Analysis of Extended List of PFASs in Human Serum/Plasma

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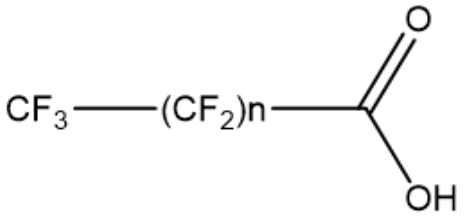
Outline

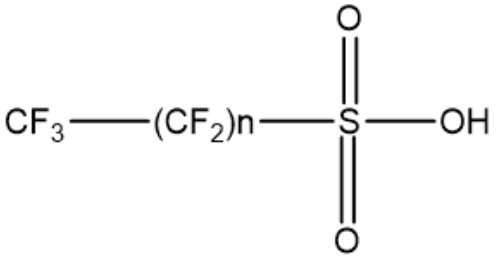
- Background and objective of method development
- Challenges in method development
- Method validation
- Intra-Program Pilot Phase 7 (IPP7) data and discussion
- Recommendations for Studying Trends in Exposures in Prenatal Samples (STEPS) study
- Summary

Need of New Analytical Method for PFAS Biomonitoring

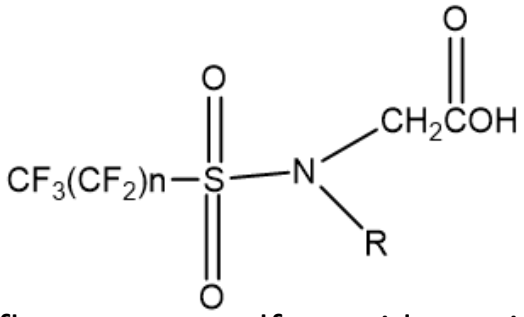
- Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a large group of man-made chemicals (thousands) that have been used in consumer products.
- PFASs are complex compounds, and they have different functional groups.
- New/replacement PFAS concerns call for new method to assess human exposure to emerging compounds.
- High demand for plasma analysis method.



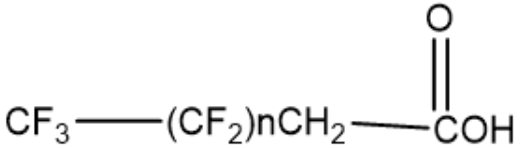
Perfluoroalkyl carboxylates



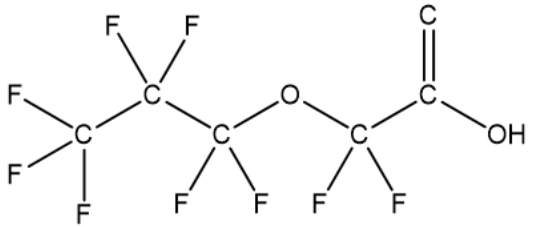
Perfluoroalkyl sulfonates



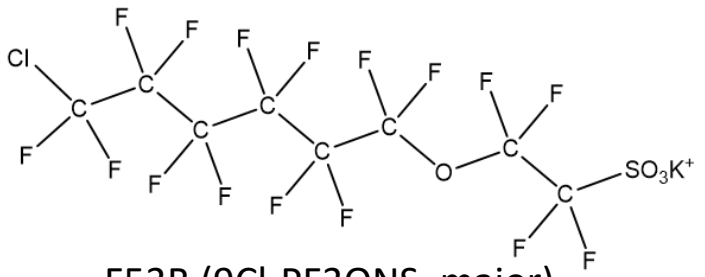
Perfluorooctanesulfonamidoacetic acids



Telomer Acids (FTOH derivatives)



HFPO-DA (Gen-X)



F53B (9Cl-PF3ONS, major)

History of Methods Development for PFASs in Serum

Year	Method	Number of Compounds	Analytes
2009	Sciex QTRAP 4000, On-line SPE LC-MS/MS for human serum	12 (reported)	**12 Legacy PFCs:** PFHpA, PFOA, PFNA, PFDeA, PFUA, PFDoA, PFBuS, PFHxS, PFOS, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH
2016	Sciex QTRAP 5500, manual SPE LC-MS/MS for human serum samples	32 (reported)	**PFASs from method in 2009 + 20 more PFASs:** PFBA, PFPeA, PFHxA, PFDS, 5:3 FTCA, 7:3 FTCA, 6:2 FTCA, 8:2 FTCA , 6:2 FTUCA, 8:2 FTUCA, 4:2 FTS, 6:2 FTS, 8:2 FTS, 8:2 PAP, 6:2 diPAP, 8:2 diPAP, 6:6 PFPiA, 6:8 PFPiA, PFHxPA and PFOPA
2022 (NEW)	Sciex Quad 6500+, On-line SPE LC-MS/MS for human serum/plasma samples	51 (investigated)	**PFASs in methods 2009 and 2016 + 19 more PFASs:** PFTrDA, PFTeDA , PFPeS, PFHpS, PFNS, PFECHS, 10:2 FTUCA, 10:2 FTS, 6:2 PAP, SAmPAP, diSAmPAP, 8:8 PFPiA, PFDPA, Gen-X, ADONA, F53B (9Cl-PF3ONS, major), F53B (11Cl-PF3OUS, minor), FBSA, N-AP-FHxSA

Challenges and Solutions in Method Development

- Diversified structures warrant different optimized experimental conditions for each compound: e.g.,
  - Short-chain carboxylic acids vs long-chain carboxylic acids retention in SPE cartridge and column
    → Different SPE cartridges: DVB, C8 (42 Comp.) and phenyl cartridges
  - Mass Spectrometry optimized conditions for 126 detection channels (quantitation & qualification)
    → Compromise conditions

- Matrix effect: e.g.,
  - Some longer-chain carboxylic acids 10 times signal depression in matrix vs in reagent
    → Labeled Internal Standards (IS, n=29) and separation of interferences by changing UHPLC conditions

- Contamination or background interference:
  - "Everywhere compounds"
    → Washing instrument system and screening for high quality solvents

- Limited time & resources including staff

Not Perfect, But Best “One Size Fits All” Method Thus Far!

Sample Pretreatment

100 µL sample is spiked with internal standard and 0.1 M formic acid

On-line SPE, UHPLC System : CHRONECT® Symbiosis Online SPE/UHPLC system (Axel Semrau®, Germany), CHROSPE C8 HD online SPE sorbents

LC Condition:

Ultra High-Performance Liquid Chromatography (UHPLC) reverse phase separation
Total run time: 12 minutes/sample

Mass Spectrometry System :

SCIEX Triple Quad™ API 6500^{+} Mass Spectrometer (Sciex, USA)
Negative detection mode (-4500V)

Injection Volume: 50 µL

Investigated Standard Curve Range: 0.01 ng/mL to 10 ng/mL in bovine serum

Acceptable QC Validation Criteria

- In-house QCs prepared in three levels (low, medium, and high):

  ---Accuracy acceptable criteria: ± 30%

  ---Precision acceptable criteria: ± 30%

- Standard curve linearity: R^2>0.95
- Stability: 6:2 PAP and 8:2 PAP warranted for further investigation
- NIST SRM 1958: PFNA, PFOA, PFOS and PFHxS
- International Performance Test (Arctic Monitoring and Assessment Program (AMAP)):
  9 compounds (PFHpA, PFHxA, PFOA, PFNA, PFDeA, PFUA, PFBuS, PFHxS, PFOS)

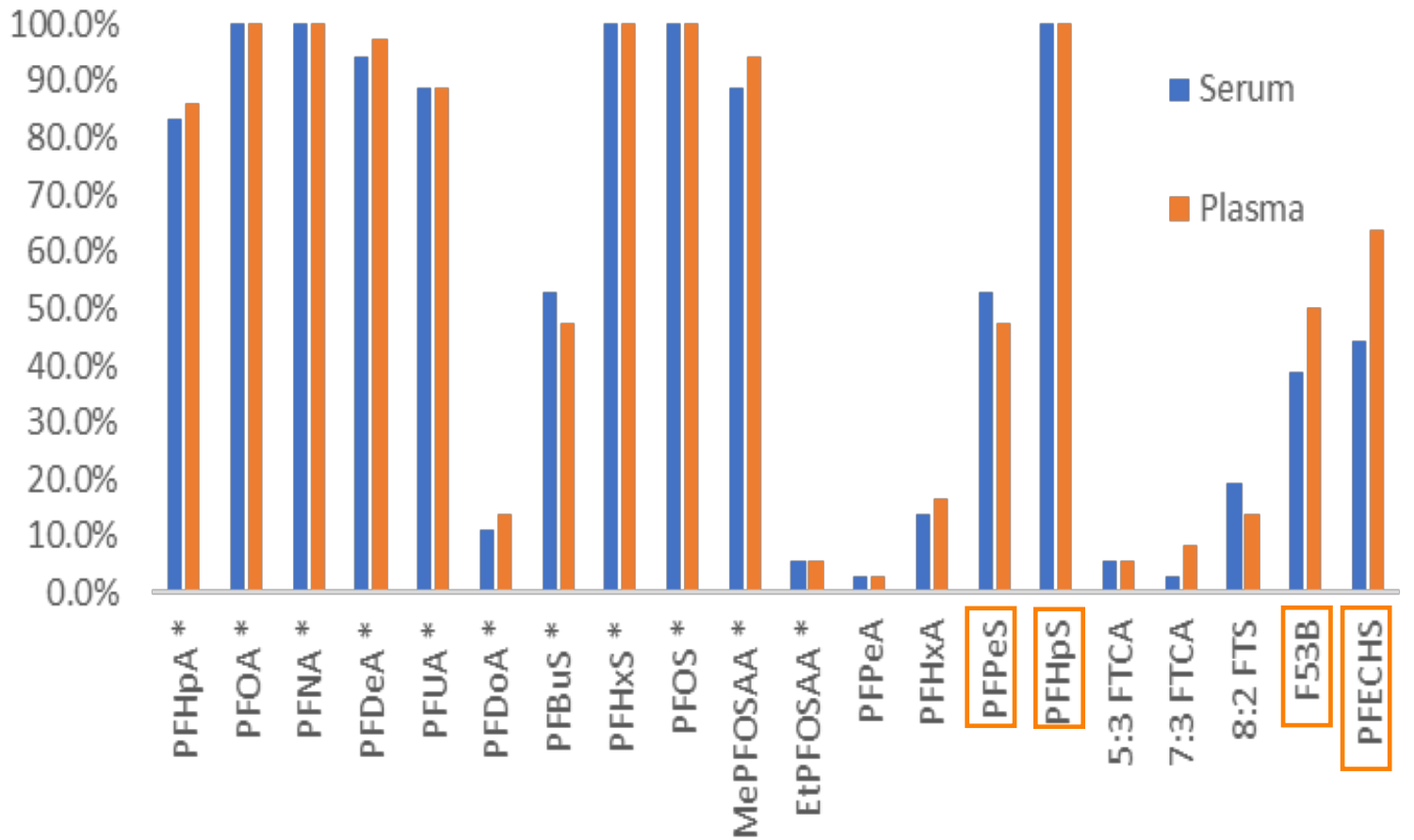
  z' score*: 2022 round 2, -0.69 to 0.48

  2023 round 1, -0.88 to 0.55

** A **z'-score** is the value of an observation expressed in standard deviation units.*
Acceptable / Satisfactory: |z'-score| ≤2.

IPP 7 Study-Detection Frequency (DF) for 20 PFASs

- 20/42 PFASs were detected.
- Legacy PFASs still have high DFs.
- PFPeS, PFHpS, PFECHS and F53B (9Cl-PF3ONS) were detected for the first time by Biomonitoring California.
- With lower MDL, PFHpA, 5:3 FTCA, 8:2 FTS had higher DFs than in previous studies
- PFBuS (a 4-carbon PFAS) is increasing in DF.



* *Legacy PFASs*

• *MDL from 0.01 to 0.25 ng/mL*

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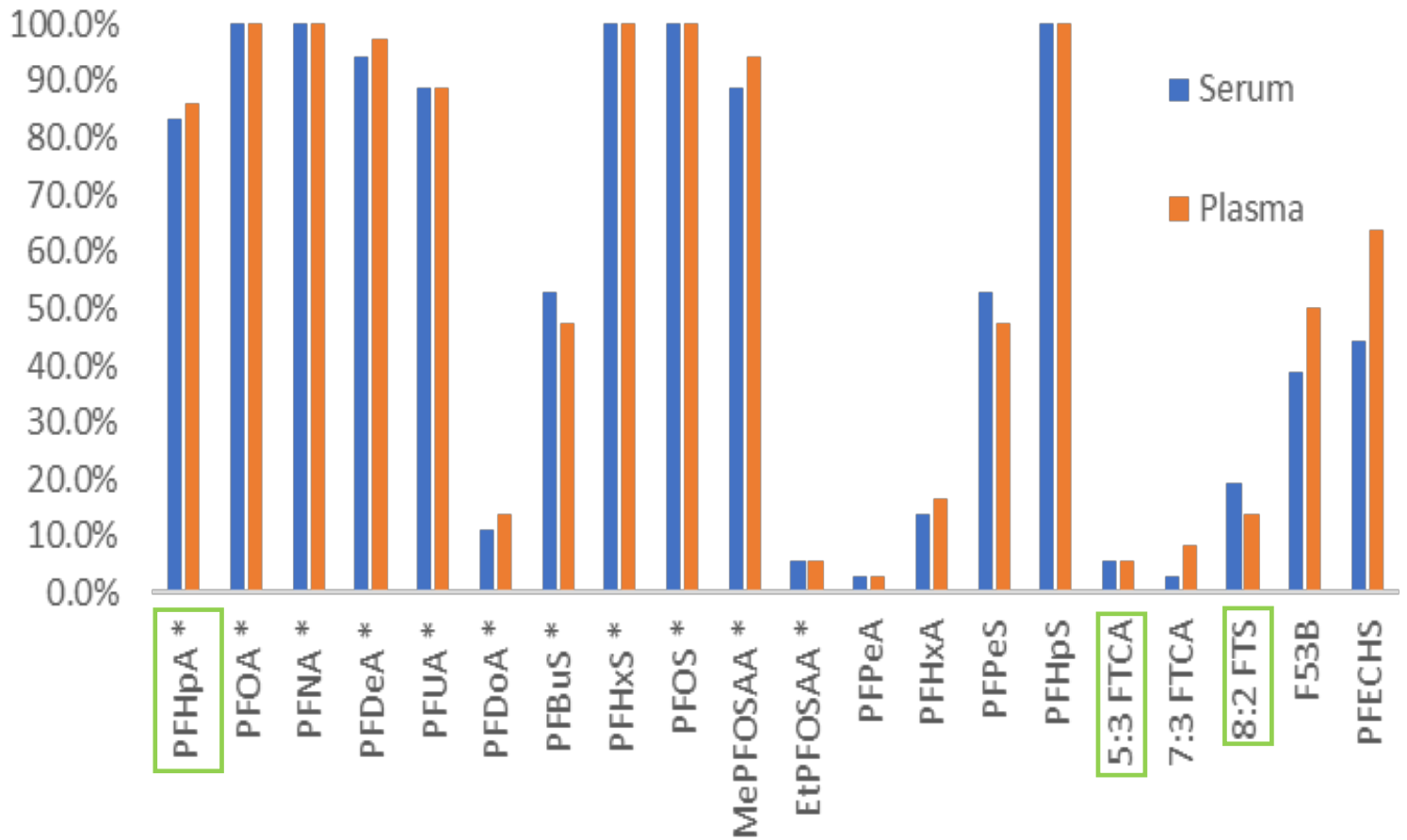

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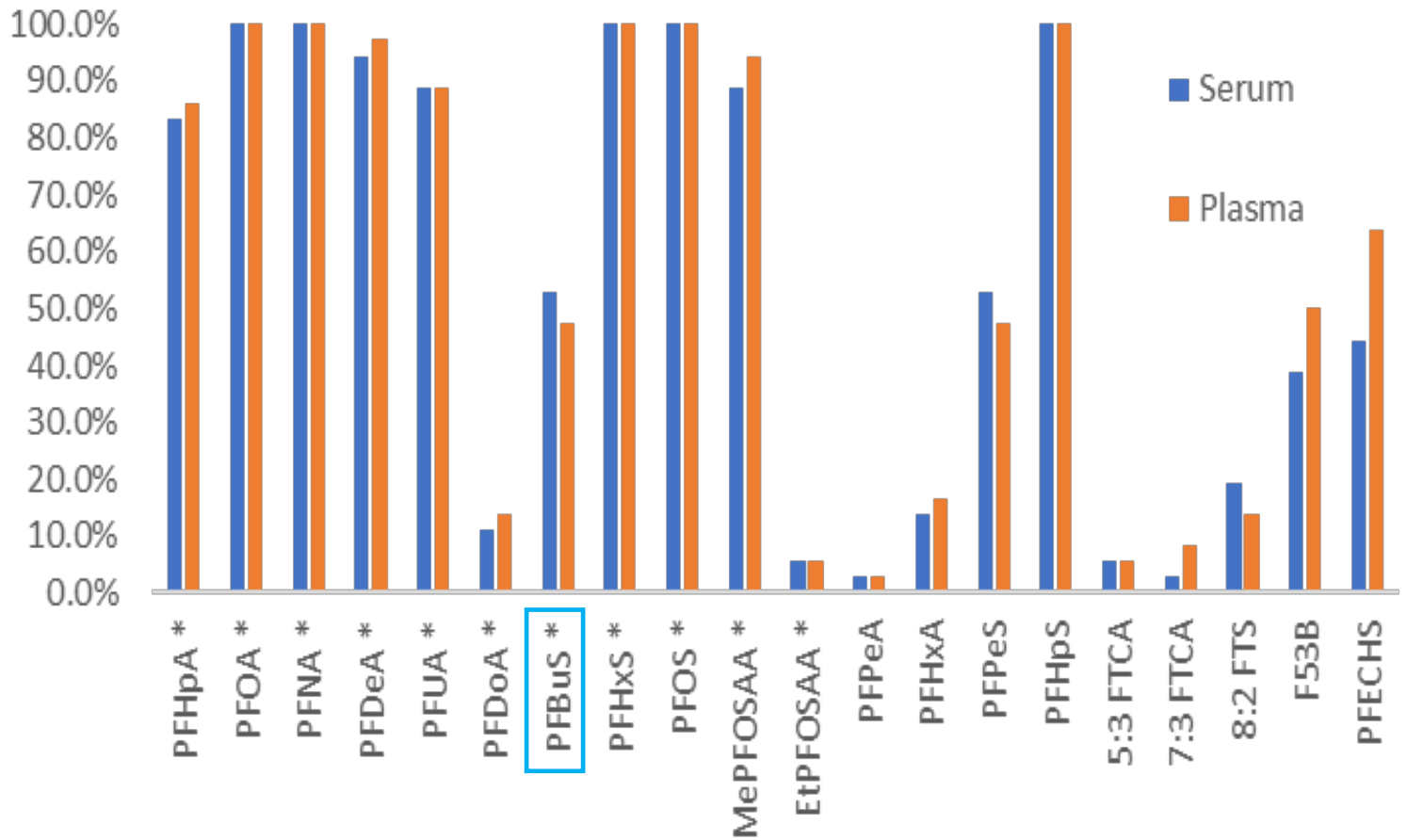

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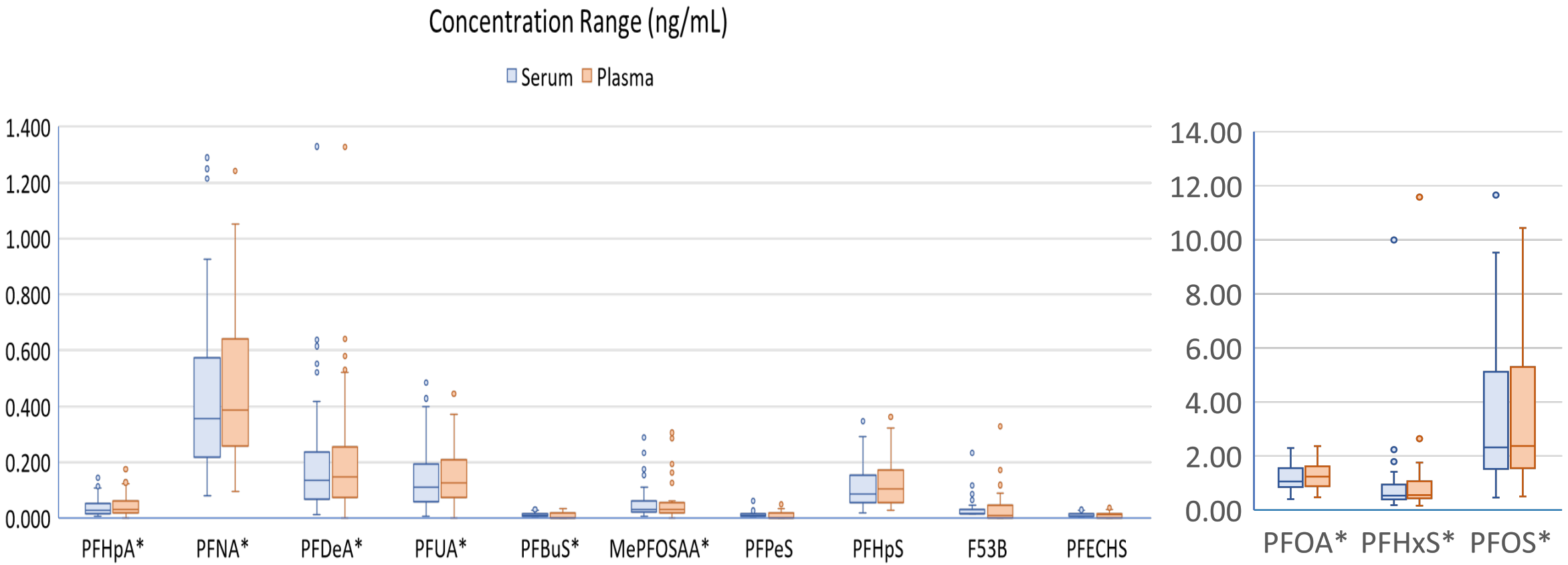
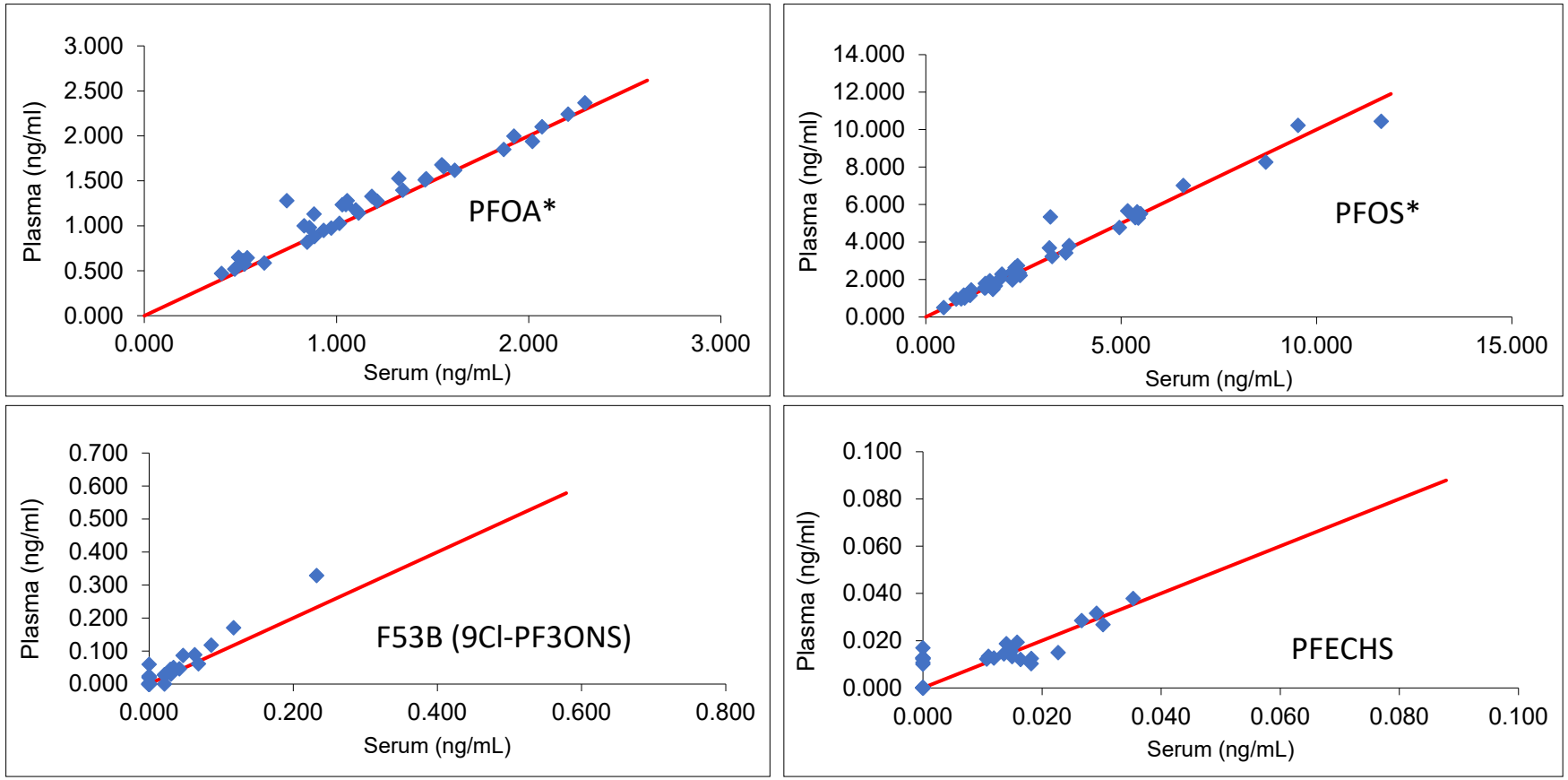
Comparison of Medians and Ranges of PFASs Concentrations in Serum and Plasma



--- Legacy PFASs* *---Only PFASs with DF > 30% are Plotted.*

Serum vs Plasma Concentration (n=36 pairs)



- Generally, plasma concentrations matched with serum concentrations.
- Significant matrix effects were observed for some compounds, e.g., PFPiAs, Perfluoroalkylphosphoic acids etc.

Recommended Monitoring List for STEPS Project

---More samples vs More compounds?

- QC Criteria following ISO17025
- Detection Frequency
- Abundance and Sensitivity
- New/replacement PFASs such as Gen-X, ADONA, short chains …
- Agreement between plasma and serum matrices

Year and /or Study	Analytes
2009	**12 Legacy PFASs:** PFHpA, PFOA, PFNA, PFDeA, PFUA, PFDoA, PFBuS, PFHxS, PFOS, PFOSA, Me-PFOSA-AcOH, Et-PFOSA-AcOH
2016 Asian/Pacific Islander Community Exposures (ACE) Project	**12 Legacy PFASs + 20 more:** **PFCA:** PFBA^, PFPeA, PFHxA **PFSA:** PFDS **Telomer Acids:** 5:3 FTCA, 6:2 FTCA^, 7:3 FTCA, 8:2 FTCA^, 6:2 FTUCA, 8:2 FTUCA **FTSs:** 4:2 FTS, 6:2 FTS, 8:2 FTS **PAPs:** 8:2 PAP^, 6:2 diPAP, 8:2 diPAP^ **PFPiA:** 6:6 PFPiA^, 6:8 PFPiA^ **Perfluoroalkylphosphonic acids:** PFHxPA^, PFOPA^
Current recommendation for STEPS Project (Replace blue^ with red)	**12 Legacy PFASs + 20 more:** **PFCA:** PFPeA, PFHxA **PFSA:** PFPeS, PFHpS, PFNS, PFDS, PFECHS **Telomer Acids:** 5:3 FTCA, 7:3 FTCA, 6:2 FTUCA, 8:2 FTUCA **FTSs:** 4:2 FTS, 6:2 FTS, 8:2 FTS **PAPs:** 6:2 diPAP **Ether Acids:** Gen-X, ADONA, F53B (9Cl-PF3ONS, major), F53B (11Cl-PF3ONS, minor) **Sulfonamide:** FBSA

Summary

- New on-line SPE LC-MS/MS analytical method was developed to measure 42 PFASs in human serum & plasma by using single method.
- The new method was greener, faster and more sensitive.
- In paired serum and plasma samples from IPP7 study, 13 PFASs were detected at > 30% and generally showed good agreement between matrices.
- Given limited resources and time, we recommend monitoring 32 PFASs in STEPS.
- We will continue to optimize the method, including adding the “not-reported PFASs” and other PFASs.

Acknowledgements

- Biomonitoring California
- Prof. Tracey Woodruff and UCSF EaRTH Program
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- iChrom Solutions
- IPP7 participants

Disclaimer The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

Questions?