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EU-China Food Safety Partnership

Analysis of PFAS in foods

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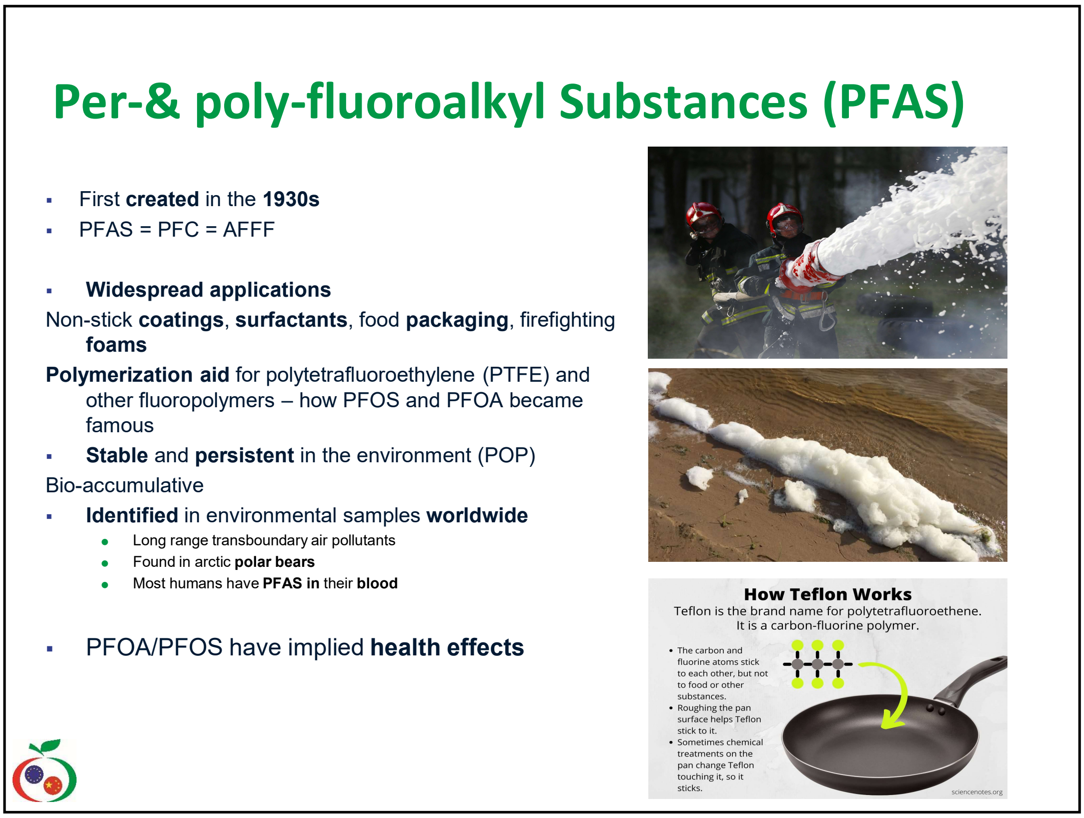
Fera Science

fera
Original thinking... applied



Per-& poly-fluoroalkyl Substances (PFAS)

- First **created** in the **1930s**
- PFAS = PFC = AFFF

- **Widespread applications**

Non-stick **coatings**, **surfactants**, food **packaging**, firefighting **foams**

Polymerization aid for polytetrafluoroethylene (PTFE) and other fluoropolymers – how PFOS and PFOA became famous

- **Stable** and **persistent** in the environment (POP)

Bio-accumulative

- **Identified** in environmental samples **worldwide**
  - Long range transboundary air pollutants
  - Found in arctic **polar bears**
  - Most humans have **PFAS in** their **blood**

- PFOA/PFOS have implied **health effects**

How Teflon Works

Teflon is the brand name for polytetrafluoroethene.
It is a carbon-fluorine polymer.

- The carbon and fluorine atoms stick to each other, but not to food or other substances.
- Roughing the pan surface helps Teflon stick to it.
- Sometimes chemical treatments on the pan change Teflon touching it, so it sticks.

sciencenotes.org

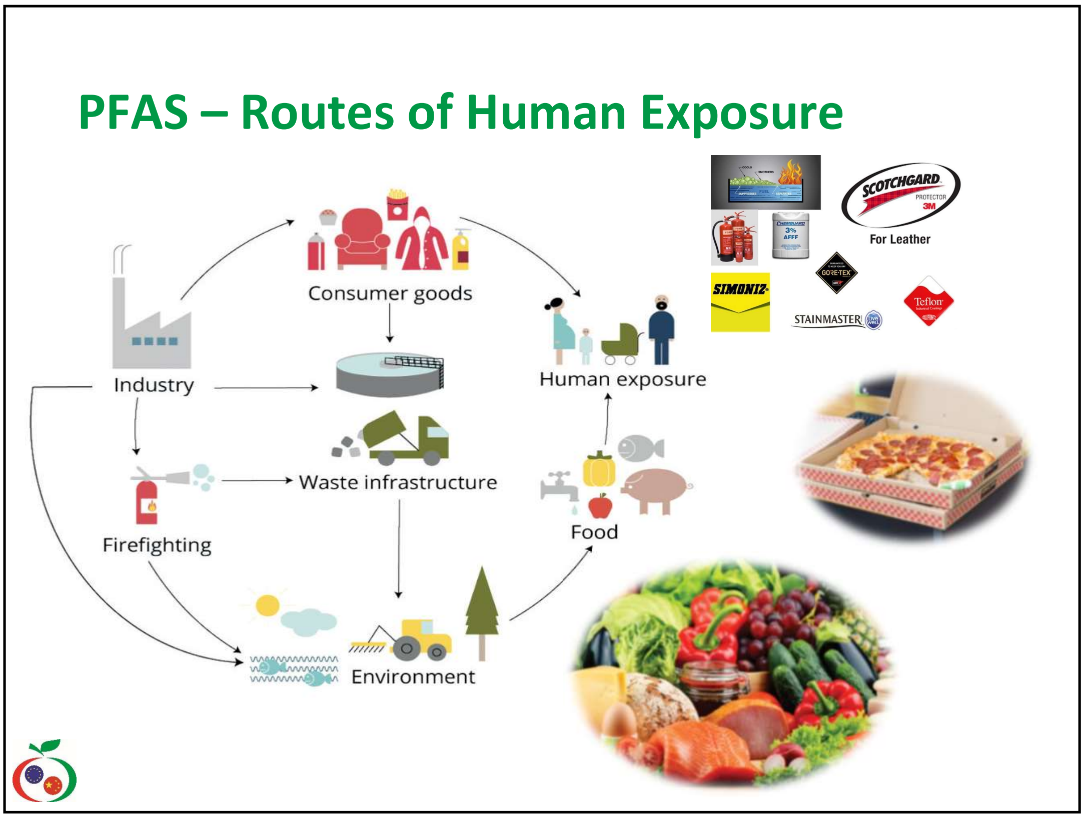
Per-and polyfluoroalkyl substances (PFASs) are a complex group of man-made chemicals including oligomers and polymers. The Organization of Economic Co-operation and Development (OECD) has identified 4730 PFAS related CAS numbers (OECD, 2018). PFASs were first introduced to the global market in the 1940's and have been widely used for a variety of applications and everyday products (Buck *et al.*, 2011). For example, they prevent food from sticking to cookware, make clothes and carpets resistant to water and stains, and create firefighting foam that is more effective. PFAS are used in industries such as aerospace, automotive, construction, electronics and military.

Perfluorinated compounds have been in use for many decades and have been known by various names throughout that time period. Perfluoroalkyl substances (PFAS) is currently the accepted name for this group of compounds, but they've also been known as perfluorinated compounds (PFCs) and even Aqueous Film Forming Foams (AFFF). PFAS are used in the production of many consumer products as nonstick and water resistant coatings, surfactants, polymerization aids, and even in fire fighting foams (where the term AFFF comes into play). AFFFs are typically used at airports or military bases where the danger of large fuel fires is greatest.

PFOS and PFOA are probably to most famous PFAS compounds, as they were the first to garner publicity with their use in the manufacturing process for Teflon (PFTE). These are only two out of thousands of potential PFAS compounds in use. Most research regarding exposure effects are from PFOS and PFOA only, but this is an ever expanding research area. PFAS have been linked to a variety of health effects including elevated cholesterol, reproductive impacts, and are potentially carcinogenic.

PFAS are environmentally important compounds as they are extremely stable and persistent in the environment and are bio-accumulative. They have been identified in environmental samples around the world, even in arctic wildlife like polar bears and arctic fox that are far removed from the industrial world. Many studies have also shown that most humans have ppb levels of PFAS in their blood.

Novel PFAS are increasingly detected in European surface waters. Several PFAS (such as fluorotelomers, GenX and PFBS) are sufficiently volatile to be considered long-range transboundary air pollutants implying that emissions outside Europe are transported into Europe where they may accumulate in cold areas such as the Arctic (EA, 2021).



PFAS – Routes of Human Exposure

PFAS are a group of man-made chemicals that are manufactured and used in a variety of industries around the world (e.g. textiles, household products, fire-fighting, automotive, food processing, construction, electronics).

Exposure to these chemicals may lead to adverse health effects.

People can be exposed to PFAS in different ways, including food, where these substances are most often found in drinking water, fish, fruit, eggs, and egg products.

Global concern about PFAS

NEW TEFLON TOXIN FOUND IN NORTH CAROLINA DRINKING WATER


NATIONAL QUEENSLAND

Chemicals in Brisbane Airport spill have sparked fears 'worldwide'

'New' perfluoroalkyl substances found in fish in China

US air bases blamed for fluorinated pollutants on Japanese island

PFAS costs Europe more than €50 billion a year in health problems

Toxicity Concerns -1

- PFOS is recognized as a POP under the UNEP Stockholm Convention (2010) and designated as a 'chemical for priority action'
- The use of PFOS in products has been restricted in Europe for some time
  - Products manufactured outside Europe must be tested for their presence.
- PFOS has been on priority substances list of the European Water Framework Directive since 2013
- HFPO-DA (it's salts & acyl halides) & perfluorobutane sulfonic acid (PFBS) and it's salts were added to the REACH Candidate List of Substances of Very High Concern (ECHA, 2019)
  - The SVHC identification was based on their persistence, mobility & toxicity which were considered to pose a threat to human health and wildlife when exposed through the environment (including via drinking water)

https://echa.europa.eu/hot-topics/perfluoroalkyl-chemicals-pfas
https://ec.europa.eu/environment/pdf/chemicals/2020/10/SWD_PFAS.pdf

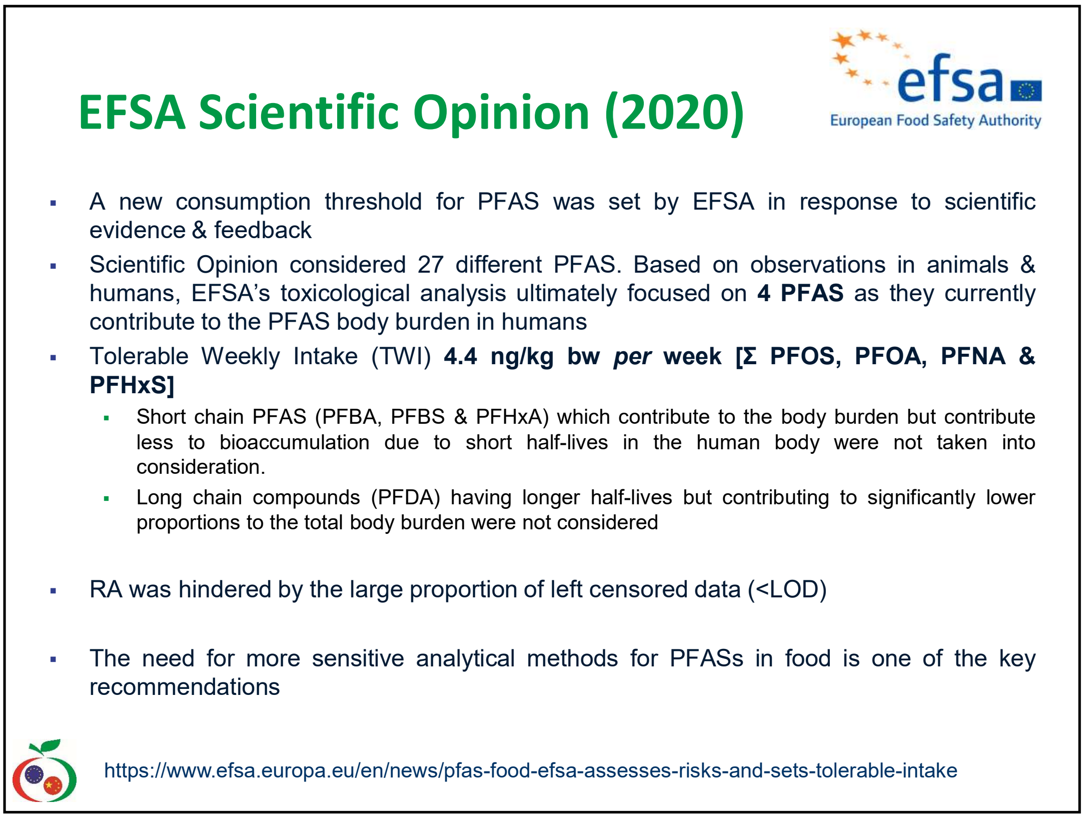
Toxicity Concerns -2

- The governments of Denmark, Germany, the Netherlands, Sweden & Norway have announced that by July 2022 they will formally propose to the ECHA that these chemicals be restricted under REACH legislation
- The proposal aims to prohibit the production, marketing & use of these substances throughout Europe
  - *Exceptions will be considered for certain established uses, such as medical applications*
- After summer 2022, ECHA's scientific bodies and socio-economic analysis committee will assess the REACH restriction dossier and deliver an opinion by 2023
  - A final agreement by EU member states could be possible **as early as 2025**

Registration, Evaulation, Authorisation and Restriction of Chemicals (REACH)

PFAS – Human Health Risks

- The health risks for various population groups have been assessed based on the 2020 EFSA Tolerable Weekly Intake (TWI) and PFASs concentration data from food surveys and patient blood samples
- Results show that the exposure of some population groups partially exceeds the TWI to an extent that may be associated with a lower concentration of vaccine antibodies in the blood, increases in serum cholesterol levels and low birth weights
- Infants during their first years of life and children (aged between 1 - 9 years) are considered the most risk with high PFAS exposure through their diet



EFSA Scientific Opinion (2020)

- A new consumption threshold for PFAS was set by EFSA in response to scientific evidence & feedback
- Scientific Opinion considered 27 different PFAS. Based on observations in animals & humans, EFSA's toxicological analysis ultimately focused on **4 PFAS** as they currently contribute to the PFAS body burden in humans
- Tolerable Weekly Intake (TWI) **4.4 ng/kg bw *per* week [Σ PFOS, PFOA, PFNA & PFHxS]**
  - Short chain PFAS (PFBA, PFBS & PFHxA) which contribute to the body burden but contribute less to bioaccumulation due to short half-lives in the human body were not taken into consideration.
  - Long chain compounds (PFDA) having longer half-lives but contributing to significantly lower proportions to the total body burden were not considered
- RA was hindered by the large proportion of left censored data (<LOD)
- The need for more sensitive analytical methods for PFASs in food is one of the key recommendations

https://www.efsa.europa.eu/en/news/pfas-food-efsa-assesses-risks-and-sets-tolerable-intake

EFSA's current opinion considered 27 different PFAS

Based on observations in animals and humans, EFSA's toxicological analysis has ultimately focused on four PFAS compounds as they currently contribute to the PFAS body burden in humans.

Short chain PFAS such as PFBA, PFBS and PFHxA which contribute to the body burden but contribute less to bioaccumulation due to short half-lives in the human body were not taken into consideration.

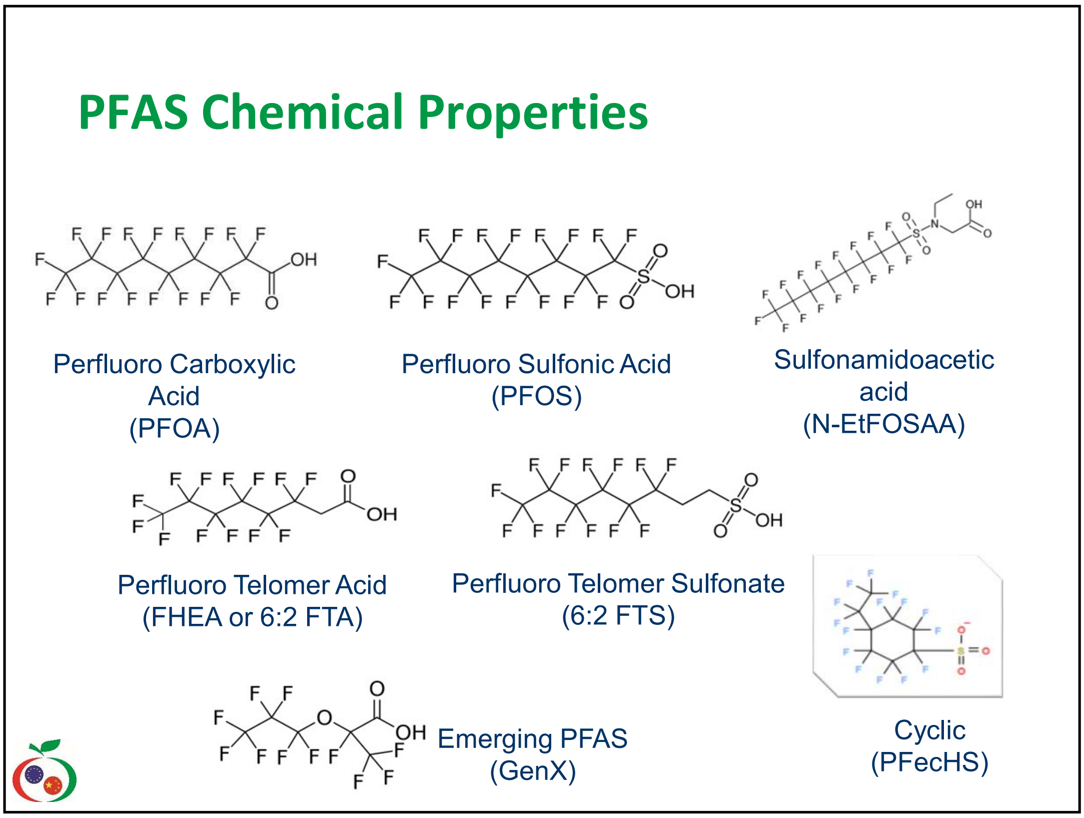
Also, the long chain compounds such as PFDA having longer half-lives but contributing to significantly lower proportions to the total body burden were not considered.

The risk assessment carried out by EFSA was hindered by the large proportion of left censored data (<LOD) and EFSA highlighted the need for more sensitive analytical methods for PFASs in food in its opinion of 2020 as one of the key recommendations.

PFAS – Regulatory Situation

- Currently, there is no legislation concerning PFASs levels in food or feed within the EU
  - *EFSA (202) Total Weekly Intake (TWI) of 4.4 ng kg^{-1} bw per week for the sum of PFOA, PFNA, PFHxS and PFOS*
- Recommended minimum limits of quantification (LOQ) for analytical methods used for risk assessment studies have been calculated by The Dutch National Health Institute (RIVM) and Wageningen University (NVWA, 2018)
- Analytical methods for risk assessment studies should at least be able to quantify
  - PFOA at 0.003 µg L^{-1} in milk and 0.03 µg kg^{-1} in egg
  - For GenX, these were 0.1 µg L^{-1} in milk and 1.2 µg kg^{-1} in egg
- "**Toxic Equivalency**" has now approach has been proposed and described in a recent publication by RIVM (Bil *et al.*, 2020)

Bil,W., *et al*., 2020 "Risk Assessment of Per- and Polyfluoroalkyl Substance Mixtures: A Relative Potency Factor Approach" *Environmental Toxicology and Chemistry* **40** (3) pp. 859-870. https://doi.org/10.1002/etc.4835



PFAS Chemical Properties

Perfluoro Carboxylic Acid
(PFOA)

Perfluoro Sulfonic Acid
(PFOS)

Sulfonamidoacetic acid
(N-EtFOSAA)

Perfluoro Telomer Acid
(FHEA or 6:2 FTA)

Perfluoro Telomer Sulfonate
(6:2 FTS)

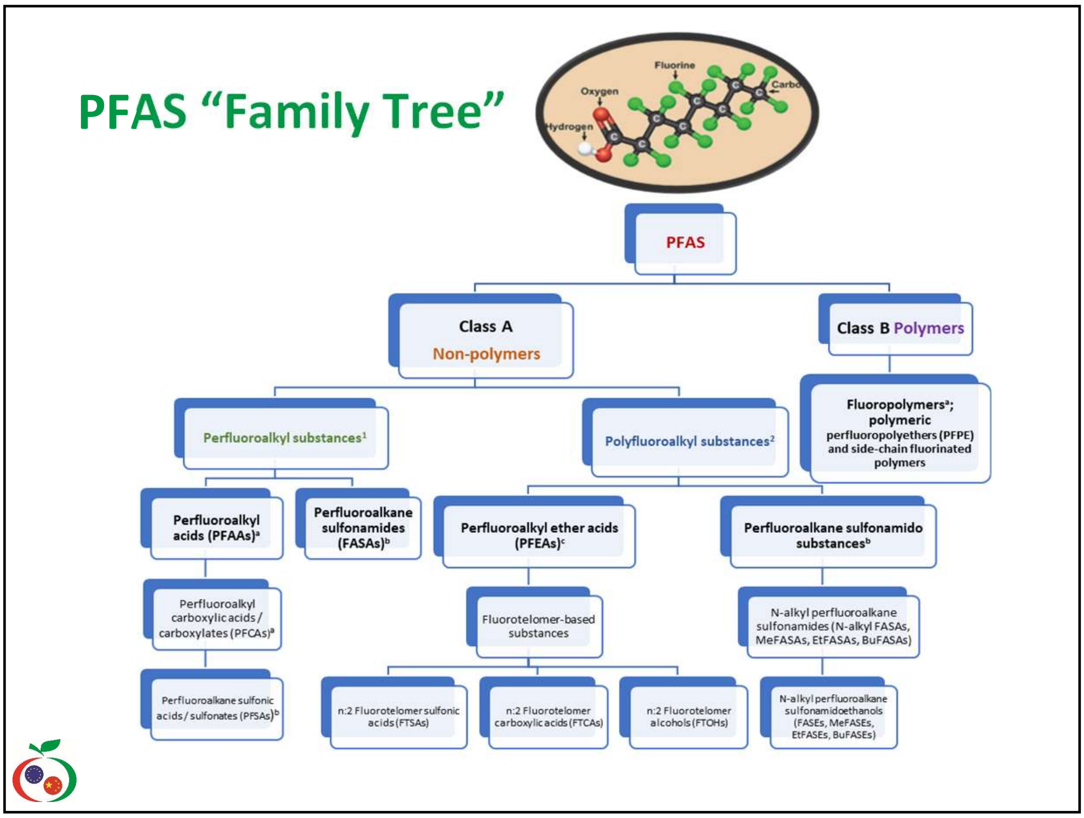
Emerging PFAS
(GenX)

Cyclic
(PFecHS)

PFASs are comprised of a wide variety of molecules with different physical and chemical properties and molecular weights with perfluoroalkyl moieties as common structural features. PFAS molecules are made up of a chain of linked carbon and fluorine atoms. As the carbon-fluorine bond is one of the strongest, these chemicals exhibit high thermal, chemical and biological stability and do not readily degrade in the environment. For many of the PFAS compounds it has still not been possible to calculate the half-life and or environmental fate.

The basic chemical structure is a hydrophobic alkyl chain (or tail) of two or more carbon atoms with a hydrophilic charged functional group (or head) attached to one end. The hydrophilic end group can be neutral, or positively or negatively charged. The resulting substances are non-ionic, cationic or anionic surface-active agents due to their amphiphilic character. In cationic PFASs the fluorinated hydrophobic part is attached to a quaternary ammonium group. Examples of neutral end groups are; -OH, -SO_3NH_2 and include the fluoroteleomer alcohols (FTOHs) and perfluoroalkane sulfonamides (FASAs).

If we look at just a few of the classes of PFAS, we can see both similarities and differences in the chemical properties. The common feature among each class are fully or partially fluorinated carbon tails. This C-F tail is connected to a head group which varies from group to group. Within each group, individual PFAS vary by the length of the C-F chain. This slide is only highlighting just a very few of the PFAS groups. The variation in chemical structure and properties across the entire suite of thousands of PFAS makes extraction and analysis challenging.



PFAS “Family Tree”

PFAS

Class A Non-polymers

Class B Polymers

Perfluoroalkyl substances[1]

Polyfluoroalkyl substances[2]

Fluoropolymers[a]; polymeric perfluoropolyethers (PFPE) and side-chain fluorinated polymers

Perfluoroalkyl acids (PFAAs)[a]

Perfluoroalkane sulfonamides (FASAs)[b]

Perfluoroalkyl ether acids (PFEAs)[c]

Perfluoroalkane sulfonamido substances[b]

Perfluoroalkyl carboxylic acids / carboxylates (PFCAs)[a]

Fluorotelomer-based substances

N-alkyl perfluoroalkane sulfonamides (N-alkyl FASAs, MeFASAs, EtFASAs, BuFASAs)

Perfluoroalkane sulfonic acids / sulfonates (PFSAs)[b]

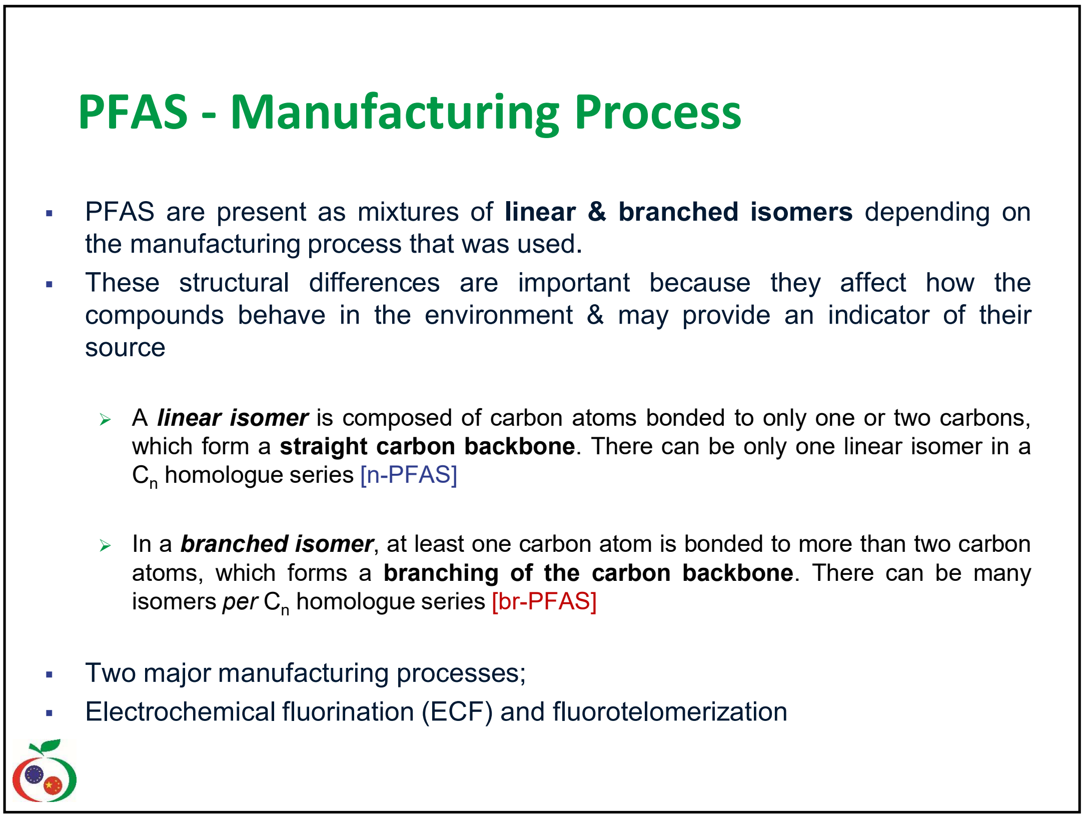
n:2 Fluorotelomer sulfonic acids (FTSAs)

n:2 Fluorotelomer carboxylic acids (FTCAs)

n:2 Fluorotelomer alcohols (FTOHs)

N-alkyl perfluoroalkane sulfonamidoethanols (FASEs, MeFASEs, EtFASEs, BuFASEs)

The variety of PFAS with diverse properties has been organised in the form of a PFAS family tree including two primary classes, the polymers and non-polymers. Each class contains many subclasses, groups and subgroups. The non-polymers are the most detected PFAS in humans, biota and the environment to date. The **non-polymers** are significant precursors that can transform to **more persistent forms.**

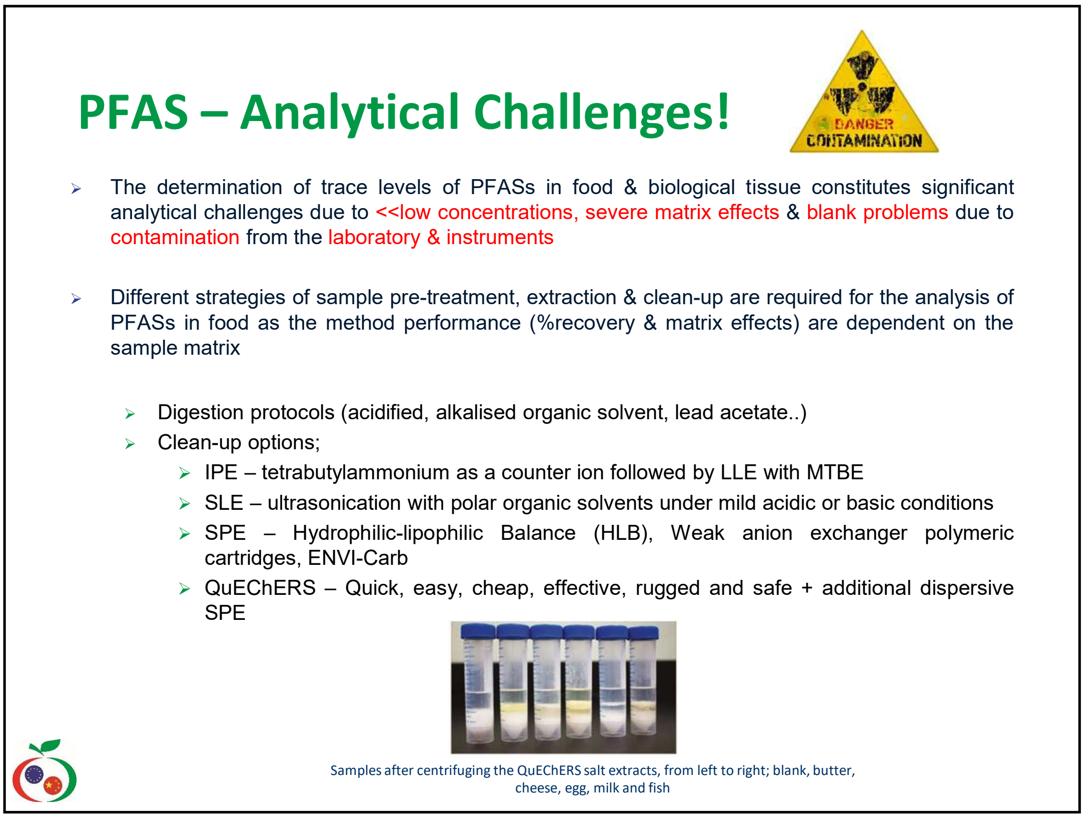


PFAS - Manufacturing Process

- PFAS are present as mixtures of **linear & branched isomers** depending on the manufacturing process that was used.
- These structural differences are important because they affect how the compounds behave in the environment & may provide an indicator of their source
  - A ***linear isomer*** is composed of carbon atoms bonded to only one or two carbons, which form a **straight carbon backbone**. There can be only one linear isomer in a C_n homologue series [n-PFAS]
  - In a ***branched isomer***, at least one carbon atom is bonded to more than two carbon atoms, which forms a **branching of the carbon backbone**. There can be many isomers *per* C_n homologue series [br-PFAS]
- Two major manufacturing processes;
- Electrochemical fluorination (ECF) and fluorotelomerization

ECF produces a mixture of branched (br-PFAS) and linear (n-PFAS) isomers whereas the telomerisation process results only in substances consisting of a linear alkyl chain with an even number of carbon atoms.

To differentiate among PFAS in understanding a conceptual site model for environmental risk assessment, it is important to know about the chemical manufacturing processes. The various manufacturing processes produce different types of PFAS, such as linear and branched isomers may affect the environmental fate, treatment, toxicology and site forensics for these chemicals. The type of PFAS that might be formed by the transformation of precursor PFAS at or related to an environmental release site also may depend on the manufacturing process.



PFAS – Analytical Challenges!

- The determination of trace levels of PFASs in food & biological tissue constitutes significant analytical challenges due to <<low concentrations, severe matrix effects & blank problems due to contamination from the laboratory & instruments
- Different strategies of sample pre-treatment, extraction & clean-up are required for the analysis of PFASs in food as the method performance (%recovery & matrix effects) are dependent on the sample matrix
  - Digestion protocols (acidified, alkalised organic solvent, lead acetate..)
  - Clean-up options;
    - IPE – tetrabutylammonium as a counter ion followed by LLE with MTBE
    - SLE – ultrasonication with polar organic solvents under mild acidic or basic conditions
    - SPE – Hydrophilic-lipophilic Balance (HLB), Weak anion exchanger polymeric cartridges, ENVI-Carb
    - QuEChERS – Quick, easy, cheap, effective, rugged and safe + additional dispersive SPE

Samples after centrifuging the QuEChERS salt extracts, from left to right; blank, butter, cheese, egg, milk and fish

Before diving into the methodologies we've been working on, it's critical to pause for a moment to acknowledge the difficulties to analysis due to the risk of sample contamination. Since PFAS can be found practically everywhere, we really must take care to reduce risks of contamination from sample collection to sample preparation to sample analysis. It's important to make sure you are using suitable laboratory consumables and solvents that have been evaluated for PFAS contamination prior to use. Another major source of potential contamination can come from the LC system…

PFASs are known to adsorb to glassware, especially when the chemicals are stored in a glass container for longer periods of time. The use of polypropylene (PP) or high-density polyethylene (HDPE) sample bottes, centrifuge tubes, autosampler vials fitted with PP or HDPE screw caps is recommended. The use of fluoropolymers such as PTFE, PFA should be avoided.

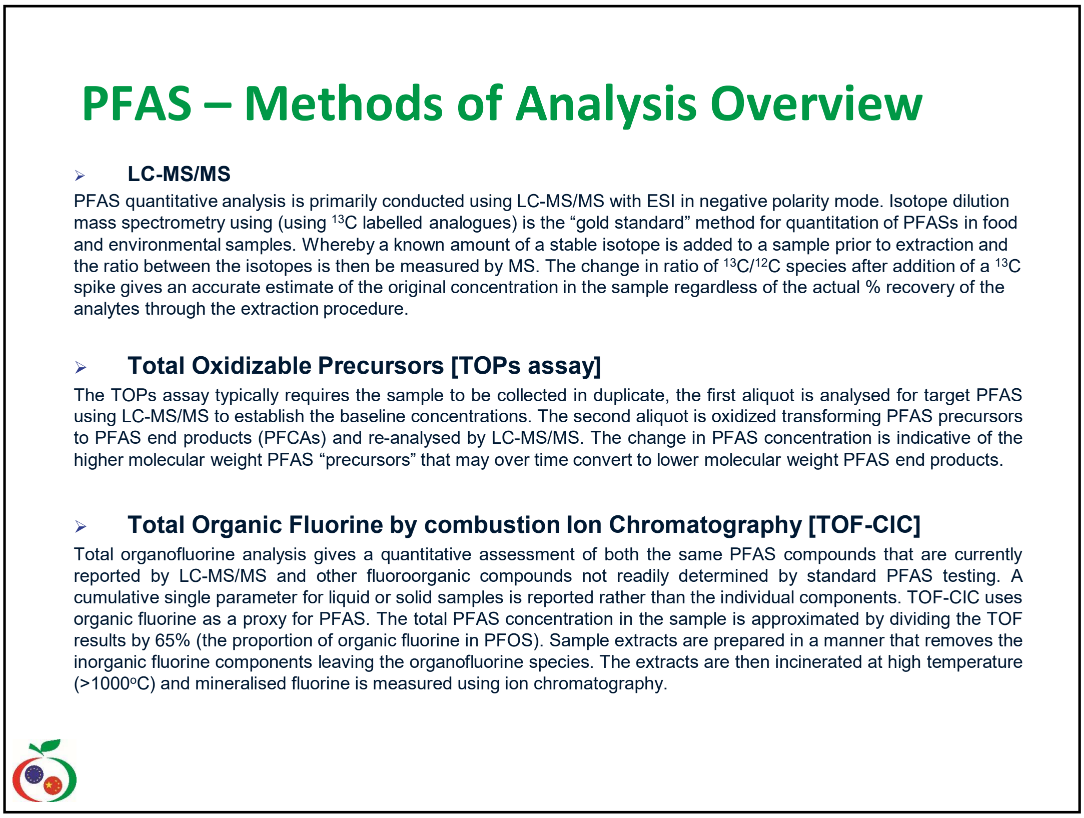
Sample collected bottles must be discarded after use to prevent cross-contamination.

When cleaning laboratory equipment personnel should avoid using decontamination soaps containing fluorosurfactants such as Decon 90.

PFAS can be present in lab water and solvents representing a potential source of contamination and should be checked for background levels prior to analysis.

Materials used in the manufacturing of supplies for SPE may also contain PFAS. To avoid pre-concentrating the background PFAS during this step of the analysis, all new SPE cartridges, solvents and vials for collecting the samples must be tested for PFAS prior to their first use.

Modification of the LC system is recommended whereby the standard fluoropolymer mobile phase tubing and ferrules are replaced with PEEK and stainless-steel parts. The use of an isolator column installed after the solvent mixer to delay any other contamination bleeding out of the LC. In combination with a stainless-steel solvent delay coil, the isolation column delays the PFAS contaminants from the LC and mobile phase to provide chromatographic resolution between the analytical PFAS components from the sample and the contamination front. PFC free mobile phase bottle caps should be used to minimize organic solvent vapors contaminating the lab environment.



PFAS – Methods of Analysis Overview

- **LC-MS/MS**

PFAS quantitative analysis is primarily conducted using LC-MS/MS with ESI in negative polarity mode. Isotope dilution mass spectrometry using (using ^{13}C labelled analogues) is the “gold standard” method for quantitation of PFASs in food and environmental samples. Whereby a known amount of a stable isotope is added to a sample prior to extraction and the ratio between the isotopes is then be measured by MS. The change in ratio of ^{13}C/^{12}C species after addition of a ^{13}C spike gives an accurate estimate of the original concentration in the sample regardless of the actual % recovery of the analytes through the extraction procedure.

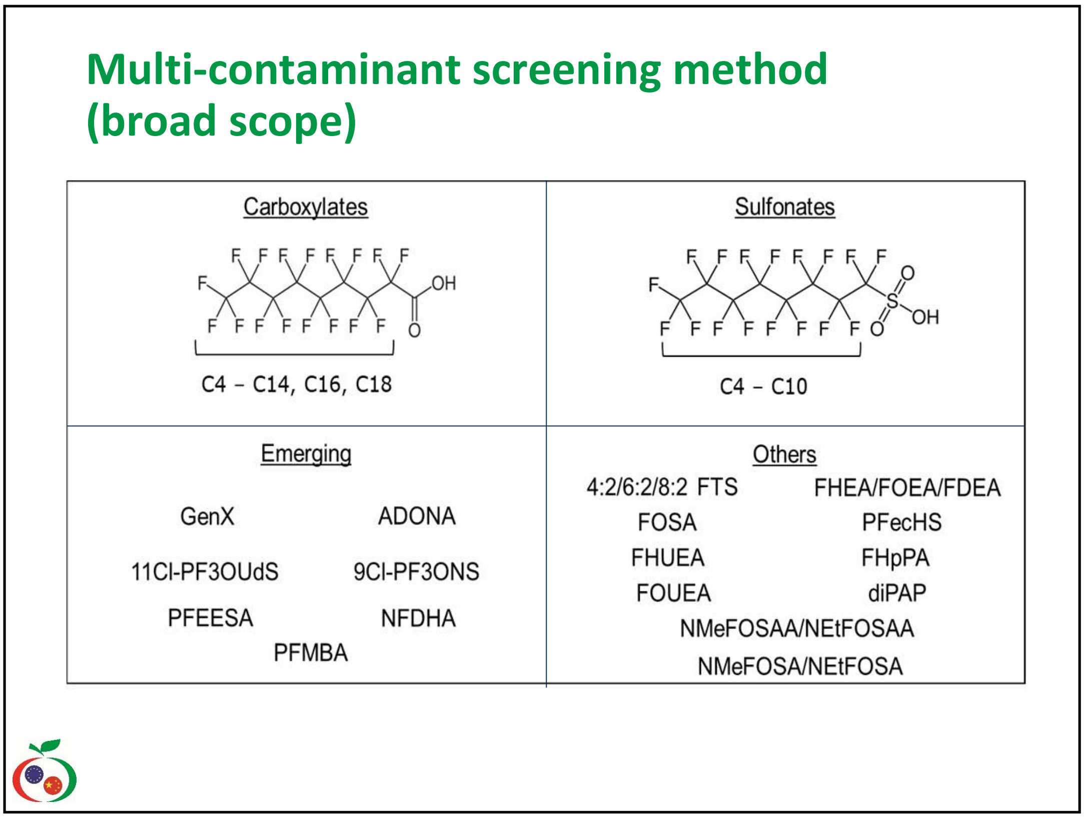
- **Total Oxidizable Precursors [TOPs assay]**

The TOPs assay typically requires the sample to be collected in duplicate, the first aliquot is analysed for target PFAS using LC-MS/MS to establish the baseline concentrations. The second aliquot is oxidized transforming PFAS precursors to PFAS end products (PFCAs) and re-analysed by LC-MS/MS. The change in PFAS concentration is indicative of the higher molecular weight PFAS “precursors” that may over time convert to lower molecular weight PFAS end products.

- **Total Organic Fluorine by combustion Ion Chromatography [TOF-CIC]**

Total organofluorine analysis gives a quantitative assessment of both the same PFAS compounds that are currently reported by LC-MS/MS and other fluoroorganic compounds not readily determined by standard PFAS testing. A cumulative single parameter for liquid or solid samples is reported rather than the individual components. TOF-CIC uses organic fluorine as a proxy for PFAS. The total PFAS concentration in the sample is approximated by dividing the TOF results by 65% (the proportion of organic fluorine in PFOS). Sample extracts are prepared in a manner that removes the inorganic fluorine components leaving the organofluorine species. The extracts are then incinerated at high temperature (>1000^{o}C) and mineralised fluorine is measured using ion chromatography.

LC-MS/MS operated as a “targeted” analytical technique the results are limited to a fixed set of Multiple Transition Monitoring (MRM) parameters. The results do not provide a comprehensive measure of the magnitude of the total pool of PFASs (>4000) that may exist in sample, nor does it measure the potential for targeted PFAS formation due to natural transformation of precursor compounds over time to the regulated end products.

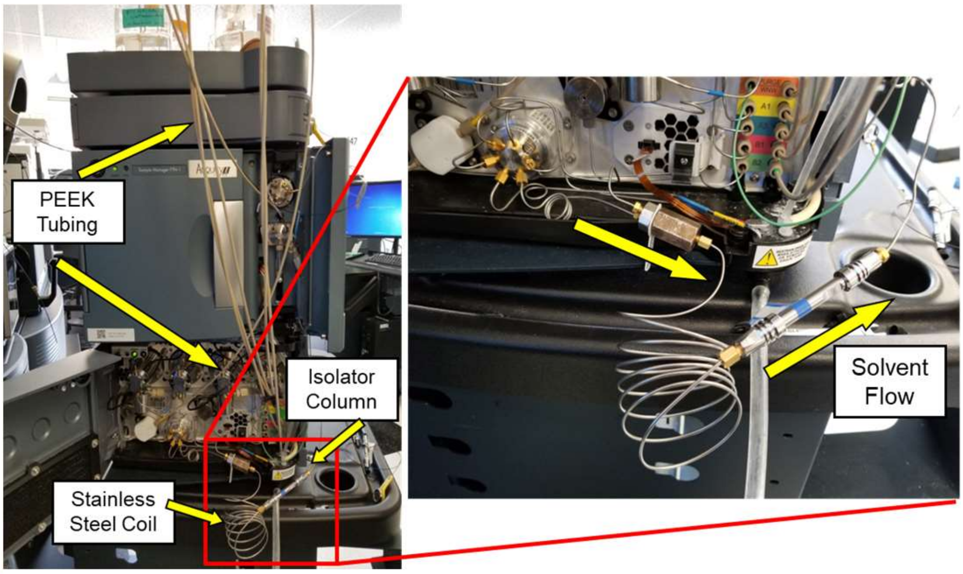


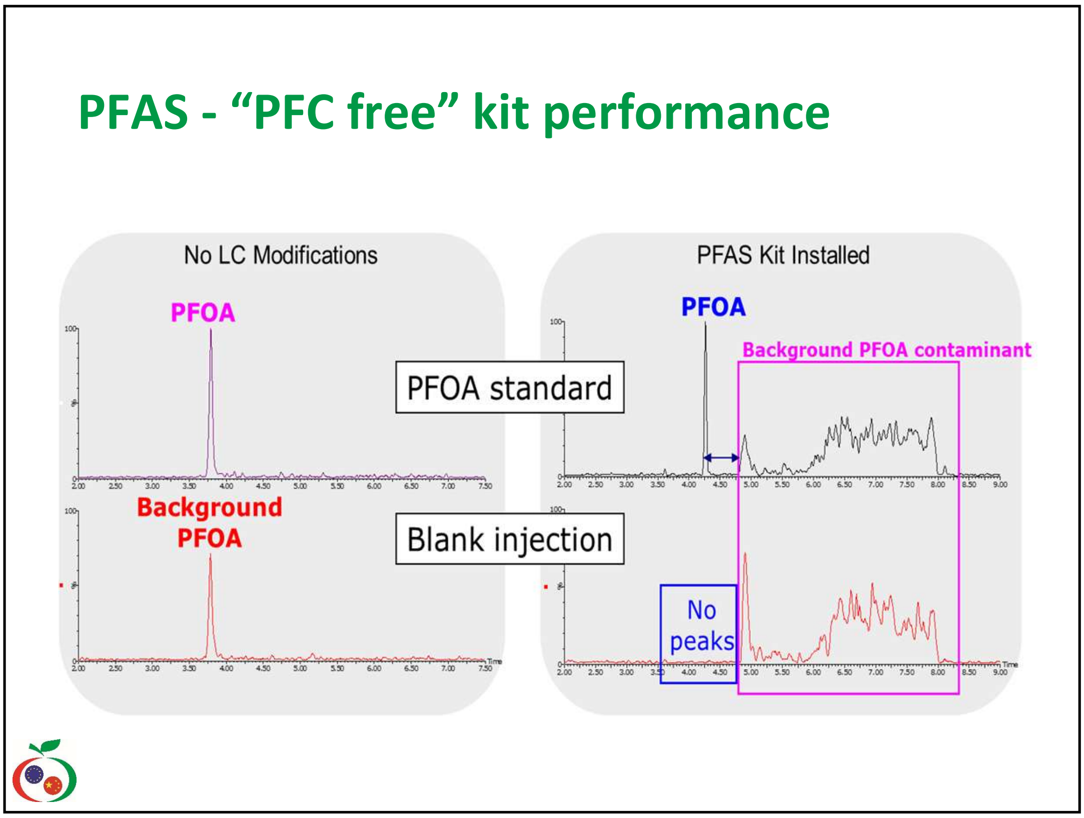
Multi-contaminant screening method (broad scope)

Carboxylates	Sulfonates
C4 – C14, C16, C18	C4 – C10
Emerging	**Others**
GenX ADONA 11Cl-PF3OUdS 9Cl-PF3ONS PFEESA NFDHA PFMBA	4:2/6:2/8:2 FTS FHEA/FOEA/FDEA FOSA PFecHS FHUEA FHpPA FOUEA diPAP NMeFOSAA/NEtFOSAA NMeFOSA/NEtFOSA

In the methods under development, we are focusing on a list of about 45 PFAS covering a wide variety of PFAS chemistries. Here is the list of compounds included in those methods which included legacy PFAS, precursors, and emerging PFAS. We are continually updating the list of PFAS as we learn of new emerging compounds.

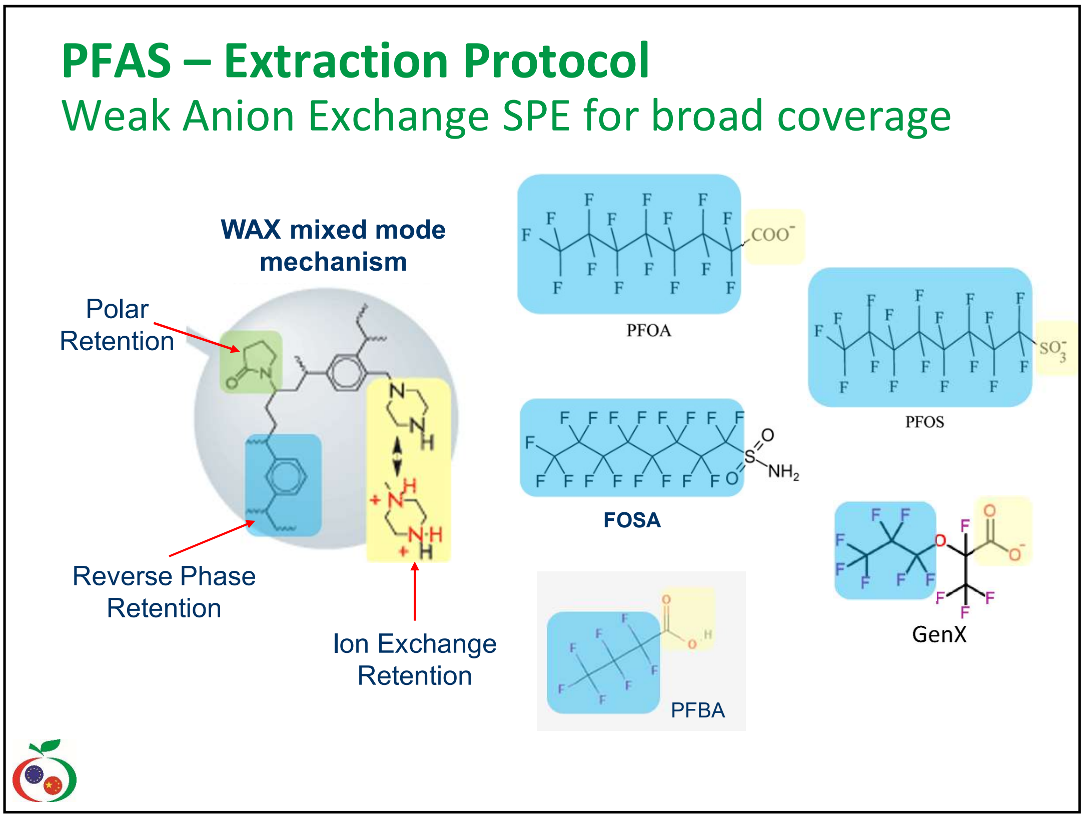
PFAS – LC modifications to control background





PFAS - “PFC free” kit performance

Here is a comparison of the effects of the PFAS kit. On the left is an example of an unmodified system where there is a large, distinct PFOA peak in the solvent blank injection which is PFOA contamination resulting from the solvents and the inherent PFOA in the LC system. On the right is an example of the same system modified with the PFAS kit. We can see now that the PFOA contamination is resolved from the analytical peak in the standard. It is important to understand that we can not completely eliminate potential PFAS contamination from the system, but we can reduce and delay it.



PFAS – Extraction Protocol

Weak Anion Exchange SPE for broad coverage

WAX mixed mode mechanism

Polar Retention

Reverse Phase Retention

Ion Exchange Retention

PFOA

PFOS

FOSA

PFBA

GenX

For this method was are also using the WAX chemistry for solid phase extraction of PFAS from food samples. The structure of WAX can be seen on the left-hand side of this slide, and you can see the structural properties that allow for each retention mechanism. If you look at some of the highlighted example structures on the right, you can see why both the reverse phase and ion exchange mechanisms are crucial for PFAS. Using both mechanisms allows for better retention on the SPE cartridge for PFAS that have both hydrophobic and ionic sites. This is important for the shorter chain compounds that may have weaker RP retention due to their shorter C-F tail. WAX also allows retention of the neutral PFAS for compounds like FOSA that have no ionic sites. This is why WAX is the best chemistry for the broadest coverage of PFAS.

PFAS – Method Performance Checks

Background control

Reagent blank → Extraction solvent taken through sample prep → Concentration must <0.5 x RL

“Double” blank → Blank matrix taken through sample prep → Concentration must <0.5 x RL

“Single” blank → Blank matrix + ^{13}C-I.S. + injection standards → Detectable recovery 70-150% [%RSD<20]

PFAS – Isotope Dilution Method

Report limit check

Blank matrix + ^{13}C-I.S. + targets + injection standards

Detectable recovery 35-150% for targets & 70-150% of I.S

Blank matrix "spiked" with target PFAS compounds pre-extraction OR reference material

Matrix matched standards

Blank matrix + ^{13}C-I.S. pre-extraction + targets + injection standards post extraction

RRF [target/I.S]

R^2 >0.97

Recovery 70-150% for I.S

Matrix matched calibrants across the reporting range 0.01–10 μg kg^{-1}

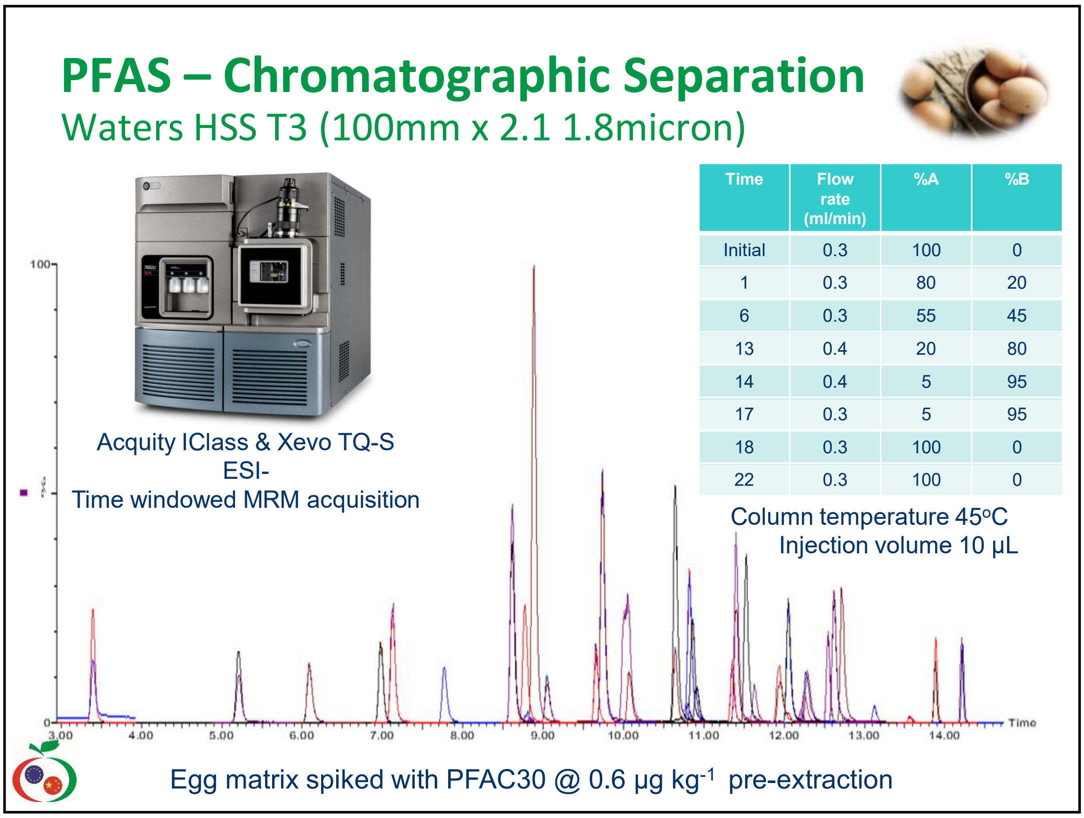
"Unknowns"

"Unknown" + ^{13}C-I.S. pre-extraction + injection standards post extraction

Calculate amount in unknowns

Detectable recovery 70-150% of I.S

Unknown concentration = (Target response/mean RRF)*(^{13}C concentration/^{13}C response)



PFAS – Chromatographic Separation

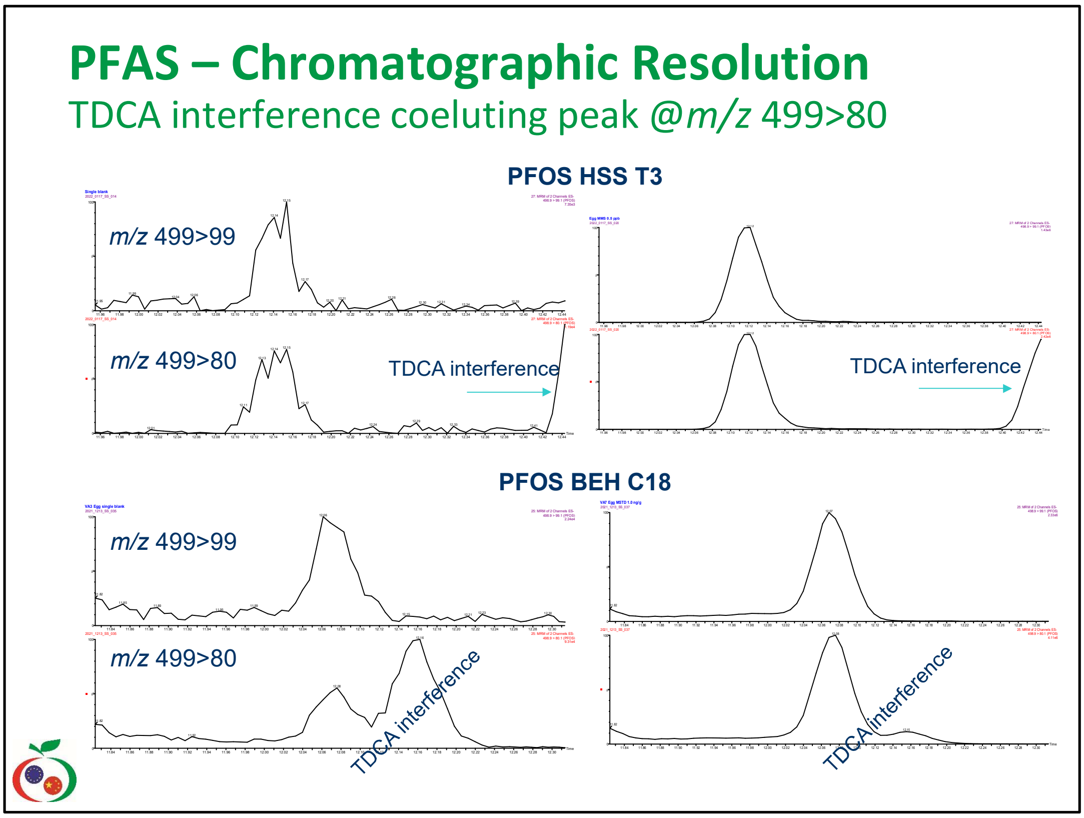
Waters HSS T3 (100mm x 2.1 1.8micron)

Time	Flow rate (ml/min)	%A	%B
Initial	0.3	100	0
1	0.3	80	20
6	0.3	55	45
13	0.4	20	80
14	0.4	5	95
17	0.3	5	95
18	0.3	100	0
22	0.3	100	0

Acquity IClass & Xevo TQ-S
ESI-
Time windowed MRM acquisition

Column temperature 45°C
Injection volume 10 µL

Egg matrix spiked with PFAC30 @ 0.6 µg kg^{-1} pre-extraction



PFAS – Chromatographic Resolution

TDCA interference coeluting peak @*m/z* 499>80

PFOS HSS T3

PFOS BEH C18

Priority Food Matrices for method development

Fish*
Meat (muscle, pork liver tissue**)
Egg**
Milk
Fruits
Vegetables
Honey**
Wheat flour

* FAPAS proficiency test material available
**Included in the EURL POPs proficiency test scheme 2021-2022
https://eurl-pops.eu/

www.euchinasafe.eu

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 727864 and from the Chinese Ministry of Science and Technology (MOST).