Behavior Effects of Structurally Diverse Per- and Polyfluoroalkyl Substances (PFAS) in Zebrafish

Journal: Chemical Research in Toxicology

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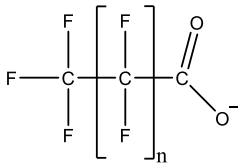
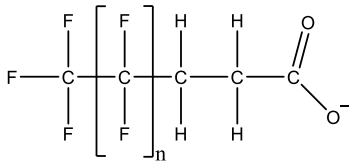
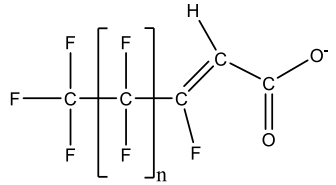
Contains:

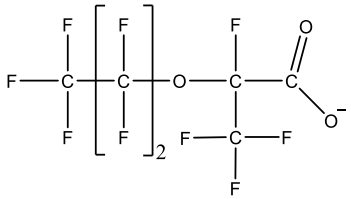
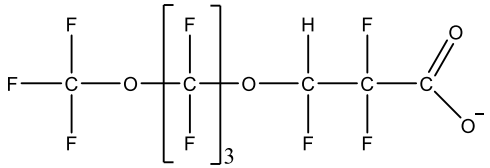
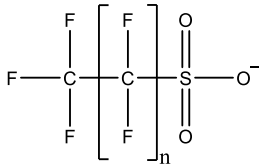
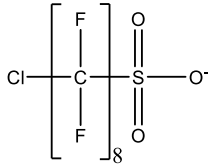
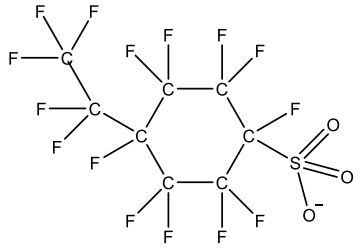
Table S1. Table of all individual PFASobtained from Wellington Laboratories as 50 µg/ml stock solutions in methanol and tested at a single concentration. CAS number is reported as obtained from Wellington Laboratories. M/C indicates multiple components.

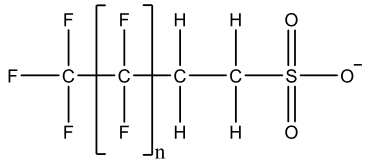
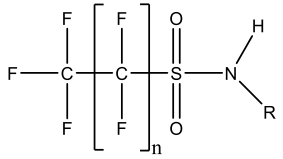
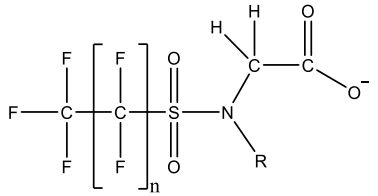
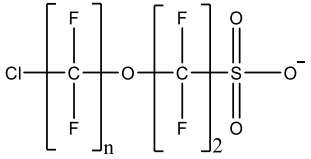
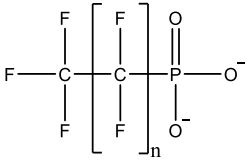
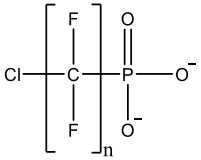
Structural Class	Number of Fluorinated Carbons	Abbreviated Name	Full Name	CAS	Molecular Weight (g/mole)
Carboxylic acids	4	PFBA	Perfluoro-n-butanoic acid	375-22-4	214.04
	5	PFPeA	Perfluoro-n-pentanoic acid	2706-90-3	264.05
	6	PFHxA	Perfluoro-n-hexanoic acid	307-24-4	314.05
	7	PFHpA	Perfluoro-n-heptanoic acid	375-85-9	364.06
	8	PFOA	Perfluoro-n-octanoic acid	335-67-1	414.07
	9	PFNA	Perfluoro-n-nonanoic acid	375-95-1	464.08
	10	PFDA	Perfluoro-n-decanoic acid	335-76-2	514.08
	11	PFUdA	Perfluoro-n-undecanoic acid	2058-94-8	564.09
	12	PFDoA	Perfluoro-n-dodecanoic acid	307-55-1	614.1
	13	PFTrDA	Perfluoro-n-tridecanoic acid	72629-94-8	664.11
	14	PFTeDA	Perfluoro-n-tetradecanoic acid	376-06-7	714.11
	15	PFHxDA	Perfluoro-n-hexadecanoic acid	67905-19-5	814.13
	17	PFODA	Perfluoro-n-octadecanoic acid	16517-11-6	914.14
Fluorotelomer carboxylic acids	3	FPrPA	3-Perfluoropropyl propanoic acid	356-02-5	242.09
	5	FPePA	3-Perfluoropentyl propanoic acid	914637-49-3	342.11
	7	FHpPA	3-Perfluoroheptyl propanoic acid	812-70-4	442.12
Unsaturated fluorotelomer carboxylic acids	6	FHUEA	2H-Perfluoro-2-octenoic acid	N/A	358.08
	8	FOUEA	2H-Perfluoro-2-decenoic acid	N/A	458.1
Ether/polyether carboxylic acids	3,2	HFPO-DA (GenX)	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	13252-13-6	330.05
	1,3,2	NaDONA	Sodium dodecafluoro-3H-4,8-dioxanonanoate	958445-44-8	400.05
Sulfonic acids	3	PFPrS	Sodium Perfluoro-1-propanesulfonate (linear)	N/A	272.07
	4	PFBS	Potassium perfluoro-1-butanesulfonate (linear)	29420-49-3	338.19
	5	PFPeS	Sodium Perfluoro-1-pentanesulfonate (linear)	630402-22-1	372.09
	6	PFHxS	Sodium perfluoro-1-hexanesulfonate (linear) with potassium salt branched isomers	M/C	438.2
	7	PFHpS	Sodium Perfluoro-1-heptanesulfonate (linear)	N/A	472.1
	8	PFOS	Potassium perfluoro-1-octanesulfonate (linear) with branched isomers	M/C	538.22
	8	8-Cl PFOS	Sodium 8-chloroperfluoro-1-octanesulfonate	N/A	538.57
	9	PFNS	Sodium perfluoro-a-nonanesulfonate (linear)	98789-57-2	572.12
	10	PFDS	Sodium perfluoro-1-decanesulfonate (linear)	N/A	622.13
	12	PFDOS	Sodium Perfluoro-1-dodecanesulfonate (linear)	1260224-54-1	722.14

Sulfonic acids (cyclic)	8	PFECHS	Potassium perfluoro-4-ethylcyclohexanesulfonate (isomeric mixture)	67584-42-3	500.22
Fluorotelomer sulfonic acids	4	4:2 FTS	Sodium 1H,1H,2H,2H-perfluorohexane sulfonate	27619-93-8	350.13
	6	6:2 FTS	Sodium 1H,1H,2H,2H-perfluorooctane sulfonate	27619-94-9	450.15
	8	8:2 FTS	Sodium 1H,1H,2H,2H-perfluorodecanesulfonate	N/A	550.16
	10	10:2 FTS	Sodium 1H,1H,2H,2H-perfluordodecanesulfonate	N/A	650.18
Sulfonamides	8	N-MeFOSA	N-methylperfluoro-1-octanesulfonamide	31506-32-8	513.17
	8	N-EtFOSA	N-ethylperfluoro-1-octanesulfonamide	4151-50-2	527.2
Sulfonamidoacetic acids	8	N-MeFOSAA	N-methylperfluoro-1-octanesulfonamidoacetic acid	2355-31-9	585.23
	8	N-EtFOSAA	N-ethylperfluoro-1-octanesulfonamidoacetic acid	2991-50-6	571.21
	8	FOSAA	Perfluoro-1-octanesulfonamidoacetic acid	2806-24-8	557.18
Chlorinated ether sulfonic acids	8,2	11-Cl PF3OUdS (F-53B)	Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	83329-89-9	670.69
	6,2	9-Cl PF3ONS (F-53B)	Potassium 9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	73606-19-6	570.67
Phosphonic acids	6	PFHxPA	Perfluorohexylphosphonic acid	40143-76-8	400.03
	6	Cl PFHxPA	6-Chloroperfluorohexylphosphonic acid	N/A	416.49
	8	PFOPA	Perfluorooctylphosphonic acid	40143-78-0	500.05
	8	Cl PFOPA	8-Chloroperfluorooctylphosphonic acid	N/A	516.5
	10	PFDPA	Perfluorodecylphosphonic acid	52299-26-0	600.06
Phosphate ethers (mono-/di-substituted)	6	6:2 PAP	Sodium 1H,1H,2H,2H-perfluorooctylphosphate	N/A	488.05
	8	8:2 PAP	Sodium 1H,1H,2H,2H-perfluorodecylphosphate	N/A	588.06
	8	SAMPAP	Sodium 2-(N-ethylperfluorooctane-1-sulfonamido)ethyl phosphate	N/A	695.19
	6,6	6:2 DiPAP	Sodium bis(1H,1H,2H,2H-perfluorooctyl)phosphate	N/A	812.15
	8,8	8:2 DiPAP	Sodium bis(1H,1H,2H,2H-perfluorodecyl)phosphate	N/A	1012.18
	8,8	DiSAM PAP	Sodium bis-[2-(N-ethylperfluorooctane-1-sulfonamido)ethyl] phosphate	N/A	1226.45
Phosphinic acids	6,6	6:6 PFPiA	Sodium bis(perfluorohexyl)phosphinate	70609-44-8	724.05
	8,8	8:8 PFPiA	Sodium bis(perfluorooctyl)phonsphinate	500776-69-2	924.08
Zwitterions	6	N-AP-FHxSA	N-(3-(dimethylaminopropan-1-yl)perfluoro-1-hexanesulfonamide	50598-28-2	484.28
	6	N-TAMP-FHxSA	N-[3-(perfluoro-1-hexanesulfonamido)propan-1-yl]-N,N,N-trimethylammonium	38850-51-0	498.3
	6	N-CMAMP 6:2-FOSA (6:2FTAB)	N-(carboxymethyl)N,N-dimethyl-N-[3-(1H,1H,2H,2H-perfluoro-1-octanesulfonamido)propan-1-yl]ammonium	34455-29-3	570.37

Table S2. Table of structural differences between PFAS classes. Structures of acid classes are in the deprotonated form to accurately represent predominant structures at environmentally relevant pH. Bolded portions of the abbreviated structures indicate the structural feature that deviates between classes. R = Methyl or Ethyl group. *PFECHS has both cis and trans isomers that are not depicted.

Structural Class	Compounds	Abbreviated Structure	Structure
Carboxylic acids (2)	PFBA PFPeA PFHxA PFHpA PFOA PFNA PFDA PFUdA PFDoA PFTrDA PFTeDA PFPFHxDA PFODA	$CF_3[CF_2]_n\mathbf{COO^-}$	
Fluorotelomer carboxylic acids (3)	FPrPA FPePA FHpPA	$CF_3[CF_2]_n\mathbf{CH_2CH_2COO^-}$	
Unsaturated fluorotelomer carboxylic acids (2)	FHUEA FOUEA	$CF_3[CF_2]_n\mathbf{CF{=}CHCOO^-}$	

Ether/polyether carboxylic acids (2)	HFPO-DA (GenX)	$CF_3[CF_2]_2\mathbf{OCF[CF3]COO^-}$	
	NaDONA	$\mathbf{CF_3O[CF_2]_3OCFHCF_2COO^-}$	
Sulfonic acids (10)	PFPrS PFBS PFPeS PFHxS PFHpS PFOS PFNS PFDS PFDOS	$CF_3[CF_2]_n\mathbf{SO_3^-}$	
	8-Cl PFOS	$\mathbf{Cl}[CF_2]_8\mathbf{SO_3^-}$	
Sulfonic acids (cyclic) (1)	PFECHS*	$\mathbf{[CF_2]_2CF[CF_2CF_3][CF_2]_2CF[SO_3^-]}$	

Fluorotelomer sulfonic acids (4)	4:2 FTS 6:2 FTS 8:2 FTS 10:2 FTS	$CF_3[CF_2]_n\mathbf{[CH_2]_2SO_3^-}$	
Sulfonamides (2)	Methyl FOSA Ethyl FOSA	$CF_3[CF_2]_n\mathbf{SO_2N[R]H}$	
Sulfonamidoacetic acids (3)	FOSAA Methyl FOSAA Ethyl FOSAA	$CF_3[CF_2]_n\mathbf{SO_2N[CH_2COO^-][R]}$	
Chlorinated ether sulfonic acids (2)	11Cl PF3OUDS (minor component F-53B) 9Cl PF3ONS (major component F-53B)	$\mathbf{Cl[CF_2]_nO[CF_2]_2SO_3^-}$	
Phosphonic acids (5)	PFHxPA PFOPA PFDPA	$CF_3[CF_2]_n\mathbf{PO3^-}$	
	Cl PFHxPA Cl PFOPA	$\mathbf{Cl}[CF_2]_n\mathbf{PO3^-}$	

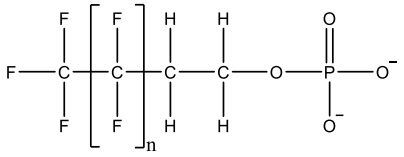
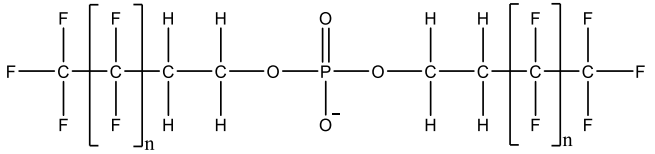
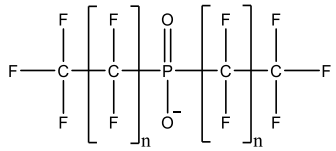
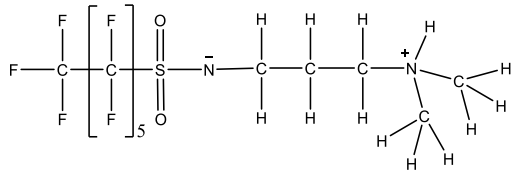
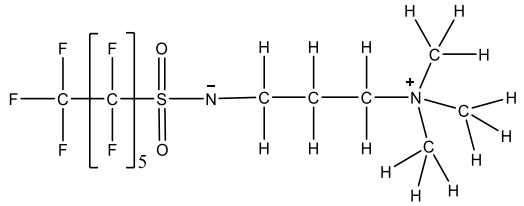
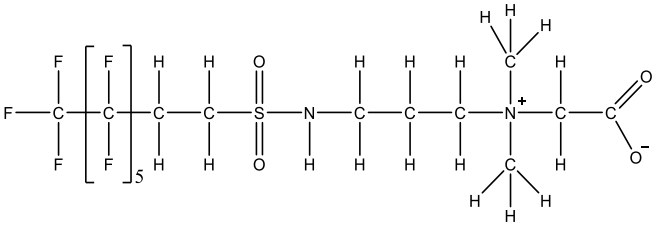
Phosphate esters (mono-/di-substituted) (6)	6:2 PAP 8:2 PAP SAMPAP	$CF_3[CF_2]_n\mathbf{CH_2CH_2OP[O][O^-]_2}$	
	6:2 DiPAP 8:2 DiPAP DiSAMPAP	$\mathbf{[CF_3[CF_2]_nCH_2CH_2O]_2PO_2^-}$	
Phosphinic acids (2)	6:6 PFPiA 8:8 PFPiA	$\mathbf{[CF_3[CF_2]_n]_2PO_2^-}$	
Zwitterions (3)	N-AP-FHxSA	$CF_3[CF_2]_5\mathbf{SO_2N^-[CH_2]_3N^+[CH_3]_2H}$	
	N-TAMP-FHxSA	$CF_3[CF_2]_5\mathbf{SO_2N^-[CH_2]_3N^+[CH_3]_3}$	
	N-CMAMP (6:2 FOSA)	$CF_3[CF_2]_5\mathbf{[CH_2]_2SO_2NH[CH_2]_3N^+[CH_3]_2CH_2CO_2^-}$	

Table S3. PFCA-MXA mixture constituents. This mixture was obtained as an analytical standards from Wellington Laboratories.

PFC-MXA Constituents	Concentration PFC-MXA (ug/mL)	CAS
PFBA	2	375-22-4
PFPeA	2	2706-90-3
PFHxA	2	307-24-4
PFHpA	2	375-85-9
PFOA	2	335-67-1
PFNA	2	375-95-1
PFDA	2	335-76-2
PFUdA	2	2058-94-8
PFDoA	2	307-55-1
PFTrDA	2	72629-94-8
PFTeDA	2	376-06-7

Table S4. Mixture B constituents. The 46 constituents in Mixture B were all obtained as analytical standards from Wellington Laboratories (CAS numbers are listed as reported by Wellington Laboratories; M/C indicates mixed components), except for MeFBSAA which was obtained from 3M. All constituents are at a concentration of 0.05 µg/mL in Mixture B.

Mixture B Constituents	CAS
PFC-MXA	M/C
PFHxDA	67905-19-5
PFODA	16517-11-6
FPrPA	356-02-5
FPePA	914637-49-3
FHpPA	812-70-4
FHUEA	N/A
FOUEA	N/A
HFPO-DA	13252-13-6
NaDONA	958445-44-8
PFPrS	N/A
PFBS	29420-49-3
PFPeS	630402-22-1
PFHxS	M/C
PFHpS	N/A
PFOS	M/C
8-Cl PFOS	N/A
PFNS	98789-57-2
PFDS	N/A
PFDOS	1260224-54-1
PFECHS	67584-42-3
4:2 FTS	27619-93-8
6:2 FTS	27619-94-9
8:2 FTS	N/A
10:2 FTS	N/A
N-MeFOSA	31506-32-8
N-EtFOSA	4151-50-2
N-MeFOSAA	2355-31-9
N-EtFOSAA	2991-50-6
MeFBSAA	159381-10-9
FOSAA	2806-24-8
11-Cl PF3OUdS	83329-89-9
9-Cl PF3ONS	73606-19-6
PFHxPA	40143-76-8
Cl PFHxPA	N/A
PFOPA	40143-78-0
Cl PFOPA	N/A
PFDPA	52299-26-0
6:2 PAP	N/A
8:2 PAP	N/A
SAMPAP	N/A
6:2 DiPAP	N/A
8:2 DiPAP	N/A
DiSAM PAP	N/A
6:6 PFPiA	70609-44-8
8:8 PFPiA	500776-69-2

Table S5. Table of manufacturer-reported impurities for PFAS obtained as analytical standards used for toxicological testing.

Structural Class	PFAS	Lot	Impurities noted in COA
Carboxylic acids	**PFBA**	PFBA1119	NA
	PFPeA	PFPeA0420	NA
	PFHxA	PFHxA1219	NA
	PFHpA	PFHpA1019	NA
	PFOA	PFOA0120	NA
	PFNA	PFNA1219	~0.2% PFOA, < 0.1% PFHpA, < 0.1% PFUdA
	PFDA	PFDA1119	~0.2% PFNA
	PFUdA	PFUdA1019	~0.1% PFDoA
	PFDoA	PFDoA0520	NA
	PFTrDA	PFTrDA0919	~0.1% PFUdA, ~0.4% PFDoA, ~0.1% PFTeDA
	PFTeDA	PFTeDA1119	~0.3% PFDoA, ~0.1% PFTrDA, ~0.1% PFHxDA
	PFHxDA	PFHxDA0319	NA
	PFODA	PFODA0419	~0.2% PFHxDA, ~0.1% PFHpDA
Fluorotelomer carboxylic acids	**FPrPA**	FPrPA0310	<1% unsaturated 3:3 telomer acid ($C_8H_3F_7O_2$)
	FPePA	FPePA0310	<1% unsaturated 5:3 telomer acid ($C_8H_3F_{11}O_2$)
	FHpPA	FHpPA1217	NA
Unsaturated fluorotelomer carboxylic acids	**FHUEA**	FHUEA0310	<0.1% FOUEA
	FOUEA	FOUEA1209	~0.15% FHUEA
Ether/polyether carboxylic acids	**HFPO-DA**	HFPODA0120	NA
	NaDONA	NaDONA0417	NA
Sulfonic acids	**PFPrS**	LPFPrS0416	NA
	PFBS	LPFBS1218	NA
	PFPeS	LPFPeS0718	NA
	PFHxS	brPFHxSK1018	~0.3% PFHxA, ~0.15% PFPeS
	PFHpS	LPFHpS0819	NA
	PFOS	br-PFOSK	NA
	8-Cl PFOS	8ClPFOS0814	NA
	PFNS	LPFNS1119	NA
	PFDS	LPFDS0419	~0.9% L-PFDoS
	PFDoS	LPFDoS1218	~0.2% PFDoA
Sulfonic acids (cyclic)	**PFECHS**	PFECHS0418	~1.5% other isomeric impurities
Fluorotelomer sulfonic acids	**4:2 FTS**	42FTS1111	NA
	6:2 FTS	62FTS0712	NA
	8:2 FTS	82FTS1111	NA
	10:2 FTS	102FTS0619	NA
Sulfonamides	**N-Methyl FOSA**	NMeFOSA0312M	NA
	N-Ethyl FOSA	NEtFOSA1112M	NA

Sulfonamidoacetic acids	**FOSAA**	FOSAA0418M	NA
	N-Methyl FOSAA	brNMeFOSAA0119	NA
	N-Ethyl FOSAA	brNEtFOSAA0718	~0.6% PFOA
Chlorinated ether sulfonic acids	**9-Cl PF3ONS**	9ClPF3ONS0518	NA
	11-Cl PF3OUdS	11ClPF3OUdS0718	NA
Phosphonic acids	**PFHxPA**	PFHxPA0418	NA
	Cl PFHxPA	ClPFHxPA0610	NA
	PFOPA	PFOPA0619	NA
	Cl PFOPA	ClPFOPA0114	NA
	PFDPA	PFDPA0413	~1% C10F21P(=O)(OH)CH2CH3
Phosphate ethers (mono-/di-substituted)	**6:2 PAP**	62PAP0418	NA
	8:2 PAP	82PAP0418	NA
	SAMPAP	SAmPAP0114	NA
	6:2 DiPAP	62diPAP0317	NA
	8:2 DiPAP	82diPAP0917	NA
	DiSAMPAP	diSAmPAP0317	NA
Phosphinic acids	**6:6 PFPiA**	66PFPi1210	NA
	8:8 PFPiA	88PFPi1210	NA
Zwitterions	**N-CMAMP**	NCMAmP62FOSA 0818	NA
	N-AP-FHxSA	NAPFHxSA0818	NA
	N-TAMP-FHxSA	NTAmPFHxSA081 8	NA
Mixtures	**PFC-MXA**	PFCMXA0718	NA
	Mixture B	NA	NA

Table S6. Table of PFCAs obtained as commercial reference standards.

Full Name	Abbreviated Name	CAS	Source	Reported Purity (%)
Perfluorobutanoic acid	PFBA	375-22-4	Sigma-Aldrich (Darmstadt, Germany)	98
Perfluoropentanoic acid	PFPeA	2706-90-3	Matrix Scientific (Columbia, South Carolina)	97
Perfluorohexanoic acid	PFHxA	307-24-4	Matrix Scientific (Columbia, South Carolina)	98
Perfluoroheptanoic acid	PFHpA	375-85-9	Sigma-Aldrich (Darmstadt, Germany)	99
Perfluorooctanoic acid	PFOA	335-67-1	Sigma-Aldrich (Darmstadt, Germany)	96
Perfluorononanoic acid	PFNA	375-95-1	Sigma-Aldrich (Darmstadt, Germany)	97
Perfluorodecanoic acid	PFDA	335-76-2	Sigma-Aldrich (Darmstadt, Germany)	98
Perfluoroundecanoic acid	PFUdA	2058-94-8	Matrix Scientific (Columbia, South Carolina)	96
Perfluorododecanoic acid	PFDoA	307-55-1	Sigma-Aldrich (Darmstadt, Germany)	95
Perfluorotridecanoic acid	PFTrDA	72629-94-8	Sigma-Aldrich (Darmstadt, Germany)	97

Table S7. PFAS acronyms with the transitions monitored, collision voltage, cone energy, and corresponding mass-labeled surrogate standard for analytical validation of stocks.

Analyte	Transitions Monitored	Collision Voltage (eV)	Cone Energy (eV)	Surrogate Standard for Quantification
PFBA	212.9->168.9	16	9	[$^{13}C_4$]PFBA
PFPeA	263 -> 219	20	8	[$^{13}C_3$]PFPeA
PFHxA	312.9->268.9 312.9 -> 118.9	20 20	33 8	[$^{13}C_2$]PFHxA
PFHpA	362.9->318.9 362.9 -> 168.9	20 20	14 8	[$^{13}C_4$]PFHpA
PFOA	412.9->368.9 412.9 -> 168.9	20 20	18 8	[$^{13}C_4$]PFOA
PFNA	462.9->418.9 462.9 -> 168.9	16 16	18 10	[$^{13}C_5$]PFNA
PFDA	513 -> 468.9 513 -> 269	22 22	18 10	[$^{13}C_2$]PFDA
PFUnDA	563 -> 518.9 563 -> 169	22 22	22 10	[$^{13}C_2$]PFUnDA
PFDoDA	613 -> 569 613 -> 169	22 22	24 10	[$^{13}C_2$]PFDoDA
PFTrDA	662.9->618.9 662.9 ->169	24 24	26 12	[$^{13}C_2$]PFDoDA
[$^{13}C_4$]PFBA	216.9 -> 171.9	16	10	N/A
[$^{13}C_3$]PFPeA	266 -> 222	20	8	N/A
[$^{13}C_2$]PFHxA	314.9 -> 269.9	20	8	N/A
[13C4]PFHpA	366.9 -> 321.9	15	11	N/A
[$^{13}C_4$]PFOA	416.9 -> 371.9	20	8	N/A
[$^{13}C_5$]PFNA	467.9 -> 422.9	22	8	N/A
[$^{13}C_2$]PFDA	515 –> 470	22	10	N/A
[$^{13}C_2$]PFUnDA	564.9 –>519.7	15	10	N/A
[$^{13}C_2$]PFDoDA	615 -> 570	22	10	N/A

Table S8. Purity of PFAS standard, nominal (target) concentration, and measured concentration of each PFAS stock solution, and the percent agreement between measured and nominal concentrations. The percent agreement between the measured PFAS concentrations was within 82-119% of the nominal (target) concentrations.

PFAS	Mass Purity %	Nominal concentration (mM)	Measured concentration (mM)	Recovery (%)
PFBA	98	30	26.7	89
PFPeA	97	30	24.6	82
PFHxA	98	30	24.9	83
PFHpA	99	30	22.2	74
PFOA	95	30	23.7	79
PFNA	97	30	25.5	85
PFDA	98	30	29.4	98
PFUdA	96	15	16.8	112
PFDoDA	95	15	17.8	119
PFTrDA	97	7.5	7.6	102

Table S9. Stock concentrations in ug/mL and uM for individual PFAS tested at single concentration.

Structural Class	Abbreviated Name	Molecular Weight (g/mole)	Stock Concentration (ug/mL)	Stock Concentration (uM)
Carboxylic acids	PFBA	214.04	50	234
	PFPeA	264.05	50	189
	PFHxA	314.05	50	159
	PFHpA	364.06	50	137
	PFOA	414.07	50	121
	PFNA	464.08	50	108
	PFDA	514.08	50	97
	PFUdA	564.09	50	89
	PFDoA	614.1	50	81
	PFTrDA	664.11	50	75
	PFTeDA	714.11	50	70
	PFHxDA	814.13	50	61
	PFODA	914.14	50	55
Fluorotelomer carboxylic acids	FPrPA	242.09	50	207
	FPePA	342.11	50	146
	FHpPA	442.12	50	113
Unsaturated fluorotelomer carboxylic acids	FHUEA	358.08	50	140
	FOUEA	458.1	50	109
Ether/polyether carboxylic acids	HFPO-DA (GenX)	330.05	50	151
	NaDONA	400.05	50	125
Sulfonic acids	PFPrS	272.07	50	184
	PFBS	338.19	50	148
	PFPeS	372.09	50	134
	PFHxS	438.2	50	114
	PFHpS	472.1	50	106
	PFOS	538.22	50	93
	8-ClPFOS	538.57	50	93
	PFNS	572.12	50	87
	PFDS	622.13	50	80
	PFDOS	722.14	50	69
Sulfonic acids (cyclic)	PFECHS	500.22	50	100
Fluorotelomer sulfonic acids	4:2 FTS	350.13	50	143
	6:2 FTS	450.15	50	111
	8:2 FTS	550.16	50	91
	10:2 FTS	650.18	50	77
Sulfonamides	N-MeFOSA	513.17	50	97
	N-EtFOSA	527.2	50	95

Sulfonamidoacetic acids	N-MeFOSAA	585.23	50	85
	N-EtFOSAA	571.21	50	88
	FOSAA	557.18	50	90
Chlorinated ether sulfonic acids	11-Cl PF3OUdS (F-53B)	670.69	50	75
	9-Cl PF3ONS (F-53B)	570.67	50	88
Phosphonic acids	PFHxPA	400.03	50	125
	Cl PFHxPA	416.49	50	120
	PFOPA	500.05	50	100
	Cl PFOPA	516.5	50	97
	PFDPA	600.06	50	83
Phosphate ethers (mono-/di-substituted)	6:2 PAP	488.05	50	102
	8:2 PAP	588.06	50	85
	SAMPAP	695.19	50	72
	6:2 DiPAP	812.15	50	62
	8:2 DiPAP	1012.18	50	49
	DiSAM PAP	1226.45	50	41
Phosphinic acids	6:6 PFPiA	724.05	50	69
	8:8 PFPiA	924.08	50	54
Zwitterions	N-AP-FHxSA	484.28	50	103
	N-TAMP-FHxSA	498.3	50	100
	N-CMAMP 6:2 FOSA	570.37	50	88

Table S10. PFCA CRS exposure concentrations adjusted for chemical purity. Gray cells indicate that this concentration was not tested for the specified PFAS.

PFCA	Molar Mass (g/mole)	Purity (%)	Nominal Stock Concentration (mM)	Exposure concentrations (µM) for each nominal concentration adjusted for purity								
				1	2.54	6.45	16.4	25	35	55	74.8	100
PFBA	214.04	98	30	0.98	2.49	6.32	16.07		34.30		73.30	98.00
PFPeA	264.05	97	30	0.97	2.46	6.26	15.91		33.95		72.56	97.00
PFHxA	314.06	98	30	0.98	2.49	6.32	16.07		34.30		73.30	98.00
PFHpA	364.06	99	30	0.99	2.51	6.39	16.24		34.65		74.05	99.00
PFOA	414.07	95	30	0.95	2.41	6.13	15.58		33.25		71.06	95.00
PFNA	464.08	97	30	0.97	2.46	6.26	15.91		33.95		72.56	97.00
PFDA	514.08	98	30	0.98	2.49	6.32	16.07		34.30		73.30	98.00
PFUdA	564.10	96	15	0.96	2.44	6.19	15.74		33.60	52.80	71.81	96.00
PFDoDA	614.10	95	15	0.95	2.41	6.13	15.58		33.25	52.25	71.06	95.00
PFTrDA	664.11	97	7.5	0.97	2.46	6.26	15.91	24.25	33.95	53.35	72.56	97.00

Figure S1. Results of methanol developmental toxicity testing: A. Embryonic Photomotor Response assay, B. Larval Photomotor Response assay, C. Startle Response assay. Results of control fish treated with 0% methanol and treated fish exposed to 0.5, 0.75, and 1.0% methanol are represented by black, red, purple, and blue lines, respectively.

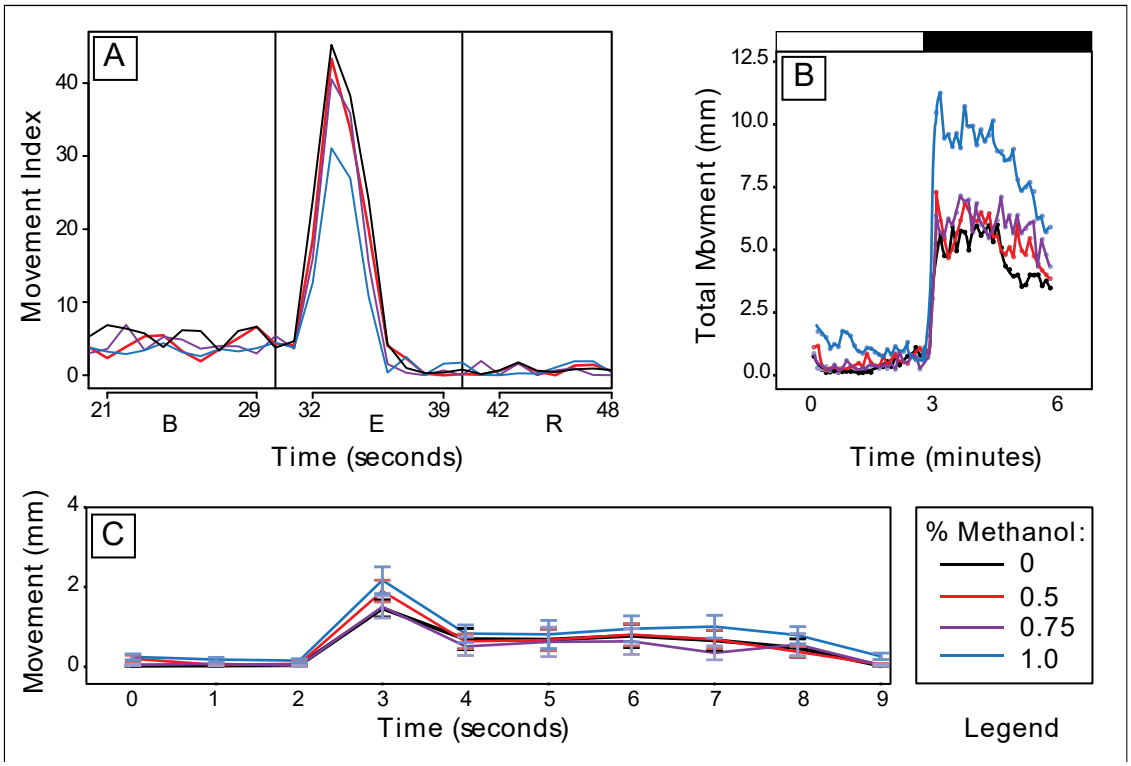


Figure S2. Results of isopropanol developmental toxicity testing: A. Embryonic Photomotor Response assay, B. Larval Photomotor Response assay, C. Startle Response assay. Results of control fish treated with 0% methanol and treated fish exposed to 0.5, 0.75, and 1.0% methanol are represented by black, red, purple, and blue lines, respectively. Treatment groups with high rates of mortality or abnormal morphology were not included in the behavior assays and are therefore not shown in the respective panel.

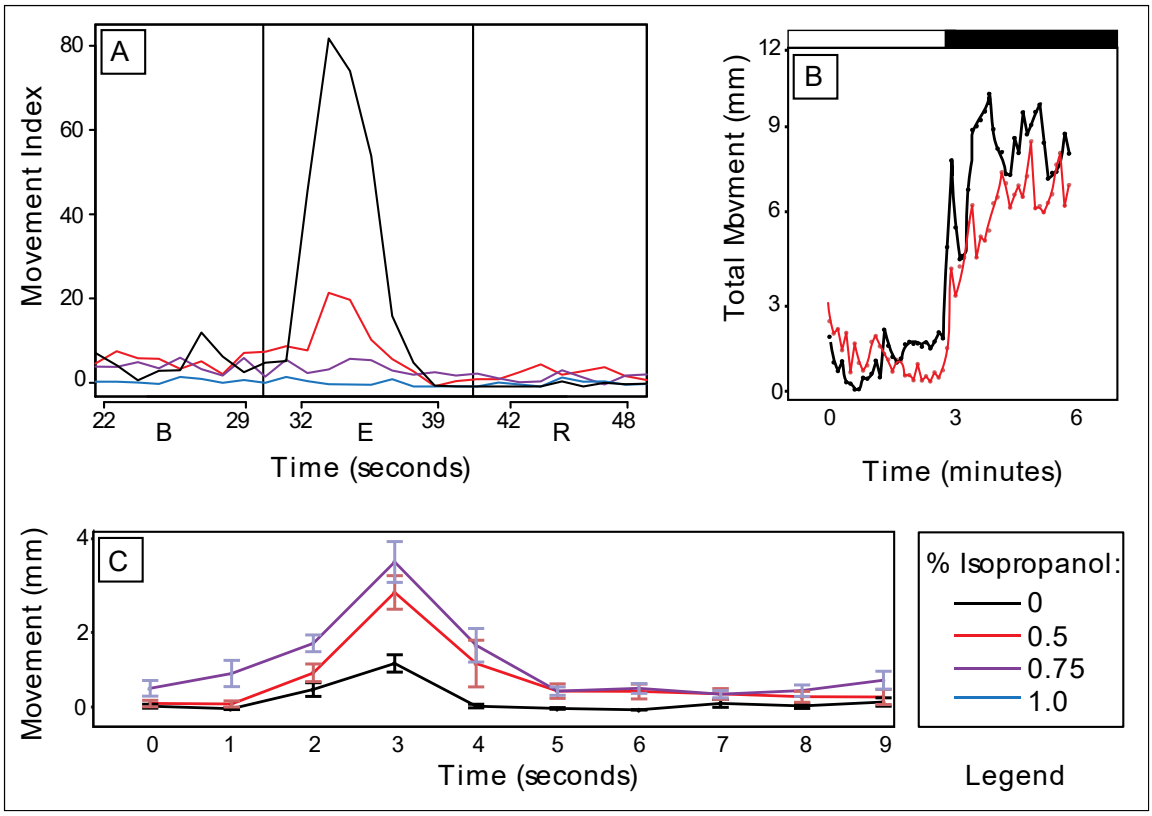
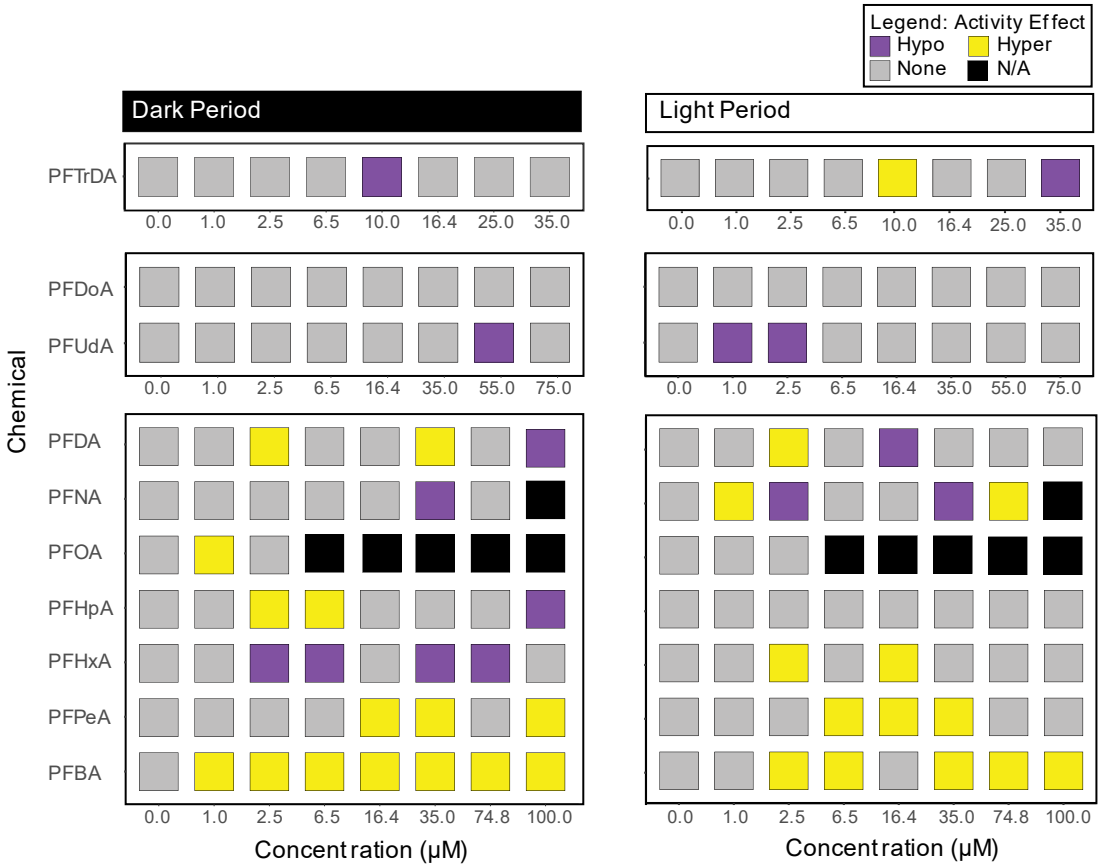


Figure S3. Mortality and morphology assessment results for PFCA CRS. Gray cells indicate no effect. Red cells indicate a morphological effect and contain the lowest concentration (µM) at which the effect was observed. Full descriptions of the mortality and morphology endpoints are available in the Raw Data Guide supporting material.

PFCAs	Mortality and Morphology Endpoints													
	24 hpf			120 hpf										Combined
	MO24	DP24	SM24	MORT	CRAN	AXIS	EDEM	MUSC	LTRK	BRN	SKIN	NC	TCHR	Any Effect
PFBA														
PFPeA														
PFHxA														
PFHpA														
PFOA	100			35										16.4
PFNA					100		100							74.8
PFDA														
PFUdA														
PFDoA														
PFTrDa														

Figure S4. LPR assay results for PFCA CRS. The concentration is the nominal concentration, not taking into account the purity of the CRS.



Section S1. Stock solution preparation.

PFCAs including C4, C7, C8, C9, C10, C12, and C13 were purchased from Sigma-Aldrich (Darmstadt, Germany), while C5, C6, and C11 were purchased from Matrix Scientific (Columbia, South Carolina). All stock solutions, except PFBA and PFPeA, were made by dissolving 0.1 - 0.4 g solid into 25 ml HPLC grade methanol and then transferred to 30 ml polypropylene bottles. For the PFBA and PFPeA, because of their liquid status, they were measured by transferring 60 – 80 ml to 30 ml PPE bottles containing 25 ml methanol. For analysis, one aliquot of stock solution was further diluted to reach the middle of the calibration curve (2000-7000 ng/L) and then spiked with 0.75 ng of mass-labelled standards.

Section S2. Quantification and quality control.

Analytical-grade native and mass-labelled standards were purchased from Wellington labs (Guelph,Canada; Table 1). The calibration curve ranged from 20 – 10,000 ng/L and each standard was required to be within 70%- 130%. The LOQ was 20 ng/L. Each PFAS had a matched mass-labeled surrogate standard, except PFTrDA, which used the surrogate of PFDoDA. The lowest calibration standard and solvent blank were spiked with surrogate mass-labeled standard and were analyzed every ten samples and the end of the total run.

Section S3. LC-MS/MS.

An Agilent 1100 series HPLC (Santa Clara, CA) and Waters Acquity TQD (Milford, MA) were employed for separation and quantification of PFAS in stock solutions. A C18 delay column 4.6x 50mm x 5μm Zorbax Eclipse was fitted between the LC pump and the autosampler where locates the stock solution dilution samples and 900 ml injection were utilized for all samples. Chromatographic separation was achieved by an Eclipse C18 analytical column (4.6x 75mm x 3.5μm). Mobile phase A consisted of HPLC-grade methanol and deionized water (5:95) and mobile phase B contained deionized water:acetonitrile: methanol (5:20:75), both mobile phase A and B were comprised of 5 mM ammonium acetate and N-methylpiperidine. The initial flow rate was 0.6 ml/min and mobile phase B was kept at 1% for 2 min and then increased to 60%, 85% and 99% at 3, 8.5 and 9 min, respectively, followed by a hold at 99% mobile phase B until 12.7 min.