



Standard operating procedures (SOPs) for new POPs

Jacob de Boer

VU Univeristy, Amsterdam, The Netherlands

Heidlore Fiedler

Örebro University

School of Science and Technology, MTM Research Centre

Örebro, Sweden

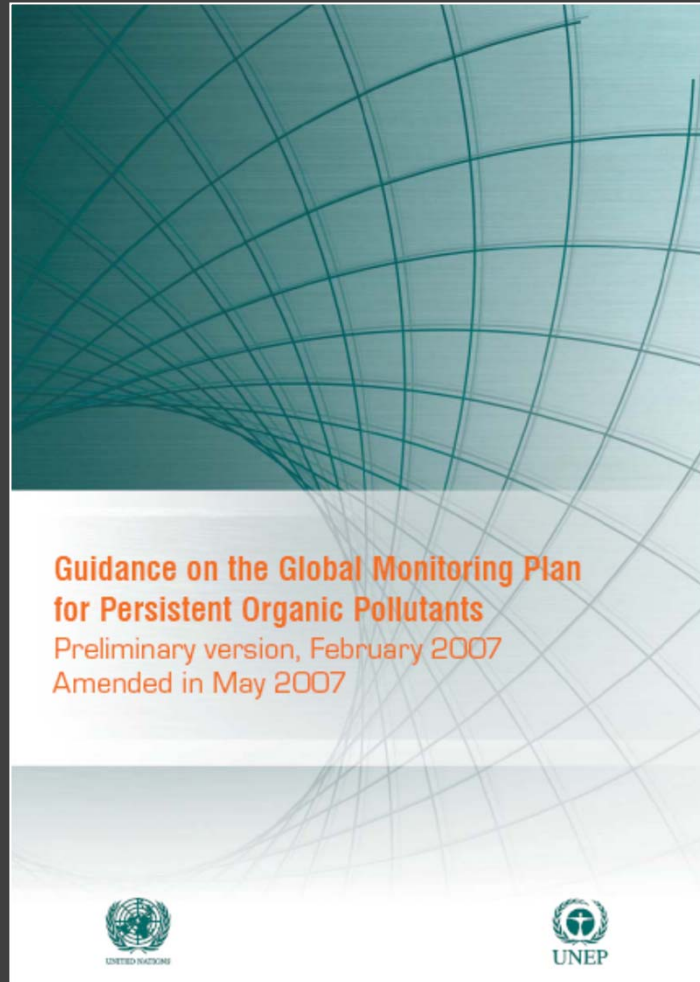


New POPs to be analysed

POP	Air	Human milk/blood	Water
Chlordecone	Chlordecone		
Endosulfan	α -, β -endosulfan; and endosulfan sulfate		
HBCD	α -HBCD, β -HBCD, γ -HBCD		
Hexachlorocyclohexanes	α -HCH, β -HCH, γ -HCH		
Hexabromobiphenyl	PBB-153		
Pentachlorobenzene	PeCBz		
Penta BDE, Octa BDE	PBDE 28, 47, 99, 153, 154, 175/183 (may co-elute)		
	Optional: PBDE 17, 100		
PFOS	PFOS (linear and sum of PFOS)		
	NMeFOSA, NEtFOSA, NMeFOSE, NEtFOSE		

Guidance for Global Monitoring Plan

Orientation and benchmark for
POPs www.pops.int



UNITED
NATIONS



SC

UNEP/POPS/COP.7/INF/39

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**Stockholm Convention
on Persistent Organic
Pollutants**

Conference of the Parties to the Stockholm
Convention on Persistent Organic Pollutants

Seventh meeting
Geneva, 4–15 May 2015

Item 5 (i) of the provisional agenda*

**Matters related to the implementation of the Convention:
effectiveness evaluation**

**Guidance on the global monitoring plan for persistent
organic pollutants**



POPs Analysis and Monitoring

Pacific Islands Region

- GMP Regional Report of Pacific Islands Region
- GMP National Report of Kiribati
- GMP National Report of Marshall Islands
- GMP National Report of Niue
- GMP National Report of Palau
- GMP National Report of Solomon Islands
- GMP National Report of Samoa

GRULAC Region

- GMP Regional Report of GRULAC Region (en, sp)
- GMP National Report of Antigua and Barbuda
- GMP National Report of Brazil
- GMP National Report of Chile
- GMP National Report of Ecuador
- GMP National Report of Jamaica
- GMP National Report of Mexico (sp)
- GMP National Report of Peru (sp)

East and South Africa

- GMP Regional Report of E+S Africa
- GMP National Report of Egypt
- GMP National Report of Ethiopia
- GMP National Report of Kenya
- GMP National Report of Mauritius
- GMP National Report of Uganda
- GMP National Report of Zambia

West Africa

- GMP Regional Report of West Africa (en, fr)
- GMP National Report of DR Congo (fr)
- GMP National Report of Ghana
- GMP National Report of Mali (fr)
- GMP National Report of Nigeria
- GMP National Report of Senegal (fr)
- GMP National Report of Togo (fr)

Cross-cuttings

- IVM Mirror samples Final Report (Africa, Pacific, Barbados)
- MTM Report. Analysis of dl POPs in PUF samples (Africa and Pacific Islands)
- MTM Report dl-POPs in National Samples
- UNEP Report: Passive air sampling (PAS)

Interlaboratory Assessments

- Biennial Global Interlaboratory Assessment on POPs – Round 1

Regional and national reports

Training reports

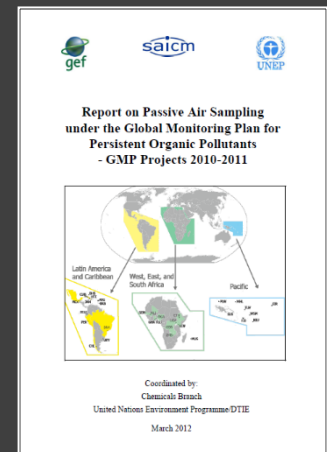
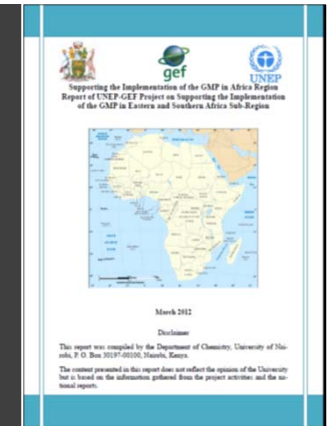
- Fiji Training Report

- Regional Report for GRULAC

- Reports of Antigua and Barbuda (en, sp); Brazil (sp); Chile (sp); Ecuador (sp); Jamaica (en, sp); Mexico (sp); Peru (sp); Uruguay (sp)

- Egypt Training Report
- Kenya Training Report
- Mauritius Training Report
- Zambia Training Report

- Ghana Training Report
- Mali Training Report
- Senegal Training Report





POPs Analysis and Monitoring SOPs and supporting materials



Pacific Islands Region

- SOP Regional Guidance for Mothers Collecting Milk Samples
- USP-IAS Instructions for PAS

GRULAC Region

- Guide for PAS (en, sp)
- SOP Cleaning of glassware (en, sp)
- SOP Collection of mothers' milk (en, sp)
- SOP Indicator PCB in air (en, sp)
- SOP Indicator PCB in fish (en, sp)
- SOP Indicator PCB in mothers' milk (en, sp)
- SOP OCP en aire (en, sp)
- SOP OCP en leche materna (en, sp)
- SOP OCP en pescado (en, sp)
- SOP OCP en sedimentos (en, sp)
- SOP PCDD PCDF dI-PCB en aire (en, sp)
- SOP PCDD PCDF dI-PCB en leche materna (en, sp)
- SOP PCDD PCDF dI-PCB en pescado (en, sp)
- SOP PCDD PCDF dI-PCB en sedimentos (en, sp)

East and South Africa

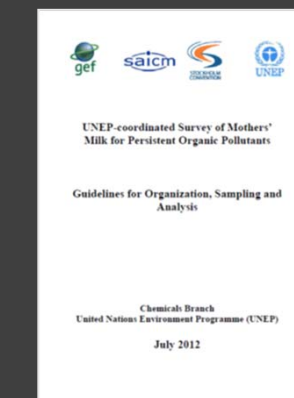
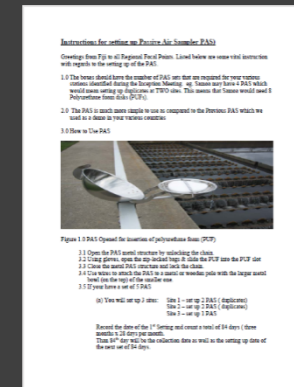
- SOP Kenya: Mothers' Milk
- SOP Recetox PAS

West Africa

- SOP in passive air sampling (PAS)

Cross-cuttings

- Guidance for organisation, sampling and analysis of human milk



Laboratory instrumentation level	Equipment	Infrastructure needs	Chemicals
5	Sample extraction and clean-up systems (manually or automated), LC-MS/MS)	Nitrogen/air conditioning/consistent power/high operational costs/personnel specifically trained to operate and troubleshoot complicated instrumentation	PFOS and other anionic PFCs , PFOSA
3	Basic sample extraction and clean-up equipment, capillary GC-ECD	Nitrogen/air conditioning/power/ personnel specifically trained to operate and troubleshoot equipment problems	PBB, most PCB and all OCPs except toxaphene
2a	Sample extraction and clean-up equipment, capillary GC-LRMS – electron ionization mode	Helium/air conditioning/ consistent power/ personnel specifically trained to operate and trouble-shoot equipment problems	PBB, most PCB and all OCPs; Also perfluoro-sulfamido alcohols in positive chemical ionization mode
2b	Sample extraction and clean-up equipment, capillary GC-LRMS – negative chemical ionization mode	Methane or other moderating gas/air conditioning/ consistent power/ personnel specifically trained to operate and trouble-shoot equipment problems	PBDE and PBB, as well as toxaphene and other highly chlorinated (≥ 4 Cl) OCPs
1	Sample extraction and clean-up equipment, capillary GC-HRMS	Helium/air conditioning/ consistent power/high operational costs /personnel specifically trained to operate and troubleshoot complicated instrumentation	PCDD/PCDF, all PCB, all OCPs, PBB, all PBDE

GC-ECD – gas chromatography/electron capture detection
GC-LRMS – gas chromatography/low resolution mass spectrometry
GC-HRMS – gas chromatography/high resolution mass spectrometry
LC-MS/MS – high performance liquid chromatography/tandem mass spectrometry
PY – Person-year

Instrumentation –
Tier

GMP guideline

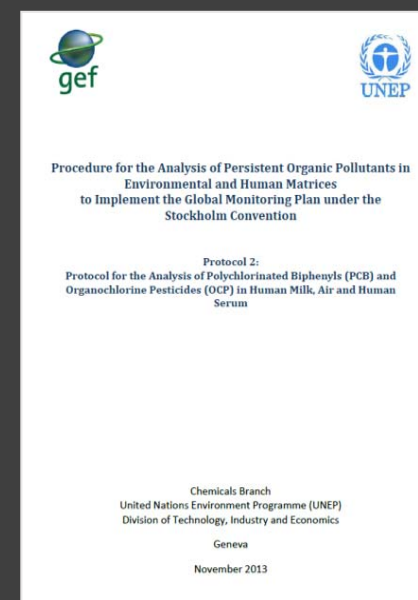
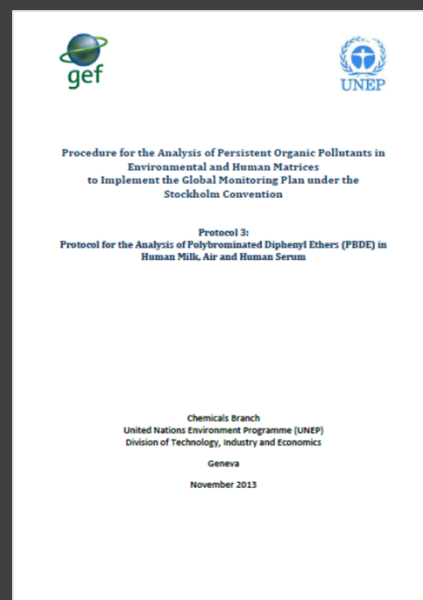
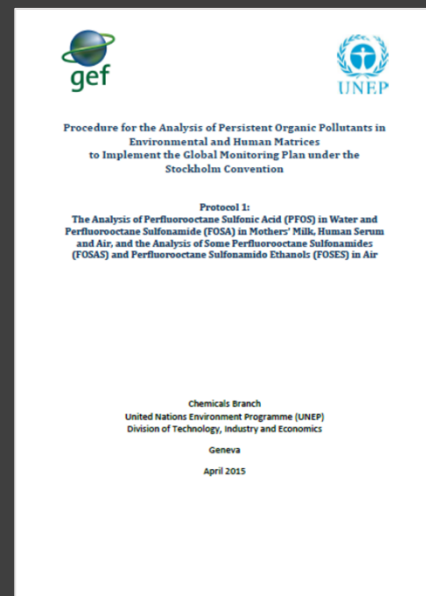
Standard operational
procedures for new POPs –
example of PFAS

How to use SOPs

- SOPs are guidelines
- If followed precisely: good results
- Other methods are possible and allowed
- However: always optimize and validate!
- Validation: in house reference materials, certified reference materials, interlab study

SOPs for POPs

- Protocol 1: Analysis of **PFOS** in **Water and FOSA** in Mothers' Milk Serum and Air, and the Analysis of some FOSAs and FOSEs in Air
- Protocol 2: Analysis of **PCB and OCP** in Human Milk, Air and Human Serum
- Protocol 3: Analysis of **PBDE** in Human Milk, Air and Human Serum
- Mirror samples



<http://www.unep.org/chemicalsandwaste/POPsandScience/AnalysisandMonitoring/MethodDevelopment/tabid/1059865/Default.aspx>

Method development

- In order to generate high quality and comparable results, the protocols and methods for sampling and analysis of all POPs in relevant types of samples have to be harmonized;
- In all regions and over time, the same basic approaches and quality criteria for acceptance of data and assessment of results should be applied.
 - Standard operating procedures (SOPs) for groups of POPs
 - General guidelines for specific matrices (types of samples):

Note: The guides and SOPs should be taken as an orientation and be transferred into daily routines by each laboratory.

<http://www.unep.org/chemicalsandwaste/POPsandScience/AnalysisandMonitoring/MethodDevelopment/tabid/1059865/Default.aspx>

Choice of analytical method

- The SOPs prepared for UNEP describe general procedures for analysis;
- However, it is possible to change certain parameters and analytical conditions described in this protocol, while still obtaining the same results;
- In any case, the entire method should be optimized and validated to ensure the comparability of data.

**INTERNATIONAL
STANDARD**

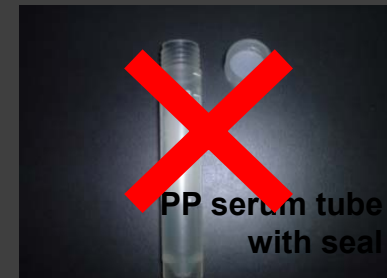
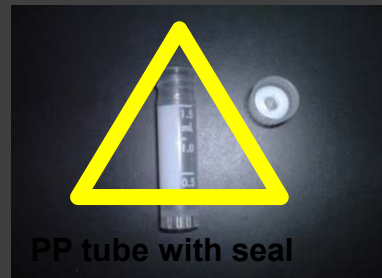
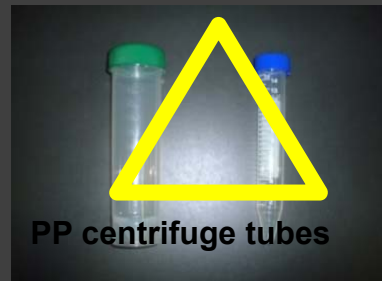
**ISO
25101**

First edition
2009-03-01

Available at ISO home page

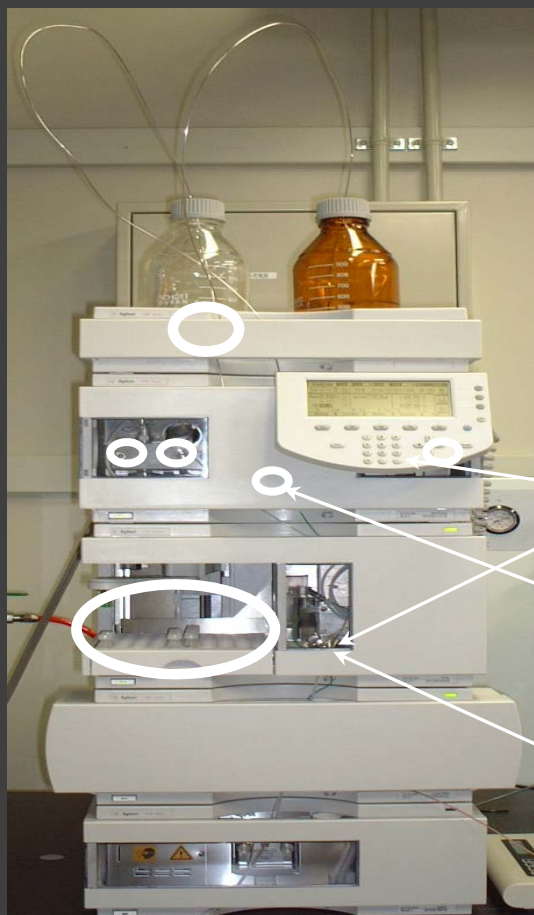
**Water quality — Determination of
perfluorooctanesulfonate (PFOS) and
perfluorooctanoate (PFOA) — Method for
unfiltered samples using solid phase
extraction and liquid
chromatography/mass spectrometry**

How to control background contamination in the laboratory?



How to control background contamination from the instrument?

How to control instrumental blank?



seal

solvent
selection valve

rotor seal



Septum blank?



POLYETHYLENE



TEFLON



VITON

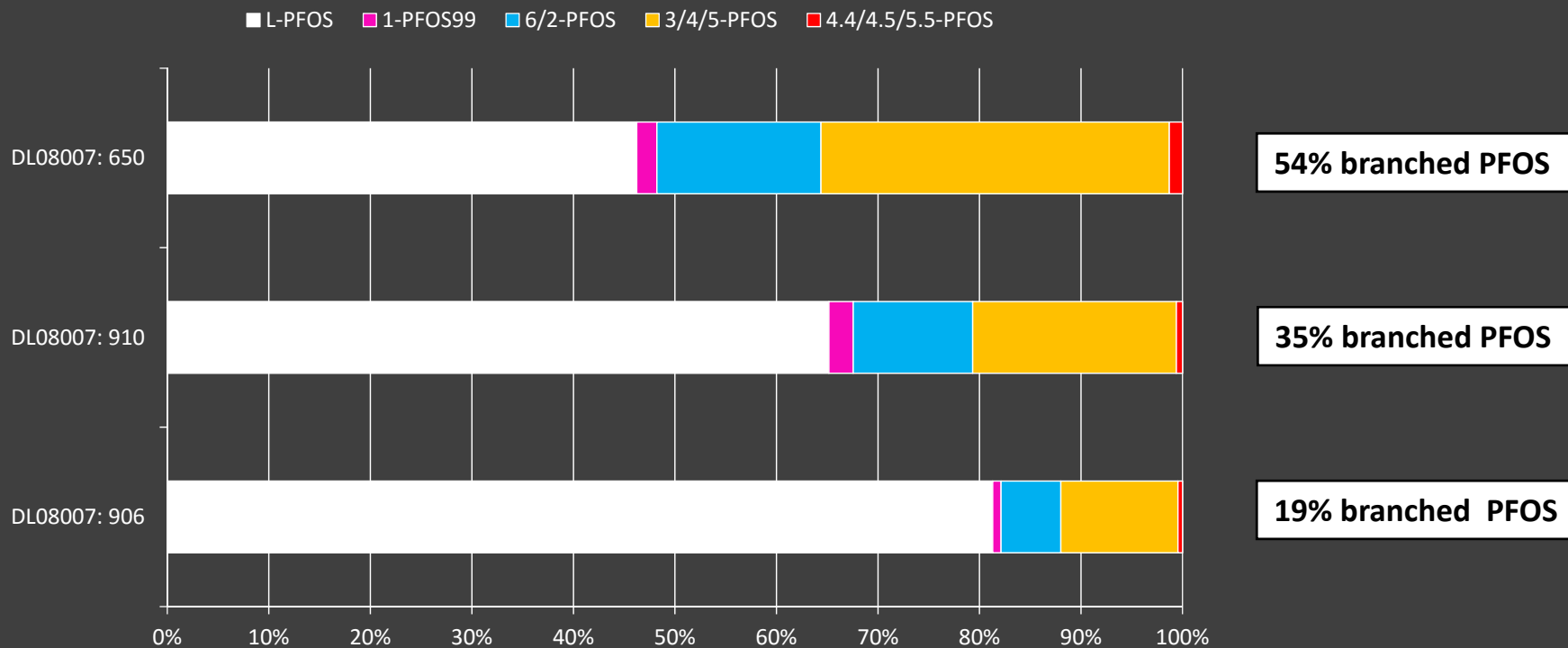
Structural isomers of PFOS identified in technical mixtures

Abbreviation	Formula	Name
L-PFOS	$\text{CF}_3\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{SO}_3^-$	<i>n</i> -perfluoro-octanesulfonate
1-PFOS	$\text{CF}_3\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}(\text{CF}_3)\text{SO}_3^-$	perfluoro-1-methyl-heptanesulfonate
2-PFOS	$\text{CF}_3\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}(\text{CF}_3)\text{CF}_2\text{SO}_3^-$	perfluoro-2-methyl-heptanesulfonate
3-PFOS	$\text{CF}_3\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}(\text{CF}_3)\text{CF}_2\text{CF}_2\text{SO}_3^-$	perfluoro-3-methyl-heptanesulfonate
4-PFOS	$\text{CF}_3\text{CF}_2\text{CF}_2\text{CF}(\text{CF}_3)\text{CF}_2\text{CF}_2\text{CF}_2\text{SO}_3^-$	perfluoro-4-methyl-heptanesulfonate
5-PFOS	$\text{CF}_3\text{CF}_2\text{CF}(\text{CF}_3)\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{SO}_3^-$	perfluoro-5-methyl-heptanesulfonate
6-PFOS	$\text{CF}_3\text{CF}(\text{CF}_3)\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{SO}_3^-$	perfluoro-6-methyl-heptanesulfonate
4,4-PFOS	$\text{CF}_3\text{CF}(\text{CF}_3)_2\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{SO}_3^-$	perfluoro-4,4-dimethyl-hexanesulfonate
3,5-PFOS	$\text{CF}_3\text{CF}(\text{CF}_3)\text{CF}_2\text{CF}(\text{CF}_3)\text{CF}_2\text{CF}_2\text{SO}_3^-$	perfluoro-3,5-dimethyl-hexanesulfonate
4,5-PFOS	$\text{CF}_3\text{CF}(\text{CF}_3)\text{CF}(\text{CF}_3)\text{CF}_2\text{CF}_2\text{CF}_2\text{SO}_3^-$	perfluoro-4,5-dimethyl-hexanesulfonate
5,5-PFOS	$\text{CF}_3\text{C}(\text{CF}_3)_2\text{CF}_2\text{CF}_2\text{CF}_2\text{CF}_2\text{SO}_3^-$	perfluoro-5,5-dimethyl-hexanesulfonate

Technical mixtures typically contain between 71% and 83% L-PFOS (Vyas et al. 2007)

Differences in PFOS isomer profiles in human plasma

Why do we see differences?
-sources of exposure
-historical vs. recent exposure
-precursors
-differences internal elimination



Choice of analytical method

Table 2. A summary of selected analytical methods for the determination of fluorinated POPs in human serum/plasma

Analytes	N analytes	Study type	Year	Sample preparation			Instrumental analysis			Ref.
				Sample amount	Pre-treatment	Extraction and clean-up	Configuration	Injection volume	Separation	
PFAAs	13	Method development	2011	100 µL	0.1 M formic acid	On-line SPE: C18	HPLC-ESI-MS/MS	400 µl	Betasil C8	[41]
PFAAs	13	Inter-laboratory comparison	2010	0.15–1.2 g	Formic acid (50/50, v/v)	SPE: Oasis WAX	HPLC-ESI-MS/MS	5–20 µl	Betasil C8	[38]
PFAAs	10	Inter-laboratory comparison	2010	0.2 mL	0.1 mol/L formic acid	On-line SPE: C18	HPLC-TIS-MS/MS	n.i.	Betasil C8	[38]
PFAAs	13	Inter-laboratory comparison	2010	0.2 mL	PP with acetonitrile	n.i.	HPLC-TIS-MS/MS	n.i.	Betasil C18, Prism RP C12	[38]
PFAAs	8	Inter-laboratory comparison	2010	1 mL	n.i.	LLE using ion pair, filtration with 0.2 µm	HPLC-ESI-MS/MS	n.i.	ACE C18	[38]
PFAAs	9									[38]
PFAAs	11									[37]
PFAAs	19									[40]
PFAAs	25	Method development	2010	0.5 mL whole blood	Formic acid (50% in water) or acetonitrile	SPE: Oasis WAX, LLE using ion pair, and LLE using acetonitrile	LC-ESI-MS/MS	10 µl	Betasil C18 and JJ-50 2D	[36]
PFAAs	18	Method development	2005	100 µL	0.1 M formic acid	On-line SPE: C18	HPLC-TIS-MS/MS	400 µl	Betasil C8	[34]
PFAAs	12	Method development	2005	0.75 mL whole blood	Formic acid (50/50, v/v)	SPE: C18, filtration with membrane 0.2 µm filter	HPLC-ESI-MS and LC-MS/MS	10 µl	Discovery HS C18	[43]
PFAAs	13	Method development	2004	1 mL	0.1 M formic acid	Off-line SPE: Oasis HLB	HPLC-TIS-MS/MS	12 µl	Betasil C8	[39]

Note: no information (n.i.).

The choice of analytical method should be needs-oriented and developed to fit the purpose of the study!

Principle

- Sample preparation of water, milk, and serum/plasma samples is similar but: different for air
- Extraction: Soxhlet extraction and SPE
- Clean-up (ENV carb): depending on the complexity of the sample matrix
- Method validation: ILSs, SRMs, CRMs, and spiking experiments
- Instrumental analysis: UPLC-ESI-MS/MS in negative ion mode

Materials and reagents

- **Materials:**

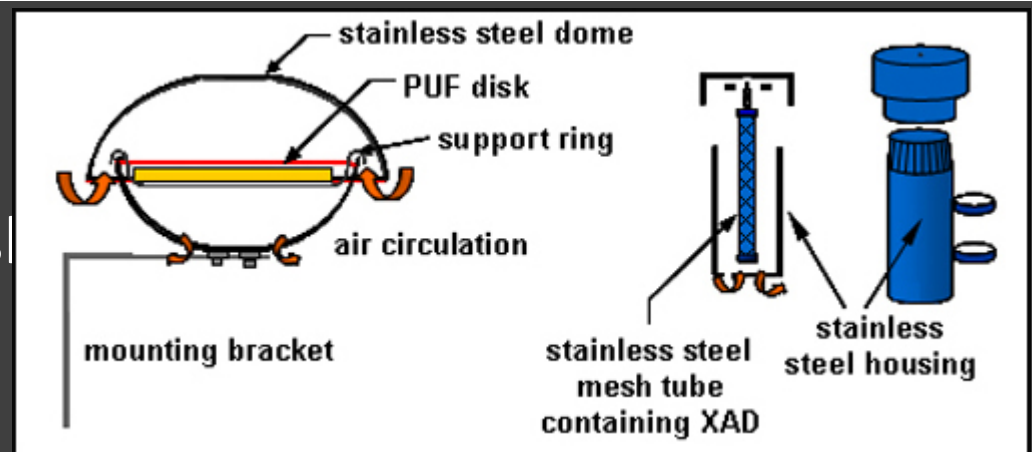
- ✓ Glass Beaker (2 L)
- ✓ Polypropylene bottle (100 mL)
- ✓ Plastic pipettes
- ✓ Polypropylene tubes (15 mL)
- ✓ Micro tubes (1.5 mL)
- ✓ Crimpcap polypropylene vial (700 µL)
- ✓ Seal, silver aluminum 11 mm, PTFE/Rubber Liner
- ✓ Capper/Decapper
- ✓ Ultrasonic bath
- ✓ Vacuum dessiccator
- ✓ Passive sampler
- ✓ Balance (precision 0.01 g)
- ✓ Pipettes (50, 100 and 200 µL)
- ✓ Centrifuge
- ✓ Oven (37 °C)
- ✓ SPE device (rinse with methanol and water prior to use)
- ✓ pH meter
- ✓ Vacuum pump
- ✓ Water bath (50 °C)
- ✓ Whirl mixer
- ✓ LC-MS/MS (LC-QQQ). Electrospray source (ESI) with negative polarity
- ✓ FluoroSEP-RP Octyl column, 15 cm x 2.1 mm, 5 µm particle size, ES Industries (132211-FO)
- ✓ 2 x Symmetry columns C18, 20 mm x 3.9 mm, 5 µm particle size, Waters (WAT054225)
- ✓ Symmetry column C18, 50 mm x 2.1 mm, 5 µm particle size, Waters (18600206)

- **Reagents:**

- ✓ Polyurethane foam (PUF) disk, 14 cm x 1.35 cm, surface area 365 cm², mass 4.40 g, volume 207 cm³, Tisch Environmental, Cleves, OH
- ✓ Aceton, Ultraresi, J.T.Baker (9254)
- ✓ Petroleum ether, J.T.Baker
- ✓ Methanol, HPLC gradient grade, J.T.Baker (8402)
- ✓ Internal standard (¹³C₄ PFOS + ¹⁸O₂ PFOSA + ²H₃ MeFOSEA + ²H₅ EtFOSEA + ²H₇ MeFOSE + ²H₉ EtFOSE) in methanol (100 ng/mL)
- ✓ Internal standard (¹³C₄ PFOS + ¹⁸O₂ PFOSA) in methanol (100 ng/mL)
- ✓ 50% Formic acid in water
- ✓ SPE Cartridge, Oasis WAX 6cc, Waters 186002493
- ✓ Ammonia 25% p.a. purity
- ✓ 0.1% NH₄OH in methanol; add 400 µL ammonia to 100 mL methanol
- ✓ 2 % NH₄OH in methanol; add 8 mL ammonia to 92 mL methanol
- ✓ HPLC water, HPLC analyzed, J.T. Baker (4218), or MilliQ purity
- ✓ Acetic acid 100 % pro analysis (p.a.) purity
- ✓ Ammonium acetate p.a. purity
- ✓ 25 mM Ammonium acetate; add 190 mg ammonium acetate to 100 mL water and adjust the pH to pH=4 with acetic acid
- ✓ Nitrogen gas. Purity 5.0
- ✓ Injection standard (1) (13C8 PFOS) in methanol/water (1:1, v/v) (150 ng/mL)
- ✓ Injection standard (2) (13C8 PFOS) in methanol/water (1:1, v/v) (50 ng/mL)
- ✓ Injection standard (3) (13C8 PFOS) in methanol/water (1:1, v/v) (25 ng/mL)
- ✓ Ammonium formate, (>99%), Fluka (09735)
- ✓ Ammonium formate buffer 5 mM: Dissolve 315 mg ammonium formate in 1 L HPLC water. Filter prior to use.
- ✓ PFAS calibration solutions (0.05, 0.25, 0.5, 5, 50, 100 ng/mL) in methanol/water (1:1, v/v)

Air

- Polyurethane foam (PUF) disk
 - Preparation of the PUF
- Cleaning of a PUF:
 - If necessary, wash the PUF in water;
 - Perform a Soxhlet extraction on the PUF with acetone (24 h), followed by petroleum ether (24 h)
 - Dry the PUF in a desiccator (24 h)
- Air sampling
 - Place PUF in passive sampler for 3 months at sampling location
- Sample preparation
 - Take PUF out of the sampler
 - Add 150 μL Internal standard (I.S.) to the PUF
- Procedural blank
 - Prepare a PUF as described above without the exposure time during the sampling



Air - analysis

- Perform a Soxhlet extraction with methanol (12 h)
- Concentrate extract to 1 mL by rotary evaporator or Kuderna-Danish
- Filter extract through a 0.2 μm glass hydrophilic polypropylene (GHP) filter into a polypropylene LC vial
- Concentrate to 200 μL under a gentle stream of nitrogen
- Add 100 μL injection standard
- Add 300 μL 2mM ammonium acetate and shake manually
- Analyze with LC-MS/MS



Water sampling and sample preparation

Water sampling described in “PFOS analysis in water for the Global Monitoring Plan of the Stockholm Convention” (UNEP GMP WG)

Sample preparation

- As soon as the sample arrives to the analytical laboratory internal standards (IS) should be added to compensate for absorbance to laboratory equipment
- The sample (incl. IS) should have time to equilibrate before analysis
- Keep the water samples (500 mL) in a high density polyethylene (HDPE) in the fridge or freezer (-20 °C) and defrost them the day before analysis
- Shake the water rigorously before subsamples are taken out;
- Weigh 100 mL of water sample in a HDPE bottle

Procedural blank

- Prepare a procedural blank sample using ultra clean (MilliQ) water as sample substitute

Human milk and serum sample preparation

Follow the UNEP/WHO protocol for sampling of human milk 'UNEP-coordinated Survey of Mothers' Milk for Persistent Organic Pollutants' (<http://www.unep.org/chemicalsandwaste/portals/9/POPs/docs/Mothers%20milk%20guide%20POPs.pdf>)

Sample preparation

- Homogenise sample (50 mL) by shaking for 1 min
- Weigh 1 mL of milk, or 0.5 mL serum in PP tube (15 mL)
- Add 50 µL I.S.
- Add 2 mL 50% formic acid and shake manually
- Place sample in an ultrasonic bath for 15 min
- Centrifuge 15 min at 3,000 rpm
- Place sample in an oven at 37 °C for 30 min

Procedural blank

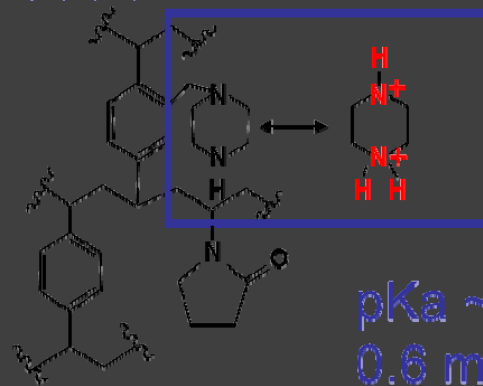
- Prepare a procedural blank sample as described above in sample description using ultra clean (MilliQ) water

Water, human milk and human serum

- Solid phase extraction (SPE) is used for the extraction of water, mothers' milk and serum.
- Install an SPE cartridge on the SPE device

- Waters Oasis® WAX SPE Column
- Mixed-mode **Weak Anion-eXchange** and reversed-phase sorbent
- Single use Oasis cartridge
- Retain and release strong acids (e.g. sulfonates)

Oasis® WAX



pKa ~6
0.6 meq/g



Method validation and performance

- Sensitivity
 - MDLs ranged 0.01 ng mL⁻¹-0.17 ng mL⁻¹
 - Linear range: 0.01 ng mL⁻¹-60 ng mL⁻¹
- Accuracy
 - Conformed well with NIST SRM 1957 (n=54)
 - 6-32% normalized difference

Repeatability (QC n=7) and reproducibility (QC n=103) ranged between 2%-20% including structural PFOS isomers



Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma



A rapid method for the determination of perfluoroalkyl substances including structural isomers of perfluorooctane sulfonic acid in human serum using 96-well plates and column-switching ultra-high performance liquid chromatography tandem mass spectrometry



Samira Salihovic^{a,*}, Anna Kärrman^a, Gunilla Lindström^a, P. Monica Lind^b, Lars Lind^c, Bert van Bavel^a

^aMTM Research Centre, School of Science and Technology, Örebro University, Örebro, Sweden

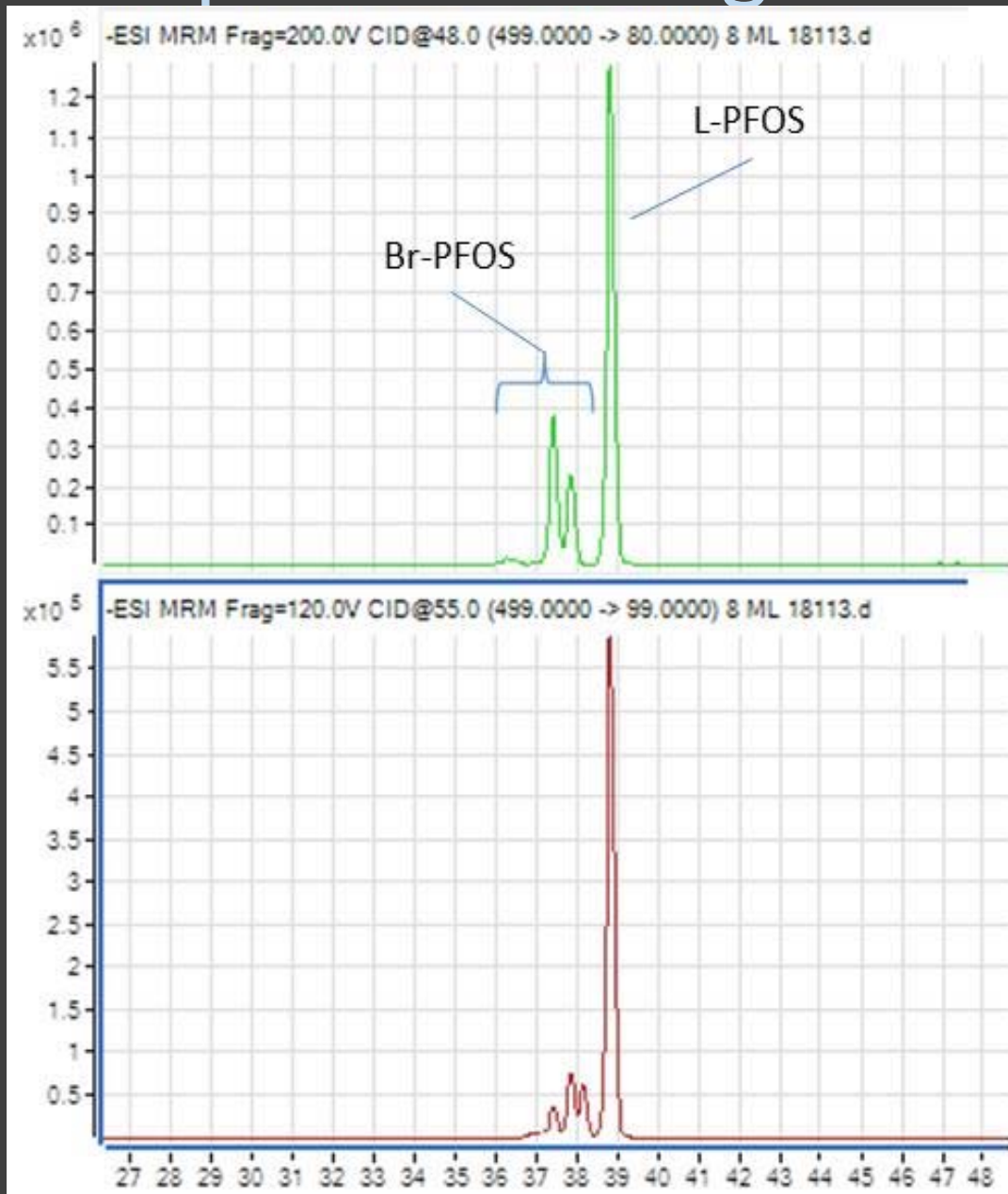
^bOccupational and Environmental Medicine, Uppsala University, Uppsala, Sweden

^cAcute and Internal Medicine, Uppsala University, Uppsala, Sweden

Instrumental analysis

- Analytical column and guard column
- Extra column (50 mm) and a guard column between the LC pump and the injector, to prevent interference of PFASs, originating from the LC system
- Purge all the mobile phase solvents through the system
- Start pump with 65% ammonium formate and 35% methanol
- Place all extracts, blanks, calibration solutions in tray of autosampler
- Make a sequence in the computer. Analyse the samples, the calibration solutions, the blank and the reference material in random order
- Inject calibration solution after pump has been running for 30 min
- Check performance of LC-MS/MS by comparing retention times and peak intensities of the calibration solution with earlier results;

Sample chromatogram



Chromatogram showing the separation of linear and branched PFOS in water (surface water sample from The Netherlands)

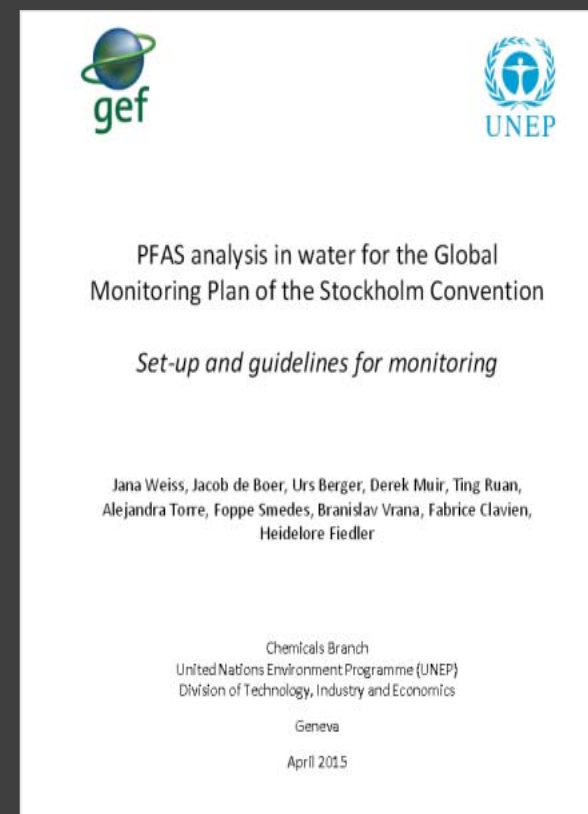
Note: PFAS concentrations should be reported on wet weight basis. However, often, results are reported on sulfonate anion basis, i.e., corrected for the molecular weight of the PFOS salt (with cation)

Mass settings for PFAS analysis

Compound		Precursor Ion (m/z)	Production (m/z)	Comment
PFOS	Target compound	499	80	Quantifier
			99	Qualifier
¹³ C ₄ PFOS	Internal standard	503	80	Quantifier
			99	Qualifier
¹³ C ₈ PFOS	Injection standard	507	80	Quantifier
			99	Qualifier
FOSA	Target compound	498	78	Quantifier
			169	Qualifier
¹⁸ O ₂ FOSA	Internal standard	502	82	Quantifier
			169	Qualifier
MeFOSA	Target compound	512	169	Quantifier
			219	Qualifier
² H ₃ MeFOSA	Internal standard	515	169	Quantifier
			219	Qualifier
EtFOSA	Target compound	526	169	Quantifier
² H ₅ EtFOSA	Internal standard	531	169	Quantifier
MeFOSE	Target compound	602	45	Quantifier
² H ₇ MeFOSE	Internal standard	609	45	Quantifier
EtFOSE	Target compound	616	45	Quantifier
² H ₉ EtFOSE	Internal standard	625	45	Quantifier

Tools and methods for new POPs

- PFAS analysis in water - Set-up and guidelines for monitoring
- Instructive movie for analysis of PFOS and precursors
- Movie with instructions for the cleaning of PUF disks for passive sampling of ambient air



<http://www.unep.org/chemicalsandwaste/POPsandScience/AnalysisandMonitoring/MethodDevelopment/tabid/1059865/Default.aspx>