Appendix 5a. Additional method information environmental samples.

Participant	L01 Water sample	L02 Water sample	L03 Water sample	L04 Water sample	L07 Water sample	L08 Water sample	L09 Water sample	L10 Water sample
Sample pretreatment	Add.of int. Stds., subsampling 100mL			-	None	-	not applicable	shaking
Extraction technique:	SPE, 1.7g Chromabond HR-P	SPE; STRATA X-AW	SPE , t-C18 (1 g)	-	SPE (Oasis HLB 6 cc)	SPE	SPE extraction	SPE
Extraction solvents:	Elution 60mL MeOH Evaporation to 1mL	MeOH; ACN/ACE (1 % AA); MeOH (0.1 % NH3)		-	MeOH	MeOH	methanol	methanol
Clean Up:	no cleanup	incl. in extraction technique		SPE w/Oasis WAX 3cc 60mg 30um		SPE (HLB Oasis, 3ml)	not applicable	filter (RC 0.2µm)
LC column:	Phonomenex Synergi Polar+Hydro 50+75mm, D=2mm	Synergy 4u Fusion RP C-18 (100 mm × 2.0 mm i.d.)	Betasil C18 2.1 x 50 mm, 5 µm linear	BEH C18 1.7um 2.1 x 100mm Methanol, water, ammoniumacetate	Waters Acquity UPLC BEH C18 1.7µm 2.1x100 mm Solvent A: H2O/ACN (95/5) + 2mM NH4Ac; Solvent B: ACN + 2mM NH4Ac	Atlantis T3 (100 mm x 4 mm i.d.; 5 µm) gradient MeOH - 2mM ammonium acetate	Zorbax Eclipse Plus C18 Narrow Bore RR 2.1*50mm 3.5µm 85% (2mM (NH4)2OAc in H2O) - 15%Methanol	C18
LC/MS(MS):	API5500QTrap	Varian Model 1200; LC-MS/MS-ESI	API2000 (Sciex) ESI negative	Waters Aquity UPLC Waters Quattro Premier/XE ESI-	Acquity UPLC system with Micromass Quattro Premier XE tandem mass spectrometer (MS/MS) from Waters Negative Electrospray ionisation	Alliance 2695/Quattro Premier XE, Waters (USA) ESI- MS/MS (triple quadrupole)	5% (2mM (NH4)2OAc in H2O) - 95%Methanol LC/MSMS	Quattro Premier XE
(Mass Labelled) Internal Standards:	PFOS-13C4, PFOA-13C2, PFHxS-18O3	7		9: HxS,OS,BA,HxA,OA,NA,DA,UnDA,DoDA	7 (13C4-PFBA, 13C2-PFHxA, 13C4-PFOA, 13C5-PFNA, 13C2-PFDA, 13C2-PFDoA, 13C4-PFOS)		5 (MPFOS, MPFOA, MPFHxS, MPFHxA, MPFBA)	-
(Mass Labelled) Recovery Standards:	MCPA-D3, MCPA-D6, Mecoprop-D3	1	13C PFOA; 13C PFOS, 13C PFDA		15 (PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTeA, PFBS, PFHxS, PFOS, PFDS, PFOSA)		5 (MPFOS, MPFOA, MPFHxS, MPFHxA, MPFBA)	13C-PFOA
Results Corrected for Recovery:	yes	No	No	No	No		yes	no
Results Corrected for Blanks:	yes	Yes	Yes	No	No			no
Standard Method:	custom	ISO 25101:2009		-	ISO/CD 25101:2006			
Comments:								

L11 Water sample	L12 Water sample	L13 Water sample	L14 Water sample	L15 Water sample	L16 Water sample	L17 Water sample	L18 Water sample	L19 Water sample
	none	---	Filtering, pH adjustment	Formic acid addition		No	-	
SPE	Yamashita et al. method using an OASIS WAX cartridge	SPE, Strata X-AW 6 mg (conditioned with 3 mL methanol and 3 mL water, washed with 1 ml methanol, eluted with 2 mL 0.1% ammonia in methanol); The extracts were evaporated at 39 ℃ under nitrogen gas to dryness and reconstituted with 250 µL of methanol:water (1:1; v:v).	SPE	SPE on HLB plus	SPE with Oasis HLB 200mg	SPE	SPE (WAX, 150 mg cartridge)	SPE
MeOH	methanol	---	Methanolic ammonium hydroxide		Methanol	Methanol	MeOH	MeOH
wash MeOH/H2O (40:60); dispersive clean up with ENVI-Carb	none, no filtration, but there is a wash step that was discarded	---	SPE	none		Water+20%Methanol	-	
C-18	Luna C8, 50 x 2 mm, 3 micron (Phenomenex, Torrance, CA, USA, part number 00B-4248-B0) for PFHxS, PFOA to PFDoA, and PFOS For PFBA, PFPeA, PFHxA, PFHpA, and PFBS, used Shodex Rspak JJ-50 2D anion exchange/reverse phase column, 15 x 2 mm, 5 um (part number J803002, Shodex, Shanghai)	BEH C18 column (1.7 µm; 2.1 x 50 mm; waters) with precolumn (BEH C18; 1.7µm; 2.1 x 5mm, waters).	C-18	Ace 3 C18, 3 um particles,	Hypersil Gold column	C18- Alltima	Nucleodur C18 Gravity	C-18
150mm x 21mm, 3µm			100 x 2.1 mm	150 mm length, 2.1mmi.d.	Thermo Electron Corp.	4.6mm x 50mm	2.0 x 70 mm (3 µm)	
2mM NH4OAc in MeOH and H2O; start 50:50; 5min 85:15; 10min 85:15; 11min 99:1; 20min 99:1; 21min 50:50; 28min 50:50;			Up to 90% CH_3CN and 12.1 mM NH_4OAc in 0.1% AcOH	Methanol/water with 2 mM NH4OAc	100 x 2.1 mm, 3 um particles	40 H2O+60MeOH->5H2O+95MeOH	30% MeOH t=O, in 2 min to 60%, in 4 min; to 77% and in 3 min to 85%	
Waters, Micromass Q-TOF micro		Aquity UPLC (waters) with MS/MS (TQ-Detector, waters)	Waters, MS/MS	Quattro II, Micromass, MS/MS	Waters Quattro Micro LC-MS-MS	Thermofisher- IT	Varian Prostar 210 HPLC, Varian 1200 MS/MS	Time of Flight (TOF)
ESI neagtive	LC is Agilent 1100 pump MS/MS was done using Sciex AB 4000 Qtrap; used calibration curve with relative response to internal standards	negative electrospray mode with MRM	ESI-	ESI neg	ESI	ESI negative mode	ESI	
2, PFOA, PFOS	C13 PFOA, PFNA, PFDA, PFUnA, PFDoA, and PFOS, and 18O PFHxS all from Wellington	6 IS (MPFBA, MPFHxA, MPFOA, MPFOS, MPFNA, MPFDA)	7; $^{13}C_4$-PFBA, 13C4-PFHxA, 13C4-PFOA, 13C4-PFNA, 13C4-PFDA, 13C4-PFDoA, 13C4-PFOS				6, MPFBA, MPFHxA, MPFOA, MPFDA, MPFHxS, MPFOS	6: C^{13}PFOS, C^{13}PFOA, C^{13}PFNA, C^{13}PFDA, C^{13}PFDoA, C^{13}PFHxA
3,7-diMe-PFOA	recovery was conducted using 13C1,2-PFOA		1; 13C2-FOUEA			3, MPFOA, MPFNA, MPFOS	-	-
no	no see comment	No	YES			Yes (only for native with labeled standard)	No	No
no	no see comment	No	NO			NO	No	No
	no						-	
	WATER method: Did not correct for blanks. Method blanks were clean with the exception of 0.006 ng PFOA, 0.0048 ng PFNA, 0.0021 ng PFUnA, 0.0045 ng PFOS. Did not correct for recovery; however, recovery corresponded to 120% for PFOA, 90% PFNA, 100% PFDA, 110% PFUnA, 95% PFDoA, 95% PFTrA, 99% PFOS and 97% PFHxS in serum. For fish tissue, results obtained with external calibration agreed with those via standard addition within 15%. Sludge recoveries via spike and recovery corresponsed to 90 to 100% for PFOA, PFNA, PFDA, PFUnA, PFDoA, 85% for PFTrA, 80% for PFHxS but was poor for PFOS (981%); For PFBA, PFPeA, PFHxA, PFHpA, and PFBS, used Shodex Rspak JJ-50 2D anion exchange/reverse phase column, 15 x 2 mm, 5 um (part number J803002, Shodex, Shanghai)							

L20 Water sample	L21 Water sample	L23 Water sample	L25 Water sample	L26 Water sample	L28 Water sample	L29 Water sample	L31 Water sample	L33 Water sample
none		none	Acid treatment, Centrifugation	None	None	Adding formic acid (pH 4)	none	/
SPE Waters OASIS WAX (30 um) 150mg/6 mL	SPE	SPE; Waters 3cc(60mg) HLB cartridges	SPE (Oasis HLB Plus)	None	SPE; Standards and QC samples were prepared by spiking 40 mL of reverse osmosis water with target analytes and internal standards at required levels. Forty microleters of a 250 ug/mL sodium thiosulfate solution was added to each standard, QC and study sample. Forty mL of sample was loaded on a pre-conditioned Water tC18 SPE cartridge. Target analytes were eluted with 5.0 nmL of methanol	SPE: OASIS WAX 150 mg	Oasis HLB, 60 mg, 3 cc	SPE
0.1% NH4OH/methanol	MeOH	Methanol	1 % NH3 in MeOH	None			ACN with 1% NH4OH	ACN
no volume reduction under nitrogen stream								
wash 1: 25 mM NH4OAc; wash 2: methanol		none	Active carbon	None	None		none	
Thermo Hypersyl Gold 100X2.1mm, 5um	Phenomenex, Synergie, polar RP, 80 A, 4µ (150 x 2mm)	Grace Vydac Genesis C18	C-18	Atlantis dC-18 (Waters)	Betasil C18	Phenomenex LUNA (PFP2)	Betasil C8	BEH C 18
A (MEOH): B (water LC-MS 2mM NH4OAc, 5%MEOH)		50mm x 2.1mm i.d. x 4um particles	2,1x50 mm	2.1 x 100mm, 3µm	2.1 x 100 mm, 5 micron	150*3.0 mm, 5µm	3um, 2.1 x 50 mm	2,1 x 50 mm
0 min (5:95);1 min (5:95); 2 min (45:55); 30 min (95:5); 35 min (95:5); 36 min (5:95); 50 min (5:95)		MeOH & H_2O (10mM Ammounim Acetate); isocratic 80:20 MeOH:H2O	Gradient	Gradient with MeOH and Water, each with 0.1% NH4Ac		A: MeOH / B: NH4Ac (PH 3.5); (A: 60% (0min) --> 100 % (12min)	0.1% formic acid in H2O and 0.1% formic acid in CAN; at 400 uL/min, 30%B, 0.25 min. Ramp to 90%B over 2 min. Hold @90%B till 7 min. 7.01 min, 30%B & 500 uL/min, hold till 8.5 min. 8.51 min, reduce flow rate to 400 ul/min	ACN + 0,1 % formic acid and (start 65%); reversed osmose water+ 0,1 % formic acid
LC-ESI-ITMS(MS)	MS/MS	ABS Sciex 4000Qtrap MS/MS	Waters UPLC-TQMS/MS	Varian 1200L, MS/MS	Agilent 1100 HPLC with heated column compartment. Applied Biosystems API 5000	Waters LC Quattro, MS/MS	Applied Biosystems 4000 Qtrap	Waters, quadrupole MS/MS, TQD
Thermo LCQ DECA XP MAX		negative ESI - MRM	ESI	ESI	Electrospray (Negative) Ionization	ESI		- ES'
no			3: 13C-PFOS, 13C-PFHxA, 13C-PFDA	0	[1,2,3,4-13C4]PFBA; [1,2-13C2]PFHxA; [1,2,3,4-13C4]PFOA; [1,2,3,4,5-13C5]PFNA; [1,2-13C2]PFDA; [1,2-13C2]PFUnA; [1,2-13C2]PFDoA; [18O2]PFBS; [18O2]PFHS; [1,2,3,4-13C4]PFOS; [18O2]FOSA	9 (PFBA, PFHxA, PFOA, PFDA, PFUDA, PFDoA, PFTeA, PFHxS, PFOS)	MPFBA, MFPHxA, MPFOA, MPFNA, MPFDA, MPFHxS, MPFOS	7; PFBA, PFHxA, PFOA, PFNA, PFDA, PFOS, PFHxS
MPFAC-MXA-100 and Perfluoro-n-[1,2,3,4-13C4]octanoic acid and Sodium perfluoro-1-[1,2,3-13C4]octanesulphonate	13C4-FBA, 13C2-PFHxA, 18O-PFHxA, 13C4-PFOA, 13C5-PFNA, 13C4-PFOS, 13C2-PFDA, 13C2-PFUdA, 13C2-PFDoA	6 - $^{13}C_4$-PFOS, $^{13}C_2$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFDoA, $^{13}C_4$-PFOA	1: 13C-PFOA	2 (13C4-PFOA, 13C4-PFOS)			0 none	none
yes	Yes	yes	Yes	Yes	No	no	no	yes (with internal standards)
no	Yes	no	Yes	Yes	No	no	no	yes
no		in-house developed method	PERFORCE, Powley method, modified	None	Internal Method	no		
					Samples were analyzed in triplicate, with duplicate lab matrix spikes at concentrations of 200 ng/L and 5000 ng/L. All lab matrix spike recoveries were within ±18% (with a single exception). Reported values reflect the average concentration ± percent relative standard deviation (%RSD); Samples were quantitated against reverse osmosis treated lab water used for the standard curve and LCS sampels.		10 mL extracted and run using a solvent based curve; matrix effects (signal enhancement) observed for PFPeA, peak detected, but not quantified.	Unextracted calibration; inclusive branched PFOS

L34 Water sample	L35 Water samples	L36 Water samples	L37 Water samples	L38 Water samples	L39 Water samples	L40 Water samples	L42 Water samples	L43 Water samples
filtration glass fiber filter	formic acid 99% to pH 3		NO	spiked with mass labelled standards, frozen	Acid treatment : pH 3 with H2SO4	sonication / acidification	Mixing, acidifying to pH 3 with H2SO4	adding internal standard in water samples; with NaCl 40 g/L, pH 4 with H2SO4
Waters Oasis WAX SPE	SPE : OASIS WAX 6cc,150 mg		NO	SPE with cartridges Oasis HLB 6cc 200mg	LLE	LLE	LLE	
	0.1% ammoniac in MeOH and MeOH		NO	MeOH and a solution of ammonia/MeOH 0.1%	MTBE	MTBE	MTBE	turbovap concentration nearly dryness, adding 1 ml MeOH - Liquid/Liquid extraction; with MTBE (methyl tert-butyl ether)
	acid water pH 3	SPE OASIS HLB	NO		none	None	None	no
same	C18	C18	Altech altima C18 150x2,1 5µm	Acquity UPLC BEH C18 1.7µm (WATERS)	C18, 2,1*50mm; 1,7µm	modified C-18	C18	C18
	50*2.1mm, 3µm	3µm (50 x 2,0 mm)	Gradient Water/ Acetonitril 0.1% HCOOH	2.1*100mm	Phases : MeOH / Water + 10mM	2.1mm*100mm*1.8µm		
	30% (2 mM acetate ammonium/ MeOH [9-1]) / 70% MeOH; 100% MeOH in 10 minutes	A : MeOH ; B : H2O/AcONH4 0.02M - 50/50 (0.5 min) until 100/0 in 6.5 min ; 100/0 for 5 min			ammonium acetate	initial 70% (Water+ACO2NH4)/30% MEOH		
same	MSMS TSQ QUANTUM	LC-MS/MS Agilent QqQ 6410	Thermo QUANTUM ULTRA MS/MS	Acquity UPLC (WATERS)	API4000, LC(ESI-)/MS/MS	WATERS MS/MS	Agilent 6410 LC-MS/MS, ESI-	LC-MS-MS : internal calibration method
	ES -	ESI	ES	MS/MS : Quattro micro (MICROMASS)		ES		with dope evian water
			Direct injection 1 ml	ES				
	3 IS : MPFHxA / MPFOA and MPFOS	8	PFAOC13 and PFOSC13	2 : MPFOA $^{13}C_4{}^{12}C_4HF_{15}O_2$ and MPFOS $^{13}C_4{}^{12}C_4F_{17}SO_3Na$	N/A	2 used (MPFOS/MPFOA)	13C4 PFBA, 13C2 PFHxA, 13C4 PFOA, 13C5 PFNA, 13C2 PFDcA, 13C2 PFUdA, 13C4 PFHxS, 13C4 PFOS, 18 O2 PFOSA	
same	MPFHxA: 115% , MPFOA: 86% and MPFOS:77%	1	Direct injection no recovery standards	2 : MPFOA $^{13}C_4{}^{12}C_4HF_{15}O_2$ and MPFOS $^{13}C_4{}^{12}C_4F_{17}SO_3Na$	N/A		N.A.	
	yes	Yes	no	yes	No	N	Yes	
	No	Yes	no	yes	No	Y	Yes	
ISO 2501	DRAFT-ISO-25101	Internal method	Internal method	ISO 25101 (march 2009)		internal	Internal	
				In water samples, the mass labelled standards mixture used is the solution sent by EIL staff (MPFAC-MXA-100). The concentration of this mixture doesn't correspond to the concentration mentionned in the Internationnal Standard ISO 25101 (march 2009), paragraph 5-6. So, for the calibration levels, we have used an other mixture of mass labelled supplied by Wellington Laboratories.				

Participant	L01	L02	L03	L04	L07	L08	L09	L10	L11	L12	L13	L14	L15
	Fish sample	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**
Sample pretreatment	1g sample, Addition of int. Stds.			No	Homogenisation	-	because the sample had 2 layers, we mixed it with a spoon	rehomogenisation		homogenization	---	Shaking with methanolic KOH	
Extraction technique:	solid/liquid, shaking, 3-times	LL	IPE	Ultrasound	LSE	solvent shaking	weighed 1 gram of sample, added 3mL 50-50 methanol/water; vortexed for 30 sec, centrifuged; supernatant collected; added 3mL 50-50 methanol/water; vortexed for 30 sec, centrifuged; supernatant collected; added 4mL 50-50 methanol/water; vortexed for 30 sec, centrifuged; supernatant collected; shake the collected fractions; mix 0.5mL fraction + 0.5mL IS ; filter trough 0.45µm	sonication	LSE	ion pairing extraction using 0.5 M TBAS and methyl tert butyl ether	1g fish sample, 2 mL ACN and 2 ml water, 30 min of mixing, 1,5 g Quicher's* Mix, 5 min centrifugation; SPE, Oasis WAX 60 mg, 3 ml (conditioned with 2 mL formic acid (0,1%) and 2 mL methanol, washed with 1 mL formic acid (0,1%) and 2 mL methanol, eluted with 2 mL 0.1% ammonia in methanol)	SPE	LSE
Extraction solvents:	ACN, 3x10mL	0.05 N KOH/MeOH	0.5 M tetrabutylammonium hydrogen sulfate (TBA);0.25 M natrium carbonat/natrium bicarbonat buffer ;MTBE	Methanol	ACN	MeOH	methanol and water	methanol	ACN	MTBE	H2O, ACN	Methanolic ammonium hydroxide	Acetonitrile
Clean Up:	Evaporation to 3mL; Freezing and centrifugation	SPE; STRATA X-AW		SPE w/Oasis WAX 3cc 60mg 30um	active carbon	activated charcoal	not applicable	filter (RC 0.2µm)	dispersive clean up with ENVI-Carb	none, no filtration	Quicher's Mix, formic acid (0,1%), methanol (see above)	SPE	Graphitised carbon
LC column:	Phonomenex Synergi Polar+Hydro 50+75mm, D=2mm	Synergy 4u Fusion RP C-18 (100 mm × 2.0 mm i.d.)	Betasil C18 2.1 x 50 mm, 5 µm linear	BEH C18 1.7um 2.1 x 100mm Methanol, water, ammoniumacetate	Waters Acquity UPLC BEH C18 1.7µm 2.1x100 mm Solvent A: H2O/ACN (95/5) + 2mM NH4Ac Solvent B: ACN + 2mM NH4Ac	Atlantis T3 (100 mm x 4 mm i.d.; 5 µm) gradient MeOH - 2mM ammonium acetate	Zorbax Eclipse Plus C18 Narrow Bore RR 2.1*50mm 3.5µm 85% (2mM (NH4)2OAc in H2O) - 15%Methanol 5% (2mM (NH4)2OAc in H2O) - 95%Methanol	C18	C-18 150mm x 21mm, 3µm 2mM NH4OAc in MeOH and H2O; start 50:50 5min 85:15; 10min 85:15; 11min 99:1; 20min 99:1; 21min 50:50; 28min 50:50;	Luna C8, 50 x 2 mm, 3 micron (Phenomenex, Torrance, CA, USA, part number 00B-4248-B0)	Luna C 18. 150x 3mm, 3 µm with precolumn	C-18 100 x 2.1 mm Up to 90% CH_3CN and 12.1 mM NH_4OAc in 0.1% AcOH	Ace 3 C18, 3 um particles, 150 mm length, 2.1mmi.d. Methanol/water with 2 mM NH4OAc
LC/MS(MS):	API5500QTrap	Varian Model 1200; LC-MS/MS-ESI	API2000 (Sciex) ESI negative	Waters Aquity UPLC Waters Quattro Premier/XE; ESI-	Acquity UPLC system with Micromass Quattro Premier XE tandem mass spectrometer (MS/MS) from Waters Negative Electrospray ionisation	Alliance 2695/Quattro Premier XE, Waters (USA) ESI-; MS/MS (triple quadrupole)	LC/MSMS	Quattro Premier XE	Waters, Micromass Q-TOF micro ESI neagtive	LC is Agilent 1100 pump; MS/MS was done using Sciex AB 4000 Qtrap; calibration curve was conducted using internal standards, also used the standard addition method with comparable results	Waters 2695 HPLC with MS/MS (Micromass Quattro micro API) negative electrospray mode with MRM	Waters, MS/MS ESI-	Quattro II, Micromass, MS/MS ESI neg
(Mass Labelled) Internal Standards:	PFOS-13C4, PFOA-13C2, PFHxS-18O3	7		9: HxS,OS,BA,HxA, OA,NA,DA,UnD A,DoDA	7 (13C4-PFBA, 13C2-PFHxA, 13C4-PFOA, 13C5-PFNA, 13C2-PFDA, 13C2-PFDoA, 13C4-PFOS)	yes 2 (Perfluoro-n-[1,2,3,4-13C4]octanoic acid-PFOA; Sodium perfluoro-1-[1,2,3,4-13C4]octasulfonate - PFOS)	5 (MPFOS, MPFOA, MPFHxS, MPFHxA, MPFBA)	-	2, PFOA, PFOS	C13 PFBA, PFHxA, PFOA, PFNA, PFDA, PFUnA, PFDoA, and PFOS, and 18O PFHxS all from Wellington	6 IS (MPFBA, MPFHxA, MPFOA, MPFOS, MPFNA, MPFDA)	7; $^{13}C_4$-PFBA, 13C4-PFHxA, 13C4-PFOA, 13C4-PFNA, 13C4-PFDA, 13C4-PFDoA, 13C4-PFOS	8, MPFHxS, MPFOS, MPFHxA, MPFOA, MPFNA, MPFDcA, MPFUnA, MPFDoA
(Mass Labelled) Recovery Standards:	MCPA-D3, MCPA-D6, Mecoprop-D3	1	13C PFOA; 13C PFOS, 13C PFDA	0	15 (PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTeA, PFBS, PFHxS, PFOS, PFDS, PFOSA)	-	5 (MPFOS, MPFOA, MPFHxS, MPFHxA, MPFBA)	13C-PFOA	3,7-diMe-PFOA	recovery was conducted using native standards spiked into serum.		1; 13C4-PFOA	1, di-methyl branched PFDcA
Results Corrected for Recovery:	yes	No	No	No	No	No	yes	no	no	no see comment	No	YES	Only via internal standard quantification, no additional correction.
Results Corrected for Blanks:	yes	Yes	Yes	No	No	No	no	no	no	no see comment	No	NO	Yes, see comments
Standard Method:	custom	inhouse method		-	ISO/CD 25101:2006		own method			no			No
Comments:	Altering to ACN as solvent to increase the extraction of long chain substances (Biota and Sediment); Methods under development for the adaption to the new much more sensitive LC-MS-MS.					Currently, the list of target PFCs has been expanded; since the validation of the LC/MS method for new analytes has not been completed, we report quantitative data only for two major PFCs present in the sample. In the addition to them we have detected following compounds (in brackets only semiquantitative results are given - fish sample ng/g): PFNA (0.9); PFDA (2.9); PFUdA (1.8); PFDoA (0.3); sludge sample (ng/g): PFHxA (0.7); PFHpA (0.4); PFNA (0.4); PFDA (0.7); std IVM PFC-MXA (ng/ml): PFBS (42); PFHxA (37); PFHxS (32); PFHpA (26); PFNA (54); PFDA (36); PFUdA (29); PFDoA (32)			FISH method: Did not correct for blanks. Triplicate method blanks were clean with the exception of PFOA (0.05 ng/ml), PFNA (0.001 ng/ml), PFHxS (0.001ng/ml) and PFOS (0.004 ng/ml); Did not correct for recovery; however, recovery corresponded to 120% for PFOA, 90% PFNA, 100% PFDA, 110% PFUnA, 95% PFDoA, 95% PFTrA, 99% PFOS and 97% PFHxS in serum. For fish tissue, results obtained with external calibration agreed with those via standard addition within 15%. Sludge recoveries via spike and recovery corresponsed to 90 to 100% for PFOA, PFNA, PFDA, PFUnA, PFDoA, 85% for PFTrA, 80% for PFHxS but was poor for PFOS (981%); For PFBA, PFPeA, PFHxA, PFHpA, and PFBS, used Shodex Rspak JJ-50 2D anion exchange/reverse phase column, 15 x 2 mm, 5 um (part number J803002, Shodex, Shanghai)			All sulfonate concentrations are given on the basis of the sulfonate anion; PFOS has been quantified in both transitions, m/z 499>80 and m/z 499>99. The water sample contained a high proportion of branched isomers, the linear isomer is valid; New parts in the LC pump led to relatively high background contamination with several PFCAs. We chose therefore to substract the blank value from all results (including sulfonates). fish and sludge samples only contained moderate amouts of isomers, the two results correspond quite well and quantification against therefore, the two results deviate substantially and the quantification against the pure linear isomer (as performed here) will be inaccurate. The

Fish method

L16	L17	L19	L20	L21	L23	L25	L28	L29	L33	L34
Fish sample	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**	**Fish sample**
	Homogenized		homogenised	grinding with silica	homogenized with tissue tearor		Residual dry ice allowed to sublime before weighing sample aliquots for matrix were accurately weighed (frozen). Aliquots were allowed to equilibrate to room temperature and then were spiked with IS/surrogates			Homog. by grinding
LSE; 2 h shacking	Sonication 30 minutes	LSE	LSE; sonication 40 min; shaking 16h	ultrasonic bath	LLE; modified 2001 Hansen ion pairing method	Sonication	Acetonitrle; Tissue/ACN homogenates placed in -20°C for at least 1 hour. Centrifuged at -5°C for 20 minutes at 3000 rpm. 1 mL of clarified supernate transferred to a 2 mL autovial spiked with 10 mL of 10%formic acid.	ASE	Solvent ectraction	vortex and sonication
Methanol	Methanol 2 times	ACN	2mL 0.2MKOH/methanol + 20 mL methanol	MeOH, H2O	MTBE, 0.25M Na2CO3, 0.5M TBAS	ACN		MeOH / H2O	ACN	Acetonitrile
30 mg Envi-Carb,Vortex mixing	Filtration - 0.2 µm	SPE	5 mL of extraction solvent (after settling) were diluted to 500 mL with MQ and extracted according to extraction procedure of the water sample	SPE	none / dilution	Active carbon		SPE: OASIS WAX 150 mg	Active Carbon	Waters Oasis WAX SPE; Supelclean ENVI-Carb
Hypersil Gold column	C18- Alltima	C-18	Thermo Hypersyl Gold 100X2.1mm, 5um	Phenomenex, Synergie, polar RP, 80 A, 4µ (150 x 2mm)	Grace Vydac Genesis C18	C-18	Thermo Scientific PRISM RP column for analysis of PFBA, PFPeA, and PFHxA; 2.1mm × 50 mm; 5 µ particle size, Thermo Scientific Betasil™ C18 analytical column (for analysis of PFCAs (C7-C12) and PFASs 2.1mm × 100 mm; 5□ particle size); Extraction Pre-Column: Oasis® HLB on-line extraction column (20 mm × 3.0 mm, 25 m particle size) with the outlet directed to a column switching valve where the first five minutes were diverted to waste. After 5 minutes effluent directed to analytical column;	Phenomenex LUNA (PFP2)	BEH C 18	Acquity BEH C18
Thermo Electron Corp.	4.6mm x 50mm		A (MEOH): B (water LC-MS 2mM NH4OAc, 5%MEOH) 0 min (5:95);1 min (5:95); 2 min (45:55); 30 min (95:5); 35 min (95:5); 36 min (5:95); 50 min (5:95) 200 µL/min		50mm x 2.1mm i.d. x 4um particles	2,1x50 mm		150*3.0 mm, 5µm	2,1 x 50 mm	50*2.1mm, 1.7um
100 x 2.1 mm, 3 um particles	40 H2O+60MeOH->5H2O+95MeOH				MeOH & H_2O (10mM Ammounim Acetate) gradient 75:25 to 90:10 MeOH:H_2O	Gradient		A: MeOH / B: NH4Ac (PH 3.5) (A: 60% (0min) --> 100 % (12min)	ACN + 0,1 % formic acid and (start 65%) reversed osmose water+ 0,1 % formic acid	
Waters Quattro Micro LC-MS-MS	Thermofisher- IT	Time of Flight (TOF)	LC-ESI-ITMS(MS)	MS/MS	ABS Sciex 4000Qtrap MS/MS	Waters UPLC-TQMS/MS	Agilent 1100 HPLC with Applied Biosystems API 4000 Q-trap; Electrospray (Negative) Ionization	Waters LC Quattro, MS/MS	Waters, quadrupole MS/MS, TQD	Acquity UPLC
ESI	ESI negative mode		Thermo LCQ DECA XP MAX		negative ESI - MRM	ESI		ESI	- ES'	Quattro Premier XE MS/MS
Provided IS-mix 13C		6: C^{13}PFOS, C^{13}PFOA, C^{13}PFNA, C^{13}PFDA, C^{13}PFDoA, C^{13}PFHxA	no		5 - $^{13}C_4$-PFOS, $^{13}C_2$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFDoA	3: 13C-PFOS, 13C-PFHxA, 13C-PFDA	[1,2,3,4-13C4]PFBA; [1,2-13C2]PFHxA; [1,2,3,4-13C4]PFOA; [1,2,3,4,5-13C5]PFNA; [1,2-13C2]PFDA; [1,2-13C2]PFUnA; [1,2-$^{13}C_2$]PFDoA; [18O2]PFBS; [18O2]PFHS; [1,2,3,4-13C4]PFOS; [18O2]FOSA	9 (PFBA, PFHxA, PFOA, PFDA, PFUDA, PFDoA, PFTeA, PFHxS, PFOS)	7; PFBA, PFHxA, PFOA, PFNA, PFDA, PFOS, PFHxS	C6,C8 sulfonates, C4,C5,C8,C9,C10;c11,C12 carboxylates
Wellington 12C mix	3, MPFOA, MPFNA, MPFOS	-	MPFAC-MXA-100 and Perfluoro-n-[1,2,3,4-13C4]octanoic acid and Sodium perfluoro-1-[1,2,3-13C4]octanesulphonate	13C4-FBA, 13C2-PFHxA, 18O-PFHxA, 13C4-PFOA, 13C5-PFNA, 13C4-PFOS, 13C2-PFDA, 13C2-PFUdA, 13C2-PFDoA	1 - $^{13}C_4$-PFOA	1: 13C-PFOA			0 none	7H-PFHpA
yes	Yes	No	yes	Yes	no	Yes	No - see below	no	yes (with internal standards)	yes
no	NO	No	no	Yes	no	No	See below	no	yes	no (blanks insignificant)
			no		in-house developed method	PERFORCE, Powley method	In house	no		
					native PFCs quantitation (calibration) standards contain branched & linear isomers; all results are reported in terms of the anion	In the UPLC we have a PFC-Isolation kit (Waters) for background elimination.	A set of LCS were spiked with BHT at a level of 200 ug/g (0.02%) and without BHT to evaluate any interference that may arise from the preservative. No effect was shown by the presence of the BHT preservative. The study sample was extracted in triplicate. Reported results reflect the average concentration ± percent relative standard deviation (%RSD). Triplicate lab matrix spikes (LMSs) of the study sample were prepared at a low concentration (~1 ng/g) and a high concentration (~10 ng/g) for all analytes except PFOS. PFOS LMS concentrations were 75 ng/g and 150 ng/g. Average LMS recoveries were within 100±20% for all reported analytes. Recoveries of the mass-labelled recovery standards (surrogates) were within 100±13% for all sample and QC replicates. Sample results were quantitated using an extracted calibration curve prepared in purchased walleye fillet control tissue. Calibration standards ranged from 0.025 ng/g to 25 ng/g. Lower limits of quantitation generally ranged from 0.05-0.25 ng/g. If PFC target analytes were detected in the control matrix, method of standard additions was used to determine the endogenous concentration. Concentrations of the calibration standards and laboratory control QC were then corrected for the endogenous levels present before the study samples and QC were quantitated. IS and recovery standard (surrogates) were spiked at a nominal concentration of 1 ng/g. Final extracts were diluted 1:25 post-extraction for PFOS analysis - diluted extract spiked with additional IS to a final concentration of ~ 1 ng/g.		Unextracted calibration	

Fish method

L36	L41	L42
Fish sample	**Fish sample**	**Fish sample**
	Dilution D10	Homogenisation
Liquid/Solid extraction	Grinding	LSE
MeOH	MeOH	MeOH
Active Carbon; (Powley's method)	Filtration	Envicarb (Powley method0
	Phenomenex Hydro RP C18 150x2 mm*4µm Formate Buffer/ Water+MeOH	C18
MS/MS ES(-)		Agilent 1200 HPLC coupled to Agilent 6410 LC-MS/MS ESI-
8	No	13C4 PFBA, 13C2 PFHxA, 13C4 PFOA, 13C5 PFNA, 13C2 PFDcA, 13C2 PFUdA, 13C4 PFHxS, 13C4 PFOS, 18 O2 PFOSA
1	No	N.A.
Yes	No	Yes
Yes	No	Yes
Internal method	-	Internal

Fish method