Comparability and Standardization of Methods for PFC Analysis in Fish Fillets

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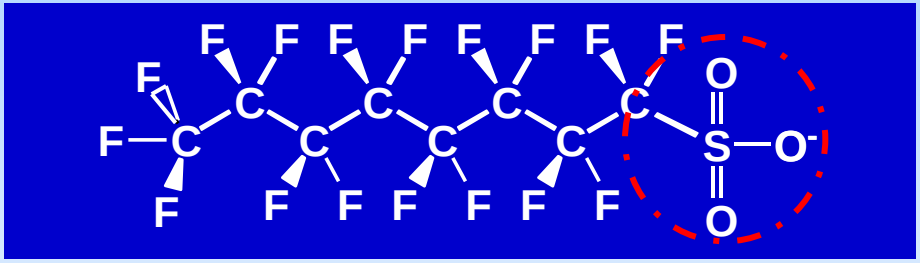
Main Points

- Historical Background
- Key Issues
  - Sources of Quantitative Bias
- 3M Method
  - Method Validation/QC
  - Mississippi River Results

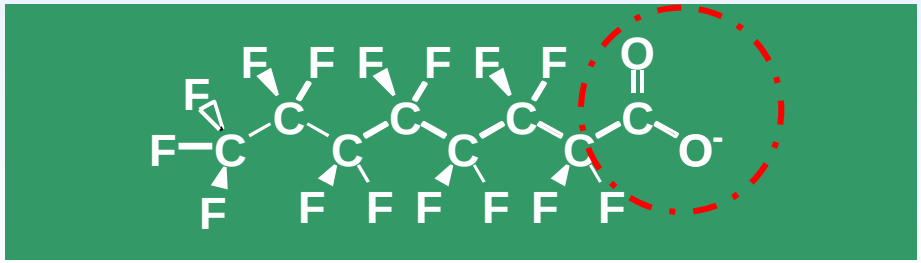
Perfluorinated Compounds (PFCs)

- Perfluoroalkane Sulfonates: PFAS



Perfluorooctane sulfonate (PFOS)

- Perfluorinated Carboxylic Acids: PFCAs



Perfluorooctanoate (PFOA, C8 Acid)

PFC Analytical Challenges

Historically there has not been

- PFC Reference Test Methods (until 2008)
  - EPA Method 537 (Drinking Water)
- Standard Reference Materials
  - e.g. NIST SRMs (serum, soil, sludge, etc..)
  - SRM 1957 (serum) and 1954 (milk) – October 2009
- Stable Isotope Internal Standards i.e. [1,2,3,4-$^{13}C_4$]PFOS
- Electrospray LC-MS/MS
  - Relatively new technology platform (1996)
  - Phys/chem properties of PFCs not amenable to traditional techniques (GC, GC-MS, LC-UV)
  - Trace level analysis (ppm>ppb>ppt)
  - Instrument/Laboratory Contamination

Method Validation Basics

*Defined Selectivity, Accuracy, Precision, Recovery, Calibration, Stability, Sensitivity, and Reproducibility

- *LC-MS/MS
  - ***Matrix-Matched Calibration*** **(Method of Standard Addition, MSA)**

- Challenges Specific to Fish Analysis
  - Suitable Control Matrix free of PFCs
  - Not all Fish are Created Equal
  - More Work

*"Guidance for Industry, Bioanalytical Method Validation", U.S. Department of Health and Human Services, FDA, May **2001**

Fish Extraction Techniques

Ion Pairing (IPE)

Generation 1
(1999-2005)

Protein Precipitation (PPT) with SPE

Generation 2
(2005-2007) – 3M

"New" Techniques

(2007-2008)

[1]Carbon clean-up, direct inject (Powley)
[2]Ion-pairing with fluorophilicity clean-up (Mabury)
[3]Basic digestion with SPE (EPA)

****Key Issues****
- **Sample Preparation**
- **Extract Clean-Up**
- **Analyte List**

(1) Powley, C. R. et al., *Chemosphere,* 2007, (70), 664-672.
(2) Furdui, V. I. et al., *Environ. Sci. Technol*. 2008, (42) 4739-4744.
(3) Ye, X. et. al., Environ. Pollut. 2008, (156) 1227-1232.

2005 PERFORCE

1st Worldwide Interlaboratory Study on PFCs in Environmental and Human Samples

**Van Leeuwen et al. Environ. Sci. Technol. (2006) Vol. 40, p. 7854-7860.*

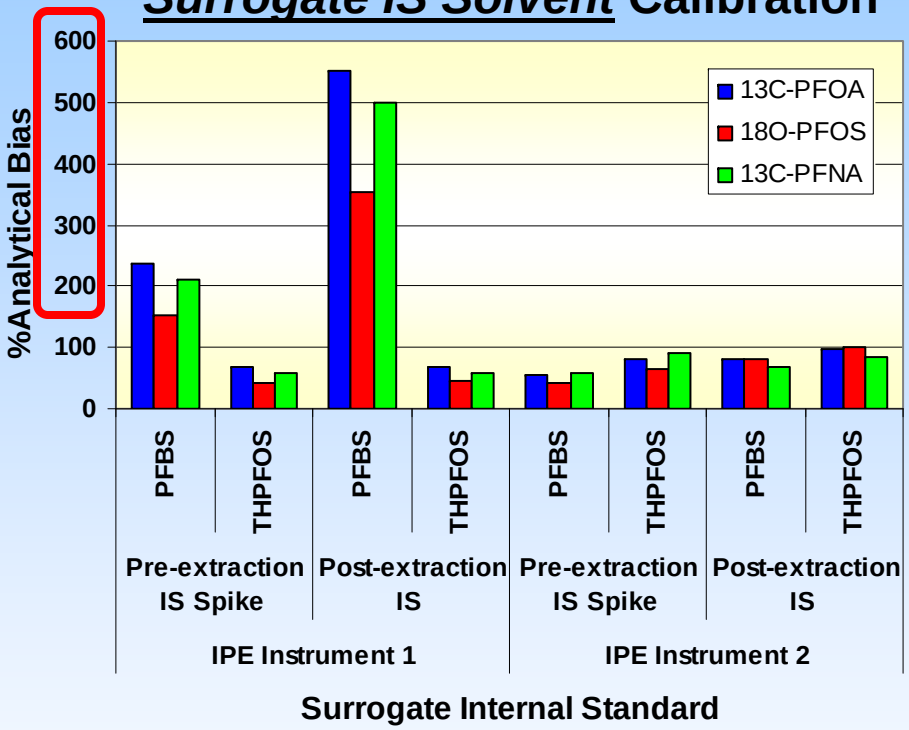
- Fish Fillet Results Most Variable, *Why???*
- Ion Pairing Extraction
- Solvent Quantitation <u>without</u> IS
- Matrix Effects

*Fish Fillet Results		PFOS	PFOA
Spiked Concentration (ng/g)		37	10
<u>Analytical Results (ng/g)</u>			
	Minimum	2.8	0.54
	Median	40	13
	Maximum	295	204
	%RSD	125	201
<u>Evaluation of Results</u>			
	%Satisfactory	17	25
	%Questionable	-	30
	%Unsatsifactory	83	45

"PFC determinations in various matrices are not yet fully mastered"

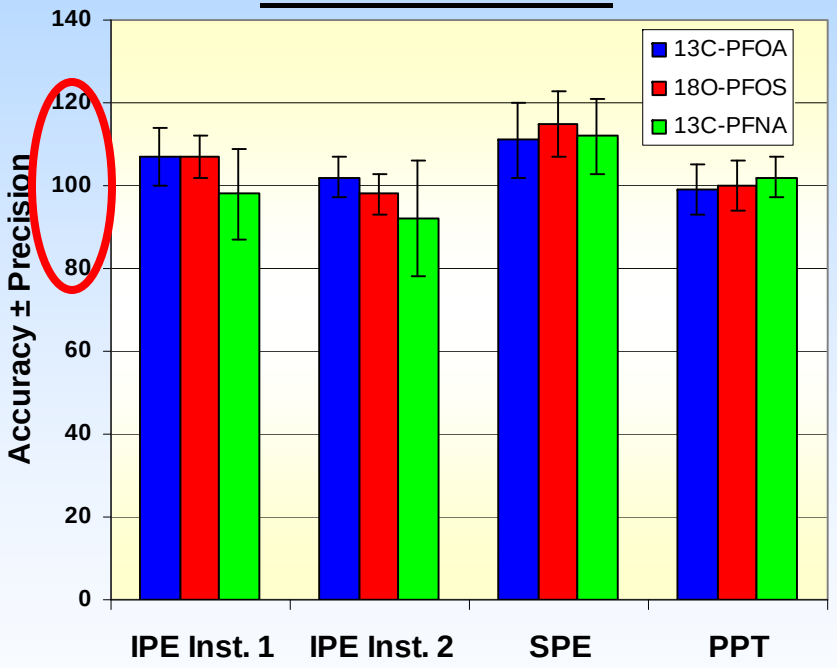
Quantitative Bias: Solvent vs. Extracted Calibration

Analytical Bias: IPE Human Sera with *Surrogate IS Solvent* Calibration



- Surrogate IS *Not* Representative of the Target Analyte
- Matrix Ionization Suppression/Enhancements

Human Sera Method Validation: Various Extraction Techniques External *Matrix-Matched* Calibration

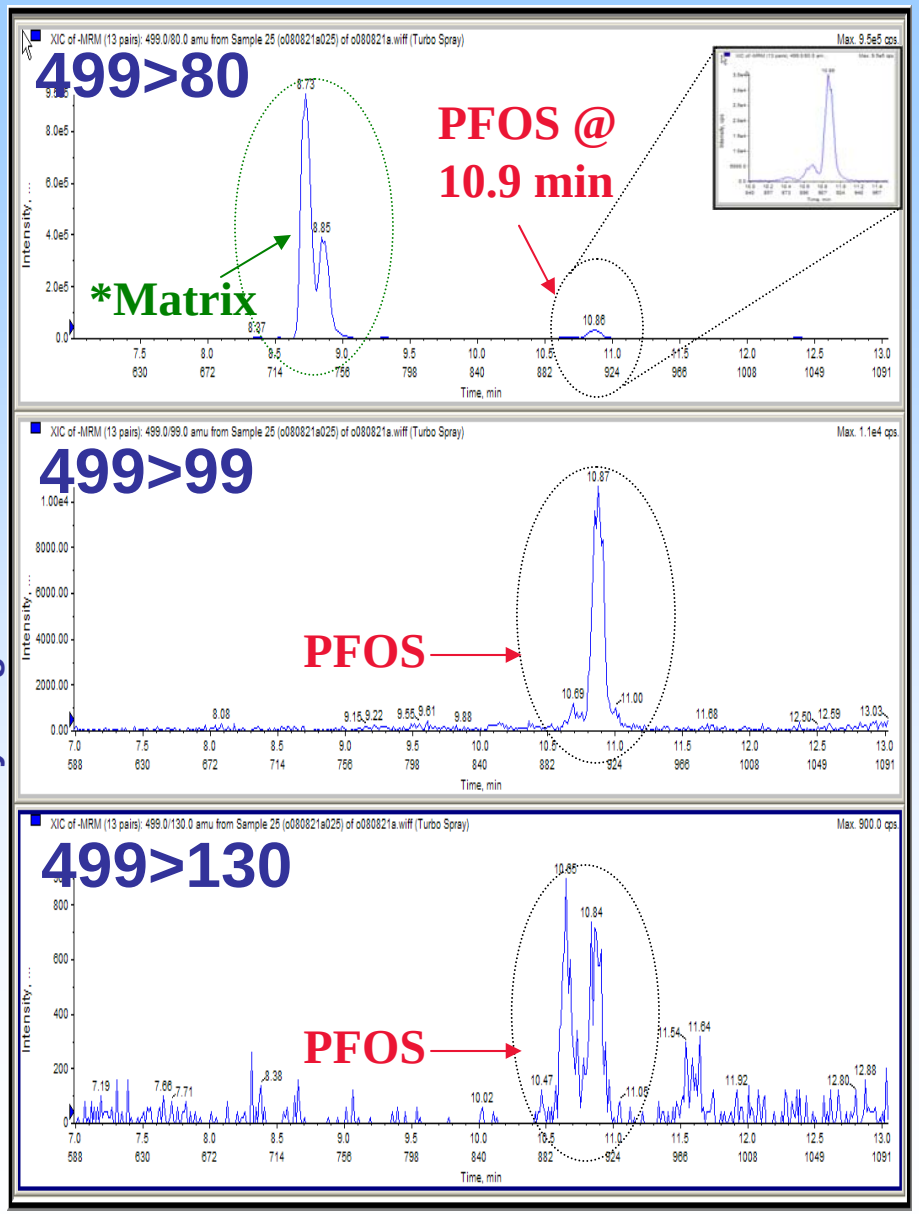


- Matrix-matched (extracted) Calibration is the great equalizer

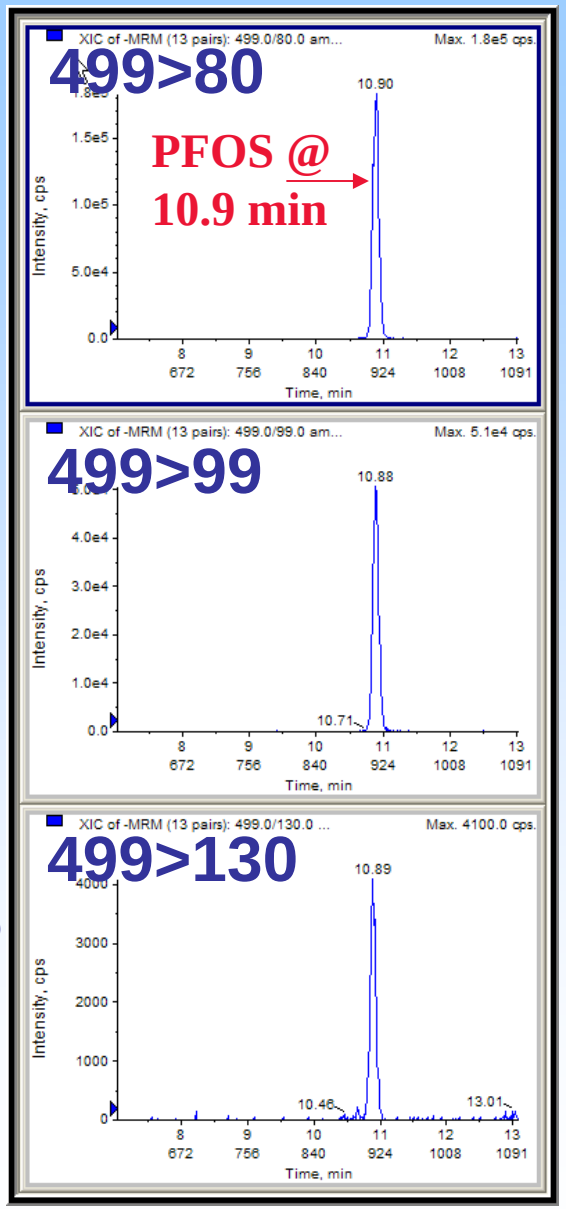
Reagen et. al *Analytica Chimica Acta* 2008 (628) 214-221.

Importance of Multiple Transitions: Matrix vs. PFOS

Whole Body Largemouth Bass Matrix Blank

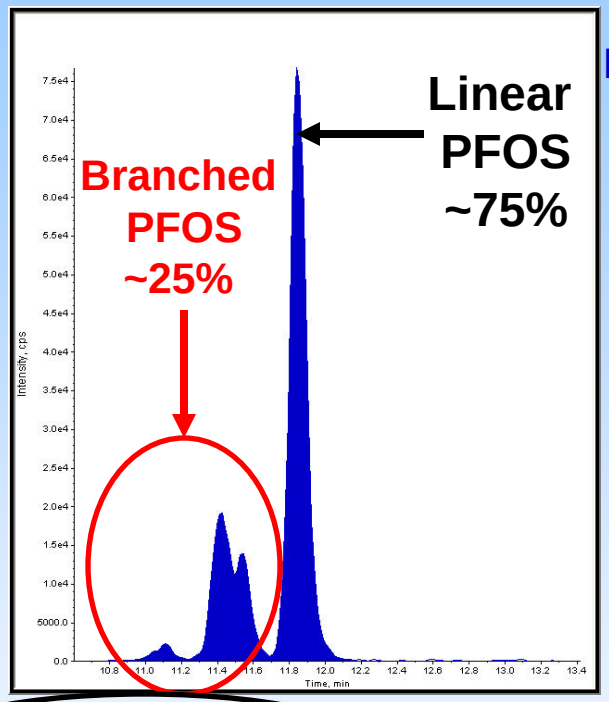


1 ng/mL Linear PFOS Solvent Standard



*Taurodeoxycholate isomers from bile salt – Benskin, J.P. et. al. Anal. Chem. 2007 (79), 6455-6464.

Quantitative Bias: Isomers



Riddell, N. et. al, *Environ Sci. Technol.* 2009 (43) 7902-7908.

- Eleven *known* isomers of PFOS in tech grade.
- 499>80 and 499>99 transitions have different relative response factors for the linear and the branched isomers.
- Quantitative biases possible depending on standard type and MRM transitions used for quantitation

(1) $CF_3CF_2CF_2CF_2CF_2CF_2CF_2CF_2SO_3$ Linear

(2) $CF_3CF_2CF_2CF_2CF_2CF_2CFSO_3$ (CF_3)

(3) $CF_3CF_2CF_2CF_2CF_2CFCF_2SO_3$ (CF_3)

(4) $CF_3CF_2CF_2CF_2CFCF_2CF_2SO_3$ (CF_3)

(5) $CF_3CF_2CF_2CFCF_2CF_2CF_2SO_3$ (CF_3)

(6) $CF_3CF_2CFCF_2CF_2CF_2CF_2SO_3$ (CF_3)

(7) $CF_3CFCF_2CF_2CF_2CF_2CF_2SO_3$ (CF_3)

(8) $(CF_3)_3CCF_2CF_2CF_2CF_2SO_3$

(9) $CF_3CF_2C(CF_3)CF_2CF_2CF_2SO_3$

(10) $CF_3CFCFCF_2CF_2CF_2SO_3$ (F_3C, CF_3)

(11) $CF_3CFCF_2CFCF_2CF_2SO_3$ (F_3C, CF_3)

2008 PERFORCE

2nd Worldwide Interlaboratory Study on PFCs in Environmental Samples (Water and Fish)

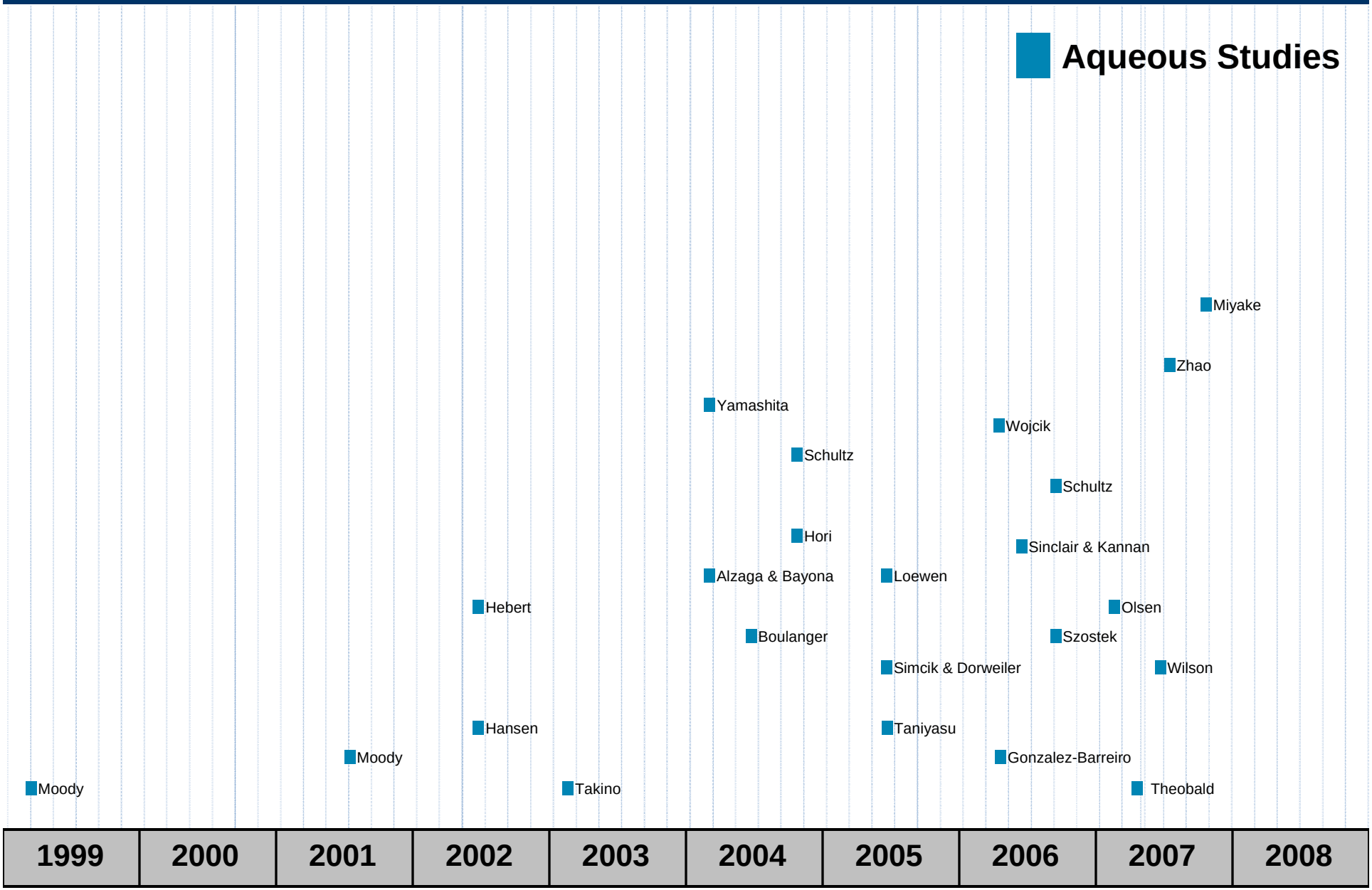
** Van Leeuwen et al. J. Chromatogr. A (1216) 2009 p.401-409.*

- Method of Standard Addition (MSA)
- Stable Isotope ISs – Solvent Calibration for most analytes
- New Extraction Techniques

Fish Results	PFOS		PFOA	
Spiked Conc. (ng/g)	145		22.6	
Analytical Results (ng/g)	Solvent	MSA	Solvent	MSA
Min	49.9	34.5	9.2	8.6
Mean	150	200	18.0	21.5
Max	230	388	23.6	41.5
%RSD	29	47	23	39

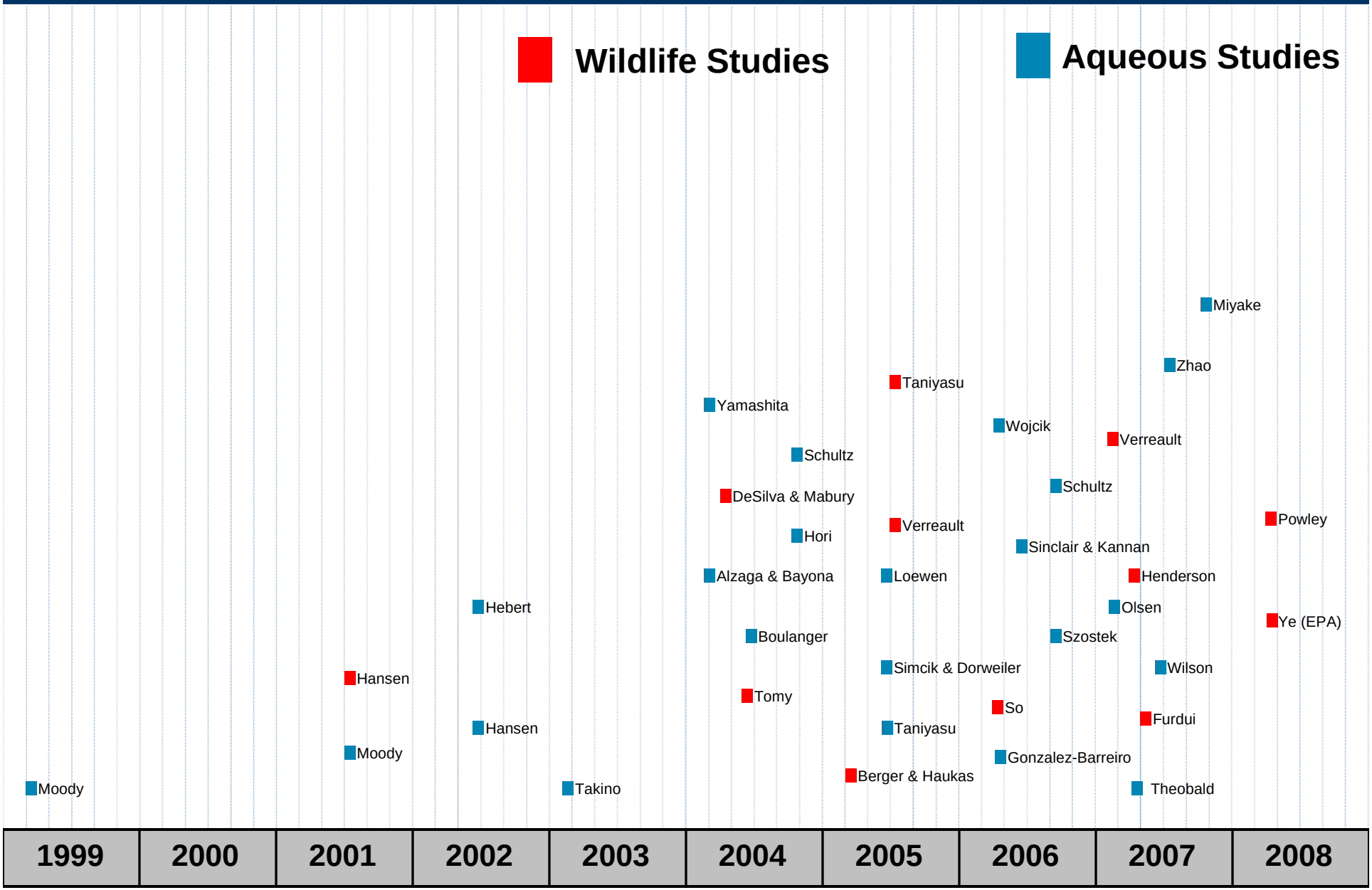
"Analytical Methods for PFCs in water and fish have improved considerably."

Analytical PFC Publications in the Open Scientific Literature



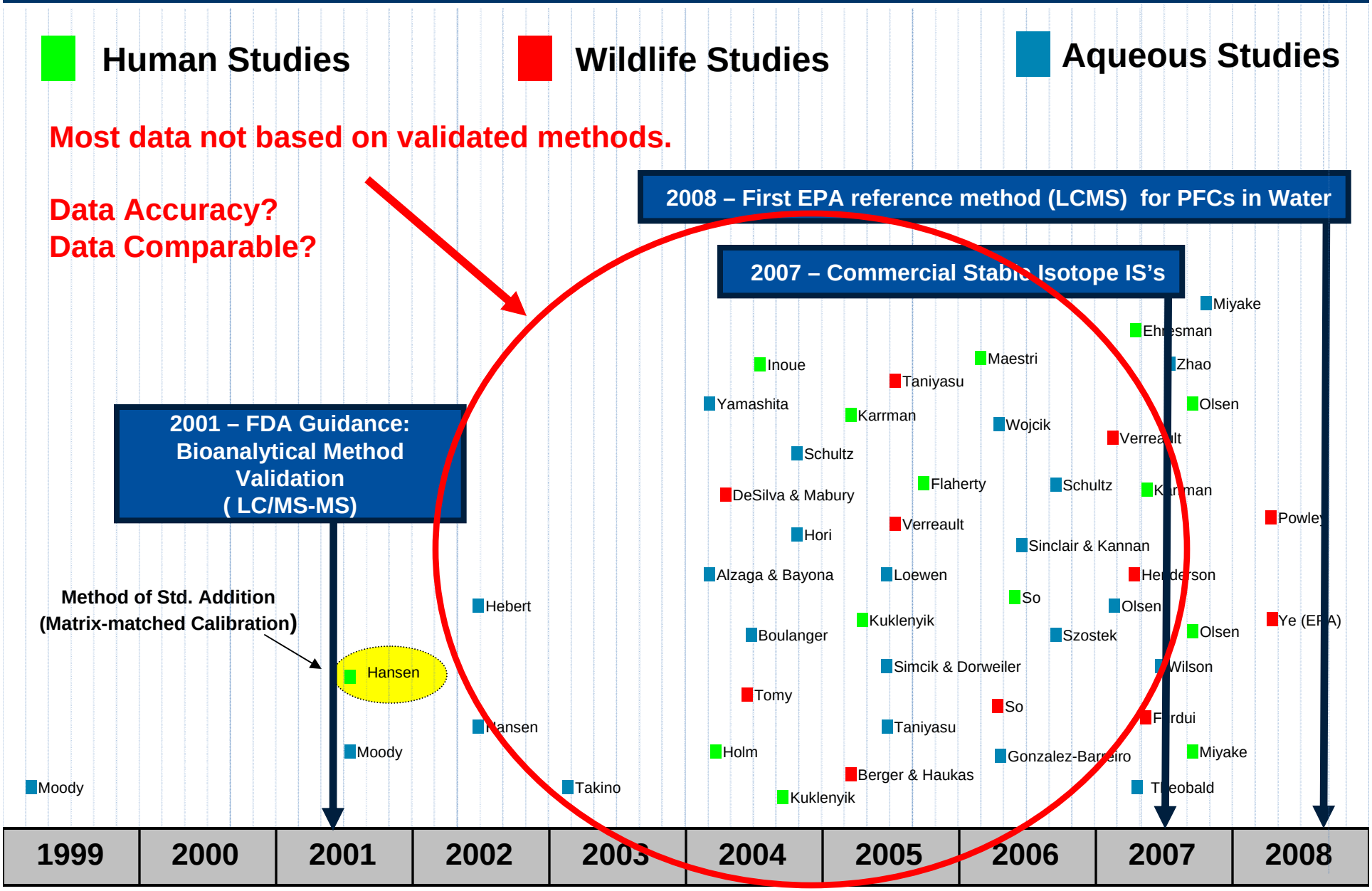
*Jahnke A, Berger U; Journal of Chromatography A 1216 (2009) 410-421

Analytical PFC Publications in the Open Scientific Literature



*Jahnke A, Berger U; Journal of Chromatography A 1216 (2009) 410-421

Analytical PFC Publications in the Open Scientific Literature*



*Jahnke A, Berger U; Journal of Chromatography A 1216 (2009) 410-421

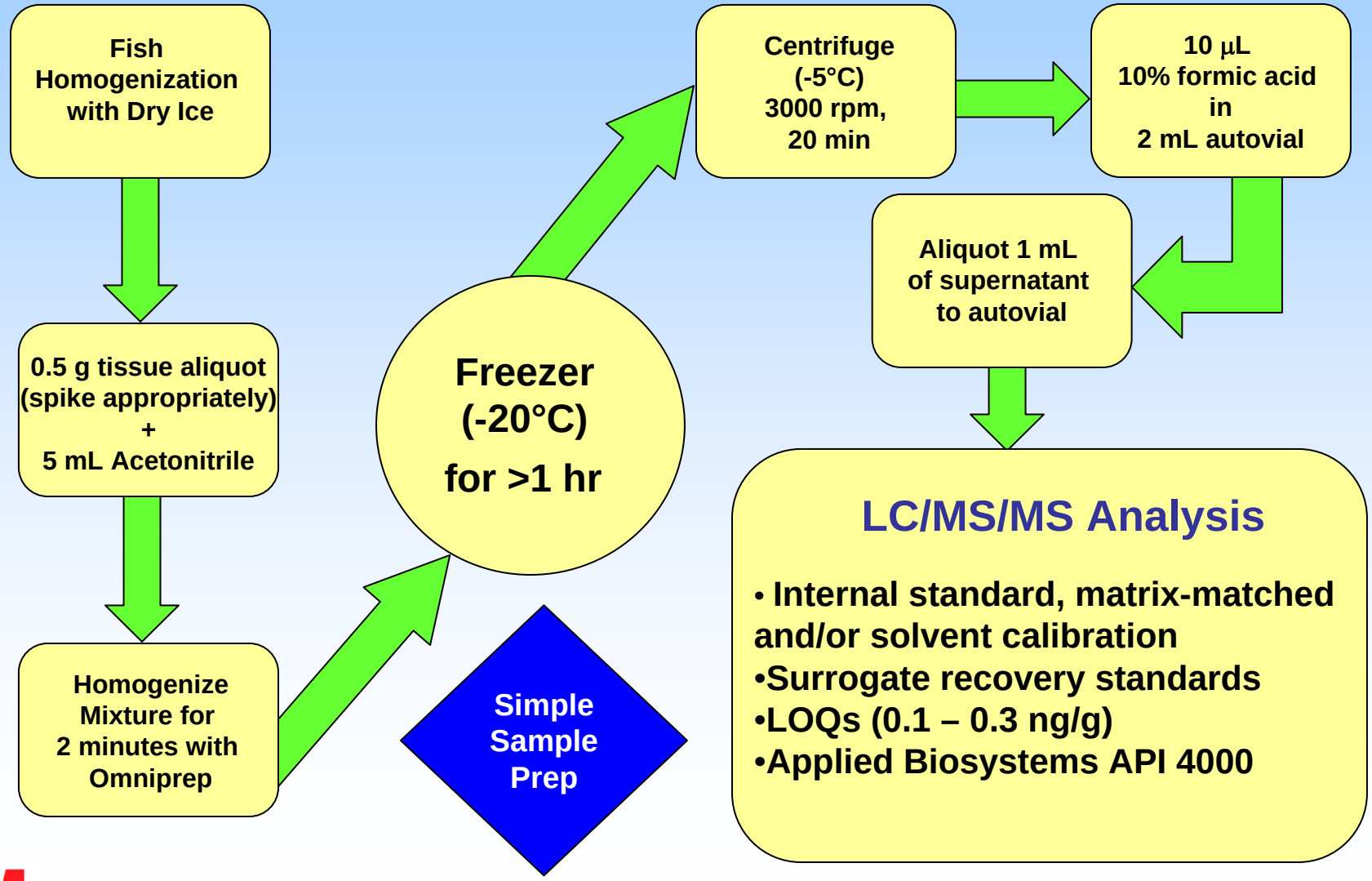
Limitations in Published PFC Methods

- Method validation *generally* not performed and/or reported.
  - Defined Selectivity, Accuracy, Precision, Recovery, Calibration, Stability, Sensitivity,and Reproducibility
- Matrix biases/interferents not fully evaluated
  - Solvent calibration without stable isotope ISs
  - Species specificity (not all fish are created equal)
  - Biological matrix components with common MRM transitions
- Complicated Sample Prep and Clean-Up procedures.

Carefully consider the analytical method before using a reported PFC value.

New 3M PFC Fish Method

Sample Preparation & Analysis



3M PFC Fish Method: Target Analytes & ISs

	Target Analyte	MRM Transition	IS	MRM	Surrogate
	PFBA (C4 Acid)	213>169	[1,2,3,4 -$^{13}C_4$]PFBA	217>172	[1,2 -$^{13}C_2$]PFOA 415>370
*	PFPeA (C5 Acid)	263>219	*[1,2,3,4 -$^{13}C_4$]PFBA	217>172	
	PFHxA (C6 Acid)	313>269, 313>119	[1,2 -$^{13}C_2$]PFHxA	315>270	
*	PFHpA (C7 Acid)	363>319, 363>169	*[1,2,3,4-$^{13}C_4$]PFOA	417>372	
	PFOA (C8 Acid)	413>369, 413>219, 413>169	[1,2,3,4-$^{13}C_4$]PFOA	417>372	
	PFNA (C9 Acid)	463>419, 463>219, 463>169	[1,2,3,4,5-$^{13}C_5$]PFNA	468>423	
	PFDA (C10 Acid)	513>469, 513>269, 513>219	[1,2 -$^{13}C_2$]PFDA	515>470	
	PFUnA (C11 Acid)	563>519, 563>269, 563>219	[1,2 -$^{13}C_2$]PFUnA	565>520	
	PFDoA (C12 Acid)	613>569, 613>319, 613>169	[1,2 -$^{13}C_2$]PFDoA	615>570	
	PFBS (C4 Sulfonate)	299>80, 299>99	[$^{18}O_2$]PFBS	303>84	[$^{18}O_2$]PFOS 503>84
	PFHS (C6 Sulfonate)	399>80, 399>99	[$^{18}O_2$]PFHS	403>84	
	PFOS (C8 Sulfonate)	499>80, 499>99, 499>130	[1,2,3,4- $^{13}C_4$]PFOS	503>80	
*	FOSA (C8 Sulfonamide)	498>70	[1,2,3,4- $^{13}C_4$]PFOS	503>80	

- 13 Target Analytes
- 10 IS
- 2 Surrogates
- 43 MRM Transitions

Two Injections

- C4-C6 Acids analyzed using different column/mobile phase
- Multiperiod method for rest

* Surrogate IS used for this compound.

LC Conditions

C4-C6 Acid

Step	Total Time (min)	Flow Rate (µL/min)	%A	%B
0	0	300	90	10
1	3.0	300	90	10
2	3.5	300	30	70
3	9.0	300	5.0	95
4	15.0	300	5.0	95
5	15.1	300	90	10
6	19.0	300	90	10

A: 5 mM Ammonium Acetate with 0.01% Acetic Acid (aq)
B: Methanol
Analytical Column: PRISM RP
50 mm x 2.1 mm; 5 µ particle size
Injection Volume: 20 µL
Divert first 3 minutes to waste

PFCAs (C7-C12),PFASs, FOSA

Step	Total Time (min)	Flow Rate (µL/min)	%A	%B
0	0	400	97	3.0
1	3.0	400	97	3.0
2	3.5	400	70	30
3	13.5	400	40	60
4	15.5	400	40	60
5	16.0	400	10	90
6	18.0	400	10	90
7	18.3	400	97	3.0
8	21.0	400	97	3.0

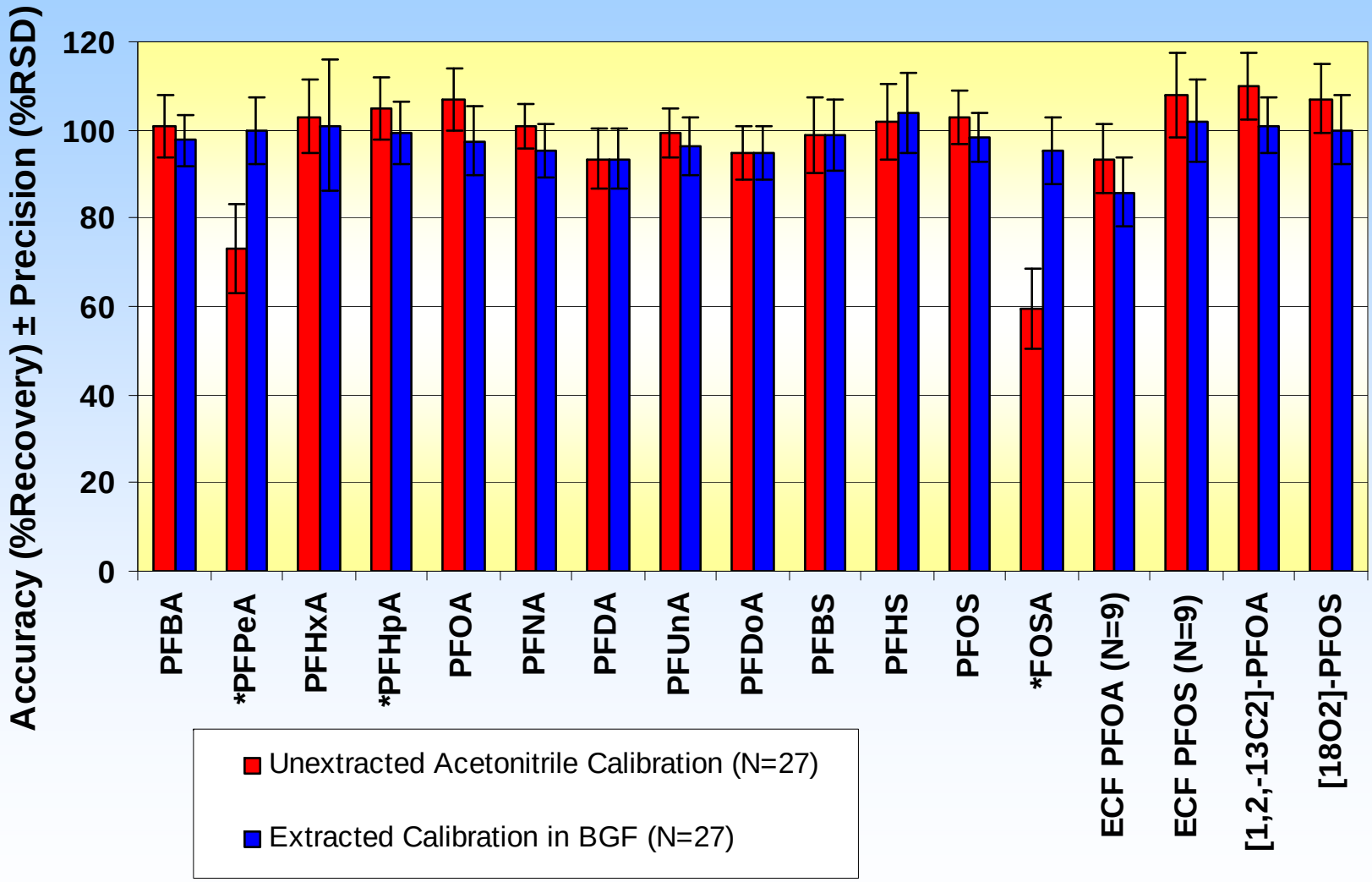
A: 2 mM Ammonium Acetate (aq)
B: Acetonitrile
Extraction Pre-Column: Waters® Oasis HLB (20 mm x 3.0 mm)
Analytical Column: Betasil C18
100 mm x 2.1 mm; 5 m particle size
Injection Volume: 25 µL
Divert first 5 minutes to waste

Method Validation

- **Accuracy & Precision – Stable IS Quantitation**
  - Method of Standard Addition (*Bluegill Fillet Control Matrix)
  - Unextracted (Solvent) Calibration
  - Triplicate lab control spikes at three levels
- **Quantitation of branched PFOS/PFOA isomers from ECF source**
- **Quantitation of low-level analytes (ppb) in the presence of high level PFOS (ppm)**
- **Specificity**

Note: All fish investigated for method validation were purchased from a supplier for scientific studies. Control fish are NOT environmental samples.

Method Validation Summary



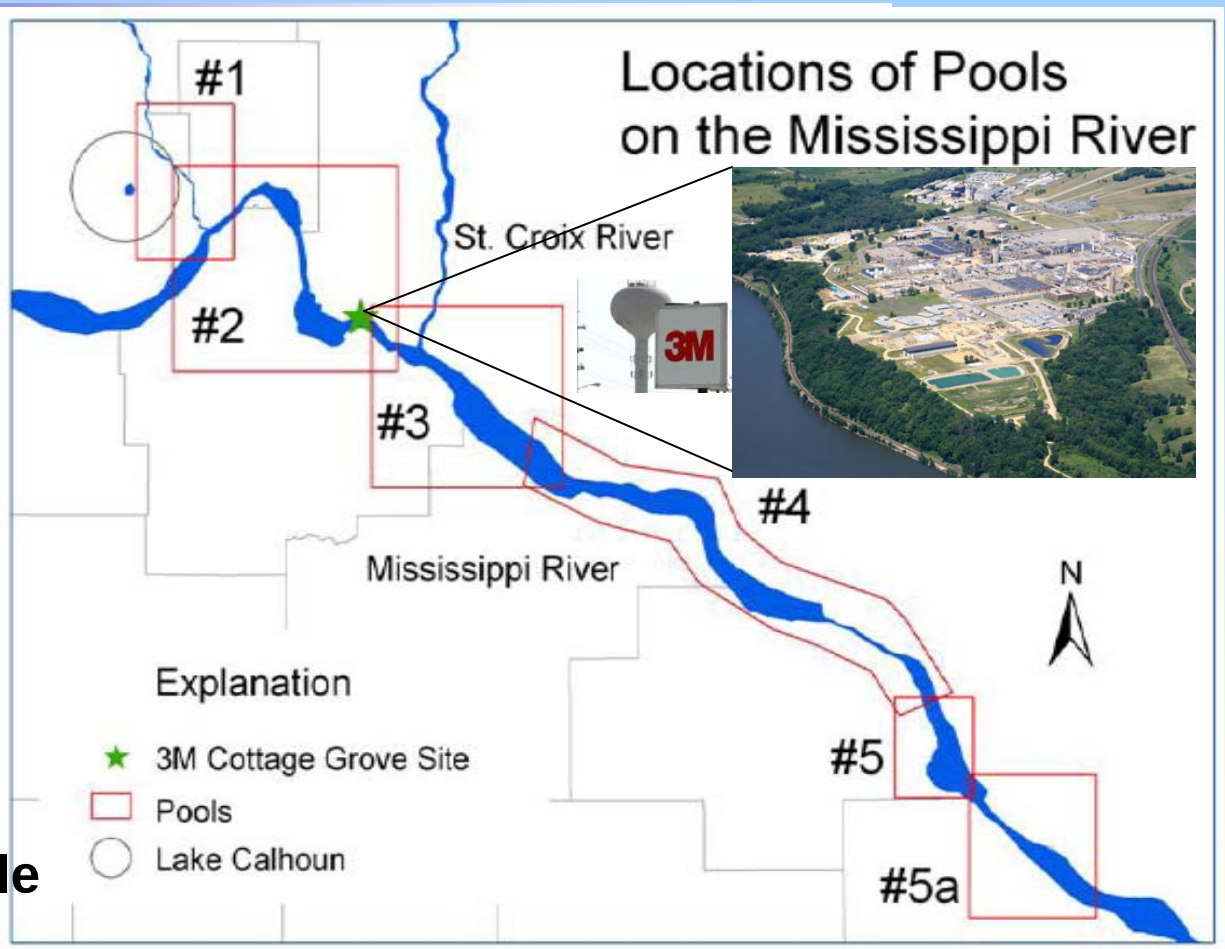
***Surrogate IS used for this compound**

Application to Environmental Samples: 2008 MPCA Mississippi River Sampling

33 Fillet Samples
- Bluegill (N=10)
- Smallmouth Bass (N=10)
- Walleye (N=9)
- Sauger (N=2)
- Black Crappie (N=1)
- Northern Pike (N=1)

Samples extracted in Duplicate

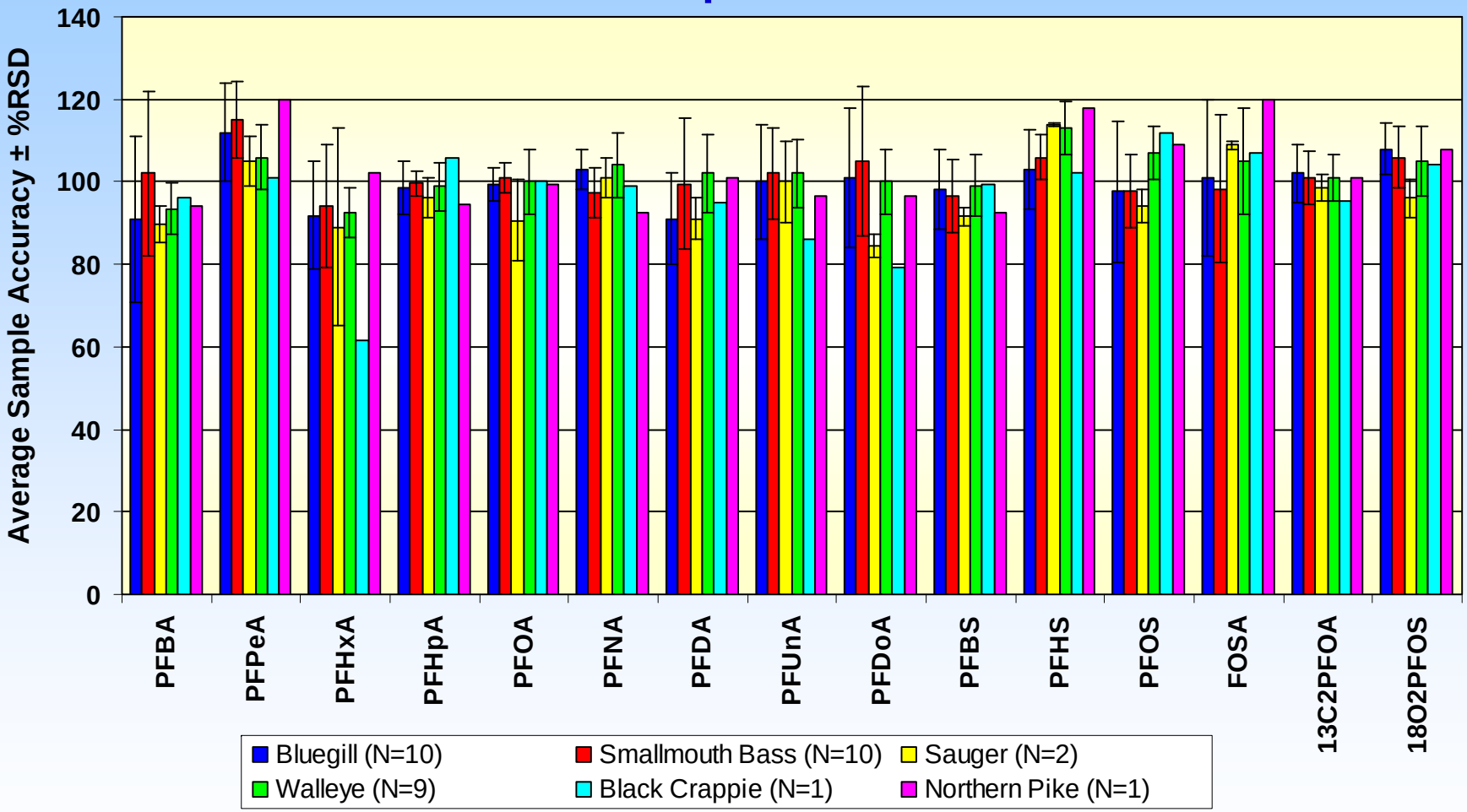
Lab Matrix Spike (LMS) prepared for each sample



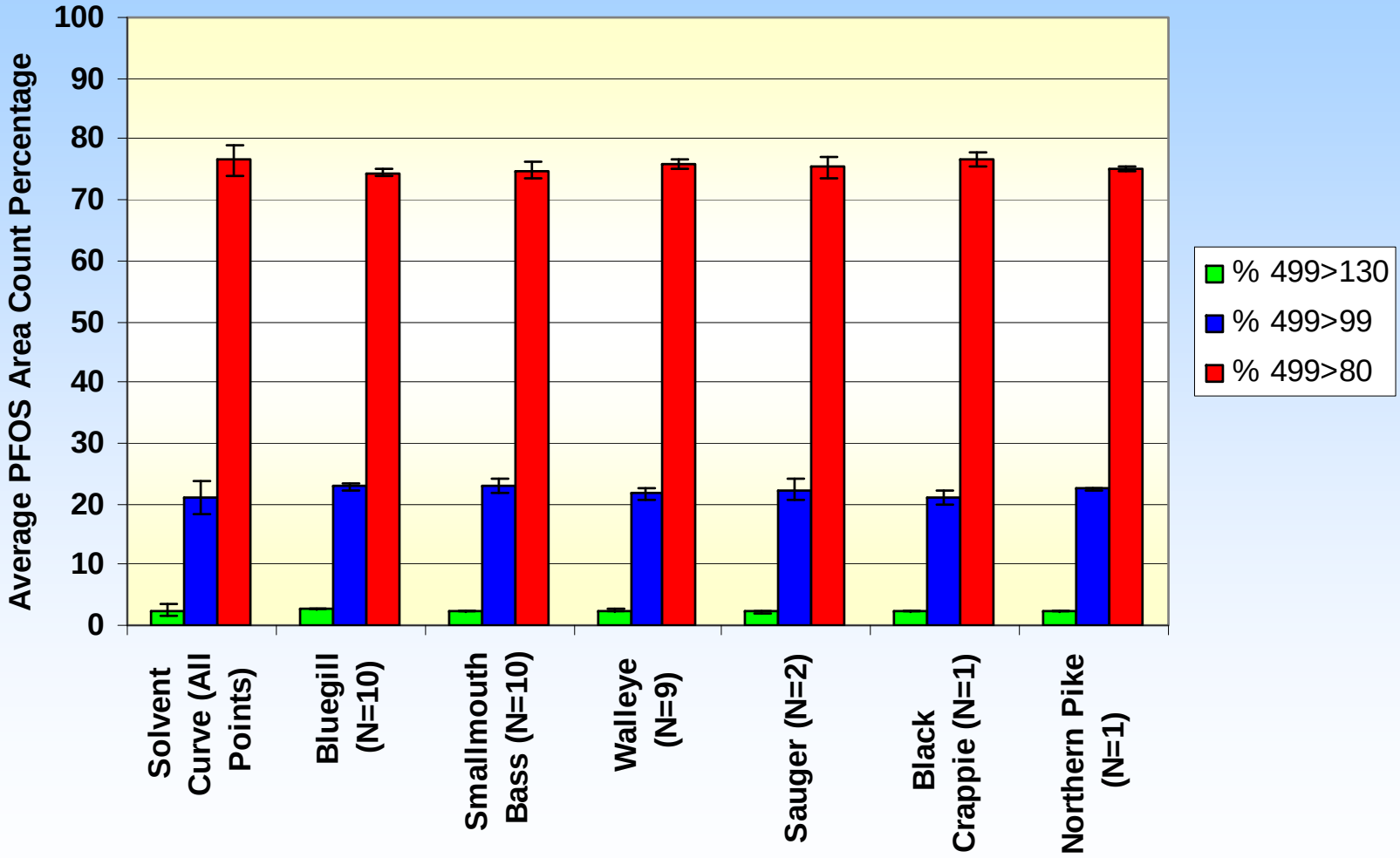
Minnesota Pollution Control Agency Home Page.
http://www.pca.state.mn.us/publications/pfc-2008mpcametrolakesfishpfcdata-final9.pdf

Mississippi River Sample Accuracy

Lab Matrix Spike Recoveries



PFOS Specificity:
MRM Transition Analysis



3M PFC Method Conclusions

- Simple Extraction Procedures
- Accuracy & Precision
  - 100±30% for most analytes
- Expanded Analyte List
- **Stable Isotope IS Quantitation**
  - MSA
  - Solvent Quantitation
- Validation in Bluegill Fillet
  - Applicable to 6+ additional freshwater species (fillet)
  - Whole-body
- Isomer Quantitation of PFOS/PFOA

QC Requirements for *Any* Performance Based PFC Methods

- Field Replicates/Lab Replicates
- Laboratory QCs in control matrix (every prep batch)
- Laboratory Matrix QCs (sample spikes at a defined frequency)
- Practical LOQs/MDLs – Defined Criteria
- Blanks/Blank Criteria
- Calibration
  - Method of Standard Addition (control matrix)
  - Unextracted Solvent Calibration with stable isotope ISs
- ***Data Uncertainty (Accuracy & Precision) - Reporting Criteria***
- Supporting Method Validation (if possible)
- Analyte Specificity
  - Multiple MRMs when possible
  - MRM area count ratio comparison to reference standards
- Isomer Evaluation

Future Directions

- **Standard Reference Materials for Method Evaluation**
  - NIST SRM 1946 (Lake Superior Trout Fillet)
  - NIST SRM 1947 (Lake Michigan Trout Fillet) – pending for certified PFCs concentrations
- **Agency Guidance**
  - QC Acceptance Criteria
  - Reporting Criteria
  - Calibration Procedures (Linear vs. Branched)
- **EPA Reference Method**