Supplementary Information

Assessment of Per- and Polyfluoroalkyl Substance Exposure Risk in Drinking Water from Kamrup Region of Assam, India: Detection of PFBS and its Potential Impact on the Metabolome of Human Kidney Cells

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Table S1 *PFAS MRM Transitions (ESI-)*

PFAS	Parent Ion	Daughter Ion	Collision Energy (eV)
PFBS	298.94	80.00*	40
	298.94	99.00	36
PFHxA	312.97	269.00*	8
	312.97	119.00	20
PFHpA	362.97	319.00*	8
	362.97	169.00	16
PFHxS	398.93	80.00*	48
	398.93	99.00	40
PFOA	412.96	370.00*	8
	412.96	169.00	16
PFNA	462.96	419.00*	8
	462.96	219.00	16
PFOS	498.93	80.00*	56
	498.93	99.00	48
PFDA	512.96	470.00*	8
	512.96	269.00	20
PFDS	598.92	80.00*	60
	598.92	99.00	52
PFuDA	562.95	519.00*	8
	562.95	269.00	20
PFOSA	497.94	80.00*	80
	497.94	99.00	48
PFDoA	612.95	568.90*	8
	612.95	169.00	28
$^{13}C_8$ PFOS	506.99	80.00*	56
	506.99	99.00	48

Table S2 *Representing Limit of detection(LOD), Limit of quantitation(LOQ) and linearity of PFAS*

Compound	R^2	Linear range ($ngmL^{-1}$)	LOD ($ngmL^{-1}$)	LOQ ($ngmL^{-1}$)
PFBS	0.99	0.1~100	0.1	0.5
PFHxA	0.99	0.5~200	0.1	0.5
PFHpA	0.99	5.0~200	0.1	5.0
PFHxS	0.99	1.0~200	0.1	1.0
PFOA	0.99	1.0~200	0.1	1.0
PFNA	0.99	0.5~200	0.1	0.5
PFOS	0.99	0.1~200	0.1	0.5
PFDA	0.99	1.0~200	0.1	1.0
PFDS	0.99	0.5~200	0.1	0.5
PFuDA	0.99	0.5~200	0.1	0.5
PFOSA	0.99	0.5~200	0.1	0.5
PFDoA	0.99	1.0~200	0.1	1.0

Table S3 *Accuracy and precision values for the developed LC-MS/MS Method (n = 3, Mean ± SD)*

Analyte	QC Samples	Nominal Conc. ($ngmL^{-1}$)	Intra-day			Inter-day		
			Calculated Conc. ($ngmL^{-1}$)	Accuracy (%)	Precision (%RSD)	Calculated Conc. ($ngmL^{-1}$)	Accuracy (%)	Precision (%RSD)
PFBS	**LQC**	0.10	0.10	100.47±0.00	2.00	0.10	98.99±0.00	1.94
	MQC	5.00	5.05	101.15±0.10	1.99	5.17	101.29±0.10	1.84
	HQC	50.00	51.24	102.48±1.03	2.00	50.98	102.35±0.92	1.79
PFHxA	**LQC**	1.00	0.88	88.35±0.02	1.96	0.88	88.32±0.02	1.90
	MQC	25.00	27.17	108.71±0.51	1.89	26.74	108.84±0.50	1.85
	HQC	100.00	102.56	102.56±2.01	1.96	104.58	102.6±1.96	1.91
PFHpA	**LQC**	10.00	11.01	110.13±0.19	1.71	10.85	110.29±0.22	1.96
	MQC	25.00	26.09	104.39±0.47	1.79	25.59	104.66±0.50	1.91
	HQC	100.00	104.23	104.23±1.90	1.83	106.41	104.31±1.82	1.74
PFHxS	**LQC**	5.00	5.33	106.69±0.07	1.31	5.36	108.69±0.11	1.95
	MQC	25.00	26.16	104.66±0.52	1.99	26.64	104.52±0.52	1.98
	HQC	100.00	99.80	99.81±1.08	1.08	100.28	100.14±0.51	0.51
PFOA	**LQC**	5.00	5.05	100.94±0.06	1.23	5.09	102.61±0.08	1.62
	MQC	10.00	12.00	120.02±0.23	1.90	12.24	120.34±0.23	1.92
	HQC	100.00	100.53	100.53±0.27	0.27	100.23	101.19±1.38	1.36
PFNA	**LQC**	1.00	1.02	101.97±0.02	1.76	1.03	102.71±0.01	0.67
	MQC	50.00	51.50	103.00±0.87	1.69	52.50	103.67±0.77	1.49
	HQC	200.00	202.67	101.33±2.40	1.18	202.99	100.67±1.49	0.74
PFOS	**LQC**	1.00	0.91	90.79±0.02	1.93	0.89	90.46±0.01	1.35
	MQC	50.00	48.56	97.11±0.88	1.82	47.54	97.25±0.96	1.97
	HQC	200.00	199.26	99.63±3.76	1.89	200.70	100.30±1.55	0.77
PFDA	**LQC**	5.00	5.09	101.86±0.10	1.95	5.19	101.39±0.11	2.08
	MQC	50.00	52.35	104.71±0.85	1.63	51.46	104.98±1.04	2.00
	HQC	100.00	101.37	101.37±1.93	1.90	100.45	100.04±0.43	0.43
PFDS	**LQC**	5.00	4.99	99.89±0.10	2.02	5.09	100.56±0.06	1.11
	MQC	50.00	50.89	101.78±0.74	1.45	50.18	101.51±0.54	1.06
	HQC	200.00	199.10	99.55±3.46	1.74	197.08	99.05±1.73	0.87
PFuDA	**LQC**	1.00	1.03	103.38±0.02	1.78	1.05	104.38±0.01	1.10
	MQC	50.00	53.42	106.83±1.01	1.89	52.90	104.55±0.97	1.86
	HQC	100.00	98.90	98.90±1.41	1.42	97.99	98.57±0.83	0.84
PFOSA	**LQC**	1.00	1.08	107.81±0.02	1.39	1.06	108.58±0.02	1.85
	MQC	25.00	25.97	103.88±0.19	0.75	25.75	104.76±0.53	2.01
	HQC	100.00	103.09	103.09±1.85	1.79	101.92	102.09±0.15	0.15
PFDoA	**LQC**	5.00	5.51	110.14±0.08	1.38	5.45	109.61±0.10	1.87
	MQC	50.00	48.72	97.45±0.18	0.37	48.93	98.11±0.52	1.07
	HQC	200.00	198.65	99.32±3.95	1.99	201.45	99.82±2.26	1.13

LQC = Lower Quality Control, MQC = Middle Quality Control, HQC = Higher Quality Control, RSD = Relative Standard Deviation (%)

Table S4 *Concentration (ngL-1) of PFAS analogues in underground water samples collected from Kamrup region of Assam, India*

Sample No.	Sample Region	PFBS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFDS	PFuDA	PFOSA	PFDoA
1	Metro	nd	1.77	17.68	8.84	21.56	1.77	10.64	8.84	1.77	1.77	1.77	8.84
2	Metro	39.48	1.77	17.68	8.84	8.84	1.77	10.17	8.84	8.24	nd	nd	8.84
3	Rural	nd	1.77	17.68	8.84	8.84	1.77	9.17	8.84	5.82	1.77	nd	8.84
4	Metro	nd	4.16	nd	8.84	8.84	1.77	10.01	8.84	6.69	1.77	nd	8.84
5	Metro	nd	1.77	17.68	8.84	0.00	1.77	9.30	8.84	4.61	1.77	nd	8.84
6	Metro	1129.21	4.47	nd	nd	15.59	1.77	7.76	8.84	nd	nd	nd	8.84
7	Metro	77.92	1.77	17.68	nd	8.84	3.09	8.56	8.84	1.77	nd	nd	nd
8	Rural	1.77	6.56	17.68	nd	8.84	1.77	10.09	8.84	1.77	1.77	nd	nd
9	Metro	1.77	8.92	17.68	8.84	19.32	1.77	9.29	8.84	1.77	nd	nd	8.84
10	Rural	1.77	9.34	nd	8.84	14.61	1.77	10.16	8.84	1.77	nd	nd	nd
11	Metro	1.77	1.77	17.68	nd	8.84	1.77	10.63	8.84	1.77	nd	nd	nd
12	Metro	119.21	1.77	17.68	8.84	17.56	1.77	9.67	8.84	nd	nd	nd	nd
13	Rural	nd	1.77	17.68	8.84	8.84	1.77	9.12	8.84	1.77	nd	nd	nd
14	Rural	nd	1.77	17.68	nd	8.84	4.38	19.80	8.84	1.77	1.77	nd	nd
15	Metro	1.77	16.04	17.68	8.84	8.84	1.77	11.71	8.84	1.77	nd	nd	nd
16	Rural	nd	1.77	17.68	nd	15.56	3.15	8.90	8.84	1.77	1.77	nd	nd
17	Metro	nd	2.69	17.68	8.84	19.63	3.06	9.47	8.84	1.77	1.77	nd	nd
18	Metro	71.52	9.53	17.68	8.84	8.84	3.23	27.94	8.84	1.77	nd	nd	nd

19	Metro	nd	3.15	0.00	0.00	14.28	1.77	8.16	0.00	1.77	nd	nd	nd
20	Metro	9.08	10.91	17.68	8.84	8.84	1.77	11.44	8.84	1.77	1.77	nd	nd
21	Metro	98.41	2.76	nd	nd	13.55	1.77	8.54	nd	3.31	nd	nd	nd
22	Metro	nd	8.04	17.68	8.84	8.84	1.77	8.67	8.84	6.99	1.77	nd	nd
23	Metro	nd	1.77	nd	8.84	8.84	3.41	10.31	nd	4.59	1.77	nd	nd
24	Metro	nd	44.04	17.68	nd	8.84	1.77	8.76	nd	4.03	1.77	nd	nd
25	Rural	119.07	9.27	17.68	nd	14.81	3.57	6.91	8.84	6.60	1.77	nd	nd
26	Rural	nd	1.77	17.68	nd	0.00	1.77	8.75	8.84	1.77	nd	nd	nd
27	Rural	154.83	10.37	17.68	8.84	8.84	1.77	8.71	nd	1.77	nd	nd	nd
28	Rural	nd	1.77	nd	nd	8.84	1.77	9.42	nd	0.87	1.77	nd	nd
29	Metro	2.16	1.77	17.68	8.84	15.11	1.77	8.22	8.84	7.12	1.77	nd	nd
30	Rural	nd	1.77	17.68	8.84	8.84	1.77	10.46	8.84	1.77	nd	nd	nd
31	Rural	nd	2.76	17.68	nd	8.84	3.19	3.26	nd	2.65	nd	nd	nd
32	Rural	192.57	1.77	17.68	8.84	8.84	1.77	9.06	nd	3.41	1.77	nd	nd
33	Rural	221.88	3.23	17.68	nd	14.11	1.77	7.68	8.84	1.77	1.77	nd	nd
34	Rural	106.46	1.77	17.68	nd	8.84	1.94	8.18	nd	2.67	1.77	nd	nd
35	Rural	237.08	1.77	17.68	nd	8.84	1.77	7.63	8.84	2.75	1.77	nd	nd
36	Rural	147.99	1.77	17.68	8.84	8.84	1.77	8.96	nd	4.96	nd	nd	nd
37	Metro	143.68	1.77	nd	nd	13.27	1.77	8.82	8.84	1.77	1.77	nd	nd
38	Rural	8.66	1.77	17.68	nd	21.72	1.77	7.13	nd	3.53	1.77	nd	nd
39	Metro	8.71	3.73	17.68	8.84	8.84	1.77	11.04	8.84	3.04	nd	nd	nd

40	Metro	7.54	15.41	17.68	8.84	8.84	1.77	9.07	nd	1.77	nd	nd	nd
41	Metro	558.03	nd	17.68	8.84	0.00	1.77	40.97	8.84	1.77	nd	nd	nd
42	Metro	107.71	1.77	nd	8.84	16.10	1.77	1.91	nd	nd	nd	nd	nd
43	Rural	nd	10.02	17.68	nd	16.25	1.77	8.35	8.84	1.77	1.77	nd	nd
44	Metro	nd	1.77	nd	8.84	25.71	1.77	1.95	8.84	1.77	1.77	1.77	8.84
45	Metro	14.67	4.42	17.68	8.84	8.84	1.77	8.95	8.84	10.76	1.77	nd	8.84
46	Rural	nd	5.71	nd	nd	8.84	1.77	9.65	8.84	4.42	nd	nd	8.84
47	Metro	1.77	5.04	17.68	nd	8.84	1.77	9.61	8.84	2.60	1.77	nd	nd
48	Metro	30.97	18.62	17.68	nd	0.00	1.77	9.47	8.84	1.77	1.77	nd	nd
49	Rural	nd	1.77	17.68	nd	25.18	1.77	9.00	8.84	1.77	nd	nd	nd
50	Rural	nd	1.77	17.68	nd	14.62	1.77	9.23	8.84	1.77	1.77	nd	nd
51	Metro	nd	1.77	nd	8.84	19.29	1.77	0.69	0.00	1.77	nd	nd	nd
52	Rural	148.39	46.72	17.68	nd	18.86	1.77	0.55	8.84	1.77	nd	nd	nd
53	Metro	182.12	0.00	17.68	8.84	8.84	1.77	10.45	8.84	1.77	nd	nd	nd
54	Metro	14.99	26.60	17.68	nd	17.08	1.77	9.10	8.84	1.77	1.77	nd	nd
55	Metro	nd	3.88	nd	nd	8.84	1.77	10.80	0.00	1.77	nd	nd	nd
56	Rural	224.47	8.55	17.68	8.84	17.02	1.77	9.31	8.84	nd	nd	nd	nd
57	Rural	nd	17.57	17.68	nd	15.25	1.77	10.12	8.84	4.98	nd	nd	nd
58	Rural	129.56	1.77	17.68	nd	8.84	1.77	7.33	8.84	1.77	nd	nd	nd
59	Rural	7.00	13.20	17.68	nd	21.77	1.77	9.07	8.84	1.77	nd	nd	nd
60	Metro	148.43	3.42	17.68	8.84	8.84	1.77	2.52	8.84	1.77	nd	nd	nd

61	Rural	160.72	1.77	nd	nd	14.84	1.77	7.23	8.84	1.77	nd	nd	nd
62	Metro	265.57	1.77	17.68	8.84	16.61	3.67	9.31	8.84	1.77	nd	nd	nd
63	Rural	nd	4.48	17.68	nd	nd	1.77	2.91	8.84	nd	nd	nd	nd
64	Metro	nd	1.77	nd	nd	21.21	1.77	2.77	8.84	1.77	nd	nd	8.84
65	Rural	149.65	1.77	nd	nd	8.84	1.77	7.42	8.84	1.77	nd	nd	0.00

Table S5 *Descriptive statistics of underground water samples from Kamrup region of Assam, India*

Compound	N total	Mean (ngL^{-1})	Minimum (ngL^{-1})	Median (ngL^{-1})	Maximum (ngL^{-1})	P95 (ngL^{-1})	DF(%)
PFBS	65	77.67	nd	2.16	1129.21	237.08	60.00
PFHxA	65	6.13	nd	1.77	46.72	18.62	96.92
PFHpA	65	13.33	nd	17.68	17.68	17.68	75.38
PFHxS	65	4.22	nd	nd	8.84	8.84	47.69
PFOA	65	11.90	nd	8.84	25.71	21.72	92.30
PFNA	65	2.00	1.77	1.77	4.38	3.41	100.00
PFOS	65	9.23	0.55	9.07	40.97	11.71	100.00
PFDA	65	6.80	nd	8.84	8.84	8.84	76.92
PFDS	65	2.64	nd	1.77	10.76	6.99	92.30
PFuDA	65	0.76	nd	nd	1.77	1.77	43.07
PFOSA	65	0.05	nd	nd	1.77	nd	3.07
PFDoA	65	1.50	nd	nd	8.84	8.84	16.92
∑ **PFAS**	/	136.23	2.32	51.90	1305.49	345.50	/

P95 = 95th Percentile, DF = Detection frequency (%), nd = non-detected

Table S6 *Descriptive statistics of underground water samples from Kamrup metro, Assam, India*

Compound	N total	Mean (ngL^{-1})	Minimum (ngL^{-1})	Median (ngL^{-1})	Maximum (ngL^{-1})	P95 (ngL^{-1})	DF(%)
PFBS	35	86.76	nd	7.54	1129.21	353.31	65.71
PFHxA	35	6.30	nd	2.76	44.04	21.01	94.28
PFHpA	35	12.12	nd	17.68	17.68	17.68	68.57
PFHxS	35	5.81	nd	8.84	8.84	8.84	65.71
PFOA	35	11.89	nd	8.84	25.71	21.31	91.42
PFNA	35	1.99	1.77	1.77	3.67	3.28	100.00
PFOS	35	9.91	0.69	9.30	40.97	16.58	100.00
PFDA	35	6.82	nd	8.84	8.84	8.84	77.14
PFDS	35	2.83	nd	1.77	10.76	7.45	91.42
PFuDA	35	0.76	nd	nd	1.77	1.77	42.85
PFOSA	35	0.10	nd	nd	1.77	0.53	5.71
PFDoA	35	2.27	nd	nd	8.84	8.84	25.71
∑ **PFAS**	/	147.56	2.46	67.34	1302.10	469.45	/

P95 = 95th Percentile, DF = Detection frequency (%), nd = non-detected

Table S7 *Descriptive statistics of underground water samples from Kamrup rural, Assam, India*

Compound	N total	Mean (ngL^{-1})	Minimum (ngL^{-1})	Median (ngL^{-1})	Maximum (ngL^{-1})	P95 (ngL^{-1})	DF(%)
PFBS	30	67.06	nd	1.77	237.08	223.30	53.33
PFHxA	30	5.93	1.77	1.77	46.72	15.61	100.00
PFHpA	30	14.73	nd	17.68	17.68	17.68	83.33
PFHxS	30	2.36	nd	nd	8.84	8.84	26.67
PFOA	30	11.91	nd	8.84	25.18	21.75	93.33
PFNA	30	2.01	1.77	1.77	4.38	3.40	100.00
PFOS	30	8.45	0.55	8.93	19.80	10.32	100.00
PFDA	30	6.78	nd	8.84	8.84	8.84	76.67
PFDS	30	2.42	nd	1.77	6.60	5.44	93.33
PFuDA	30	0.77	nd	nd	1.77	1.77	43.33
PFOSA	30	nd	nd	nd	nd	nd	nd
PFDoA	30	0.59	nd	nd	8.84	4.86	6.67
∑ **PFAS**	/	123.01	4.09	51.37	385.73	321.82	/

P95 = 95th Percentile, DF = Detection frequency (%), nd = non-detected

Table S8 *Estimated Daily Intake (EDI, ngkg-1bw d-1) of PFAS in five age groups via drinking water*

Compound	Men	Women	Children	Boys	Girls
PFBS	3.50	3.07	4.66	4.16	3.42
PFHxA	0.28	0.24	0.37	0.33	0.27
PFHpA	0.60	0.53	0.80	0.71	0.59
PFHxS	0.19	0.17	0.25	0.23	0.19
PFOA	0.54	0.47	0.71	0.64	0.52
PFNA	0.09	0.08	0.12	0.11	0.09
PFOS	0.42	0.36	0.55	0.49	0.41
PFDA	0.31	0.27	0.41	0.36	0.30
PFDS	0.12	0.10	0.16	0.14	0.12
PFuDA	0.03	0.03	0.05	0.04	0.03
PFOSA	0.00	0.00	0.00	0.00	0.00
PFDoA	0.07	0.06	0.09	0.08	0.07
∑PFAS	6.13	5.38	8.17	7.29	5.99

Table S9 *Hazard Quotient (HQ) of each PFAS for the five age groups*

Compound	Men	Women	Children	Boys	Girls
PFBS	0.17	0.15	0.23	0.21	0.17
PFHxA	0.01	0.01	0.02	0.02	0.01
PFHpA	0.03	0.03	0.04	0.04	0.03
PFHxS	0.01	0.01	0.01	0.01	0.01
PFOA	0.03	0.02	0.04	0.03	0.03
PFNA	0.00	0.00	0.01	0.01	0.00
PFOS	0.02	0.02	0.03	0.02	0.02
PFDA	0.02	0.01	0.02	0.02	0.01
PFDS	0.01	0.01	0.01	0.01	0.01
PFuDA	0.00	0.00	0.00	0.00	0.00
PFOSA	0.00	0.00	0.00	0.00	0.00
PFDoA	0.00	0.00	0.00	0.00	0.00
∑HQ	0.31	0.27	0.41	0.36	0.30

Table S10 *Top significant metabolite observed in volcano plot*

Metabolites	log2(FC)	p.adjusted
PE(18:3(6Z,9Z,12Z)/P-18:1(11Z))	-20.71	0.000228
PS(18:0/20:0)	-20.611	0.000228
PE(14:0/22:2(13Z,16Z))	-19.84	0.000228
LysoPC(18:0)	-21.129	0.000283
Varanic acid	-21.333	0.000378
D-Erythroascorbic acid 1'-a-D-xylopyranoside	-22.286	0.000591
LysoPC(17:0)	-21.228	0.000591
Ganglioside GM3 (d18:1/18:1(11Z))	-21.174	0.000591
10,20-Dihydroxyeicosanoic acid	-20.373	0.000591
4-Guanidino-1-butanol	-21.83	0.000628
Docosanedioic acid	-20.735	0.000686
MG(18:0/0:0/0:0)	-20.909	0.000715
3-(2,4-Cyclopentadien-1-ylidene)pregn-4-en-20-one	-21.397	0.001066
3-(Pyrazol-1-yl)-L-alanine	-22.053	0.001789
C16 Sphinganine	-20.893	0.014051
3L,7D,11D-phytanic acid	-20.274	0.017948
dTTP / 2'-Deoxythymidine 5'-triphosphate	2.2511	0.020979

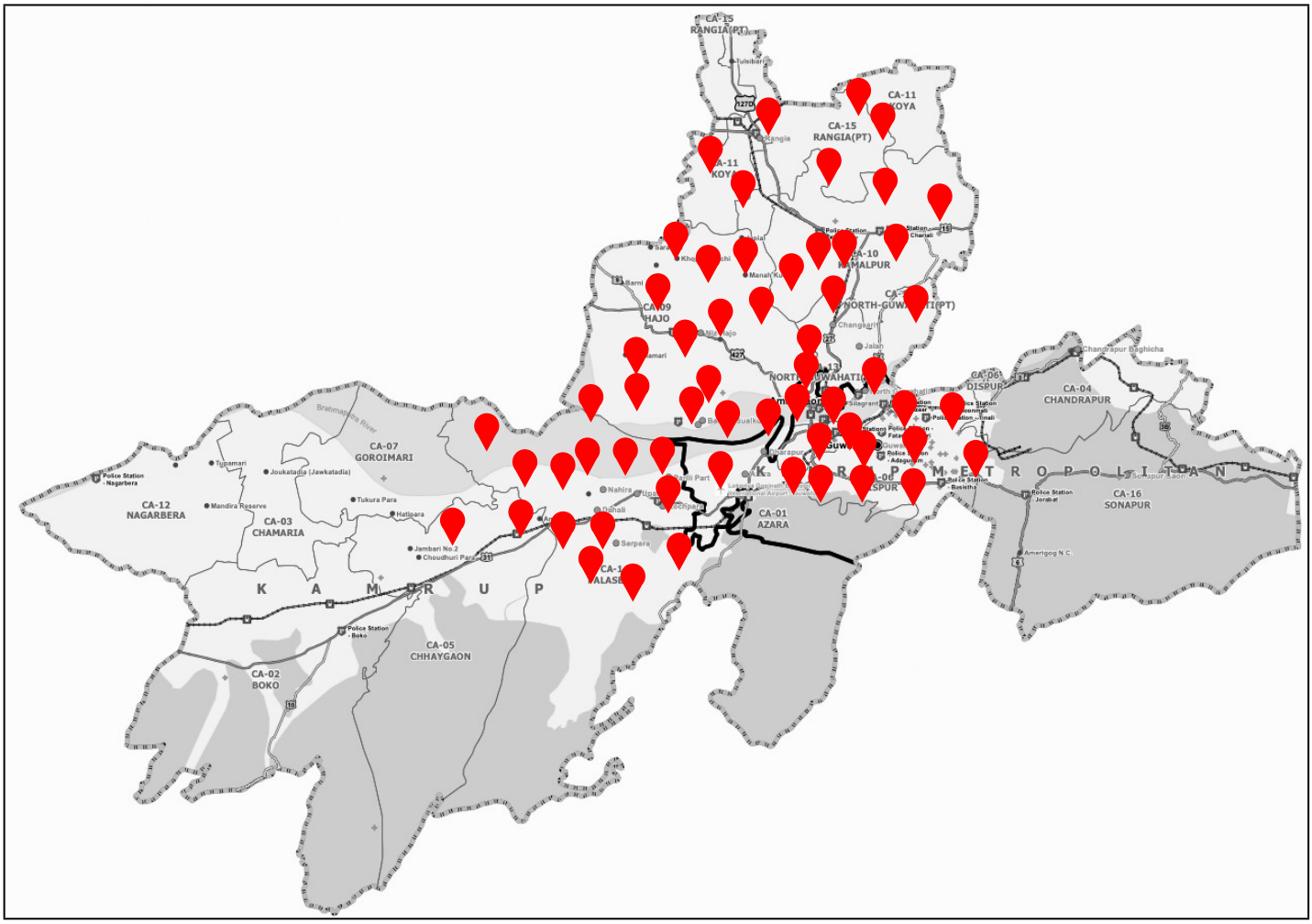

Figure S1 *Water Sample Collection sites from Kamrup region of Assam, India*

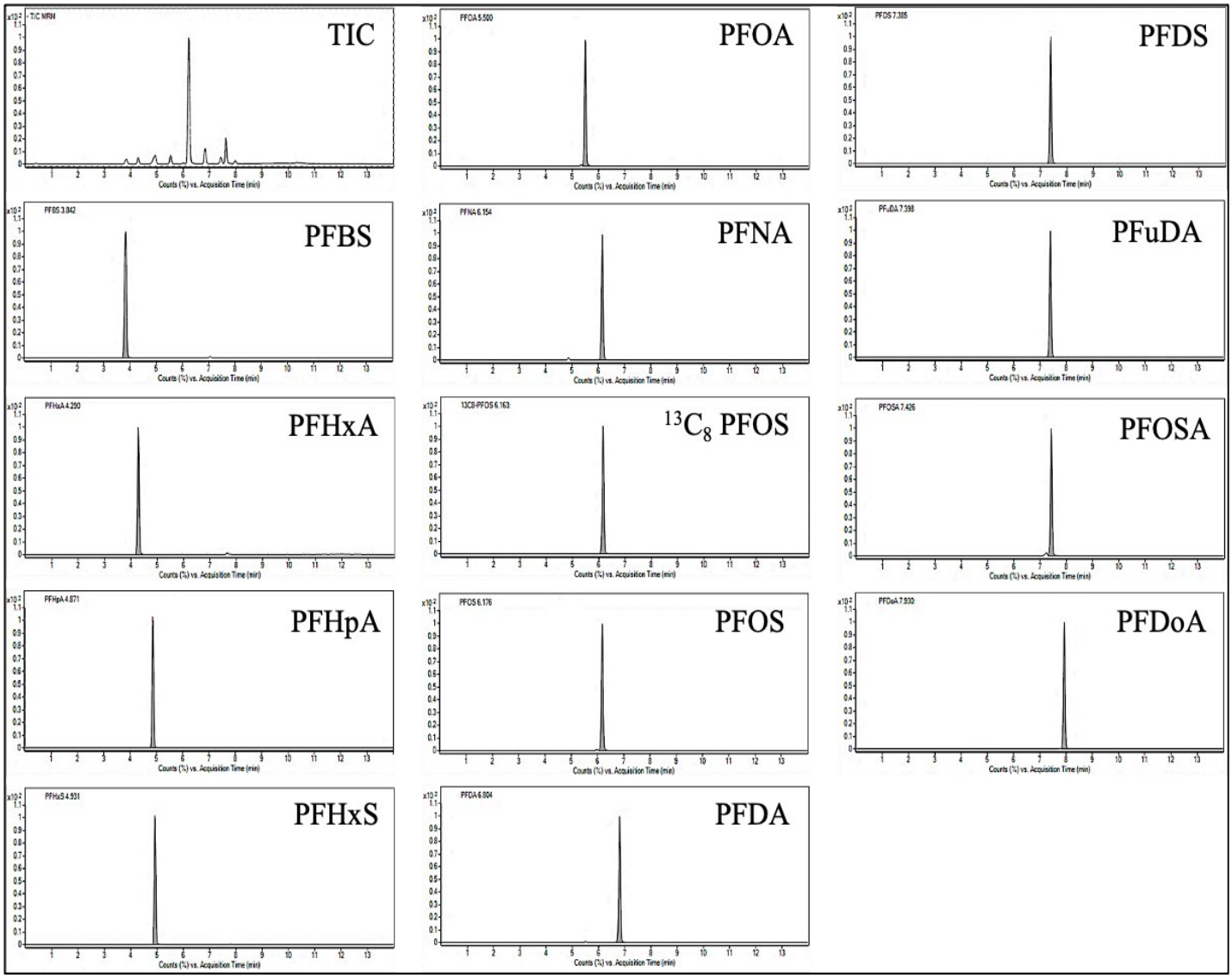

Figure S2 *LC-MS/MS chromatograms of PFAS analogues*

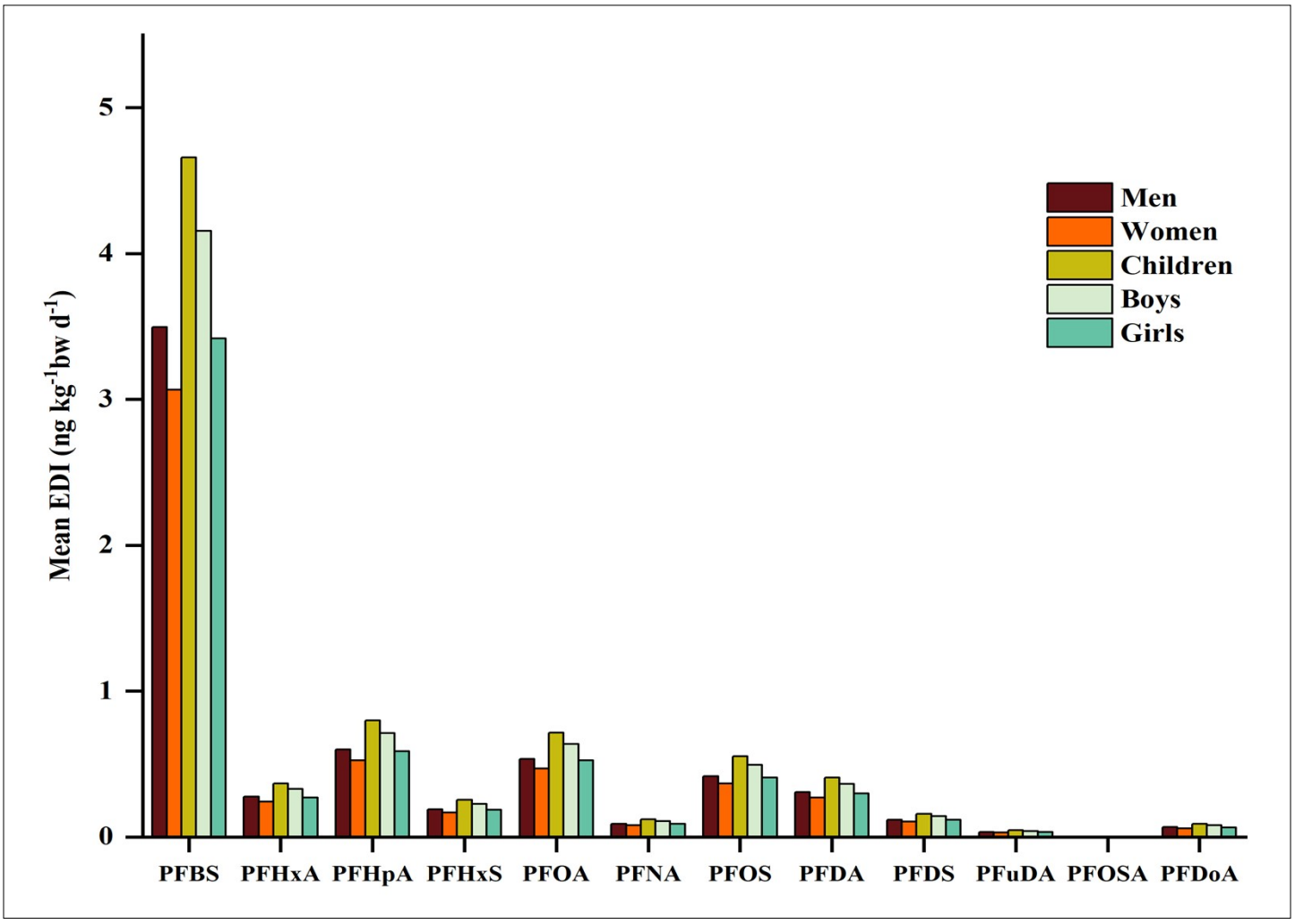

Figure S3 *Comparison of EDI, ng kg-1 bw d-1 of PFAS among the five age groups*

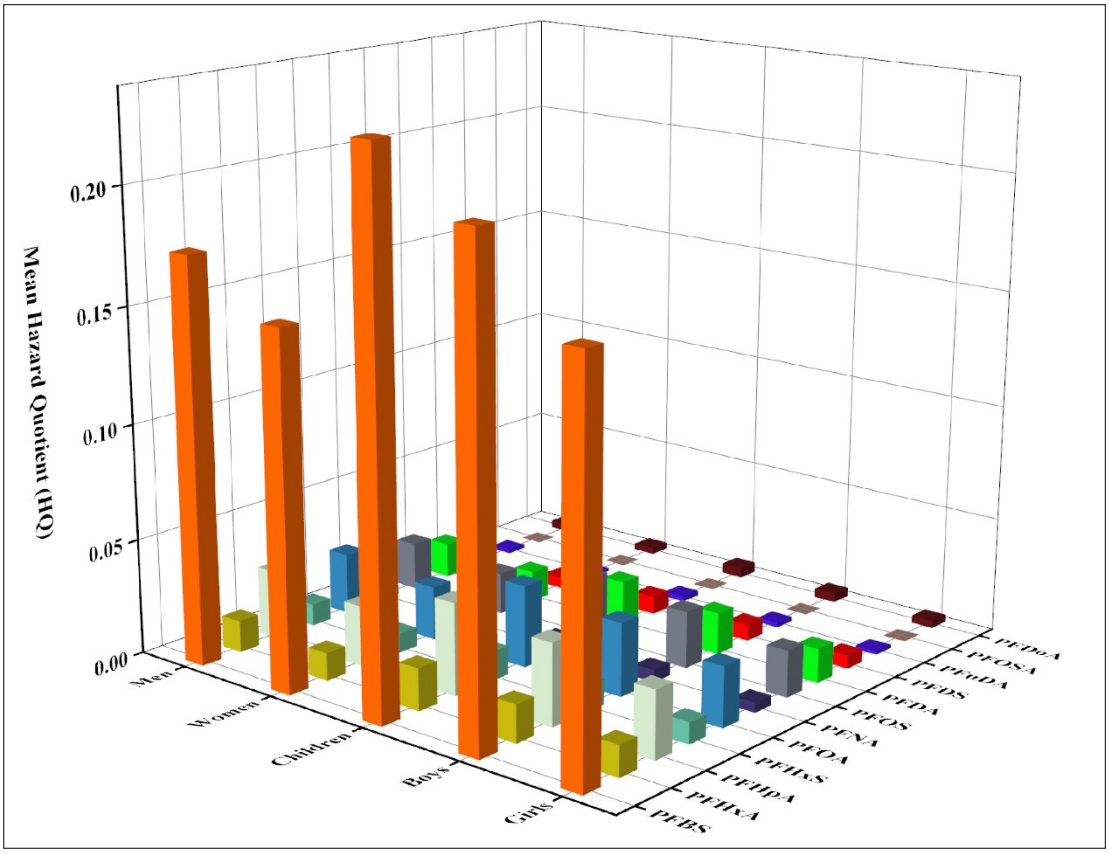

Figure S4 *Mean Hazard Quotient (HQ) for the five age groups*