)	I10a, I11a, I12a	Glucuronidation of Carboxylic Acids	C17H14F17NO10S	747.0067	747	1.94	Phase II	UGT	EQUIVOCAL

Table SM13. Individual concentrations (ng/mL) of PFOS and PFOS precursors in serum samples (n = 60)

Sample	PFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
01	4.509	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.079	<LOQ
02	6.657	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
03	3.938	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
04	2.918	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.107	<LOQ
05	3.688	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
06	4.313	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.057	<LOQ
07	4.029	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
08	3.296	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
09	1.303	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.016
10	7.012	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
11	2.403	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
12	4.618	<LOQ	0.013	<LOQ	1.167	<LOQ	<LOQ	<LOQ	<LOQ
13	2.785	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
14	4.083	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
15	16.525	<LOQ	<LOQ	<LOQ	<LOQ	1.113	<LOQ	<LOQ	<LOQ
16	3.328	<LOQ	<LOQ	<LOQ	<LOQ	0.614	<LOQ	0.225	<LOQ
18	5.168	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
19	5.397	<LOQ	<LOQ	<LOQ	<LOQ	3.739	<LOQ	<LOQ	<LOQ
20	0.965	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
21	3.073	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
22	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
23	4.330	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Sample	PFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
24	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
25	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
26	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
27	9.875	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.189	<LOQ
28	11.598	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.100	<LOQ
29	4.047	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
30	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
31	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.136	<LOQ
32	2.573	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
33	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
34	4.768	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
35	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	14.717
36	11.504	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
40	8.508	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.379	<LOQ
41	4.163	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
37	14.308	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
38	8.725	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
39	16.229	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.180	<LOQ
42	15.661	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
43	5.450	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
44	4.290	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
45	4.103	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.015	<LOQ
46	4.928	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Sample	PFOS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	FOSAA	MeFOSAA	EtFOSAA
47	12.641	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
48	3.767	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
49	4.996	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
50	6.890	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
51	6.156	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
52	5.356	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.150	<LOQ
53	12.173	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
54	3.718	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.159	<LOQ
55	3.185	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
56	6.230	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.108	<LOQ
58	2.884	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
59	2.469	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
60	7.481	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.108	<LOQ
61	8.975	0.003	0.005	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
62	5.482	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Table SM14. Individual ratios of linear:branched PFOS isomers in serum samples (n = 40)

Sample	n-PFOS / ΣBr-PFOS	n-PFOS (%)	ΣBr-PFOS (%)
01	1.22	54.87	45.13
02	1.06	51.41	48.59
03	4.49	81.79	18.21
04	2.59	72.15	27.85
05	2.40	70.62	29.38
06	2.64	72.56	27.44
07	1.99	66.56	33.44
08	4.60	82.15	17.85
09	2.15	68.23	31.77
10	3.02	75.12	24.88
11	1.26	55.74	44.26
12	2.09	67.64	32.36
13	2.88	74.20	25.80
14	2.14	68.14	31.86
15	1.75	1.99	36.35
16	2.62	2.17	27.64
18	2.37	1.82	29.66
19	2.21	2.42	31.17
20	2.51	1.75	28.49
21	3.53	77.93	22.07
22	1.22	54.87	45.13
23	1.06	51.41	48.59
24	4.49	81.79	18.21
25	2.59	72.15	27.85
26	2.40	70.62	29.38
27	2.64	72.56	27.44
28	1.99	66.56	33.44
29	4.60	82.15	17.85
30	2.15	68.23	31.77
31	3.02	75.12	24.88
32	1.26	55.74	44.26
33	2.09	67.64	32.36
34	2.88	74.20	25.80
35	2.14	68.14	31.86
36	1.75	63.65	36.35
37	2.62	72.36	27.64

Sample	n-PFOS / ΣBr-PFOS	n-PFOS (%)	ΣBr-PFOS (%)
38	2.37	70.34	29.66
39	2.21	68.83	31.17
40	1.82	64.51	35.49
41	3.53	77.93	22.07

Table SM15. Individual daily intakes of ΣPFAS (ng/day and ng/kg bw/day) through dust and food ingestion (n = 46)

Sample	Sum dust + food (ng/day)	Sum dust + food (ng/kg bw/day)
01	0.497	0.008
02	0.468	0.007
04	0.494	0.009
05	0.746	0.012
08	0.877	0.012
10	0.880	0.010
11	0.589	0.010
12	0.504	0.008
13	0.514	0.007
14	1.231	0.022
17	1.176	0.017
21	57.391	0.883
22	18.923	0.270
23	0.468	0.006
24	0.917	0.017
25	64.287	1.169
26	0.530	0.010
27	0.738	0.008
28	24.861	0.303
29	9.093	0.157
32	1.084	0.016
33	0.569	0.009
34	140.286	2.551
35	0.679	0.009
36	0.577	0.008
37	0.764	0.014
39	0.862	0.009
40	0.666	0.007
41	0.713	0.008
42	25.734	0.271
43	58.794	0.933
44	8.841	0.111
45	0.735	0.011
46	93.366	1.796

Sample	Sum dust + food (ng/day)	Sum dust + food (ng/kg bw/day)
47	40.527	0.335
48	83.199	1.434
49	44.853	0.712
50	1.448	0.019
51	5.019	0.063
52	0.794	0.011
54	1.183	0.020
55	27.667	0.461
56	17.050	0.299
58	0.968	0.016
59	0.995	0.008
60	7.881	0.102

SUPPLEMENTARY FIGURES

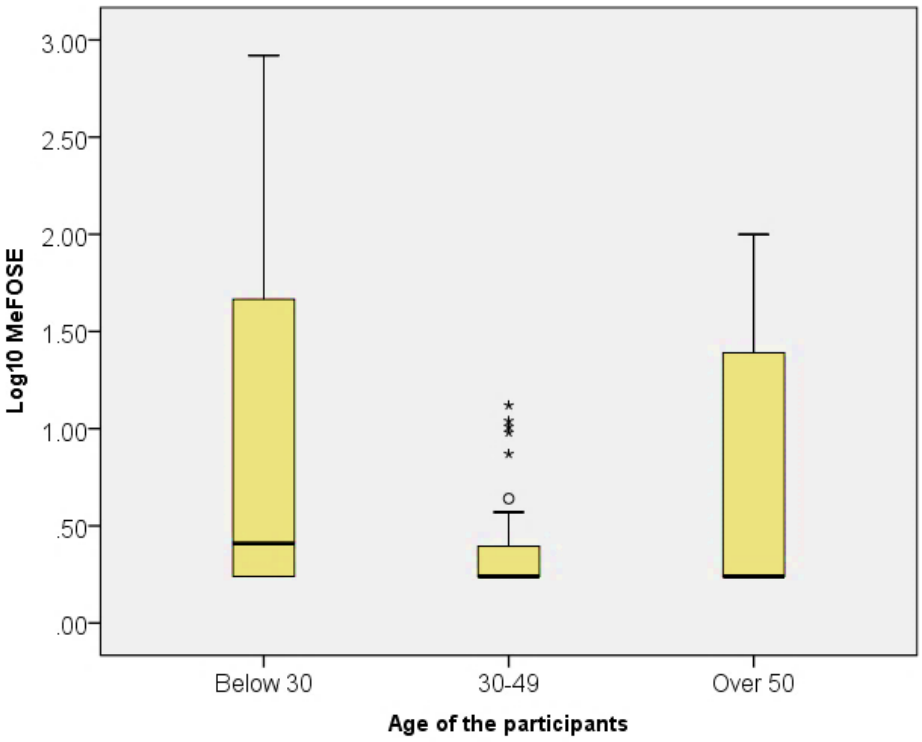


Figure SM01. Box plot showing significant differences ($p < 0.05$) found among the three groups of ages when the concentrations of individual MeFOSE in dust was considered

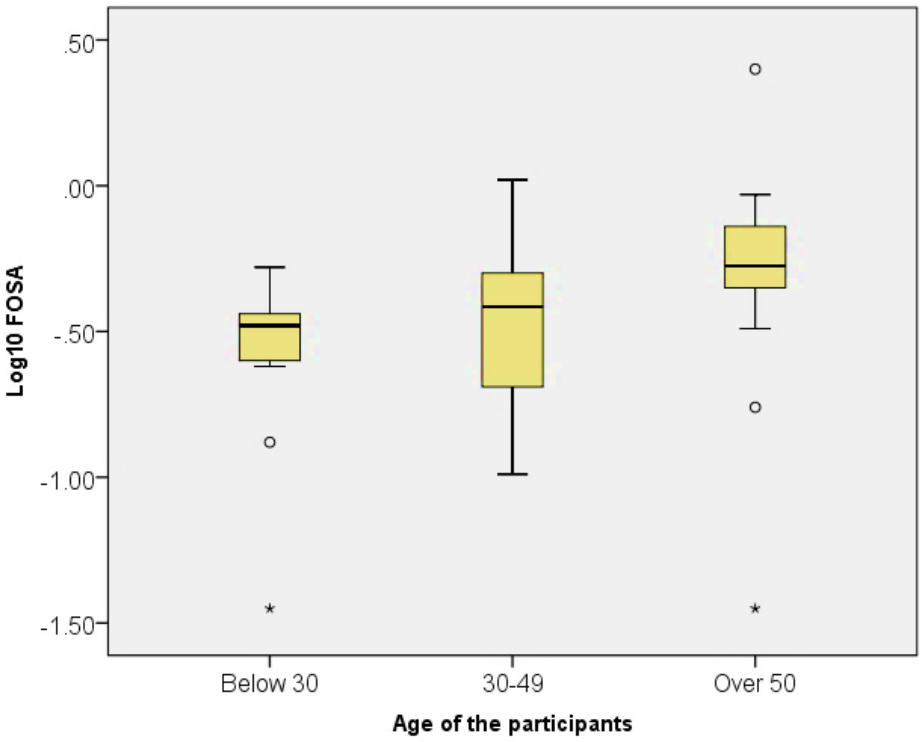


Figure SM02. Box plot showing increasing concentrations of FOSA according to the age of the participants in dust samples, even though no significant differences were found

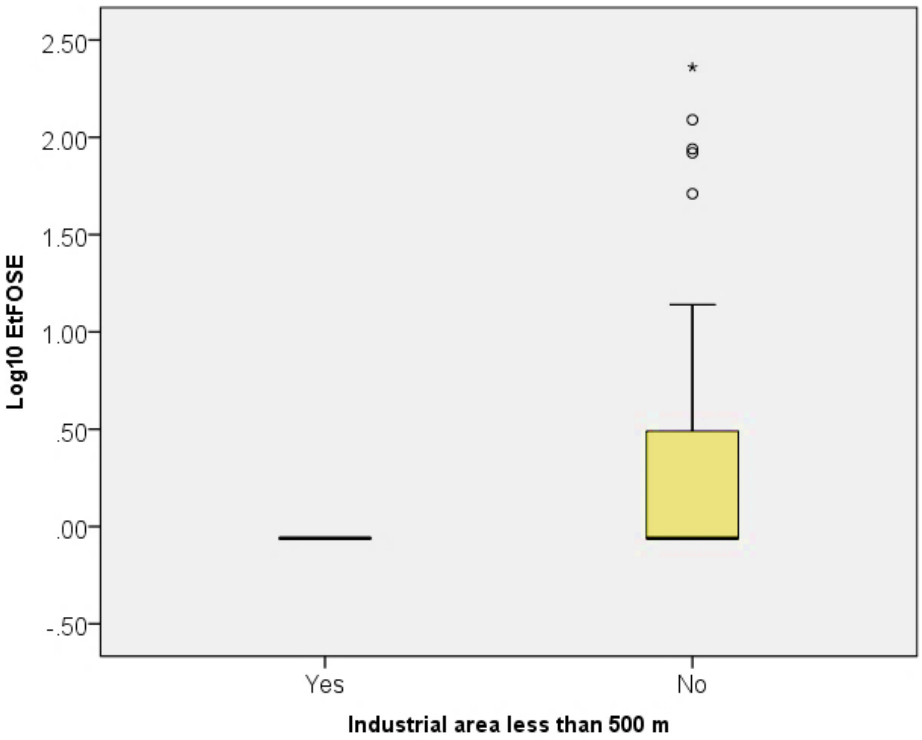


Figure SM03. Box plot showing significant differences ($p < 0.05$) between the participants whose residences were close to industrial areas the ones not when the concentrations of individual EtFOSE in dust samples were considered

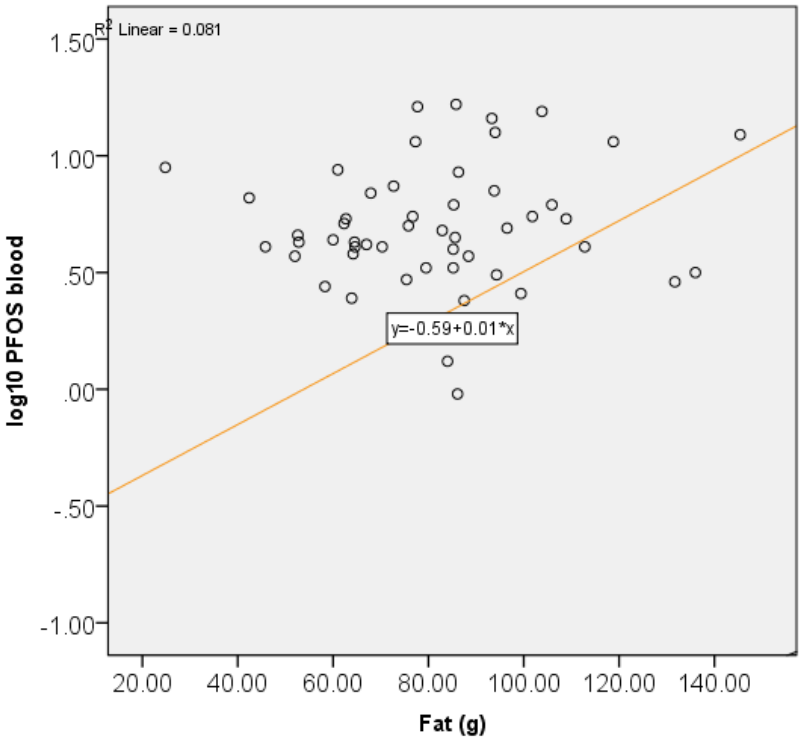


Figure SM04. Scatter plot with trends of levels of PFOS in blood for female participants of the cohort according to age

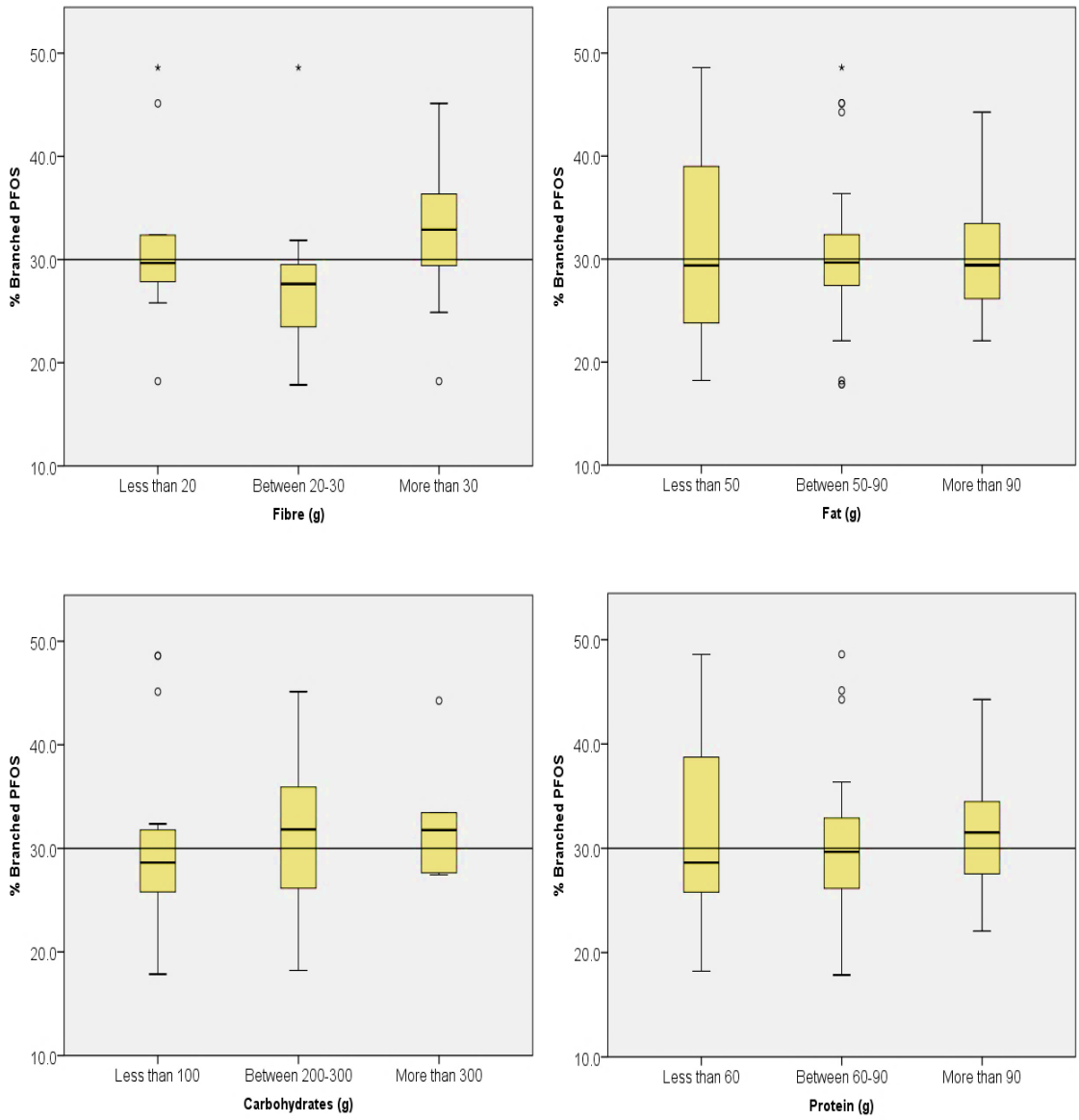
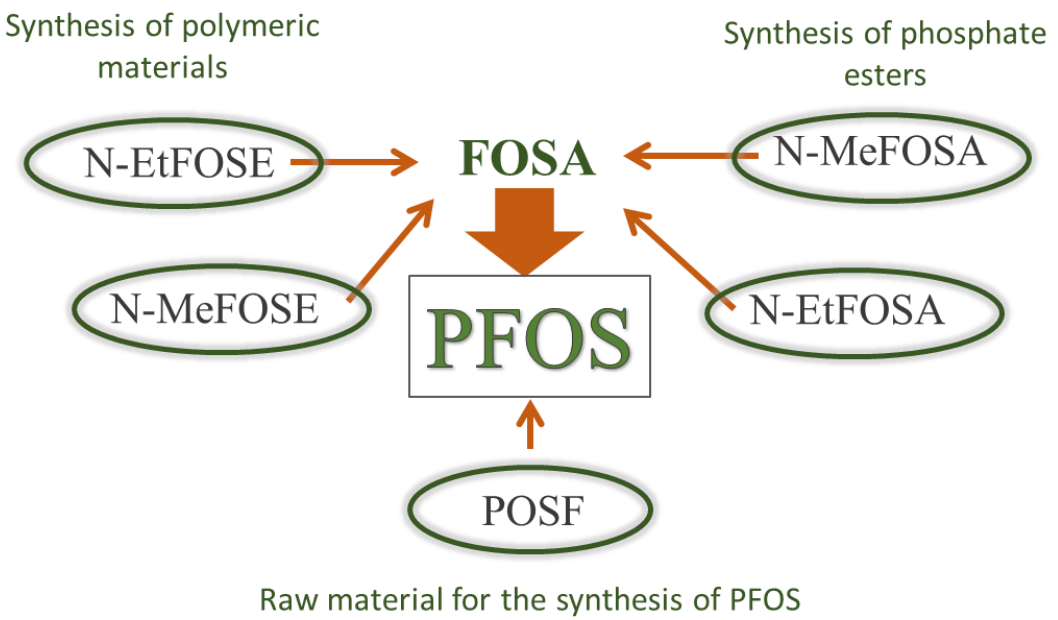


Figure SM5. Scatter plots with trends of percentages of branched PFOS in blood according to their fat, fibre, carbohydrates and proteins from the FFQ. No significant differences were identified



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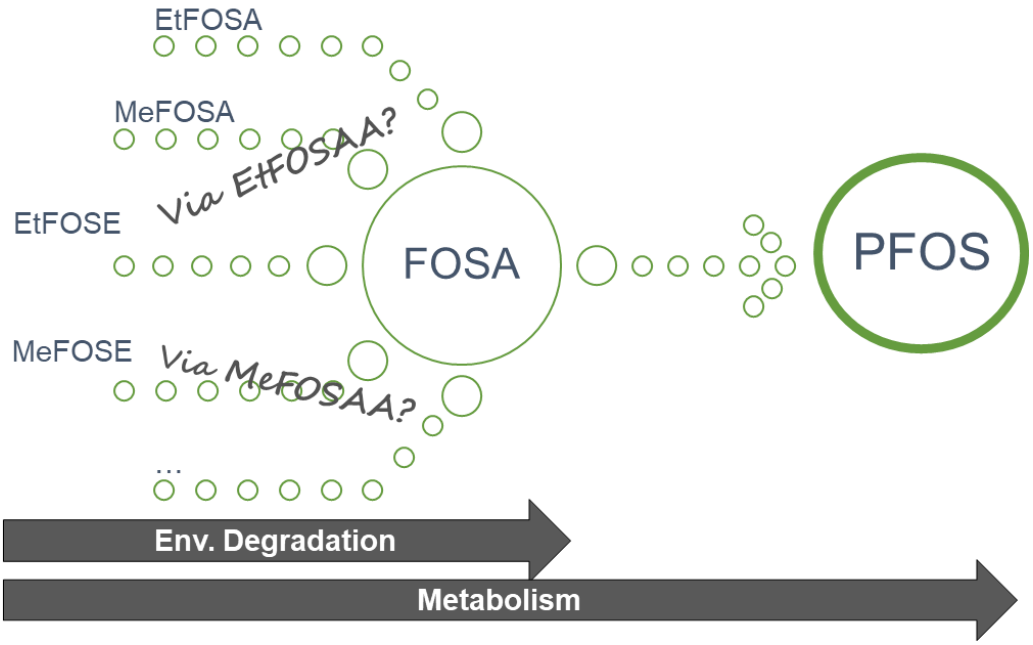

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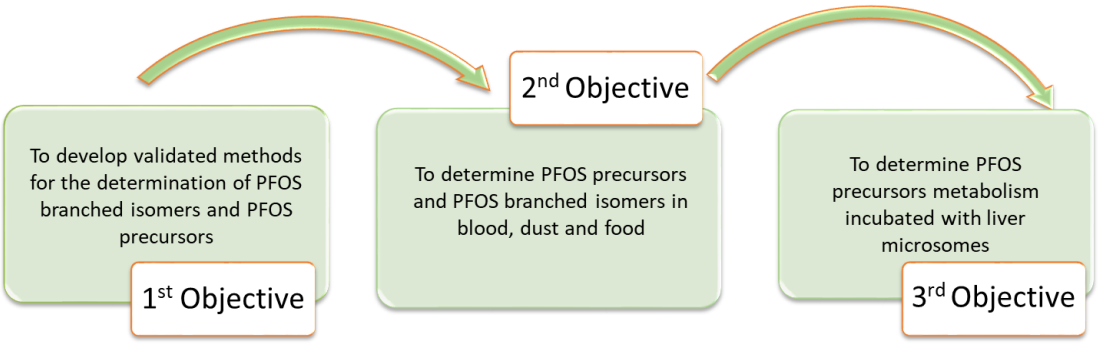

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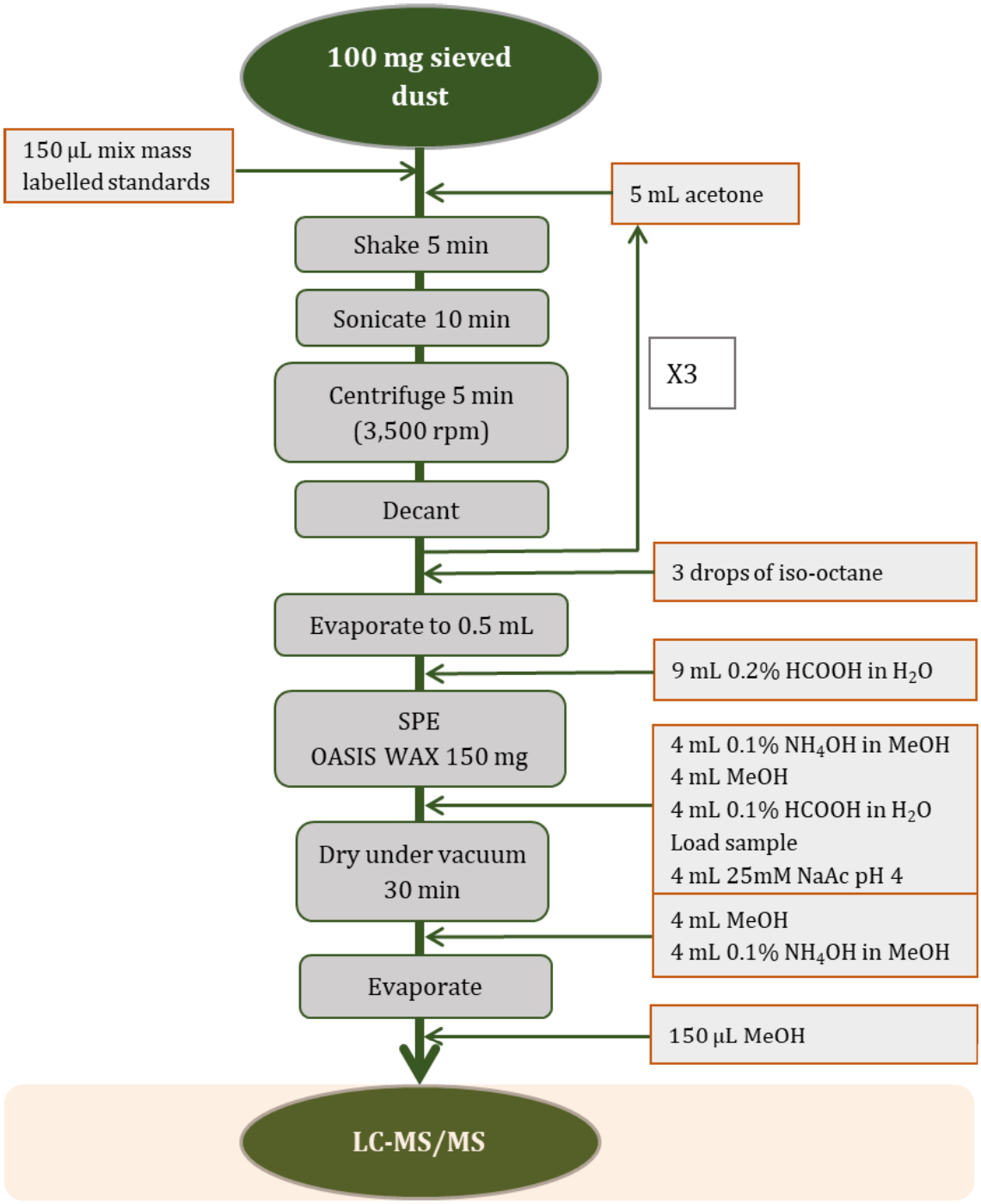

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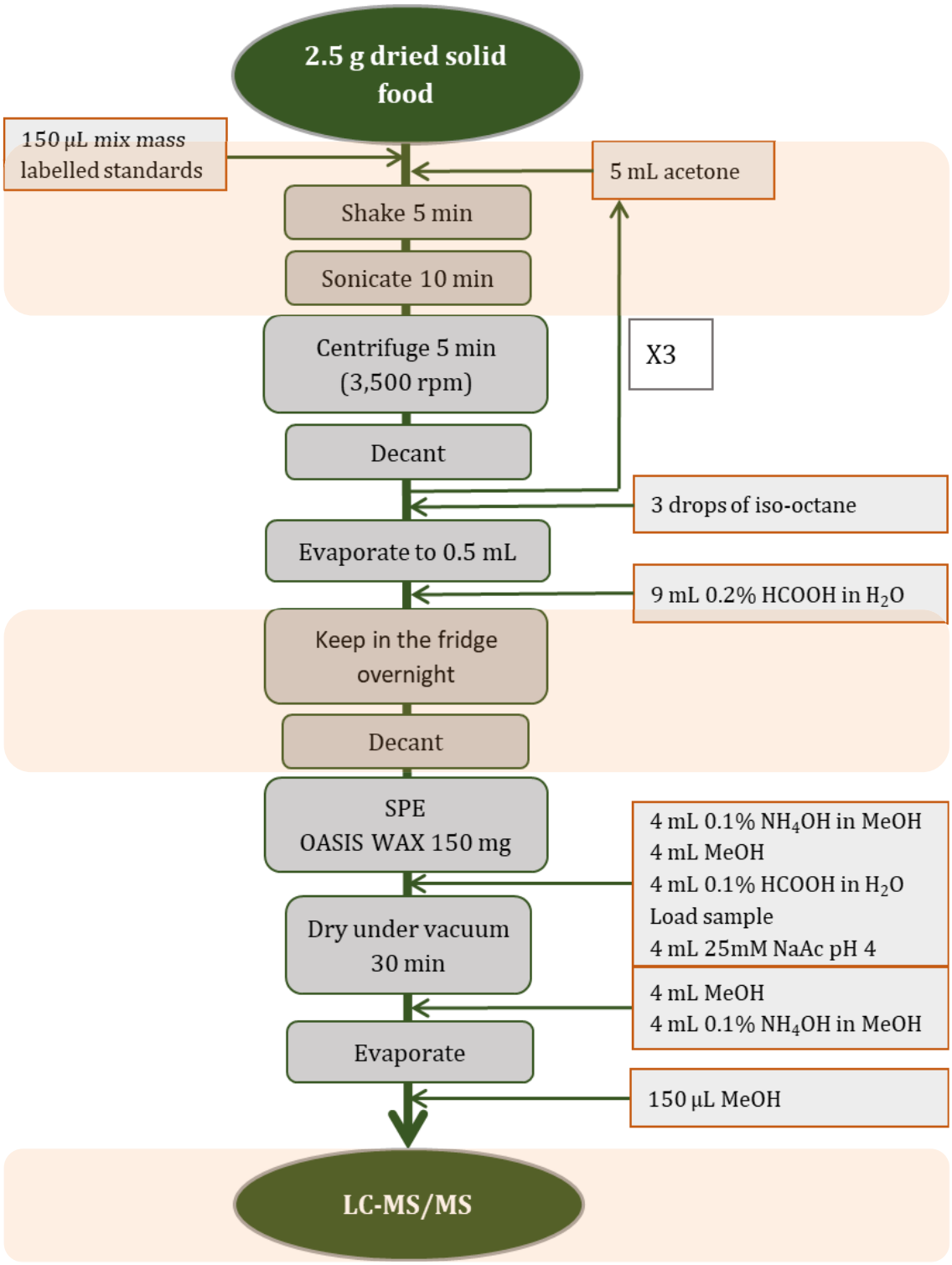

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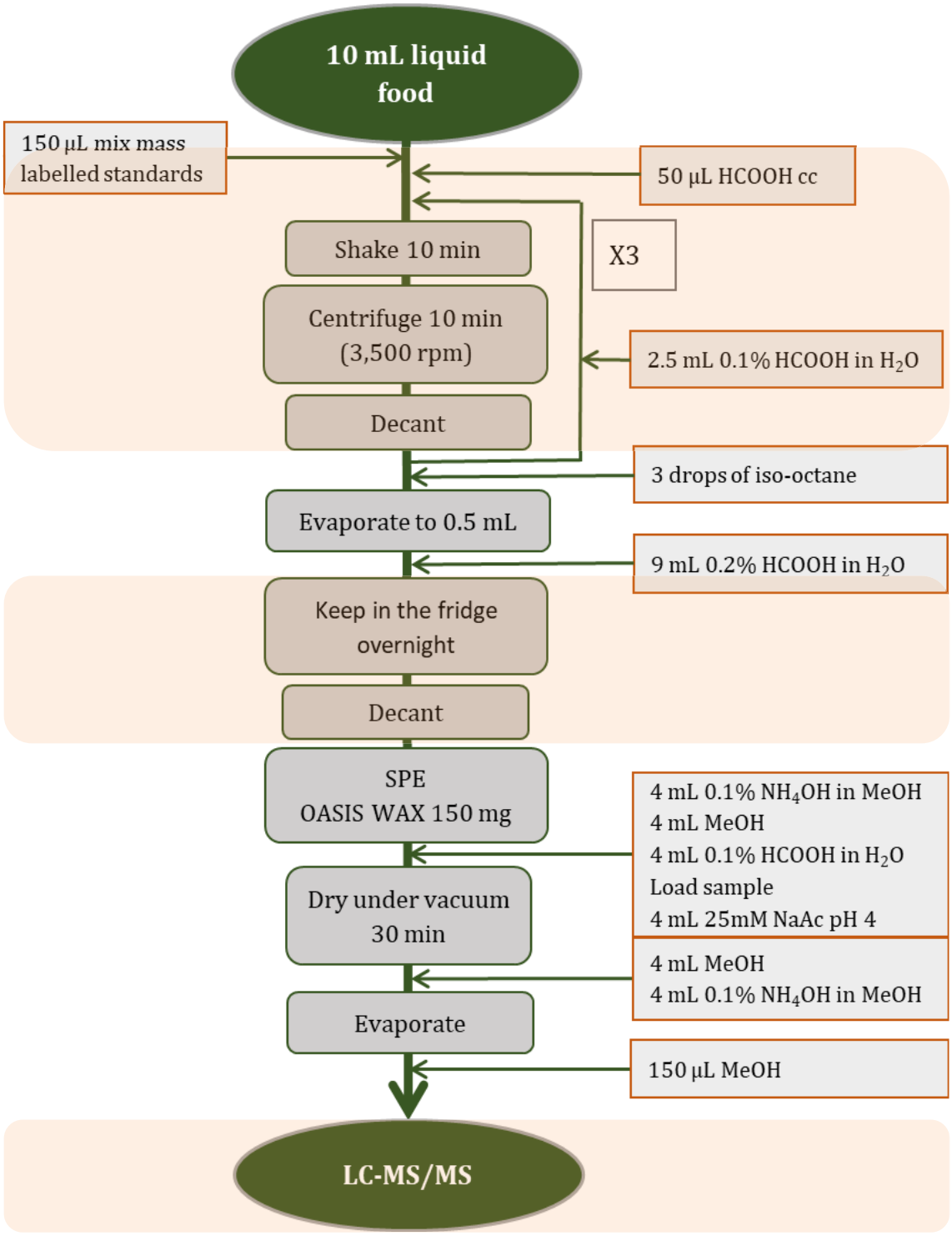

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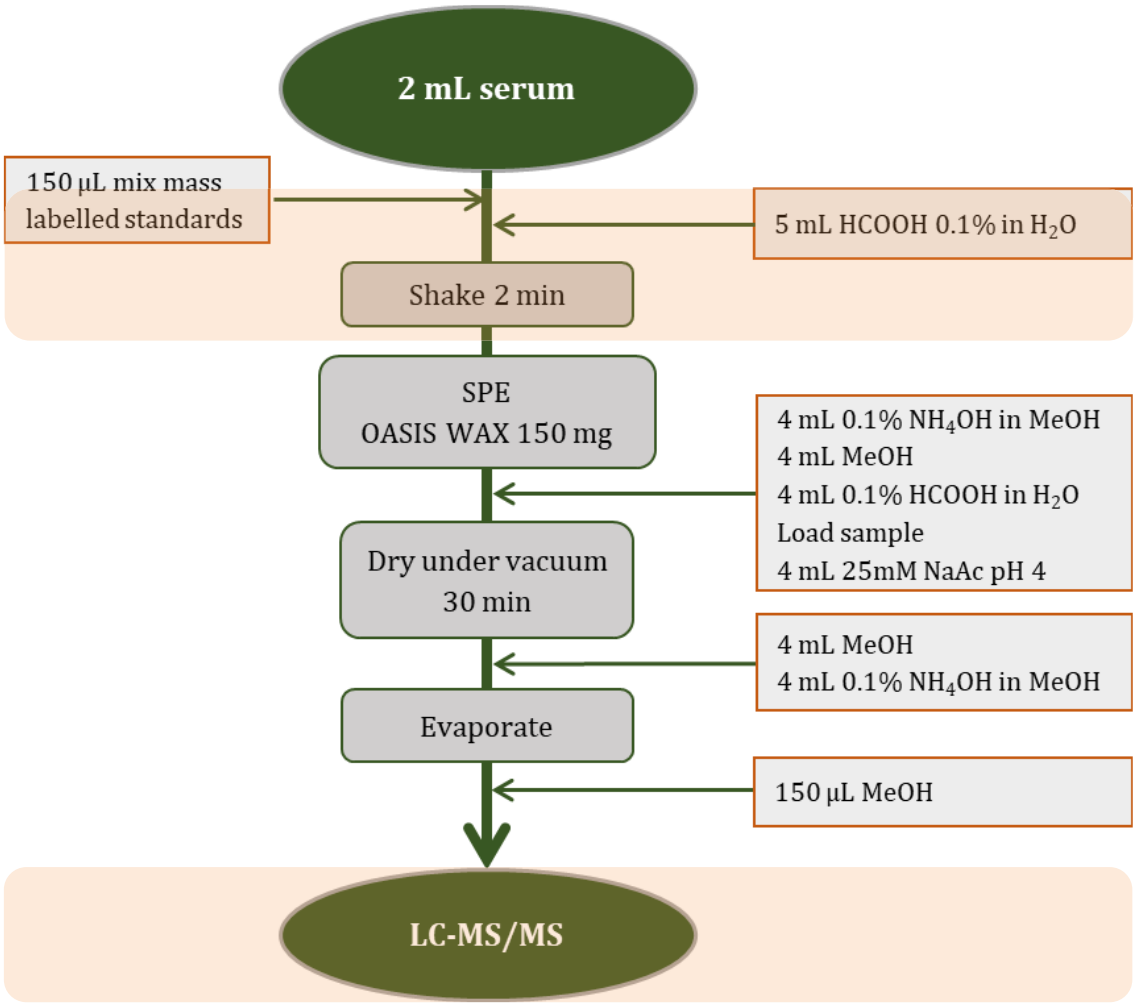

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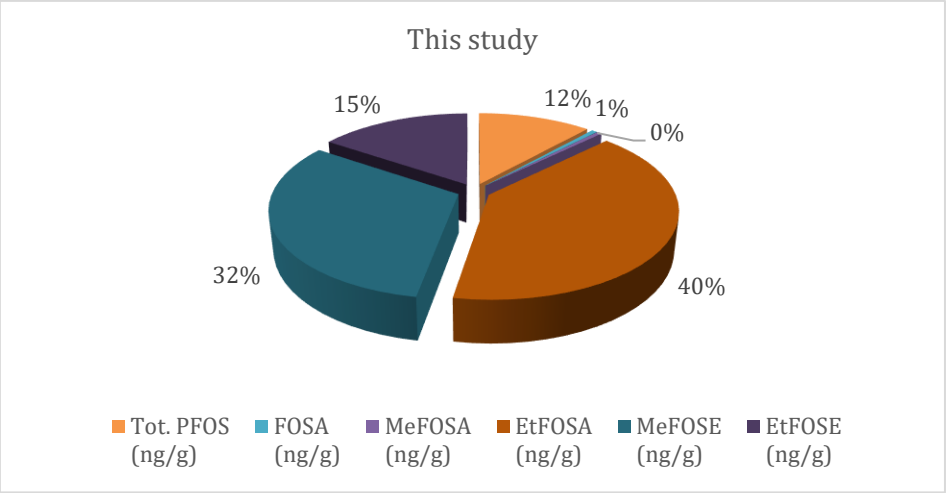

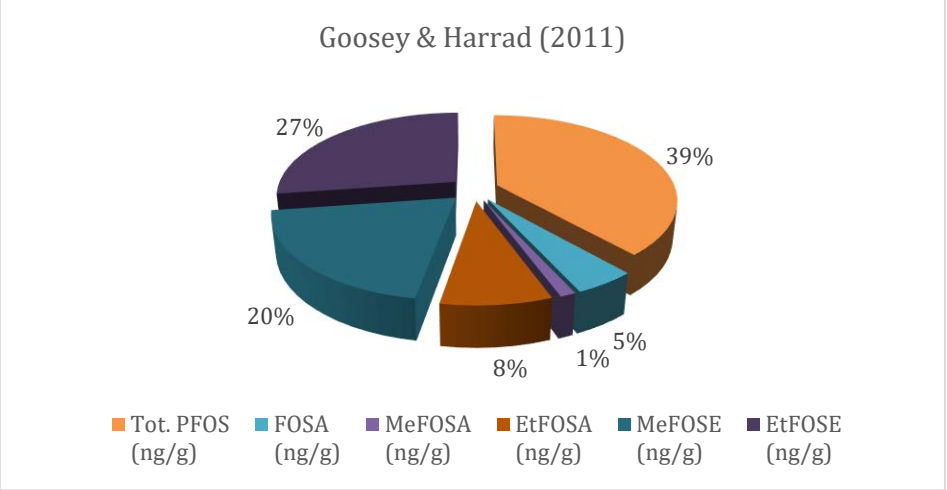

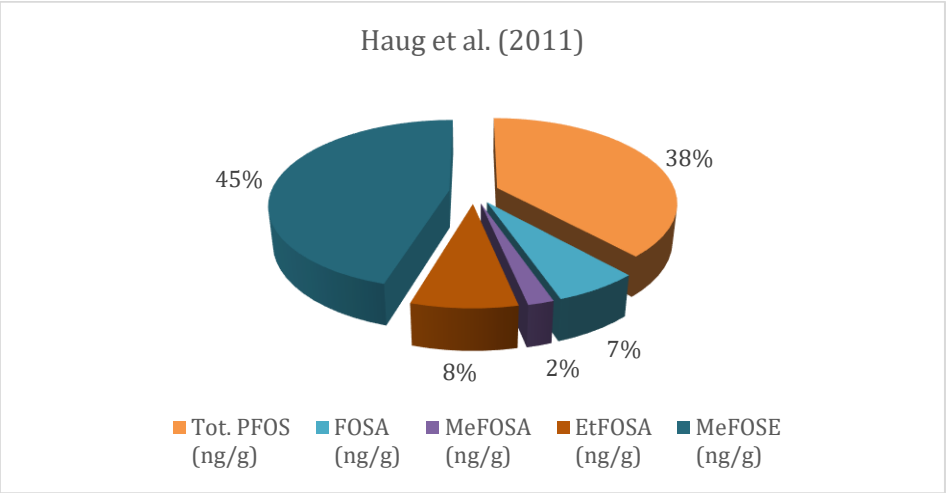

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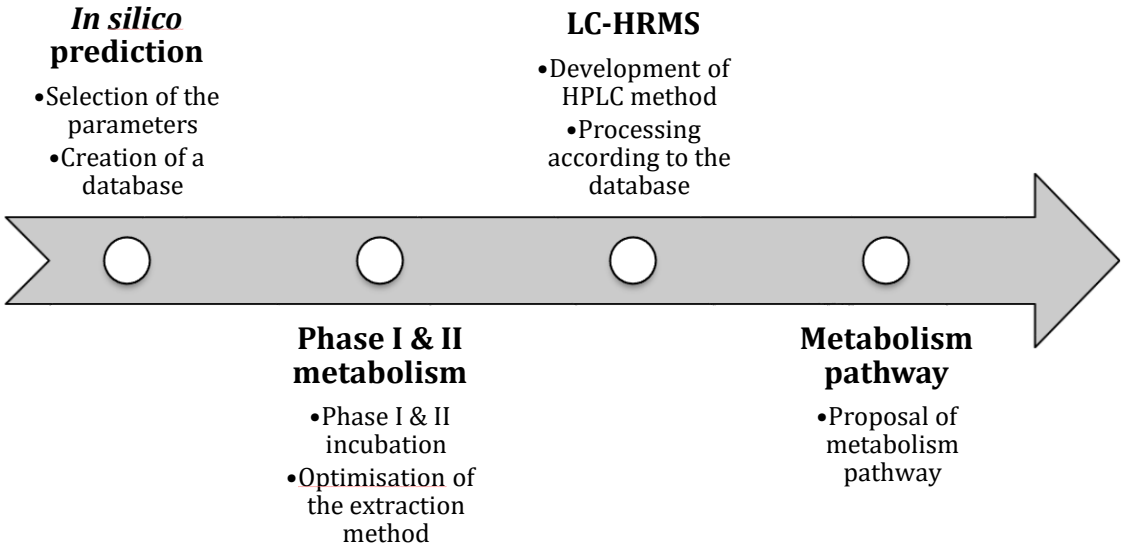

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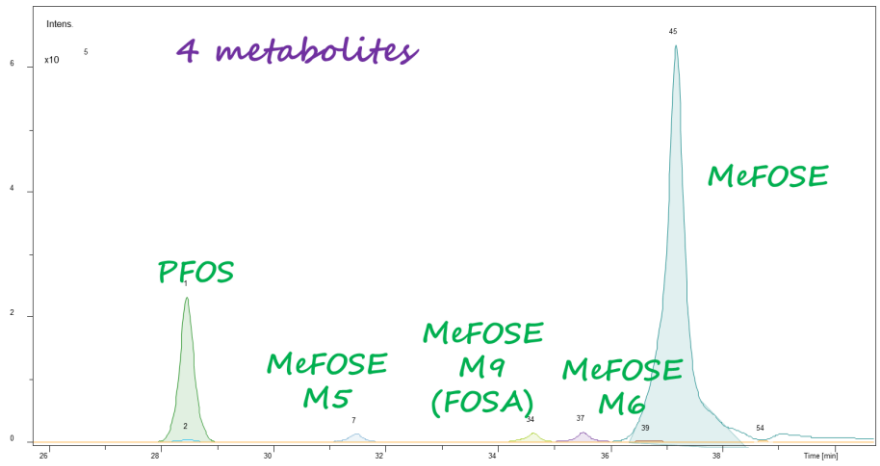

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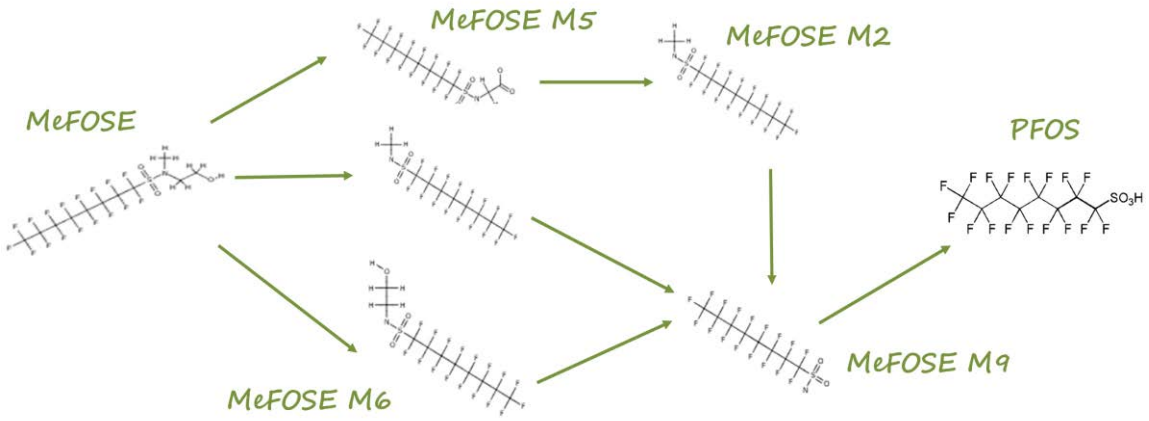

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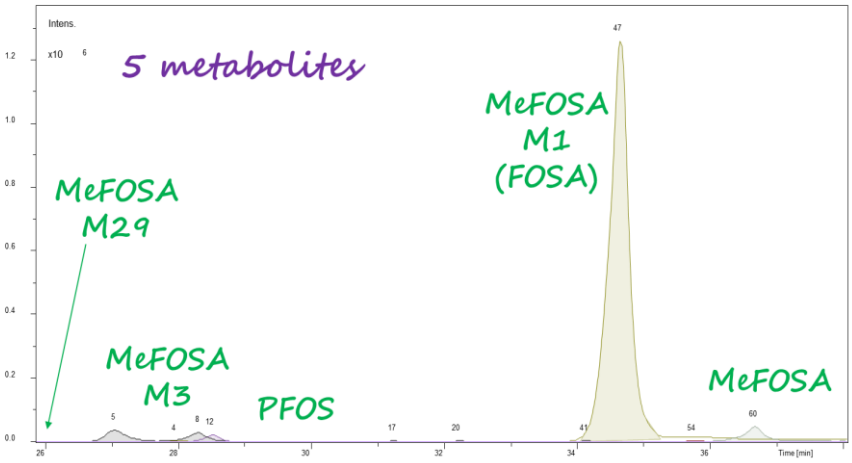

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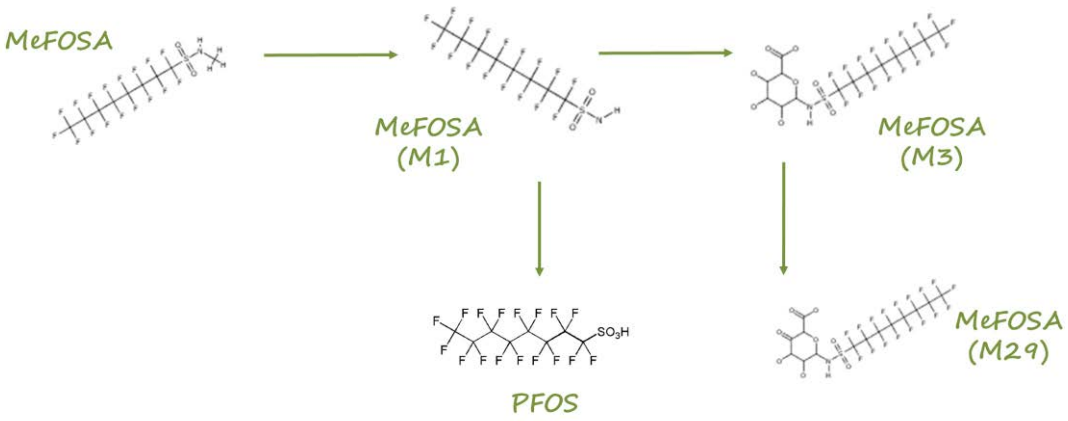

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Figure 29 shows the scheme of the two suggested routes for MeFOSA metabolism, one of them leading to PFOS as end-product, and the second one leading to a second product M29, without possibility to track its possible conversion to PFOS. For the

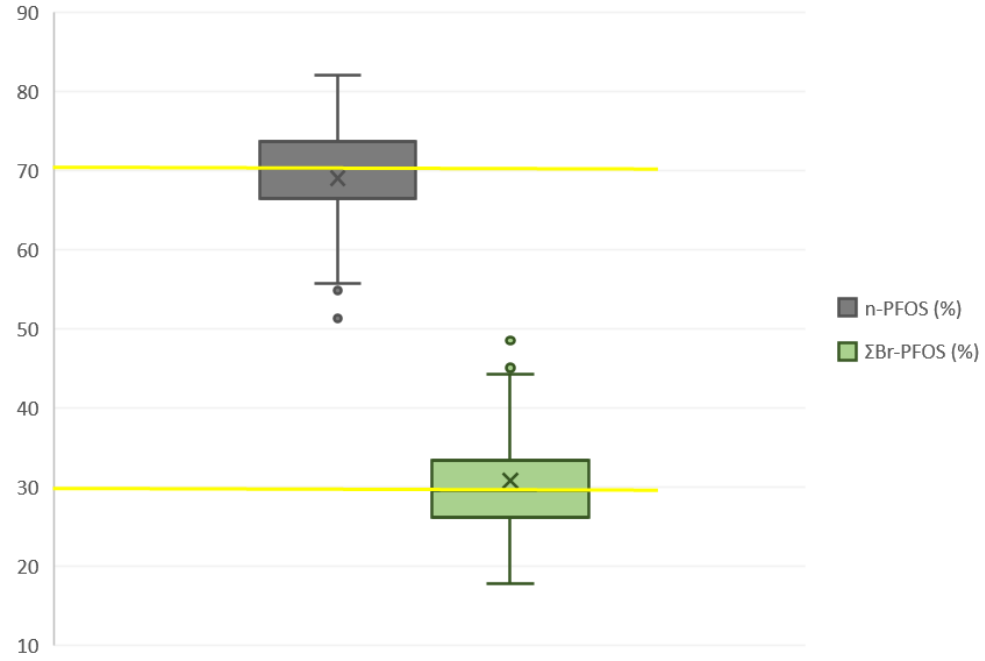


Figure 34. Box plots representing the percentages of linear and branched PFOS isomers identified in the serum samples (n = 40), where the yellow lines represent the theoretical percentage of the commercial PFOS mixture (70 % linear and 30 % branched isomers)

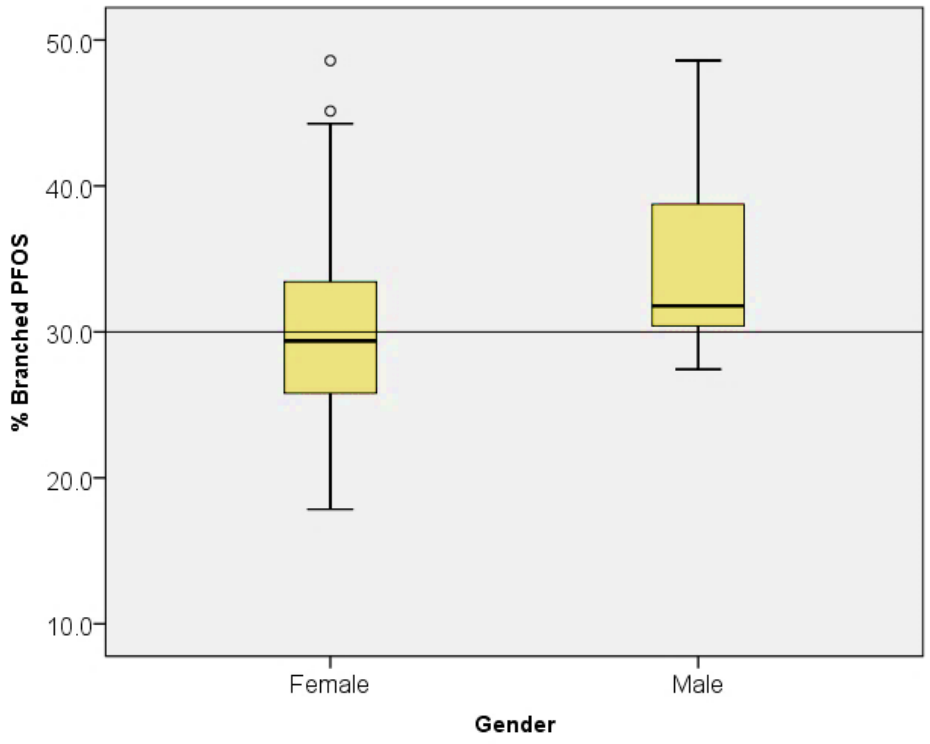
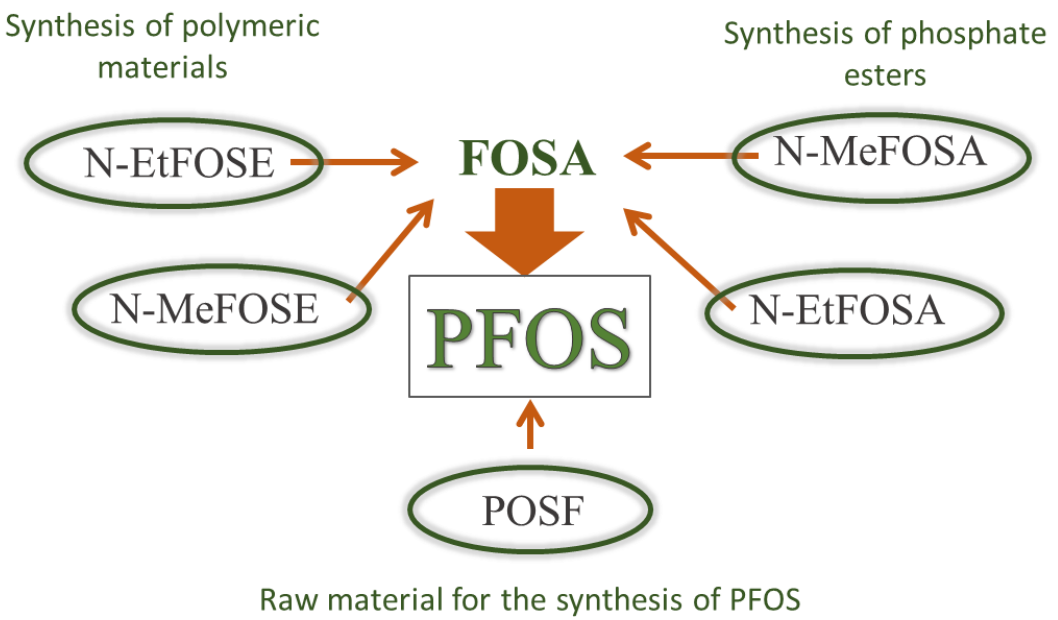


Figure 35. Box plots showing the percentages of branched PFOS isomers for male and female participants identified in the serum samples



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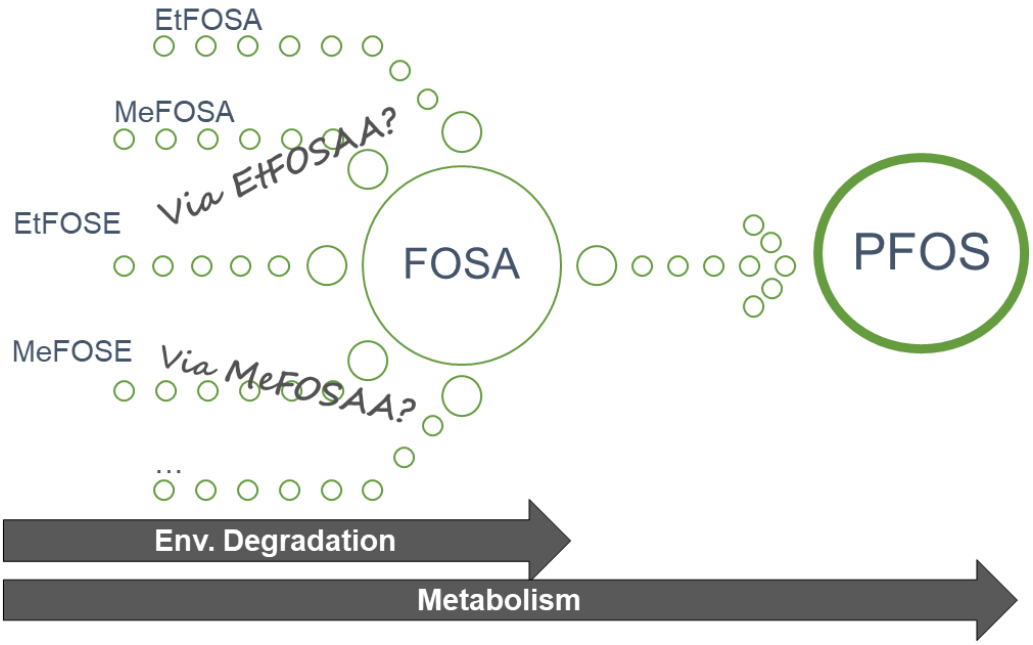

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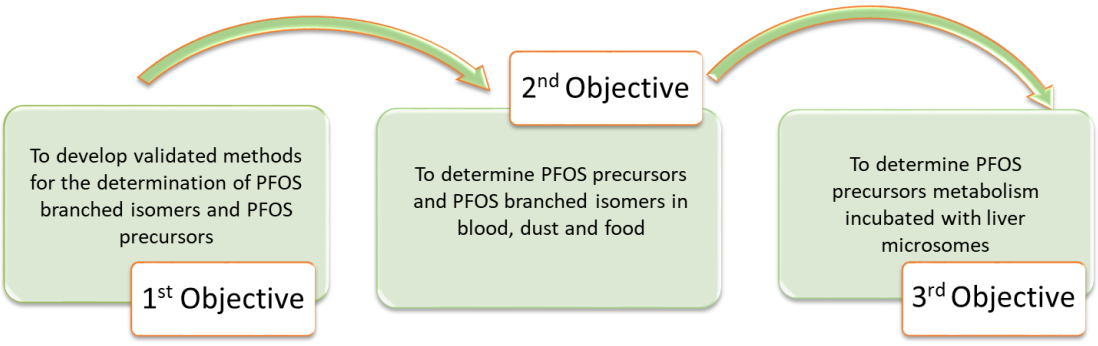

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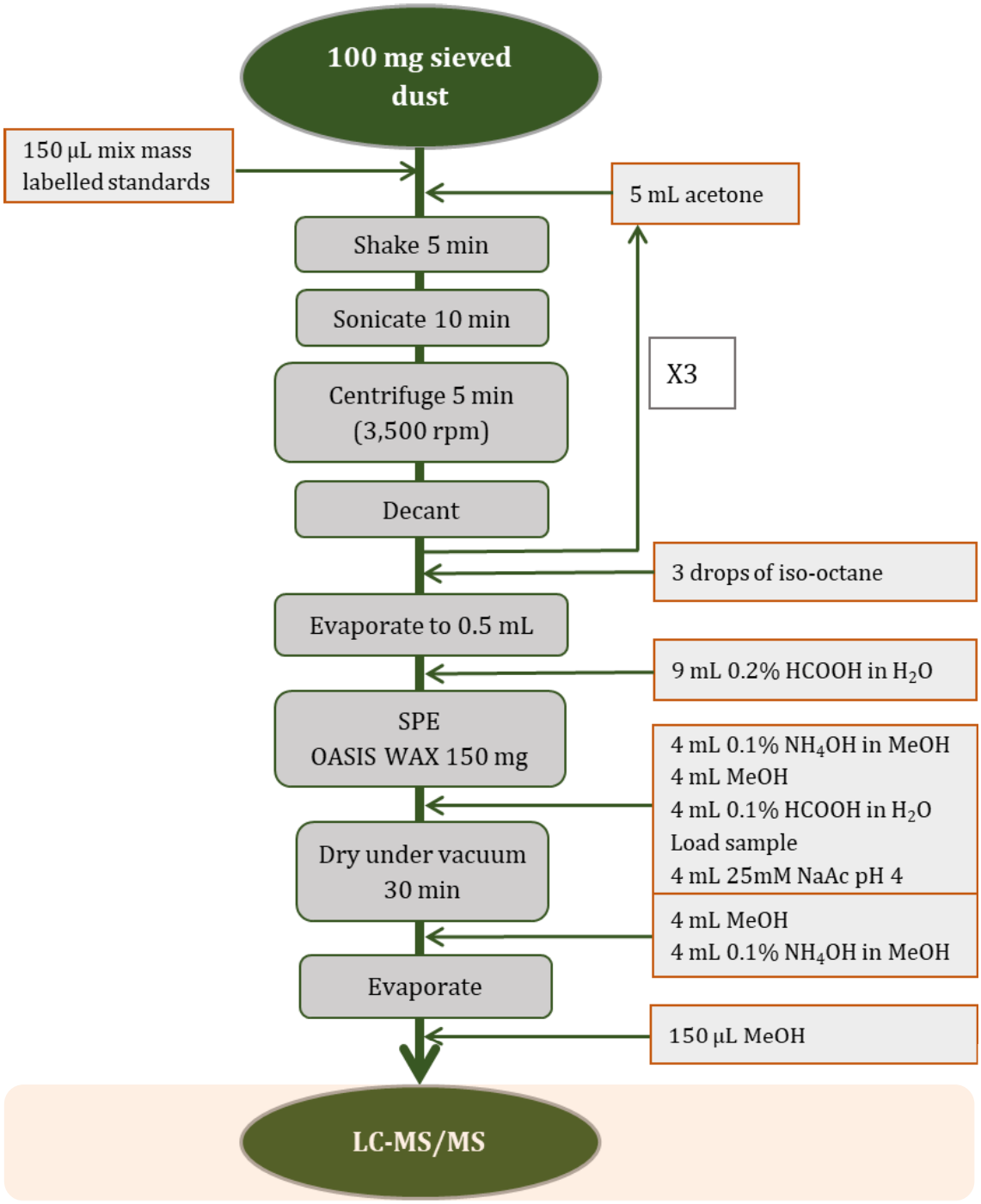

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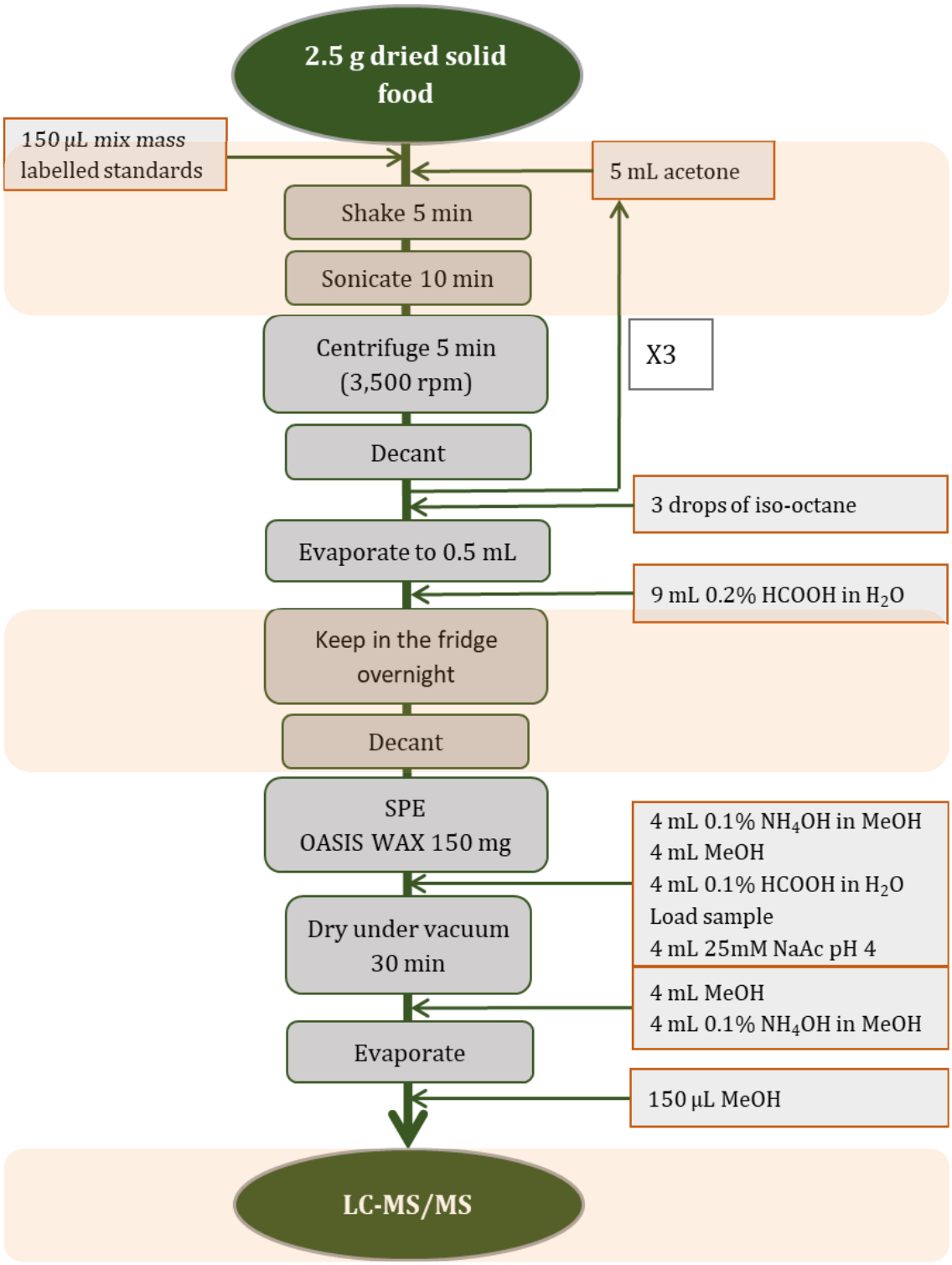

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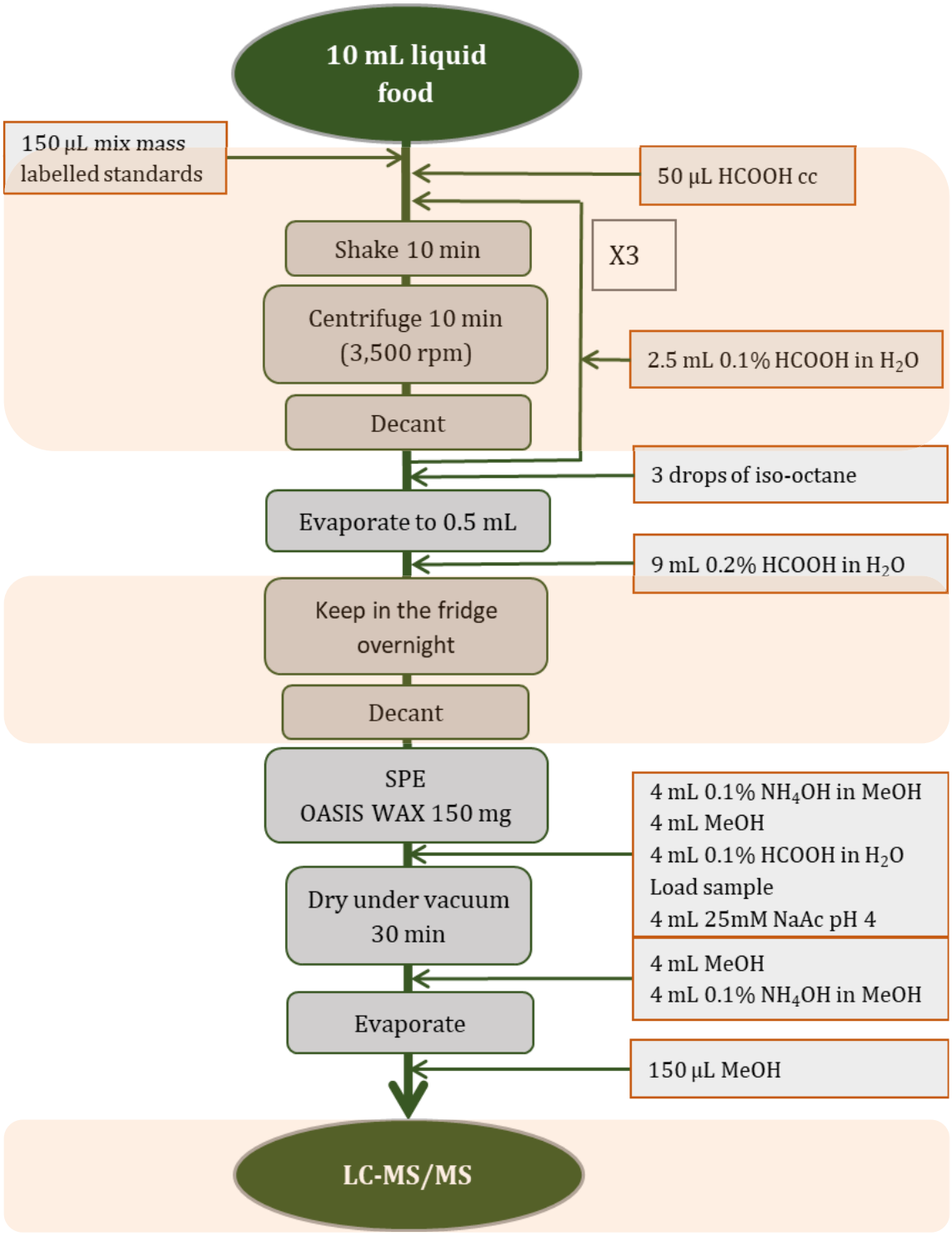

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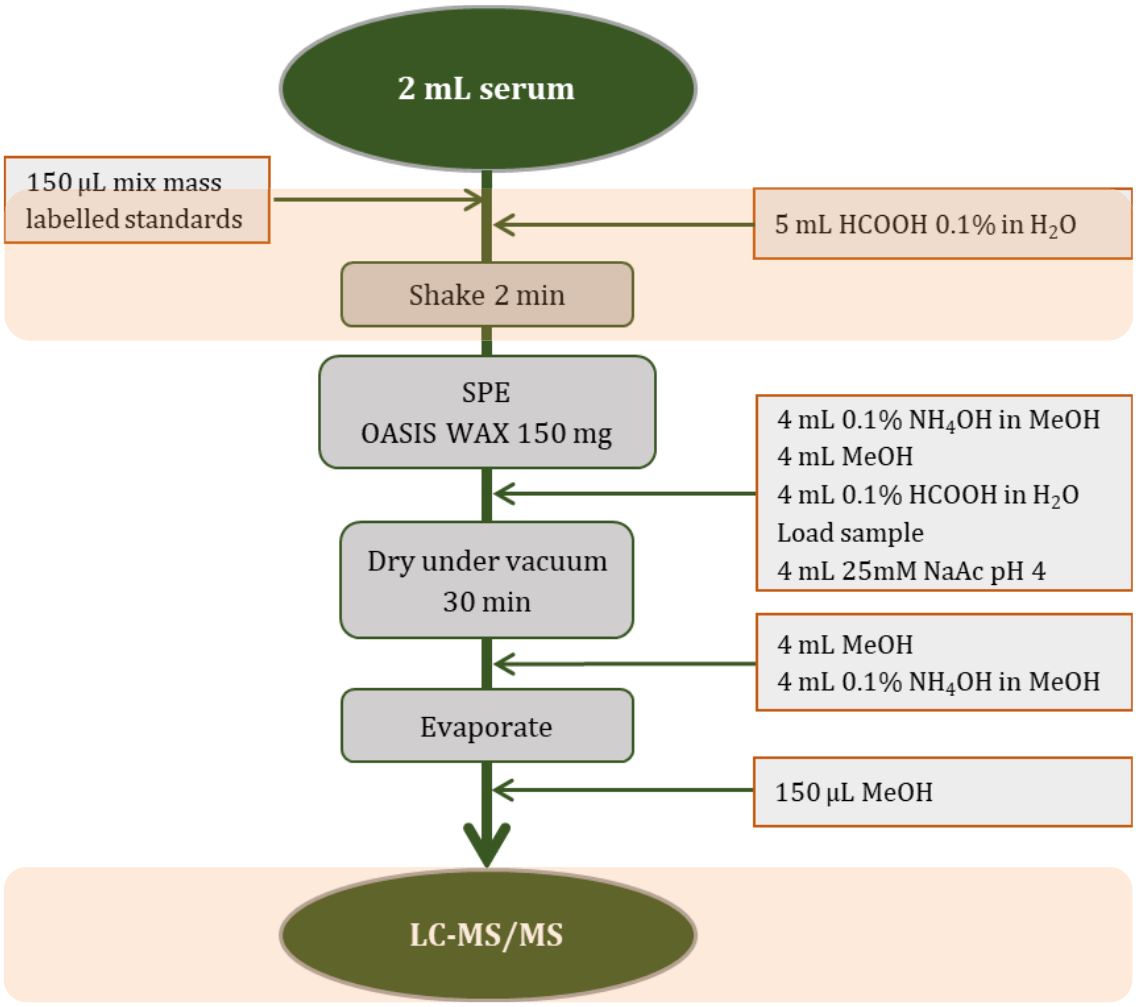

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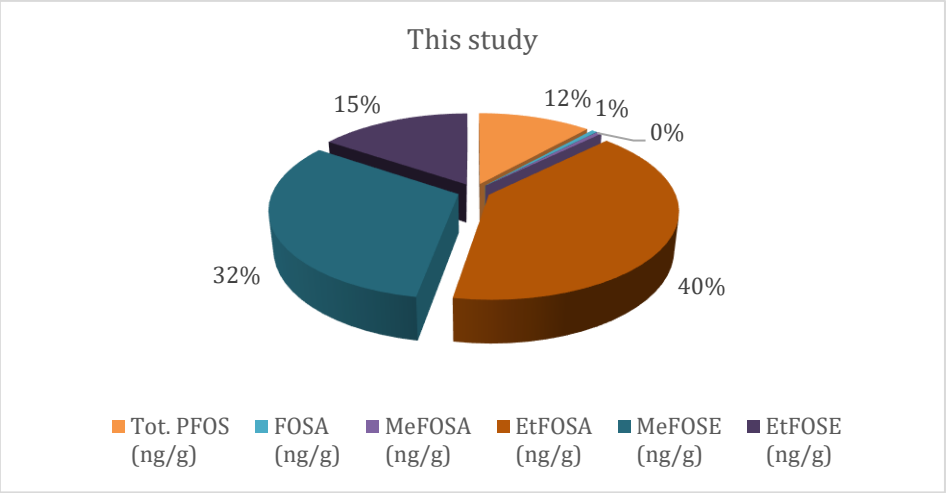

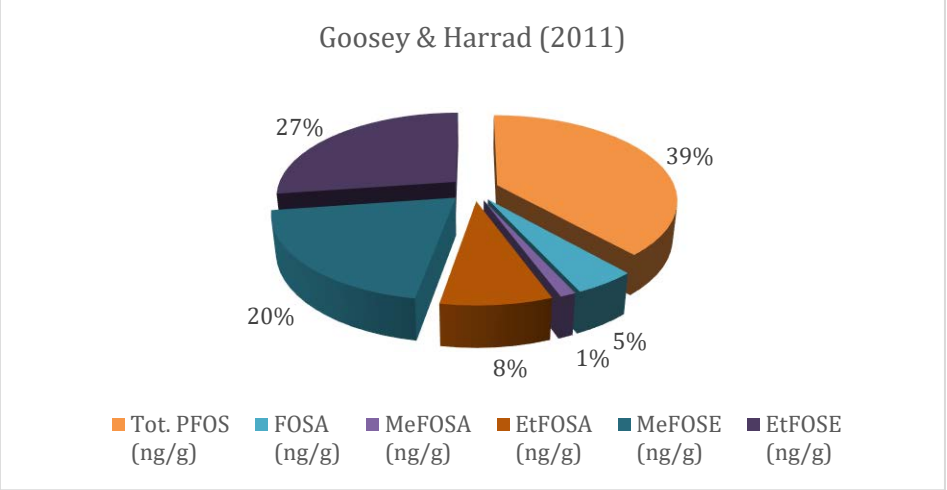

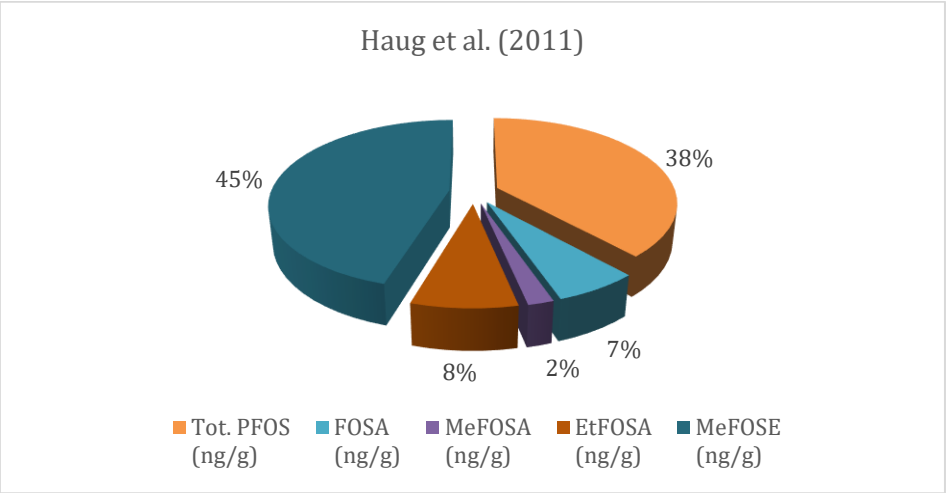

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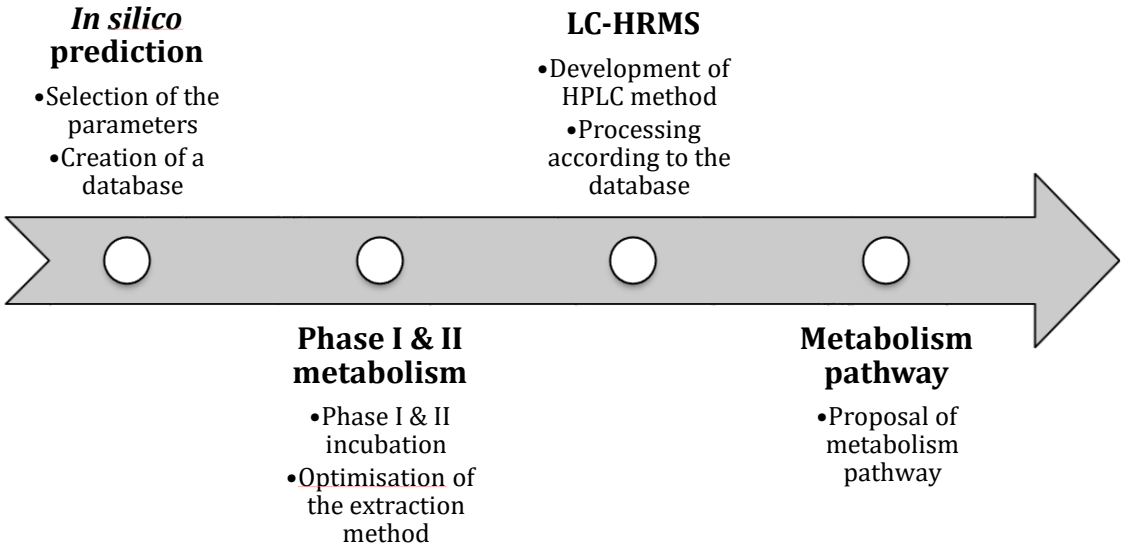

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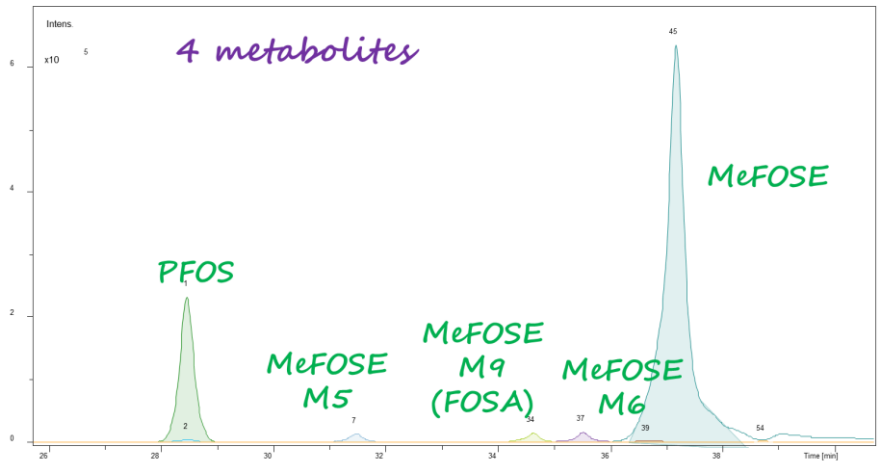

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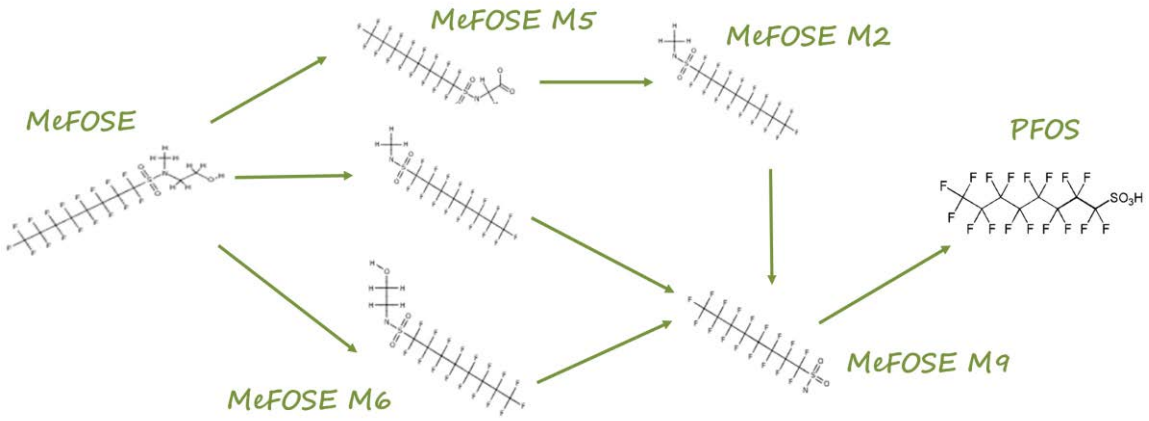

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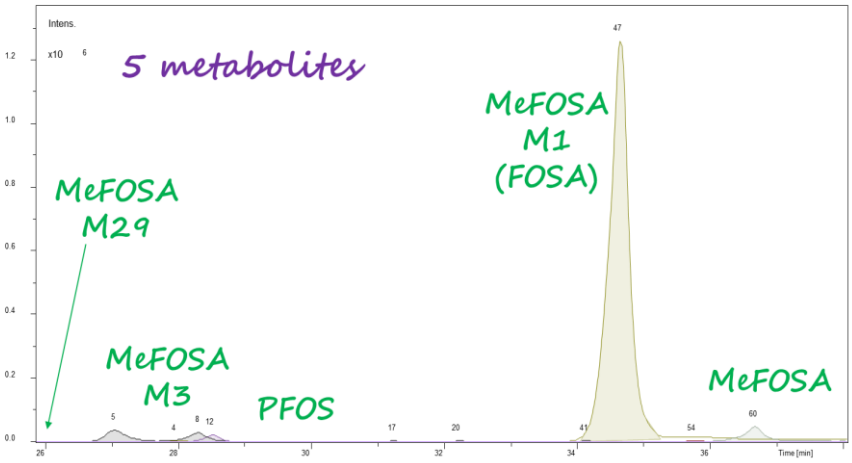

Figure 28 Overlapped XIC chromatograms of the main metabolites detected for MeFOSA

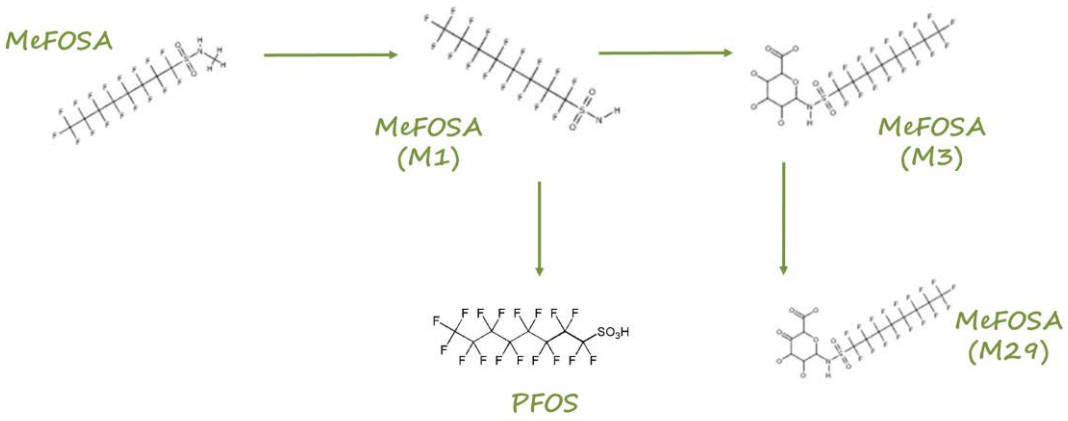

Figure 29. Suggested possibilities for the observed metabolism of MeFOSA

Figure 29 shows the scheme of the two suggested routes for MeFOSA metabolism, one of them leading to PFOS as end-product, and the second one leading to a second product M29, without possibility to track its possible conversion to PFOS. For the

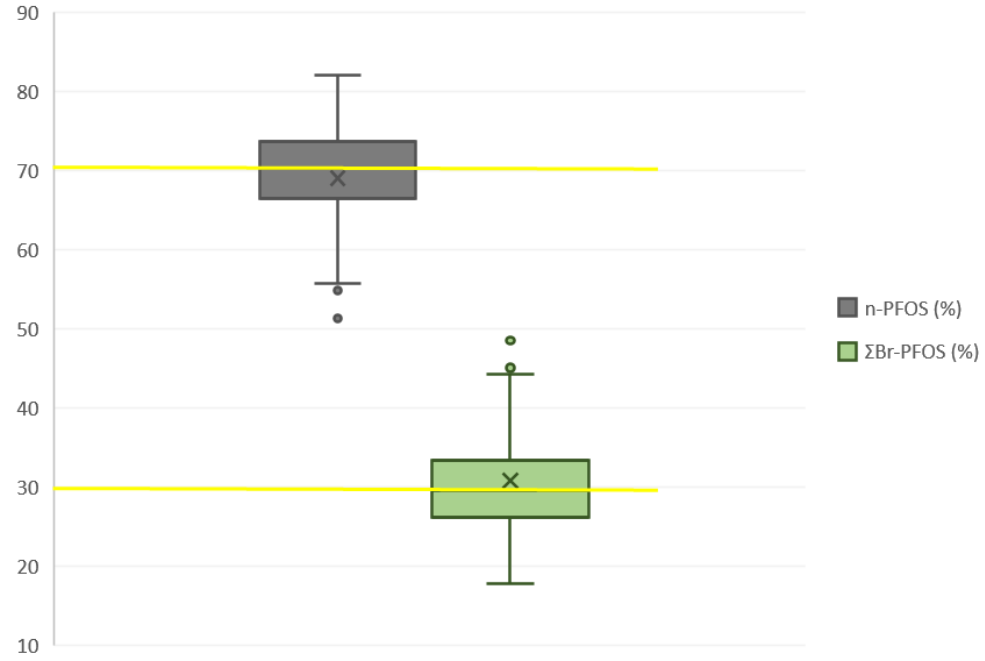

Figure 34. Box plots representing the percentages of linear and branched PFOS isomers identified in the serum samples (n = 40), where the yellow lines represent the theoretical percentage of the commercial PFOS mixture (70 % linear and 30 % branched isomers)

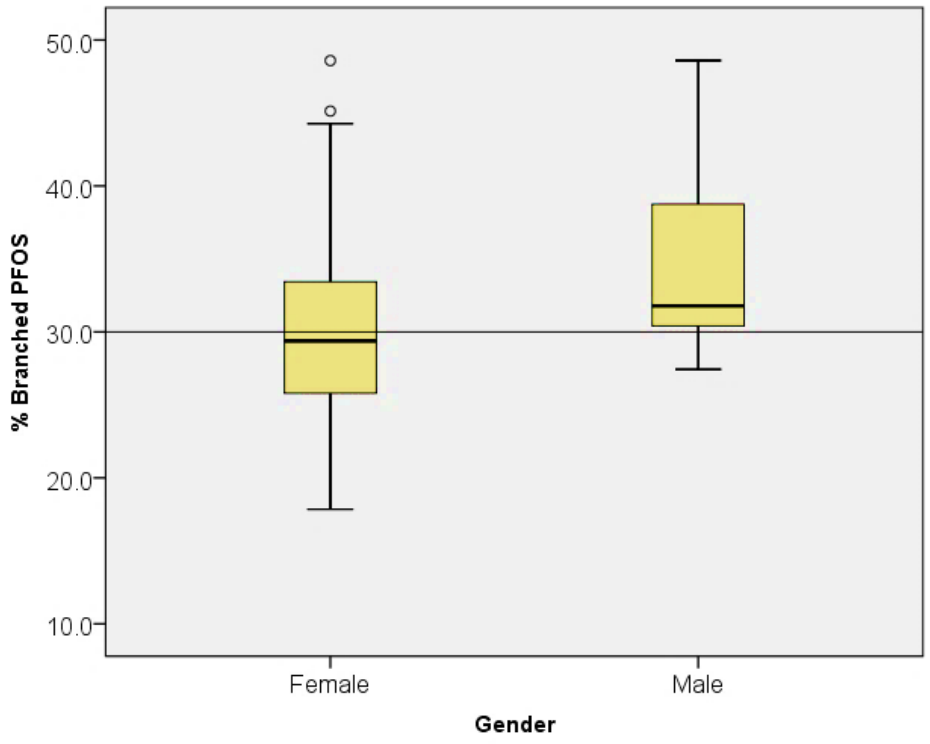

Figure 35. Box plots showing the percentages of branched PFOS isomers for male and female participants identified in the serum samples