Per- and polyfluorinated compounds (PFC) analysis of various matrices using UPLC-MS/MS and GC-MS/MS

Human exposure to PFCs through food, drinking water, house dust and indoor air

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Outline of presentation

• General background on PFCs
  • Sources of exposure
  • Human exposure and pathways of exposure

• Methodology
  • Sample preparation techniques
    • Human whole blood
    • Food
    • Water
    • Dust
    • Indoor air
  • Instrumental analysis

• Developments in methodology

• Total human exposure to PFCs
  • Internal exposure – blood levels
  • External exposure sources:
    • Food
    • Drinking water
    • Indoor environment: dust and air
Per- and polyfluorinated compounds (PFCs)

Global distribution in humans and wildlife

Unique properties
- Oil, water and grease repellency

PFOS
- Persistent, Bioaccumulative, Toxic
- Annex B, Stockholm Convention

PFOA
- Persistent
- Present in humans and wildlife in ppb-levels

Fluorotelomers (FTOHs), FOSA/Es, surfactants (PAPs), polymers

Numerous industrial and commercial applications
- Surfactants and surface protectors in carpets, leather, paper, food containers, fabric and upholstery
- Used in waxes, polishes, paints, varnishes and cleaning products
Human exposure

- General population – low ng/mL levels

- Pathways for human exposure
  - Food
  - Drinking water
  - Air
  - Dust
  - Contact exposure
  - Occupationally exposure
  - From contaminated sites
PFC exposure in a selected region

Biomonitoring of PFC in humans - blood levels (internal exposure)

PFC levels in food samples
Consumption of various food stuffs

PFC in drinking water
Drinking water consumption: 1.41 L/day, 0.40 L/day

Indoor sources: house dust and indoor air
Dust ingestion rate, inhalation rate
Methodology

• Sample matrices: human whole blood, food, water, air, house dust

• Method validation
  – Spiking experiments
  – Sample preparation
    • Extraction and clean-up
  – Instrumental analysis
Sample preparation — whole blood

- **Sample**
  - 0.5 ml blood (serum)
  - Spiked with internal std
  - Vortex mixing
  - Add formic acid/water (1:1)
  - Sonicate 15 min
  - Centrifuge at 10 000 x g, 30 min

- **Extraction on Waters Oasis® WAX SPE column** (60 mg / 3 mL)
  - Extract the supernatant on WAX (conditioned with 2 ml MeOH, 2 ml water)
  - Wash with 40 % MeOH
  - Dry columns under vacuum suction
  - Elute PFCs with 1 ml 1 % NH₄OH in MeOH

- **Filtration**
- Addition of recovery standard
- Instrumental analysis on LC-MS

- **Similar procedure for analysis of human milk samples**
- **Additional pre-concentration steps**
  - Large volume injection column switch
  - Reducing extract volume to 10 %

Solid phase extraction

- 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).

- Alternative methods for blood analysis
  - Simple extraction with MeOH or ACN – clean up using dispersive carbon (ENVI-Carb)
  - Large volume injection and column switching

**Oasis® WAX**

```
PKa ~6
0.6 meq/g
```
WAX – Recovery

![Graph showing recovery percentages for various compounds compared to WAX and C18 HF.](image-url)
Sample preparation — food samples

• **Sample types**: composite and individual food samples including processed, packaged, raw and cooked food
• 1 g freeze-dried sample
• Spike with internal standards
• Alkaline digestion (2 mL 0.2 mM NaOH in MeOH) and extraction with MeOH (10 mL)
• SPE-WAX (150mg/6cc)
  – Pre-cleaned with 2 mL water, 2 mL MeOH
  – Eluted with 2 mL 2% NH₄ in MeOH
• ENVI-Carb clean up
• Filtration
• Final volume of 500 ul including recovery standards
Sample preparation — water

- **Sample types:** drinking, river water and bottled water
- Pre-treatment: acidification to pH 4, stored refrigerated, filtration (glass microfiber)
- 500 mL
- Spike with internal standards
- Concentration on SPE-WAX (150mg/6cc)
  - Pre-cleaned with 2 mL water, 2 mL MeOH
  - Eluted with 2 mL 2% NH$_4$ in MeOH
- Filtration
- Final volume of 500 ul including recovery standards
Sample preparation – PFCs in house dust

- Sampling of vacuum cleaner dust bags (n=10)
- 1 g dust (<150 µm)
- Spike with internal standards
- Repeated extraction with methanol followed by sonication and centrifugation
- Clean up by ENVI-Carb dispersive carbon (25 mg)

- Final volume of 500 µl including recovery standards
  - Extracts split
    - GC-MS/MS analysis of neutral compounds
    - LC-MS/MS analysis of ionic compounds
Sample preparation – PFC in indoor air

- High volume sampling (>1 m over the floor) on pre-cleaned Isolute ENV+ cartridges (n=10, 2 replicates)
  - Sampling spikes: $^{13}$C$_4$-8:2 FTOH, $^{13}$C$_8$-PFOA
  - Volume sampled: 2820-3280 dm$^3$

- Stored air tight in -20 C and analyzed within 2 months of sampling

- Addition of labelled extraction standards (IS)
- Extraction with methanol

- Final volume of 500 µl including recovery standards
  - Extracts split
    - GC-MS/MS analysis of neutral compounds
    - LC-MS/MS analysis of ionic compounds
Alternative sample preparation techniques used at MTM

- **Sample types**: soil and dry sediment, various biota samples (mink liver, fish liver, fish muscle, pig liver, mussel, limpet)
  - Alkaline digestion
  - Extraction with ACN
  - Hexane shake: repeated (3 times) addition of hexane (1:2 of hexane:extract) followed by vigorous shaking and removal of hexane fraction (for lipid removal)
  - ENVI-Carb
Instrumental analysis

• Ionic PFCs
  – LC-SQ-MS for whole blood, food and water samples
  – UPLC-MS/MS for food, drinking water, house dust and indoor air

• Non-ionic PFCs
  – GC-MS/MS for house dust and indoor air samples
LC-MS

- **HP 1100 LC/MSD**
  - Analytical column: Discovery HS C18 (50 x 2.1 mm, 3 um)
  - Guard column as analytical column
  - Electrospray (negative mode)

- **Mobile phases**
  - 2 mM NH$_4$Ac in Water (A)
  - 2 mM NH$_4$Ac in MeOH (B)
    - Flow rate 0.3 ml/min
    - Column temperature: 40 ºC

- **Gradient program**
  - 0 min, 35 % B
  - 20 min, 90 % B, 2 min hold
  - 25 min, 100 % B
  - 5 min stabilization time to initial conditions

UPLC-MS/MS

• Waters ACQUITY UPLC System
  – Pre-column: Symmetry C18, 2.1 x 100mm, 3.5μm
  – Analytical column: ACQUITY BEH C18, 2.1 x 50mm, 1.7μm

• Mobile Phases
  – A : Aqueous + 2mM ammonium acetate
  – B : Methanol + 2mM ammonium acetate
    » Flow rate = 0.4 mL/min
    » Injection volume = 10 μL
    » Column temp = 50°C

• UPLC solvent run
  » 0.0 min: 70% A  30% B
  » 0.5 min: 70% A  30% B
  » 5.0 min: 10% A  90% B
  » 5.1 min: 0% A  100% B
  » 6.0 min: 0% A  100% B

• MRM of molecular ion [M-H]⁻ for PFCAs and [M]⁻ for PFSAs with products ions [M-COOH]⁻ and [FSO₃]⁻

• QA/QC: 5-7 point calibration curves, internal standards for sampling and extraction, extraction and field blanks, spiked samples, S/N of 3, recovery > 50 %, in-house or certified reference samples, instrumental blank injections, participation in interlaboratory comparisons
Comparison of run-time & peak width obtained using UPLC and traditional HPLC
GC-MS/MS

• Nonionic PFCs:
  – flurotelomer alcohols (FTOH)
  – fluorooctane sulfonamides (FOSAs) and sulfonamidoethanols (FOSEs)

• Waters Quattro Micro GC system
  – FTOHs: Cl⁺, FOSA/Es: Cl⁻
  – 1 ul injection, pulsed splitless mode (40 psi), 250 C
  – Carrier: He 1.0 ml/min, Reagent: Methane
  – Separation: Supelcowax10 (30m, 0.25 mm i.d., 0.25 um)
  – Linear calibration range of 2-1250 pg/ul
  – RSD <30 % over 100 real sample injections
SIR vs MRM

- IDL ~1 pg/ul on column FTOHs and FOSA/Es in SIR and ~2 pg/ul in MRM
- Difficulties with positive confirmation in real samples (SIR)
- Increased specificity using MRM
  - S/N of 2.2 in SIR and 6.7 in MRM for 10:2 FTOH
SIR vs MRM

- IDL ~1 pg/ul on column FTOHs and FOSA/Es in SIR and ~2 pg/ul in MRM
- Difficulties with positive confirmation in real samples (SIR)
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### MS details

<table>
<thead>
<tr>
<th>compound</th>
<th>abbreviation</th>
<th>m/z</th>
<th>labeled standards</th>
<th>UPLC-MS/MS</th>
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Analytical difficulties

- Known issue in the analysis of PFCs
  - 1) Procedure (major contamination)
    - Glassware
    - Vials, vial tops, etc
  - 2) Instrument
    - Solvents in LC run
    - Parts on the instrument pre-injector
    - Injector (minor contamination)
- Key contaminating compounds include
  - PFOA
  - PFNA

- Hard to achieve!
- For instrument:
  - Find solvents that have low levels
- Install a column post-pump and pre-injector
  - Separate the contaminating peak from the analytical peak
PFC exposure in a selected region

Biomonitoring of PFC in humans - blood levels (internal exposure)

PFC levels in food samples
Consumption of various food stuffs

PFC in drinking water
Drinking water consumption: 1.41 L/day, 0.40 L/day

Indoor sources: house dust and indoor air
Dust ingestion rate, inhalation rate
Total exposure from food, drinking water, house dust and indoor air

**PFOS**
- Dietary intake: 74
- Water: 5.2
- Air PFOS: 0.11
- Dust PFOS: 0.17
- Dust FOSA/E: 0.027

**PFOA**
- Air PFOA: 0.59
- Air FTOH: 1.3
- Water: 6.4
- Dust PFOA: 0.48
- Dust FTOH: 0.03
- Dietary intake fish, seafood: 24

**External exposure**
- 80 ng PFOS/day
- 33 ng PFOA/day

**Internal exposure**
- 103 ng PFOS/day
- 30 ng PFOA/day
THANK YOU

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Formas

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