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### **Supplemental Material**

#### **Chemical Characterization of a Legacy Aqueous Film-Forming Foam Sample and Developmental Toxicity in Zebrafish (*Danio rerio*)**

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**Figure S1.** Swim bladder inflation in 120 hours post fertilization (hpf) larvae following developmental exposure to aqueous film-forming foam (AFFF). N = 6 vials, each containing 6-10 larvae. Average percent inflation determined for each vial.

**Figure S2.** Representative images of 96 hours post fertilization (hpf) larvae exposed to 0 - 40.91 mg/L perfluorooctanesulfonic acid: perfluorohexanesulfonic acid PFOS:PFHxS mixture, 0 - 22.5 mg/L PFHxS and 0-35.28 mg/L PFOS.

**Figure S3.** . Representative images of 96 hours post fertilization (hpf) larvae exposed to 0 - 4 mg/L sodium dodecyl sulfate and 0 - 0.5 mg/L sodium tetradecyl sulfate.

Table S1. Unidentified fluorinated compounds detected in the aqueous film-forming foam (AFFF) sample by Orbitrap-HRMS with relative intensity percent values greater or equal to 0.1% of the total perfluorooctanesulfonic acid (PFOS) area.

	Name	Formula	Molecular Weight	RT (min)	Area	Relative intensity (%) based on the PFOS area	Relative intensity (%) based on the area sum
1		C6 H F13 O3 S	399.9435	6.48	12874456	14.6	8.1
2		C13 H20 F5 N9 O4 P2 S2	587.0473	6.78	5254931	6.0	3.3
3		C5 H F11 O3 S	349.9468	6.17	2378510	2.7	1.5
4		C17 H37 F P2 S	354.2076	8.21	2350105	2.7	1.5
5		C16 H32 F2 N4 O6	414.2288	7.28	2111143	2.4	1.3
6		C12 H16 F10 N8 O5 S2	606.0528	6.03	2048981	2.3	1.3
7		C7 H F15 O3 S	449.9406	6.83	1961854	2.2	1.2
8	Similar to: 3-(4-Methoxyphenyl)-N-(1-naphthyl)-3-(propylamino)-2-propenethioamide	C14 H29 F2 N2 O P S	342.1713	6.43	1887928	2.1	1.2
9		C6 H F13 O3 S	399.9435	6.42	1802043	2.0	1.1
10		C14 H24 F9 N5 O8 P2 S2	687.0400	7.55	1771504	2.0	1.1
11	Similar to: 4-Dodecylbenzenesulfonic acid	C14 H29 F O4 S	312.1762	7.63	1727805	2.0	1.1
12		C12 H14 F4 N10 O P2 S	484.0488	6.27	1648276	1.9	1.0
13		C3 H4 F O10 P	249.9534	5.26	1533768	1.7	1.0
14		C16 H26 F9 N6 O8 P S3	728.0567	5.89	1443969	1.6	0.9
15		C4 H F9 O3 S	299.9503	5.69	1343110	1.5	0.8
16		C15 H10 F14 N6 O	556.0695	5.97	1247397	1.4	0.8
17		C15 H16 F3 N9 O10 S2	603.0417	6.22	1123615	1.3	0.7
18		C17 H36 F N2 O7 P	430.2244	6.58	987977	1.1	0.6
19		C20 H47 F2 N8 P3	530.3120	8.90	904015	1.0	0.6
20		C12 H21 F9 N2 O3 P2 S	506.0599	5.49	873468	1.0	0.6

	Name	Formula	Molecular Weight	RT (min)	Area	Relative intensity (%) based on the PFOS area	Relative intensity (%) based on the area sum
21		C21 H34 F3 N2 O2 P S2	498.1750	6.55	868974	1.0	0.5
22		C16 H28 F10 N2 O2 P2 S3	628.0631	5.36	805730	0.9	0.5
23		C12 H19 F9 N7 O13 P S	703.0356	6.79	779274	0.9	0.5
24		C13 H10 F10 N6 O	456.0766	5.45	771289	0.9	0.5
25		C20 H40 F5 N10 P	546.3077	7.51	697598	0.8	0.4
26		C22 H51 F2 N8 O P3	574.3391	8.98	649004	0.7	0.4
27		C9 H22 F N2 O2 P3 S	334.0585	5.30	638464	0.7	0.4
28		C15 H28 F2 O5	326.1916	8.16	615004	0.7	0.4
29	4-Bromo-2-fluorobiphenyl	C12 H8 Br F	249.9803	0.03	548003	0.6	0.3
30		C18 H45 F N10 O2 P2	514.3177	6.63	508834	0.6	0.3
31		C11 H16 F3 N10 O P S2	456.0625	5.12	498489	0.6	0.3
32		C12 H23 F6 N10 P3 S2	578.0669	5.06	495020	0.6	0.3
33		C18 H43 F2 N8 O P3	518.2761	6.67	492092	0.6	0.3
34		C8 H F15 O2	413.9738	6.38	448748	0.5	0.3
35		C22 H44 F5 N10 O P	590.3341	7.54	443525	0.5	0.3
36		C24 H55 F2 N8 O2 P3	618.3653	9.03	427560	0.5	0.3
37		C9 H F19 O3 S	549.9357	7.46	426706	0.5	0.3
38		C7 H8 F9 N10 P	434.0520	5.99	418571	0.5	0.3
39		C6 H7 F5 N6 O16 P2 S	607.9035	8.00	414410	0.5	0.3
40		C15 H22 F14 N6 P2 S2	678.0600	5.61	413429	0.5	0.3
41	Similar to: Perfluorodecanoic acid (PFDA)	C2 H7 F3 N4 O4 P2	269.9904	5.67	411938	0.5	0.3
42		C8 H19 F6 N6 P3	406.0799	5.15	396114	0.4	0.2
43		C7 H F15 O3 S	449.9406	6.75	393516	0.4	0.2

	Name	Formula	Molecular Weight	RT (min)	Area	Relative intensity (%) based on the PFOS area	Relative intensity (%) based on the area sum
44		C17 H24 F16 N6 P2 S	710.1001	6.10	389075	0.4	0.2
45		C8 H6 Cl F11 N2 O3 P2 S	515.9087	7.16	379704	0.4	0.2
46	Similar to: Perfluorooctanoic acid (PFOA)	C5 H3 F7 N8 P2	369.9841	6.38	362842	0.4	0.2
47		C14 H25 F8 N10 P3 S	610.1071	5.58	362577	0.4	0.2
48		C22 H51 F2 N8 P3	558.3440	6.90	355867	0.4	0.2
49		C11 H13 F13 N2 O2 S	484.0488	5.98	350772	0.4	0.2
50		C20 H40 F5 N10 O P	562.3028	6.70	338722	0.4	0.2
51		C7 H13 F5 N8 O S2	384.0556	5.68	321652	0.4	0.2
52		C24 H57 F N10 O6 P2	662.3916	9.07	289465	0.3	0.2
53		C24 H48 F5 N10 P	602.3710	7.03	289136	0.3	0.2
54		C9 H17 F13 N10 O S	560.1099	5.28	288418	0.3	0.2
55		C6 H18 F3 N8 P3 S	384.0556	5.45	266850	0.3	0.2
56		C7 H6 F3 N2 O16 P	461.9411	6.92	263218	0.3	0.2
57		C23 H51 F7 N8 P2	634.3602	7.56	255918	0.3	0.2
58		C6 H2 F13 N O2 S	398.9603	6.68	237690	0.3	0.1
59		C16 H41 F N10 O P2	470.2917	6.23	229796	0.3	0.1
60		C44 H88 F O3 P S	746.6177	9.93	227319	0.3	0.1
61		C10 H17 F10 N3 O5 P2	511.0488	6.93	213307	0.2	0.1
62		C42 H80 F4 N6 O	760.6331	9.93	207806	0.2	0.1
63	1H-Perfluorohexane	C6 H F13	319.9874	6.06	201751	0.2	0.1
64		C5 H5 F11 N2 O5 P2	443.9504	6.76	194308	0.2	0.1

	Name	Formula	Molecular Weight	RT (min)	Area	Relative intensity (%) based on the PFOS area	Relative intensity (%) based on the area sum
65		C21 H47 F7 N8 P2	606.3290	6.72	192686	0.2	0.1
66		C17 H24 F16 N6 P2 S	710.1001	6.01	191339	0.2	0.1
67		C6 H7 F9 N6	334.0585	5.12	189199	0.2	0.1
68		C6 H7 F5 N6 O16 P2 S	607.9035	7.78	183897	0.2	0.1
69		C12 H20 F7 N10 O6 P S	596.0918	5.58	176314	0.2	0.1
70		C5 H13 F4 N4 O7 P	348.0454	5.50	164132	0.2	0.1
71		C4 H6 F6 N4 O12 P2	477.9361	6.95	155919	0.2	0.1
72	Similar to: 4-tert-Butylcalix[4]arene tetraacetic acid	C26 H61 F N10 O7 P2	706.4174	9.11	153157	0.2	0.1
73	1,1,2,2-Tetrafluoro-2-hydroxyethanesulfonyl fluoride	C2 H F5 O3 S	199.9567	3.61	153155	0.2	0.1
74		C44 H83 F7 O	760.6331	7.47	148283	0.2	0.1
75		C32 H71 F4 N8 O4 P	738.5261	5.01	145664	0.2	0.1
76		C24 H51 F8 N10 O P	678.3864	7.59	141418	0.2	0.1
77		C13 H14 F11 N10 O2 P	582.0862	6.52	138658	0.2	0.1
78		C2 H3 F2 N4 O2 P	183.9958	5.17	135267	0.2	0.1
79		C13 H29 F6 N8 O10 P3 S	696.0846	6.10	129967	0.1	0.1
80	Similar to: Perfluorooctanoic acid (PFOA)	C5 H3 F7 N8 P2	369.9841	6.28	123226	0.1	0.1
81		C8 H19 F N P S	211.0958	6.03	120863	0.1	0.1
82	3,3,3-Trifluoropropanoic acid	C3 H3 F3 O2	128.0083	0.85	120383	0.1	0.1
83		C3 H3 F10 N4 O3 P	363.9771	6.06	117503	0.1	0.1
84		C7 H3 F12 N4 O3 P S	481.9476	6.98	116061	0.1	0.1

	Name	Formula	Molecular Weight	RT (min)	Area	Relative intensity (%) based on the PFOS area	Relative intensity (%) based on the area sum
85		C13 H27 F O4 S	298.1605	7.30	115591	0.1	0.1
86	1,1,1,2,2,3,3,4,4-Nonafluorobutane	C4 H F9	219.9935	5.30	113822	0.1	0.1
87		C15 H34 F6 N10 O3	516.2720	4.80	113647	0.1	0.1
88		C12 H18 F11 N8 P S	546.0943	5.28	113461	0.1	0.1
89		C5 H F9 O2	263.9834	5.30	113445	0.1	0.1
90		C14 H34 F5 N8 O2 P	472.2457	4.85	112589	0.1	0.1
91		C22 H47 F8 N10 O P	650.3549	6.73	111345	0.1	0.1
92		C10 H23 F8 N6 O P3	488.1032	5.54	111162	0.1	0.1
93		C5 H12 F5 N5 O7 P2 S	442.9864	6.76	110960	0.1	0.1
94		C9 H25 F N10 O S	340.1922	7.58	110013	0.1	0.1
95		C6 H F11 O2	313.9800	5.66	109067	0.1	0.1
96		C6 H8 F12 N9 O2 P	497.0346	6.94	108137	0.1	0.1
97		C29 H57 F11 N7 O3 P3 S	885.3278	6.01	107114	0.1	0.1
98	Similar to: N-(3-(Dimethylamino) propyl) tridecafluorohexanesulphonamide	C8 H17 F9 N8 O6 S2	556.0565	5.78	103670	0.1	0.1
99		C16 H33 F O4 S	340.2083	8.93	100956	0.1	0.1
100		C10 H22 F N2 O2 P	252.1397	6.81	100429	0.1	0.1

RT = retention time

Table S2. Summary of percentages of fragmented and normal pancreatic beta cell islets observed in 96 hours post fertilization (hpf) *Tg(ins:GFP)* larvae exposed to aqueous film-forming foam (AFFF).

	<b>0% AFFF</b>		<b>2.20e-4% AFFF</b>		<b>4.40e-4% AFFF</b>	
	Normal	Fragmented	Normal	Fragmented	Normal	Fragmented
Vial 1	83% (5/6)	17% (1/6)	57% (4/7)	43% (3/7)	50% (3/6)	50% (3/6)
Vial 2	67% (4/6)	33% (2/6)	40% (4/10)	60% (6/10)	50% (4/8)	50% (4/8)
Vial 3	100% (6/6)	0% (6/6)	100% (6/6)	0% (0/6)	83% (5/6)	17% (1/6)
Vial 4	88% (7/8)	13% (1/8)	83% (5/6)	17% (1/6)	86% (6/7)	14% (1/7)
Vial 5	100% (9/9)	0% (0/9)	83% (5/6)	17% (5/6)	67% (4/6)	33% (2/6)
Vial 6	80% (8/10)	20% (2/10)	71% (5/7)	29% (2/7)	50% (3/6)	50% (3/6)
MEAN	86%	14%	73%	27%	64%	36%

Each vial represents an experimental replicate containing 6-10 larvae. Parentheses contain number of either normal or fragmented islets out of the total number of GFP-positive larvae within each vial. Adult populations contained heterozygous and homozygous individuals, so some GFP-negative embryos were observed.

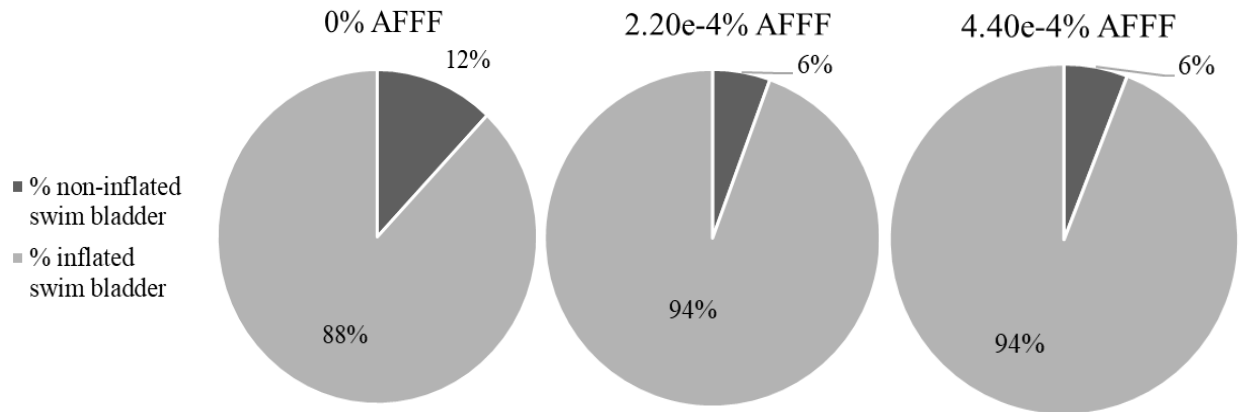


Figure S1. Swim bladder inflation in 120 hours post fertilization (hpf) larvae following developmental exposure to aqueous film-forming foam (AFFF). N = 6 vials, each containing 6-10 larvae. Average percent inflation determined for each vial.



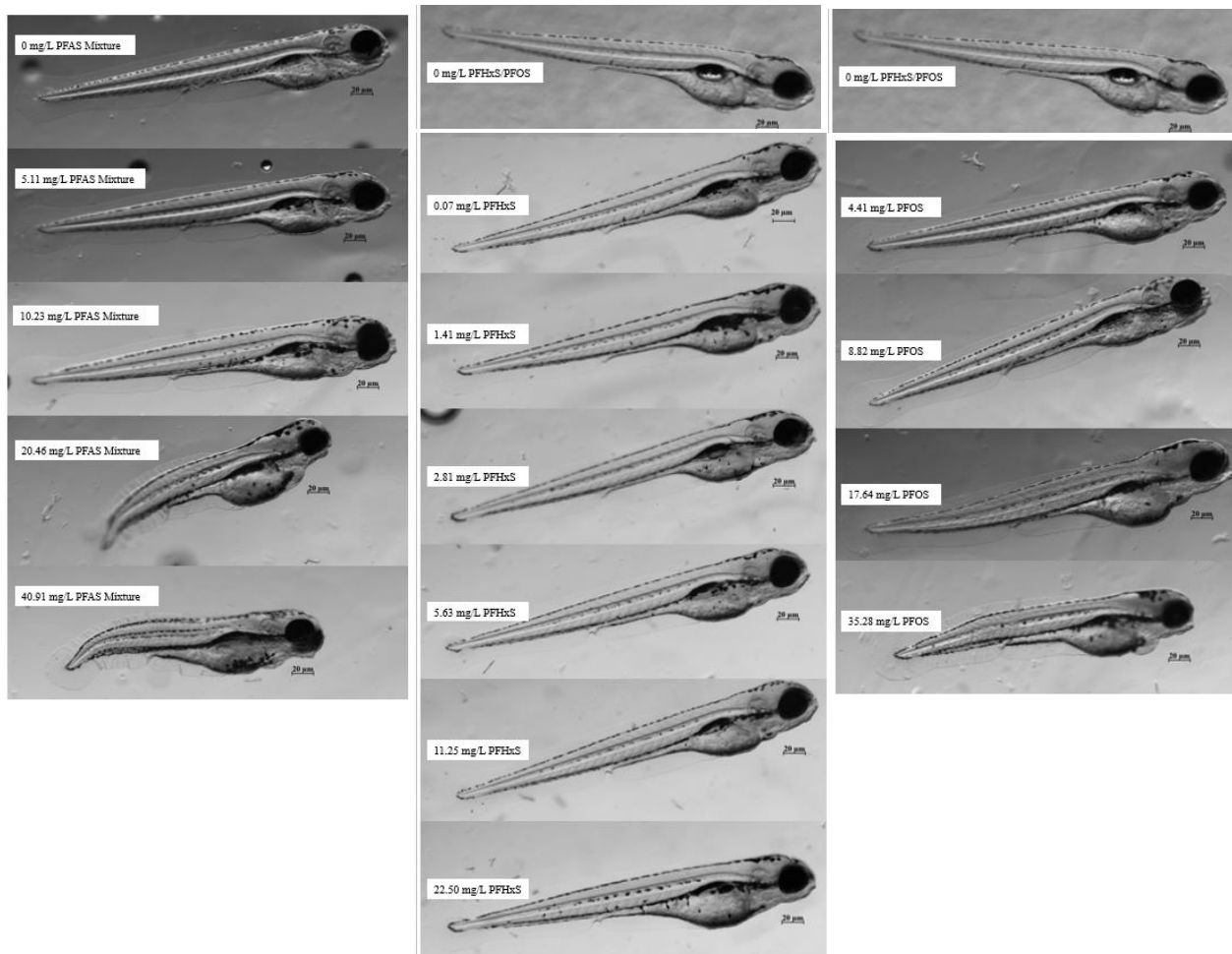


Figure S2. Representative images of 96 hours post fertilization (hpf) larvae exposed to 0 - 40.91 mg/L perfluorooctanesulfonic acid: perfluorohexanesulfonic acid PFOS:PFHxS mixture, 0 - 22.5 mg/L PFHxS and 0-35.28 mg/L PFOS.

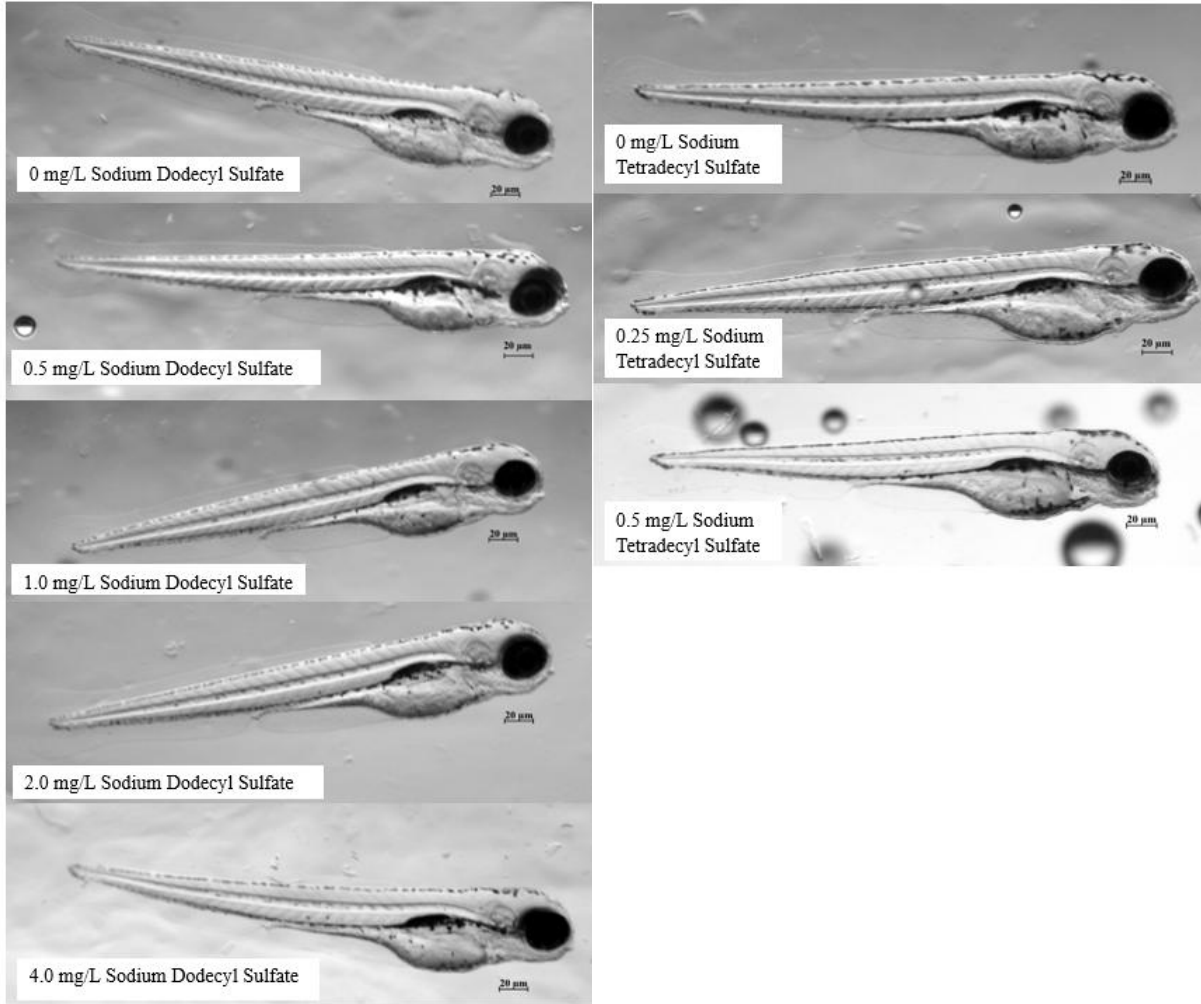


Figure S3. Representative images of 96 hours post fertilization (hpf) larvae exposed to 0 - 4 mg/L sodium dodecyl sulfate and 0 - 0.5 mg/L sodium tetradecyl sulfate.