Determination of Perfluorinated Alkyl Acid Concentrations in Biological Standard Reference Materials

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Abstract

Standard Reference Materials (SRMs) are homogeneous, well-characterized materials that are used to validate measurements and improve the quality of analytical data. The National Institute of Standards and Technology (NIST) has a wide range of SRMs that have mass fraction values assigned for a number of legacy pollutants. These SRMs can also serve as test materials for method development, method validation, and measurement for contaminants of emerging concern. Since inter-laboratory comparison studies have shown considerable variability with measurements of perfluoroalkyl acids (PFAAs), future analytical measurements will benefit from the determination of consensus values of PFAAs in SRMs to provide a means to demonstrate methods specific performance. To that end, NIST, in collaboration with multiple groups, has been measuring concentrations of PFAAs in a variety of SRMs. Here we report on levels of PFAAs and perfluorooctane sulfonamide (PFOSA) determined in four biological SRMs: fish tissue (SRM 1946 Lake Superior Fish Tissue, SRM 1947 Lake Michigan Fish Tissue), bovine liver (SRM 1577c), and mussel tissue (SRM 2974a). We also report concentrations for three in-house quality control materials: beluga whale liver, pygmy sperm whale liver, and white-sided dolphin liver. Measurements in SRMs show an array of PFAAs, with perfluorooctane sulfonate (PFOS) being the most frequently detected. Reference and information values are reported for PFAAs measured in these biological SRMs.

Keywords Perfluoroalkyl acids, Standard Reference Materials, Fish tissue, Bovine liver, Mussel tissue, Intercomparison exercise

Introduction

Perfluoroalkyl acids (PFAAs) comprise a group of fluorinated compounds considered to be ubiquitous and persistent in the environment. Included in this class of compounds are perfluoroalkyl sulfonates (PFSAs), the most recognized compound in this class being perfluoroooctane sulfonate (PFOS), and perfluorocarboxylic acids (PFCAs). The release of PFOS into the environment, from 1970 to 2002, was estimated at 96,000 t [1] and the total emissions of PFCAs, from 1951 to 2004, was estimated to be between 3,200 and 7,300 t [2]. As a result of their widespread applicability, PFAAs are found in a wide range of consumer and industrial products, including textiles, varnishes, carpets, and fire-fighting foams [2].

Over the last decade, the occurrence of PFAAs has been documented in many environmental matrices including fish, birds, and marine mammals [3-7] and reviews have observed their prevalence in biota worldwide [8-10]. While most of the biomonitoring studies have been performed by a handful of laboratories, these reviews bring into question the comparability of the data among labs. Since 2005, interlaboratory comparison studies of PFAAs have been conducted in environmental and human matrices [11-14]. Improvements in PFAA measurements have been made, but van Leeuwen et al. [11] and Riddell et al. [15] emphasize that for accurate and precise measurements of PFAAs, several analytical criteria still need to be addressed. An important criterion emphasized in all the interlaboratory studies is the availability and use of reference materials with known concentrations of analytes of interest, to help validate laboratory results.

The National Institute of Standards and Technology (NIST) has provided natural matrix Standard Reference Materials (SRMs) to validate measurements of organic and inorganic compounds and to aid with analytical method development [16]. These SRMs include human serum, human plasma, human milk, fish tissue, mussel tissue, and bovine liver. During the past years, NIST SRMs of human serum, human plasma, and human milk have been characterized for PFAAs, resulting in the assignment of reference values of some PFAAs to their Certificates of Analysis [14,17]. For the past few years, several biological SRMs, including SRM 1946 Lake Superior Fish Tissue, SRM 1947 Lake Michigan Fish Tissue, SRM 1577c Bovine Liver, and SRM 2974a Organics in Freeze-Dried Mussel Tissue (*Mytilus edulis*), and three in-house quality control materials, QC97LH02 Beluga Liver, QC03LH3 Pygmy Sperm Whale Liver, and QC04LH4 White-Sided Dolphin Liver have been examined for PFAAs by NIST. In addition to the NIST analysis, six outside laboratories submitted PFAA measurement data for some of these SRMs. The aims of this study are to compare the measurements of PFAAs in biological SRMs by all the laboratories and to add reference and information values of PFAAs to these existing NIST SRMs. The values of PFAAs in these SRMs will support future PFAA measurements in the analytical community. In the larger context, the overall intention of this study is to provide data which are useful for improving the quality of PFAA measurements made by this research community while also providing information that may help with the interpretation of previously published results.

Material and methods

Sixteen PFAAs were examined in this study. These include perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTriA), perfluorotetradecanoic acid (PFTA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), PFOS, perfluorodecane sulfonate (PFDS) and perfluorooctane sulfonamide (PFOSA).

The SRMs were prepared by NIST using methods described in their respective Certificates of Analysis and have previously been certified for concentrations of persistent organic pollutants and metals (http://www.nist.gov/srm/). For this study, PFAAs were measured in SRM 1946 Lake Superior Fish Tissue, SRM 1947 Lake Michigan Fish Tissue, SRM 1577c Bovine Liver, SRM 2974a Organics in Freeze-Dried Mussel Tissue (*Mytilus edulis*), and three in-house quality control materials, QC97LH02 Beluga Liver, QC03LH3 Pygmy Sperm Whale Liver, and QC04LH4 White-Sided Dolphin Liver.

Seven laboratories including NIST, US Environmental Protection Agency (EPA), 3M, Environment Canada, University of Toronto, Bundesamt fuer Seeschiffahrt und Hydrographie (Federal Maritime and Hydrographic Agency of Germany), and Wageningen IMARES (Institute for Marine Resources and Ecosystem Studies) participated by analyzing selected SRMs for the PFAAs routinely measured in their laboratories. In all cases the laboratories used their existing extraction and cleanup methods coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS) for the quantification of PFAAs. ~~SRMs were analyzed for PFAAs at the NIST laboratories in Charleston, SC and Gaithersburg, MD.~~

Analytical Methods

Participating laboratories were asked to determine the concentrations of the PFAAs they currently measure in their laboratory. They were asked to measure at least three replicates of the SRMs using their current methods and own standards. A brief description of sample extraction, cleanup, and instrumental technique was provided by the participating laboratories along with the results. In this study all laboratories measured PFAAs in the fish tissue SRMs (1946 and 1947). Three laboratories participated in the measurement of PFAAs in SRM 1577c and SRM 2974a. Two laboratories provided measurements of PFAAs in QC97LH02, QC03LH3, and QC04LH4.

The extraction and cleanup methods used included alterations of established methods (Figure 1). Extraction methods included an ion-pairing extraction method, acetonitrile precipitation, and basic methanol (potassium hydroxide or sodium hydroxide) extraction. Some participants choose no further cleanup after extraction; while other participants choose to use different solid-phase extraction columns (i.e. Oasis WAX or Supelco ENVI-Carb) or the addition of activated carbon to the extraction solution for the cleanup of their extracts. All laboratories used the internal standard approach with selected mass-labeled internal standards, and LC-MS/MS was used for quantification. The branched and linear isomers of PFOA, PFHxS, and PFOS were integrated together and the concentrations of these compounds are reported as totals of all isomers.

Previous studies of PFOS have reported matrix interferences in biological samples [18,19,15]. When the endogenous compound taurodeoxycholic acid (TDCA) is not removed during the extraction and cleanup process, it can coelute with PFOS, causing an over-reporting of PFOS concentration in a sample. Besides coelution with PFOS, TDCA interferes with the 499→80 PFOS transition [19], resulting in some laboratories to avoid this transition altogether and using the 499→99 transition exclusively. Included in Figure 1 are the PFOS transitions monitored by each laboratory in this study.

Determining reference values

The method that has previously been used for value assigning organic contaminants in SRMs was used for value assigning PFAAs in these SRMs. This method combines the data from at least two different analytical methods~~.~~, other producers of reference materials similar approaches for value assignment. In the present study PFAA measurements were obtained using combinations of the extraction methods by NIST with results from participant values of PFAAs from the interlaboratory study. The PFOS results reported in this study were used to assign reference values for SRMs 1946, 1947, and 1577c. -The reference value is a weighted mean of the results from the analytical methods [20]. -The expanded uncertainties about the mean were calculated according to Rukhin [21] using a coverage factor equal to 2 (approximately 95 % confidence), calculated by combining a pooled within method variance with a between method variance [22] following the ISO Guide [23,24]. The PFNA, PFDA, PFUnA, and PFTriA values reported in this study were used to assign information values for SRMs 1946 and 1947.

Results and discussion

PFAAs were detected in all the SRMs and quality control materials studied, with each laboratory's results summarized in Tables 2-8. The total PFAA concentrations ranged over two orders of magnitude depending on the matrix examined. In general there was good agreement among the data from all the laboratories for measurements of PFOS in all the SRMs. Results for the other analytes were less consistent, with relative standard deviations (RSDs) greater than 15 %.

The concentrations of PFAA measured in the fish tissue SRMs are similar to concentrations measured in biological samples collected in the field. The PFOS levels measured in SRMs 1946 and 1947 (Tables 2 and 3) are within the range of PFOS concentrations being measured in freshwater fish from around the globe [25-29]. SRMs 1946 and 1947 were prepared from adult lake trout collected in 1997 from Lake Superior and Lake Michigan, respectively. Furdui et al. [28] examined the spatial distribution of PFAAs in whole lake trout collected from the Great Lakes in 2001. Similar to the measurements of PFAAs in SRMs 1946 and 1947, Furdui et al. [28] determined the concentration of PFOS to be higher in the trout collected from Lake Michigan compared to the trout collected in Lake Superior. The PFAA concentrations determined in the fish tissue SRMs showed similar patterns to one another, with the most abundant PFAA consistently detected being PFOS, contributing between 49 % and 75 % of the total PFAAs measured (Figure 2). The long-chained PFCAs with odd numbers of carbon were detected at higher concentrations compared to even number, long-chained PFCAs.

SRMs 1577c and 2974a, although not matrices routinely measured for PFAAs, were also examined (Tables 4 and 5). In these SRMs the only consistently quantifiable PFAA measured by at least two laboratories was PFOS. Despite the differences in analytical methods, the reported total PFOS concentrations in SRM 1577c were in relatively good agreement (RSD of the means from each laboratory were 1~~8~~5 %). Only two of the three laboratories were able to measure PFOS above the reporting limit in SRM 2974a. These two laboratories showed a 40 % difference between their measurements for SRM 2974a. While the analysis of PFOS in fish tissue produced more consistent results in this study, the high percent difference in measurements of PFOS in the mussel tissue highlights the fact that there are still concerns with measurement consistency in certain matrices.

The three marine mammal liver quality control materials, QC97LH02, QC03LH3, and QC04LH4, were analyzed as part of the interlaboratory comparison exercise between NIST and 3M. The PFAA concentrations were much higher in these marine mammal livers compared to the other SRMs. PFOSA and PFOS showed the highest concentrations, making up greater than 70 % of the compounds measured in these samples. The most abundant compound detected in the marine mammal livers QC97LH02, QC03LH3, and QC04LH4 was PFOSA, contributing between 51 % and 63 % of the total PFAAs measured. This finding is consistent with other studies reporting relatively high concentrations of PFOSA in some Arctic mammals [30-32]. Additionally, longer chain PFCAs were also detected in these materials and it should be noted that PFCAs with odd numbers of carbons, PFUnA and PFTriA, were detected at higher concentrations compared to even numbered, long-chained PFCAs. The patterns of PFAAs in the three quality control materials were fairly similar (Figure 3), despite the fact that these are three different species and from different locations. Interestingly, these patterns are similar because QC97LH2 was produced from beluga whale collected in 1996 from the Alaskan Arctic Ocean, while QC03LH3 was produced from pygmy sperm whales collected in 1994 from the Southwestern Atlantic Ocean and QC04LH4 was produced from white-sided dolphins collected in 2004 from the Northwestern Atlantic Ocean.

Reference values, along with the expanded uncertainties, for PFOS measured in SRMs 1946, 1947, and 1577c can be found in Table 9. The reference values were calculated using the results from this interlaboratory study. Information values are provided for PFAAs in which values were reported by at least four laboratories (PFNA, PFDA, PFUnA, and PFTriA); however, these values showed an RSD of more than 15 %. For comparison, the reference and information values are found in the same range as concentrations of legacy pollutants (polychlorinated biphenyls, polybrominated diphenyl ethers) previously measured in the SRMs.

Conclusions

This study showed that participating laboratories were able to produce more consistent data for PFOS in fish tissue reference materials compared with earlier interlaboratory studies. However, with a rarely analyzed matrix (as compared to fish tissues), as in this case mussel tissue, there are still inconsistencies in the data. As a result of this interlaboratory exercise, reference values for PFOS have been added to the Certificates of Analysis for SRMs 1946, 1947, and 1577c. Information values for some PFCAs have also been added to the Certificate of Analysis for SRMs 1946 and 1947. Additionally values were reported for three quality control materials. These materials, representative of current day PFAA environmental concentrations, provide much needed reference materials for environmental and biological studies.

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Disclaimer

Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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Table 1. List of perfluorinated alkyl acids with their abbreviations and participating laboratories along with the SRMs analyzed and compounds measured.

Matrix	SRM	Abbreviation	NIST	U. Toronto	Env. Canada	EPA	3M	Maritime & Hydrographic	Inst. Marine Resources Eco.
			Number of Replicates						
Fish Tissue	1946		27[a]	3	8	3	9	3	2
Fish Tissue	1947		36[a]	3	17[b]	3	9	3	2
Bovine Liver	1577c		11[a]	3	3				
Mussel Tissue	2974a		3			3			2
Beluga Liver	QC97LH2		26				3		
Pygmy Sperm Whale Liver	QC03LH3		6				3		
White Sided Dolphin Liver	QC04LH4		6				3		
Compounds Targeted (✓)									
Perfluorobutanoic acid		PFBA	✓				✓		
Perfluoropentanoic acid		PFPeA	✓		✓		✓	✓	
Perfluorohexanoic acid		PFHxA	✓	✓	✓	✓	✓	✓	
Perfluoroheptanoic acid		PFHpA	✓	✓	✓	✓	✓	✓	
Perfluorooctanoate acid		PFOA	✓	✓	✓	✓	✓	✓	✓
Perfluorononanoic acid		PFNA	✓		✓	✓	✓	✓	
Perfluorodecanoic acid		PFDA	✓	✓	✓	✓	✓	✓	
Perfluoroundecanoic acid		PFUnA	✓	✓	✓	✓	✓	✓	
Perfluorododecanoic acid		PFDoA	✓	✓	✓	✓	✓	✓	
Perfluorotridecanoic acid		PFTriA	✓		✓			✓	
Perfluorotetradecanoic acid		PFTA	✓		✓			✓	
Perfluorobutane sulfonate		PFBS	✓			✓	✓	✓	
Perfluorohexane sulfonate		PFHxS	✓	✓	✓	✓	✓	✓	
Perfluorooctane sulfonate		PFOS	✓	✓	✓	✓	✓	✓	✓
Perfluorodecane sulfonate		PFDS			✓			✓	
Perfluorooctane sulfonamide		PFOSA	✓		✓		✓	✓	

[a]NIST used two different methods for the analysis of PFAAs in SRM 1946, SRM 1947, and SRM 1577c.
[b]Environment Canada used two different methods for the analysis of PFAAs in SRM 1947.

Table 2. Concentrations of PFAAs (ng/g as received) measured in SRM 1946 (Lake Superior Fish Tissue) by seven laboratories using different methods. Values represent the mean and one standard deviation. Range is reported for n=2.

Compound	NIST Method 1	NIST Method 2	U. Toronto	Env. Canada Method 1	EPA	3M	Maritime & Hydrographic	Inst. Marine Resources Eco.
	(n=15)	(n=12)	(n=3)	(n=8)	(n=3)	(n=9)	(n=3)	(n=2)
PFBA	<2.22~~<RL~~	<3.55~~<RL~~	NM	NM	NM	NM~~<RL~~	NM	NM
PFPeA	<1.11~~<RL~~	<0.770~~<RL~~	NM	1.83 ± 0.56	NM	NM~~<RL~~	<0.100~~<RL~~	NM
PFHxA	<0.844~~<RL~~	<1.13~~<RL~~	ND~~<RL~~	0.302 ± 0.094	<1.89~~<RL~~	NM~~<RL~~	<0.100~~<RL~~	NM
PFHpA	<0.120~~<RL~~	<0.969~~<RL~~	0.165 ± 0.044	0.239 ± 0.061	<5.21~~<RL~~	<0.256~~<RL~~	<0.100~~<RL~~	NM
PFOA	<2.59~~<RL~~	<0.710~~<RL~~	ND~~<RL~~	0.367 ± 0.059	<0.770~~<RL~~	<0.253~~<RL~~	<0.200~~<RL~~	<0.300~~<RL~~
PFNA	0.222 ± 0.04	<0.767~~<RL~~	NM	0.410 ± 0.142	<1.88~~<RL~~	0.251 ± 0.035	0.194 ± 0.011	NM
PFDA	<0.213~~<RL~~	<0.733~~<RL~~	0.274 ± 0.074	0.311 ± 0.033	<1.11~~<RL~~	<0.253~~<RL~~	0.166 ± 0.010	NM
PFUnA	<0.178~~<RL~~	<0.799~~<RL~~	0.385 ± 0.053	0.594 ± 0.146	<1.05~~<RL~~	0.442 ± 0.047	0.350 ± 0.004	NM
PFDoA	<0.326~~<RL~~	<0.740~~<RL~~	0.269 ± 0.040	0.304 ± 0.029	<0.720~~<RL~~	<0.253~~<RL~~	0.155 ± 0.005	NM
PFTriA	0.158 ± 0.016	<0.993~~<RL~~	NM	0.591 ± 0.089	NM	NM	0.422 ± 0.012	NM
PFTA	<0.316~~<RL~~	<0.723~~<RL~~	NM	0.232 ± 0.065	NM	NM	0.138 ± 0.002	NM
PFBS	<0.178~~<RL~~	<0.906~~<RL~~	NM	NM	<0.480~~<RL~~	<0.251~~<RL~~	<0.100~~<RL~~	NM
PFHxS	<0.0553~~<RL~~	<0.789~~<RL~~	0.0895 ± 0.0878	0.0933 ± 0.0384	<0.0100~~<RL~~	<0.252~~<RL~~	<0.0500~~<RL~~	NM
PFOS	2.42 ± 0.10	2.14 ± 0.06	4.34 ± 1.67	2.45 ± 0.74	1.59 ± 0.14	2.68 ± 0.25	1.84 ± 0.01	1.12 - 1.34
PFDS	NM	NM	NM	0.139 ± 0.020	NM	NM	0.0596 ± 0.0044	NM
PFOSA	<1.60~~<RL~~	<0.865~~<RL~~	NM	0.124 ± 0.063	NM	<0.253~~<RL~~	0.0817 ± 0.0014	NM

Values shown as "<" a specified number describe the actual reporting limit ~~<RL = less than the reporting limit~~
NM = not measured
ND = not detected

Table 3. Concentrations of PFAAs (ng/g as received) measured in SRM 1947 (Lake Michigan Fish Tissue) by seven laboratories using different methods. Values represent the mean and one standard deviation. Range is reported for n=2.

Compound	NIST Method 1	NIST Method 2	U. Toronto	Env. Canada Method 1	Env. Canada Method 2	EPA	3M	Maritime & Hydrographic	Inst. Marine Resources Eco.
	(n=24)	(n=12)	(n=3)	(n=13)	(n=4)	(n=3)	(n=9)	(n=3)	(n=2)
PFBA	<0.861~~<RL~~	<2.83~~<RL~~	NM	NM	NM	NM	NM~~<RL~~	NM	NM
PFPeA	<0.441~~<RL~~	<0.388~~<RL~~	NM	0.234 ± 0.087	NM	NM	NM~~<RL~~	<0.100~~<RL~~	NM
PFHxA	<0.917~~<RL~~	<1.04~~<RL~~	ND~~<RL~~	0.0984 ± 0.0158	NM	<1.89~~<RL~~	NM~~<RL~~	<0.100~~<RL~~	NM
PFHpA	<0.0689~~<RL~~	<0.0676~~<RL~~	0.158 ± 0.033	0.109 ± 0.013	NM	<5.21~~<RL~~	<0.255~~<RL~~	<0.100~~<RL~~	NM
PFOA	<0.297~~<RL~~	<0.676~~<RL~~	ND~~<RL~~	0.189 ± 0.064	0.0260 ± 0.0026	<0.770~~<RL~~	<0.252~~<RL~~	<0.200~~<RL~~	0.0776 - 0.117
PFNA	0.179 ± 0.013	<0.765~~<RL~~	NM	0.246 ± 0.064	0.146 ± 0.013	<1.88~~<RL~~	0.279 ± 0.029	0.206 ± 0.010	NM
PFDA	0.282 ± 0.062	<0.731~~<RL~~	0.296 ± 0.030	0.273 ± 0.085	0.262 ± 0.053	<1.11~~<RL~~	<0.252~~<RL~~	0.179 ± 0.005	NM
PFUnA	0.212 ± 0.024	<0.128~~<RL~~	0.273 ± 0.013	0.324 ± 0.055	0.281 ± 0.058	<1.05~~<RL~~	0.298 ± 0.027	0.236 ± 0.014	NM
PFDoA	<0.137~~<RL~~	<0.156~~<RL~~	0.225 ± 0.108	0.150 ± 0.032	NM ~~<RL~~	<0.720~~<RL~~	<0.252~~<RL~~	0.137 ± 0.004	NM
PFTriA	0.154 ± 0.020	<0.738~~<RL~~	NM	0.251 ± 0.043	NM ~~<RL~~	NM	NM	0.216 ± 0.004	NM
PFTA	0.198 ± 0.069	<0.108~~<RL~~	NM	0.128 ± 0.031	NM ~~<RL~~	NM	NM	0.148 ± 0.004	NM
PFBS	<0.194~~<RL~~	<1.71~~<RL~~	NM	NM	NM	<0.480~~<RL~~	<0.250~~<RL~~	<0.100~~<RL~~	NM
PFHxS	<0.0490~~<RL~~	<0.0556~~<RL~~	0.143 ± 0.145	0.0384 ± 0.0266	NM ~~<RL~~	<0.0100~~<RL~~	<0.251~~<RL~~	<0.0500~~<RL~~	NM
PFOS	6.17 ± 0.60	5.66 ± 0.28	5.97 ± 0.62	5.35 ± 1.05	5.40 ± 0.24	4.48 ± 0.10	6.41 ± 0.55	4.84 ± 0.09	4.03 - 4.04
PFDS	NM	NM	NM	0.0852 ± 0.0226	NM ~~<RL~~	NM	NM	0.0629 ± 0.0052	NM
PFOSA	<0.151~~<RL~~	<0.171~~<RL~~	NM	0.179 ± 0.044	NM	NM	0.162 ± 0.022	0.218 ± 0.002	NM

Values shown as "<" a specified number describe the actual reporting limit ~~<RL= less than the reporting limit~~
NM= not measured
ND = not detected

Table 4. Concentrations of PFAAs (ng/g as received) measured in SRM 1577c (Bovine Liver) by three laboratories using different methods. Values represent the mean and one standard deviation.

Compound	NIST Method 1	NIST Method 2	U. Toronto	Env. Canada Method 1
	(*n*=6)	(*n*=5)	(*n*=3)	(*n*=3)
PFBA	<1.37~~<RL~~	<0.308~~<RL~~	NM	NM
PFPeA	<0.259~~<RL~~	<0.631~~<RL~~	NM	NM~~<RL~~
PFHxA	<0.695~~<RL~~	<0.821~~<RL~~	~~<RL~~ND	NM~~<RL~~
PFHpA	<0.0452~~<RL~~	<0.0534~~<RL~~	0.798 ± 0.555	<0.608~~<RL~~
PFOA	<0.511~~<RL~~	<0.655~~<RL~~	~~<RL~~ND	<0.678~~<RL~~
PFNA	<0.155~~<RL~~	<0.438~~<RL~~	NM	<1.07~~<RL~~
PFDA	<0.552~~<RL~~	<0.620~~<RL~~	ND~~<RL~~	<0.824~~<RL~~
PFUnA	<0.0862~~<RL~~	<0.166~~<RL~~	ND~~<RL~~	<0.481~~<RL~~
PFDoA	<0.104~~<RL~~	<0.515~~<RL~~	ND~~<RL~~	<0.847~~<RL~~
PFTriA	<0.0550~~<RL~~	<0.121~~<RL~~	NM	NM~~<RL~~
PFTA	<0.0728~~<RL~~	<0.161~~<RL~~	NM	NM~~<RL~~
PFBS	<0.147~~<RL~~	<0.173~~<RL~~	NM	NM
PFHxS	<0.0371~~<RL~~	<0.0824~~<RL~~	ND~~<RL~~	<0.680~~<RL~~
PFOS	4.02 ± 0.44	5.98 ± 1.20	5.60 ± 0.99	4.64 ± 0.78
PFDS	NM	NM	NM	<0.721~~<RL~~
PFOSA	<0.114~~<RL~~	<0.135~~<RL~~	NM	<0.850~~<RL~~

Values shown as "<" a specified number describe the actual reporting limit ~~<RL= less than the reporting limit~~
NM= not measured
ND = not detected

Table 5. Concentrations of PFAAs (ng/g as received) measured in SRM 2974a (Organics in Freeze-Dried Mussel Tissue) by three laboratories using different methods. Values represent the mean and one standard deviation.

Compound	NIST Method 1	EPA	Inst. Marine Resources Eco.
	(*n*=3)	(*n*=3)	(*n*=2)
PFBA	<1.65~~<RL~~	NM	NM
PFPeA	<0.312~~<RL~~	NM	NM
PFHxA	<0.836~~<RL~~	<1.89~~<RL~~	NM
PFHpA	<0.0544~~<RL~~	<5.21~~<RL~~	NM
PFOA	<0.615~~<RL~~	<0.770~~<RL~~	~~<RL~~<0.400
PFNA	0.139 ± 0.001	<1.88~~<RL~~	NM
PFDA	0.289 ± 0.025	<1.11~~<RL~~	NM
PFUnA	0.360 ± 0.001	<1.05~~<RL~~	NM
PFDoA	0.916 ± 0.026	<0.720~~<RL~~	NM
PFTriA	1.42 ± 0.05	NM	NM
PFTA	<0.117~~<RL~~	NM	NM
PFBS	<0.177~~<RL~~	<0.480~~<RL~~	NM
PFHxS	<0.0447~~<RL~~	<0.0100~~<RL~~	NM
PFOS	3.50 ± 0.19	1.97 ± 0.19	~~<RL~~<0.600
PFDS	NM	NM	NM
PFOSA	22.1 ± 0.2	NM	NM

Values shown as "<" a specified number describe the actual reporting limit ~~<RL= less than the reporting limit~~
NM= not measured

Table 6. Concentrations of PFAAs (ng/g as received) measured in QC97LH2 (Beluga Liver) by two laboratories using different methods. Values represent the mean and one standard deviation.

Compound	NIST Method 1 (*n=26*)	3M (*n=3*)
PFBA	≤1.53~~<RL~~	2.66 ± 0.28
PFPeA	≤0.290~~<RL~~	≤0.993~~<RL~~
PFHxA	≤0.777~~<RL~~	≤0.351~~<RL~~
PFHpA	≤0.0505~~<RL~~	≤0.357~~<RL~~
PFOA	≤0.571~~<RL~~	≤0.256~~<RL~~
PFNA	1.06 ± 0.20	0.896 ± 0.028
PFDA	2.32 ± 0.15	2.98 ± 0.26
PFUnA	9.75 ± 3.13	8.90 ± 0.52
PFDoA	1.30 ± 0.21	1.33 ± 0.14
PFTriA	3.22 ± 0.53	NM
PFTA	1.22 ± 0.59	NM
PFBS	≤0.164~~<RL~~	≤0.100~~<RL~~
PFHxS	0.147 ± 0.107	≤0.253~~<RL~~
PFOS	10.1 ± 1.25	9.86 ± 0.83
PFOSA	42.9 ± 2.7	35.4 ± 1.3

Values shown as "<" a specified number describe the actual reporting limit ~~<RL= less than the reporting limit~~

NM= not measured

Table 7. Concentrations of PFAAs (ng/g as received) measured in QC03LH3 (Pygmy Sperm Whale Liver) by two laboratories using different methods. Values represent the mean and one standard deviation.

Compound	NIST Method 1 (*n*=6)	3M (*n*=3)
PFBA	<3.49~~<RL~~	2.32 ± 0.10
PFPeA	<0.0958~~<RL~~	<1.02~~<RL~~
PFHxA	<0.0570~~<RL~~	<0.362~~<RL~~
PFHpA	<0.971~~<RL~~	<0.368~~<RL~~
PFOA	<0.677~~<RL~~	<0.264~~<RL~~
PFNA	3.52 ± 0.49	4.14 ± 0.31
PFDA	1.94 ± 1.38	1.78 ± 0.23
PFUnA	3.52 ± 2.07	6.43 ± 0.51
PFDoA	1.53 ± 1.17	1.16 ± 0.06
PFTriA	8.94 ± 2.11	NM
PFTA	2.65 ± 0.75	NM
PFBS	<0.124~~<RL~~	<0.103~~<RL~~
PFHxS	0.491 ± 0.110	<0.261~~<RL~~
PFOS	8.04 ± 0.19	10.7 ± 0.5
PFOSA	24.5 ± 3.7	19.8 ± 1.8

Values shown as "<" a specified number describe the actual reporting limit ~~<RL= less than the reporting limit~~
NM= not measured

Table 8. Concentrations of PFAAs (ng/g as received) measured in QC04LH4 (White-Sided Dolphin Liver) by two laboratories using different methods. Values represent the mean and one standard deviation.

Compound	NIST Method 1 (*n*=6)	3M (*n*=3)
PFBA	<3.49~~<RL~~	4.98 ± 0.70 <0.991~~<RL~~
PFPeA	<0.0958~~<RL~~	
PFHxA	<0.0570~~<RL~~	0.359 ± 0.019 <0.356~~<RL~~
PFHpA	0.174 ± 0.042 <0.677~~<RL~~	
PFOA		0.401 ± 0.020
PFNA	2.06 ± 0.20	2.26 ± 0.21
PFDA	8.67 ± 0.64	8.09 ± 0.51
PFUnA	47.4 ± 2.6	39.9 ± 2.4
PFDoA	7.01 ± 0.39	6.58 ± 0.23
PFTriA	36.3 ± 3.4	NM
PFTA	6.64 ± 0.53	NM
PFBS	<0.124~~<RL~~	<0.100~~<RL~~
PFHxS	0.656 ± 0.052	0.612 ± 0.018
PFOS	145 ± 4	162 ± 10
PFOSA	409 ± 34	227 ± 7

Values shown as "<" a specified number describe the actual reporting limit ~~<RL= less than the reporting limit~~
NM= not measured

Table 9. Reference (± expanded uncertainties) and information values (ng/g, as received) for selected PFAAs in biological SRMs.

Compound	SRM 1946 Lake Superior Fish Tissue	SRM 1947 Lake Michigan Fish Tissue	SRM 1577c Bovine Liver
		Reference[a]	
PFOS[b]	2.19 ± 0.08	5.90 ± 0.39	4.96 ± 1.18
		Information[c]	
PFNA		0.20	
PFDA		0.26	
PFUnA		0.28	
PFTriA		0.20	

[a] The reference value is a weighted mean of the results from the interlaboratory exercise [20]. The expanded uncertainties about the mean were calculated according to Rukhin [21] using a coverage factor of equal to 2 (approximately 95 % confidence).

[b]PFOS values are inclusive of branched and linear isomers.

[c]The information value is a mean of the results from the interlaboratory exercise.

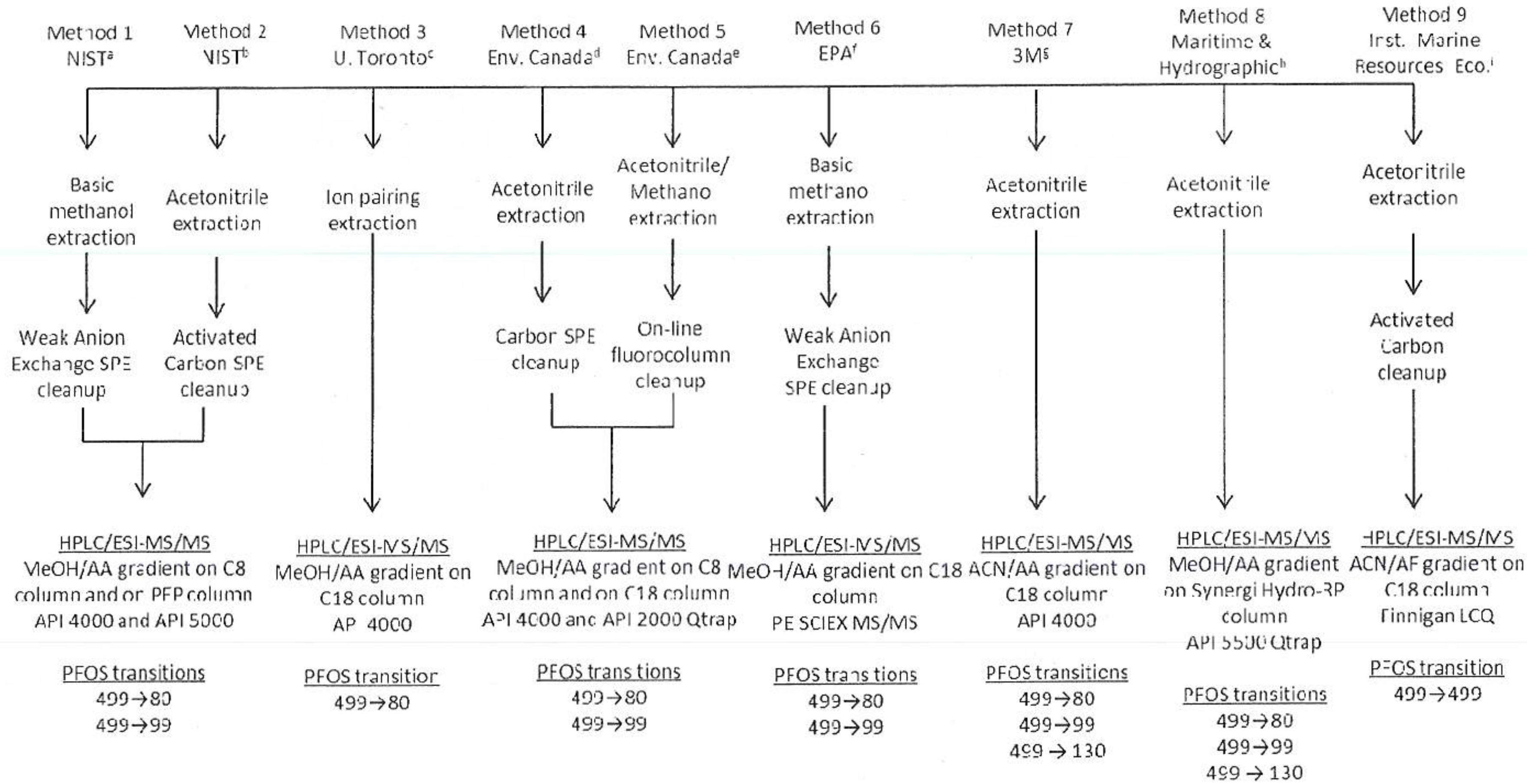

Figure 1. Methods used for the determination and quantification of PFAAs in SRMs and quality control materials. Abbreviations: solid-phase extraction (SPE); methanol-ammonium acetate mobile phase (MeOH/AA); acetonitrile-ammonium acetate mobile phase (ACN/AA); acetonitrile-ammonium formate mobile phase (ACN/AF). [a]Method modified from Taniyasu et al. [33]. [b]See Reiner et al. [17] for method details. [c]Method similar to Hansen et al. [34]. [d]Method similar to Muller et al. [35]. [e]See De Silva et al. [36] for method details. [f]See Delinsky et al. [26] for method details. [g]See Malinsky et al. [37] for method details. [h]Method adapted from Powley et al. [38]. [i]See Kwadijk et al. [39] for method details.

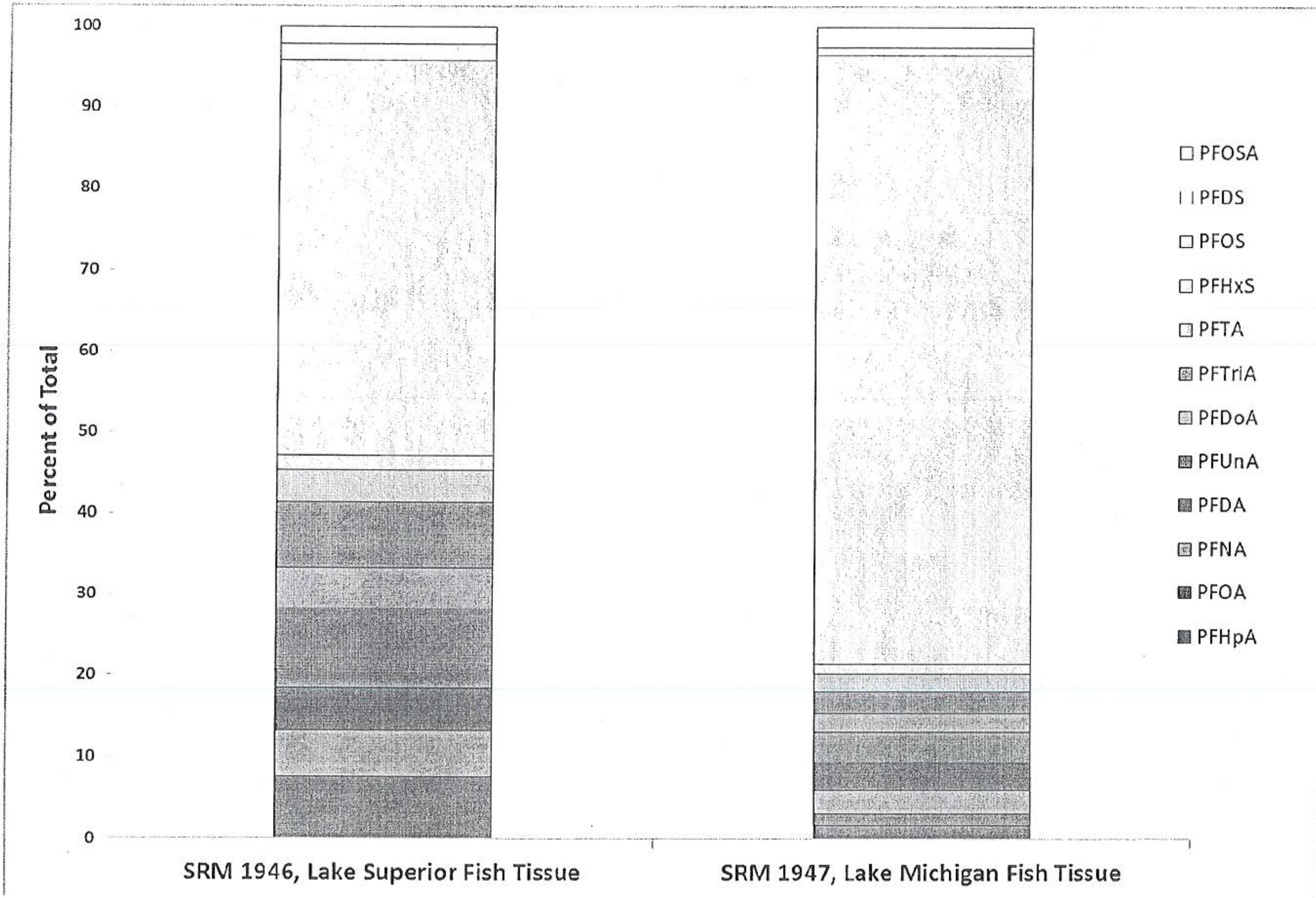

Figure 2. PFAA composition in fish tissue SRMs based on average measurements from at least two laboratories.

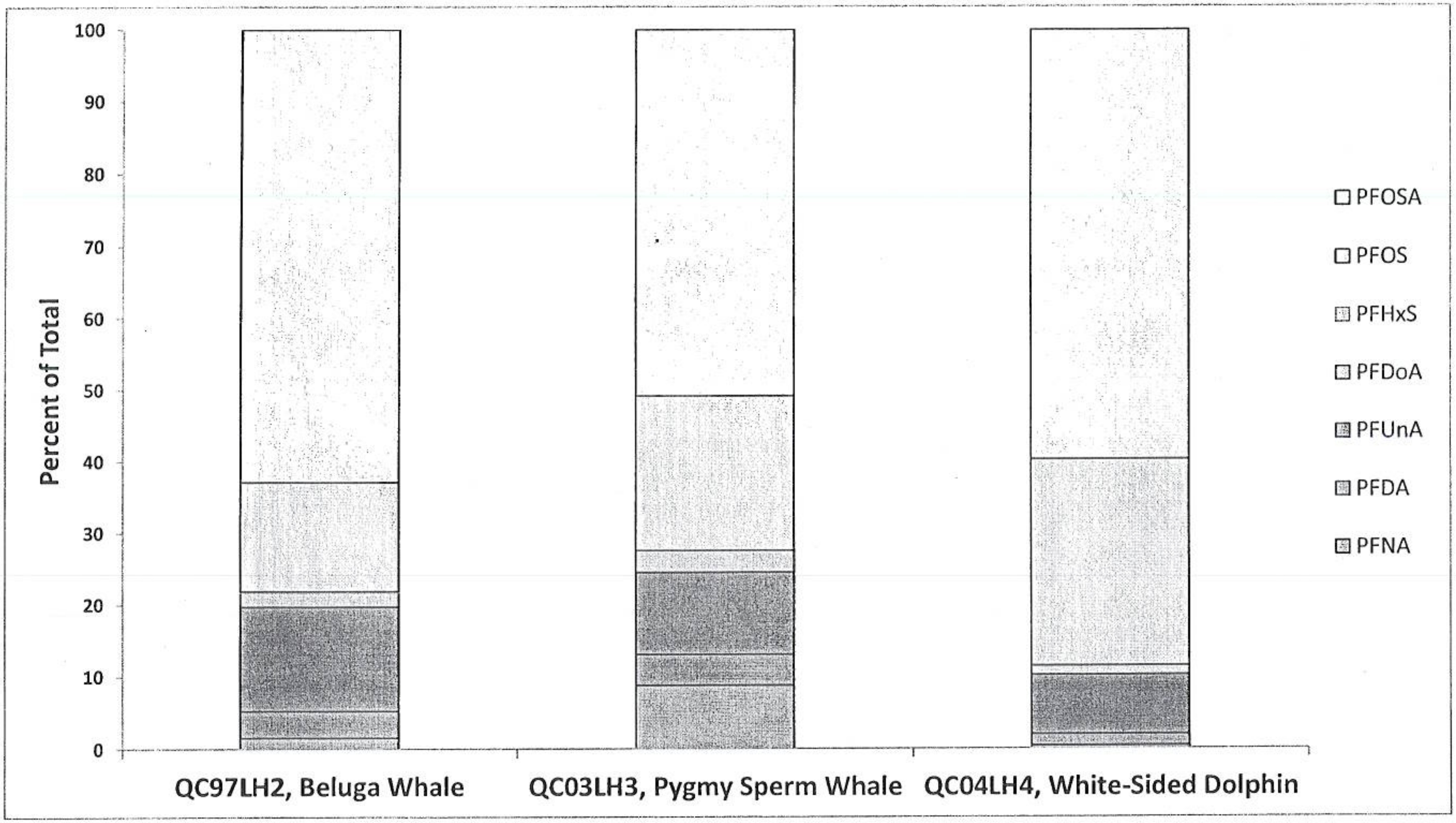

Figure 3. PFAA composition in marine mammal liver quality control materials based on average measurements from two laboratories.