

Supplemental Information for

**Perfluoroalkyl Acid Precursor Discharge from Engineered Water Systems:  
Composition and Treatment Impacts**

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Limits of quantification were defined as the lowest concentration within the calibration range exceeding twice the blank response area. Wastewater values represent average LOQs across two sampling phases (n=2) at six treatment plants; stormwater values represent single events at eight sites across three cities. Both SPE and TOP assay methods are included.

**Table S1.** Limits of Quantification (LOQ) for Target PFAS in Wastewater and Stormwater Samples

Compound	Wastewater		Stormwater		Standard Type
	LOQ (ng/L) (SPE, n=2)	LOQ (ng/L) (TOP Assay, n=2)	LOQ (ng/L) (SPE)	LOQ (ng/L) (TOP Assay)	
PFBA	3.39	9.29	74.50	129.60	ESI-
PFPeA	4.03	5.07	65.70	31.60	ESI-
PFHxA	0.08	2.15	69.80	11.60	ESI-
PFHpA	0.08	0.84	3.60	3.70	ESI-
PFOA	0.06	0.20	4.40	3.20	ESI-
PFNA	0.06	0.80	11.60	4.40	ESI-
PFDA	0.13	0.52	3.3	4.56	ESI-
PFUdA	0.28	0.79	N. A	4.30	ESI-
PFDoA	0.20	1.04	48.50	6.50	ESI-
PFTTrDA	0.07	0.38	0.60	3.40	ESI-
PFTeDA	0.20	0.68	3.10	7.40	ESI-
FOSA	0.05	1.55	14.20	30.10	ESI-
br-NMeFOSAA	0.81	3.01	33.10	10.80	ESI-
br-NEtFOSAA	0.93	19.62	77.50	68.00	ESI-
PFBS	0.07	0.32	5.9	3.00	ESI-
PFPeS	0.12	0.38	0.80	61.80	ESI-
PFHxS	0.21	0.57	0.50	11.10	ESI-
PFHpS	0.09	1.15	3.40	3.00	ESI-
PFOS	0.24	0.99	2.90	1.20	ESI-
PFNS	0.20	0.87	2.60	35.10	ESI-
PFDS	0.45	2.06	N. A	N. A	ESI-
PFDoS	0.16	0.92	73.10	64.40	ESI-
4:2 FTS	0.31	0.98	41.50	13.30	ESI-
6:2 FTS	2.33	17.54	158.20	169.20	ESI-
8:2 FTS	0.83	1.62	146.10	27.30	ESI-
HFPO-DA	N. A	N. A	114.80	368.00	ESI-
NaDONA	0.07	0.23	1.80	0.80	ESI-
9Cl-PF3ONS	0.59	4.73	621.00	32.70	ESI-
11Cl-PF3OUdS	0.21	1.90	109.80	14.50	ESI-
PMPA	0.18	0.78	8.20	2.50	ESI-

PMBA	0.44	0.48	0.80	3.50	ESI-
NFDHA	0.22	1.32	7.30	13.40	ESI-
PFEESA	0.09	0.43	0.90	0.30	ESI-
N-MeFOSA	0.15	2.01	0.30	0.90	ESI-
N-EtFOSA	0.18	1.11	40.30	33.70	ESI-
N-MeFOSE	0.15	0.49	4.00	1.60	ESI-
N-EtFOSE	0.10	0.27	2.00	8.00	ESI-
3:3 FTCA	N.A	N.A	N.A	N.A	ESI-
5:3 FTCA	0.41	3.03	41.90	13.40	ESI-
7:3 FTCA	0.74	4.26	20.1	22.3	ESI+
AmPr-FHxSA	0.16	1.15	4.70	3.80	ESI+
6:2FTAB	0.27	1.04	5.50	4.20	ESI+
5:3FTB	0.06	0.59	4.00	1.63	ESI+
M3PFBA					Internal
M2PFHxA					Internal
M2PFOA					Internal
M2PFDA					Internal
M3HFPO-DA					Internal
M4PFOS					Internal
d5-N-EtFOSAA					Internal
Atrazine-d5					Surrogate
M8FOSA					Surrogate
M8PFOA					Surrogate
M8PFOS					Surrogate

**Table S2.** WWTP Flows and Sample Dates

Plant	Sampling Phase	Date	Day of Week	Flow (MGD)
A	1	10-28-2022	Friday	43.1
	2	9-21-2023	Thursday	46.5
B	1	8-19-2022	Friday	20.5
	2	8-10-2023	Thursday	21.3
C	1	8-18-2022	Thursday	7.33
	2	8-9-2023	Wednesday	6.41
D	1	8-3-2022	Wednesday	5.43
	2	6-24-2023	Saturday	6.48
E	1	8-19-2022	Friday	1.8
	2	8-9-2023	Wednesday	1.75
F	1	1-30-2023	Monday	1.02
	2	9-29-2023	Friday	0.942

**Table S3.** Stormwater sampling data. (Precipitation data from the National Weather Service)<sup>1</sup>

City	Site	Land Use Category	Date	Sampling	Total storm
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ID				Time	rainfall (in.)
C	C-1	Residential	2023-09-11	9:09 AM	0.67
C	C-2	Commercial/downtown	2023-09-11	9:33 AM	0.67
D	D-1	Residential	2023-06-24	4:45 PM	0.91
D	D-2	University campus	2023-06-24	5:15 PM	0.91
D	D-3	Educational facility	2023-06-24	5:30 PM	0.91
D	D-4	Creek outfall	2023-06-24	6:00 PM	0.91
E	E-1	Residential	2023-08-11	5:15 AM	1.06
E	E-2	Residential	2023-08-11	5:30 AM	1.06

**Table S4.** Quality Assurance/Quality Control measures.

QA/QC	Frequency	Stage
Field blank	1 per sample location	Sampling
Trip blank	1 per sampling location per trip	Sampling
Field replicate	Every 10 samples	Sampling
Method blank	Every 20 injections	Analysis
Instrument blank	Every 20 injections	Analysis
Continuing calibration verification	Every 10 injections	Analysis

**Table S5.** Aqueous sample preparation.

Component	Volume ( $\mu$ l)
Sample	890
80:20 methanol-water mix	235
Trifluoroethanol	150
Isopropyl alcohol	130
Basic-water solution	45
Dilute surrogate standard mix	50

### **Text S1. Chromatographic Eluents**

The aqueous phase was made of 2% LCMS-grade methanol, 2 mM LCMS-grade ammonium acetate, and 0.1% acetic acid in Milli-Q water ( $R = 18.2 \text{ M}\Omega$ ). The organic phase was 2% Milli-Q water, 2 mM LCMS-grade ammonium acetate, and 0.1% acetic acid in methanol.

**Table S6.** Eluent gradient.

Time (min)	% Aqueous solvent	% Organic solvent
0	80	20
1	50	50
15	0	100
16.5	0	100
16.7	80	20
20	80	20

**Table S7.** MS Source Parameters

Parameter	ESI- setpoint	ESI+ setpoint
Spray Voltage	1000 V	3500 V
Ion Transfer Tube Temperature	250 °C	250 °C
Vaporizer Temperature	225 °C	225 °C
Sheath Gas	50	50
Aux Gas	12	12
Sweep Gas	0.5	0.5

**Text S2. Solid-Phase Extraction (SPE) Procedure***Cartridge Preparation and System Setup*

Each SPE cartridge was packed approximately halfway with silanized glass wool to reduce the potential for clogging during sample processing, particularly for samples containing suspended solids. The cartridges were mounted onto a vacuum manifold positioned inside an SPE-designated fume hood. All manifold valves remained fully closed prior to extraction to maintain consistent vacuum pressure and ensure controlled flow rates throughout the procedure.

Reservoirs were constructed by modifying 30 mL polypropylene syringes (removal of the external Luer lock) and were attached to the cartridges using reservoir adapters to create a sealed flow path. Both the vacuum pump and manifold outlets were connected to a single waste collection vessel. Prior to extraction, samples were fortified with a known mass of an internal standard not included in the calibration-standard mixture. This spike allowed calculation of SPE recoveries (Table S8).

*Cartridge Conditioning*

Before introducing samples, cartridges were conditioned with 15 mL of 1% methanolic ammonium hydroxide followed by 5 mL of 0.3 M formic acid. Conditioning solutions were pipetted directly into the cartridge in 5 mL aliquots and drawn through using low vacuum. All conditioning waste was collected in the SPE waste vessel.

*Sample Loading*

With the vacuum engaged, samples were transferred into the reservoir and pulled through each cartridge at a controlled flow rate of approximately 5 mL min<sup>-1</sup>. Original sample bottles were retained and allowed to air-dry for later rinsing. When clogging occurred, the remaining sample volume was passed through a second, preconditioned cartridge to ensure complete processing.

Following sample percolation, the reservoir was rinsed twice with 5 mL of ultrapure water and then with 5 mL of a 1:1 (v/v) 0.1 M formic acid/methanol solution. The cartridge was dried for 15 s by drawing ambient air through the system. Rinse solutions were periodically discarded from the manifold or attached waste vessel depending on fluid accumulation.

*Recovery Rinse and Elution*

A 15 mL polypropylene collection tube was placed beneath each manifold port to capture the eluate. The original sample bottle was rinsed with 5 mL of 1% methanolic ammonium hydroxide, and this rinse was transferred into the reservoir to wash and elute target compounds from the SPE cartridge. Vacuum was applied to pull the solution through at approximately 5 mL min<sup>-1</sup>.

*Extract Clean-Up and Final Processing*

Collected eluates were acidified by adding 25 µL of concentrated acetic acid (equal to the stock strength). Ten milligrams of powdered carbon were added to each collection tube to capture particulate material; the mixture was shaken by hand for 15–30 s until phase separation was visible, followed by vortexing for 30 s. Samples were centrifuged for 10 min at 2800 rpm with balanced tube masses.

After centrifugation, 5 mL of supernatant was withdrawn using a syringe fitted with a 0.2 µm nylon filter and transferred into labeled LC-compatible vials. Extracts were stored under refrigeration until LC-MS/MS analysis.

**Table S8.** Recovery rates of M4PFOA for SPE (Pre-TOP Assay).

<b>Wastewater Treatment Plant</b>		
A	Mean	78%
	Standard Deviation	11%
B	Mean	90%
	Standard Deviation	10%
C	Mean	88%
	Standard Deviation	13%
D	Mean	94%
	Standard Deviation	13%
E	Mean	83%
	Standard Deviation	10%
F	Mean	82%
	Standard Deviation	10%
<b>Stormwater Site</b>		
C-1 Intersection (Residential)	Mean	59%
	Standard Deviation	8%
C-2 Intersection (Downtown)	Mean	51%
	Standard Deviation	12%
D-1 Creek (Residential)	Mean	89%
	Standard Deviation	10%
D-2 Creek (University)	Mean	58%
	Standard Deviation	59%
D-3 Highschool Outfall	Mean	65%
	Standard Deviation	20%

D-4 Golf Course	Mean	59%
	Standard Deviation	7%
E-1 Agricultural Drain	Mean	51%
	Standard Deviation	20%
E-2 Urban Pond	Mean	125%
	Standard Deviation	33%

**Table S9.** Recovery rates of M8PFOA from M8FOSA for TOP assay.

**Wastewater Treatment Plant**

A	Mean	75.1%
	Standard Deviation	11.8%
B	Mean	66.0%
	Standard Deviation	7.7%
C	Mean	76.0%
	Standard Deviation	18.8%
D	Mean	78.7%
	Standard Deviation	15.0%
E	Mean	64.0%
	Standard Deviation	11.6%
F	Mean	73.3%
	Standard Deviation	12.8%

**Stormwater Site**

C-1 Intersection (Residential)	Mean	56%
	Standard Deviation	14%
C-2 Intersection (Downtown)	Mean	55%
	Standard Deviation	8%
D-1 Creek (Residential)	Mean	46%
	Standard Deviation	3%
D-2 Creek (University)	Mean	44%
	Standard Deviation	3%
D-3 High School Outfall	Mean	41%
	Standard Deviation	1%
D-4 Golf Course	Mean	49%
	Standard Deviation	1%
E-1 Agricultural Drain	Mean	45%
	Standard Deviation	6%
E-2 Urban Pond	Mean	72%
	Standard Deviation	6%

M8FOSA was below the LOQ in all post-TOP samples.

**Table S10.** Target PFAS Removal Efficiencies

WWTP	Sampling Phase	$\Sigma$ Target PFAS in influent (ng/L)	$\Sigma$ Target PFAS in effluent (ng/L)	Removal Efficiency (%)
A	1	43.6	70.8	-62
	2	66.2	48.1	27
B	1	71.1	39.2	45
	2	66.6	37.1	44
C	1	73.1	182.7	-150
	2	76.2	60.8	20
D	1	34.9	45.2	-29
	2	78.2	62.1	21
E	1	108.1	230.9	-114
	2	51.0	109.3	-114
F	1	25.3	19.9	21
	2	26.8	53.1	-98

**Table S11.** Summary distribution of PFAS classes (ng/L) in primary/raw influent samples of wastewater treatment plants.

WWTP	Sampling Phase	$\Sigma$ PFCA	$\Sigma$ PFSA	$\Sigma$ FTCA	$\Sigma$ FTSA	$\Sigma$ PFECA	$\Sigma$ FASA
A	1	16.40	15.99	3.37	7.48	0.33	0.00
	2	29.36	23.17	0.00	13.66	0.00	0.00
B	1	46.89	14.14	0.00	8.57	1.49	0.00
	2	35.22	13.69	16.33	0.00	0.63	0.74
C	1	48.73	4.69	19.68	0.00	0.00	0.00
	2	52.76	5.31	18.13	0.00	0.00	0.00
D	1	16.59	12.75	3.16	0.00	0.00	2.40
	2	52.11	15.71	8.56	0.00	0.00	1.83
E	1	99.29	3.99	4.85	0.00	0.00	0.00
	2	25.99	4.44	1.14	4.84	0.15	14.45
F	1	25.14	0.00	0.18	0.00	0.00	0.00
	2	24.43	2.42	0.00	0.00	0.00	0.00

**Table S12.** Summary distribution of PFAS classes (ng/L) in secondary/final effluent samples of wastewater treatment plants.

WWTP	Sampling Phase	$\Sigma$ PFCA	$\Sigma$ PFSA	$\Sigma$ FTCA	$\Sigma$ FTSA	$\Sigma$ PFECA	$\Sigma$ FASA	$\Sigma$ FOSAA
A	1	51.44	16.76	0.00	0.00	2.58	0.00	0.00
	2	33.05	14.84	0.00	0.00	0.22	0.00	0.00
B	1	27.91	10.60	0.00	0.00	0.67	0.00	0.00
	2	25.90	10.85	0.00	0.00	0.63	0.00	0.00
C	1	157.73	14.08	7.05	0.00	0.00	1.21	2.89
	2	49.97	5.90	0.00	4.97	0.00	0.00	0.00
D	1	25.74	13.34	0.00	0.00	0.00	6.11	0.00

E	2	44.21	12.65	2.04	0.00	0.00	5.69	0.00
	1	222.77	8.14	0.00	0.00	0.00	0.00	0.00
F	2	102.91	6.35	0.00	0.00	0.00	0.00	0.00
	1	19.88	0.00	0.00	0.00	0.00	0.00	0.00
	2	38.82	14.24	0.00	0.00	0.00	0.00	0.00

**Table S13.** Summary of estimated mass of PFAS discharged from WWTPs.

WWTP	Phase	Flow (MGD)	Target PFAS (ng/L)	Precursor PFAS (ng/L)	Total PFAS (ng/L)	Target Mass (g/yr)	Precursor Mass (g/yr)	Total Mass (g/yr)
A	1	43.10	70.79	60.29	131.08	4215.46	3590.22	7805.68
	2	46.50	48.12	14.43	62.54	3091.40	926.93	4018.33
B	1	20.50	39.17	93.34	132.51	1109.59	2643.81	3753.40
	2	21.30	37.09	56.64	93.73	1091.52	1666.83	2758.35
C	1	7.30	182.65	135.38	318.03	1842.26	1365.46	3207.72
	2	6.40	60.83	62.99	123.83	537.94	557.03	1094.96
D	1	5.40	45.19	64.37	109.56	337.17	480.25	817.42
	2	6.50	62.11	71.85	133.96	557.81	645.27	1203.07
E	1	1.80	230.91	81.34	312.26	574.28	202.30	776.59
	2	1.75	109.26	54.68	163.94	264.19	132.21	396.40
F	1	1.00	19.88	47.49	67.37	27.46	65.61	93.08
	2	0.90	53.06	26.89	79.95	73.32	37.15	110.47

**Table S14.** Summary of estimated mass of PFAS discharged from Stormwater Sites Sampled.\*

Site ID	Rainfall (in)	Drainage Area (ac)	Runoff Coefficient	Runoff Depth (in)	Runoff Volume (ac-ft)	Runoff Volume (m <sup>3</sup> )	Estimated Precursor (ng/L)	Estimated Precursor PFAS (g)	Total Estimated PFAS (ng/L)	Total Estimated PFAS (g)
C-1	0.67	25	31.55	21.14	44.04	54300	13.00	0.71	22.00	1.20
C-2	0.67	15	67.55	45.26	56.57	69800	39.00	2.72	59.00	4.12
D-1	0.91	50	27.05	24.62	60	12700	24.00	3.04	38.00	4.81
D-2	0.91	100	40.55	36.90	0	37900	23.00	8.72	29.00	11.00
D-3	0.91	40	36.05	32.81	5	13500	1.00	0.13	15.00	2.02
D-4	0.91	60	45.05	41.00	8	25300	9.00	2.28	29.00	7.33
E-1	1.06	80	25.25	26.77	3	22000	6.00	1.32	26.00	5.72

E-2	1.06	80	25.25	26.77	3	178.4	22000	0	20.00	4.40	143.00	31.47
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\*Notes and Assumptions:

1. Drainage area estimates are based on typical urban catchment sizes for each land use type
2. Impervious % based on literature values<sup>2,3</sup>
3. Runoff coefficient ( $R_v$ ) calculated using Simple Method<sup>4</sup>:  $R_v = 0.05 + 0.009 \times I$  (where  $I = \%$  impervious)
4. Runoff volume = Rainfall  $\times$  Drainage Area  $\times R_v$
5. These calculations are event-based estimates.

**Table S15.** Summary Molar Concentrations of PFAS in Wastewater Influent and Effluent Samples

WWTP	Sampling Phase	Influent Concentrations (nM)		Effluent Concentration (nM)	
		Untargeted PFAA Precursors	Total Estimated PFAS	Untargeted PFAA Precursors	Total Estimated PFAS
A	1	1.28	1.31	0.23	0.23
	2	0.30	0.32	0.05	0.05
B	1	0.21	0.23	0.38	0.38
	2	0.64	0.69	0.24	0.24
C	1	1.01	1.06	0.46	0.48
	2	2.46	2.51	0.23	0.25
D	1	0.35	0.37	0.23	0.24
	2	0.50	0.53	0.28	0.29
E	1	0.60	0.61	0.99	0.99
	2	0.57	0.61	0.22	0.22
F	1	0.41	0.41	0.21	0.21
	2	1.11	1.11	0.12	0.12

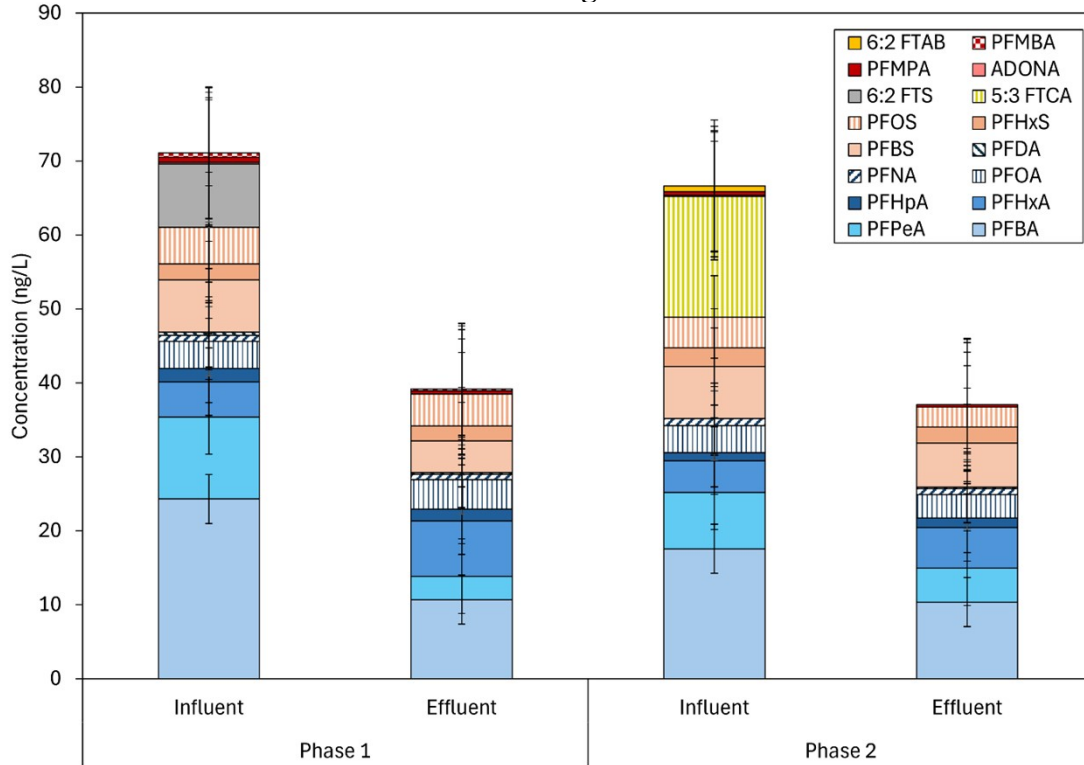
**Table S16.** Summary Molar Concentrations of PFAS in Stormwater Samples.

Site ID	PFAS Concentration (nM)	
	Precursor PFAS	Total Estimated PFAS
C-1	0.05	0.09
C-2	0.12	0.19
D-1	0.09	0.15
D-2	0.10	0.12
D-3	0.003	0.07
D-4	0.03	0.12
E-1	0.02	0.10
E-2	0.09	0.51

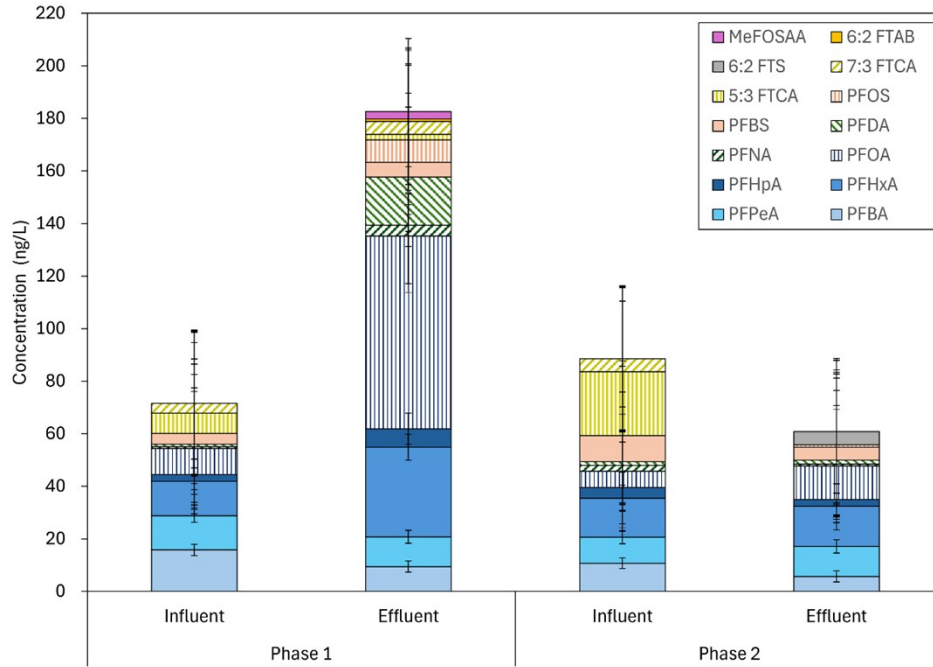
**Table S17.** Solids Retention Times (SRTs) of the six municipal WWTPs studied.

WWTP	SRT (days)
A	12
B	11
C	14
D	3
E	0.7
F	4

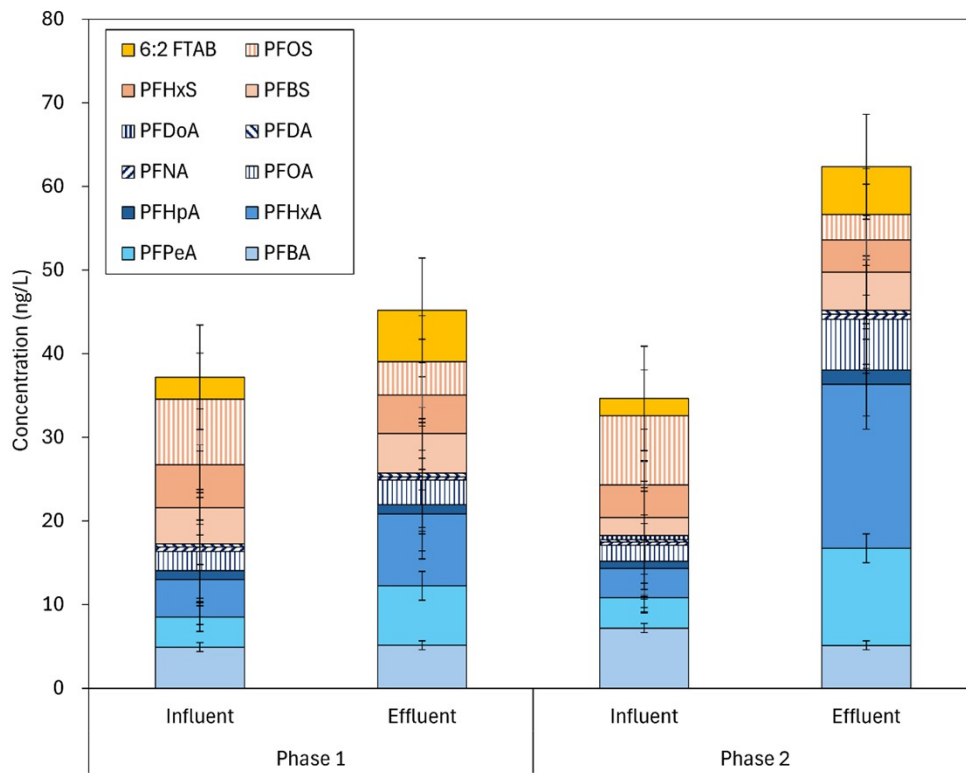
**Results: Concentrations and distributions of Target PFAS in WWTPs**



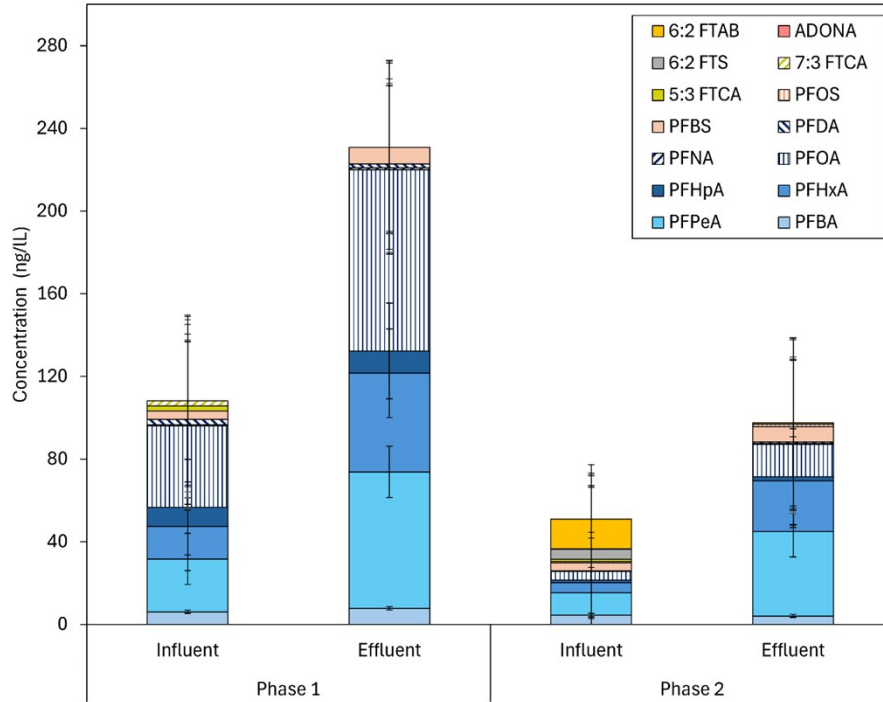
**Figure S1.** Target PFAS in WWTP B. Mean of triplicate samples; error bars represent standard errors.



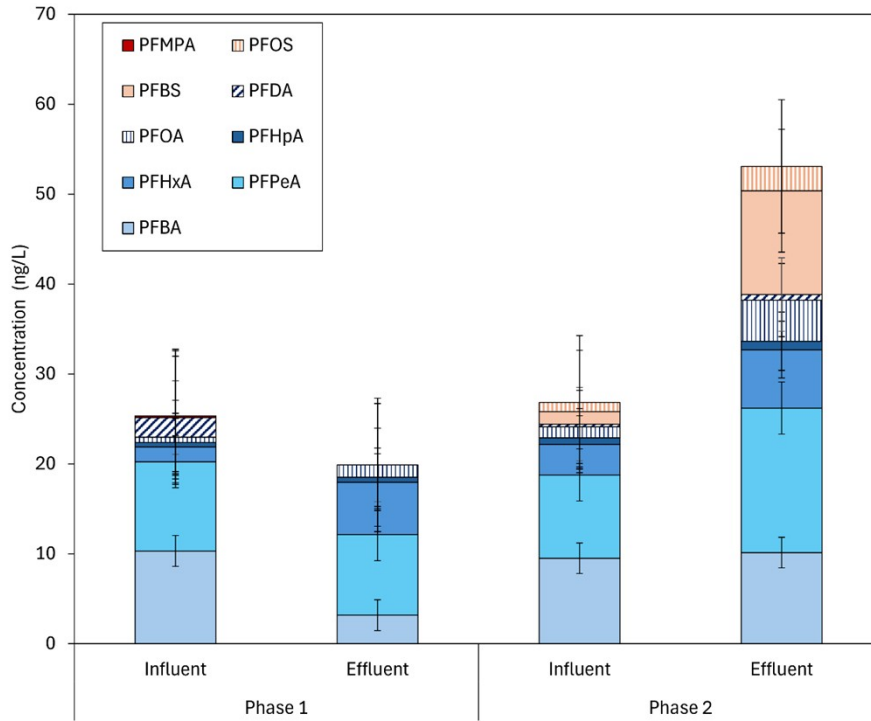
**Figure S2.** Target PFAS in WWTP C. Mean of triplicate samples; error bars represent standard errors.



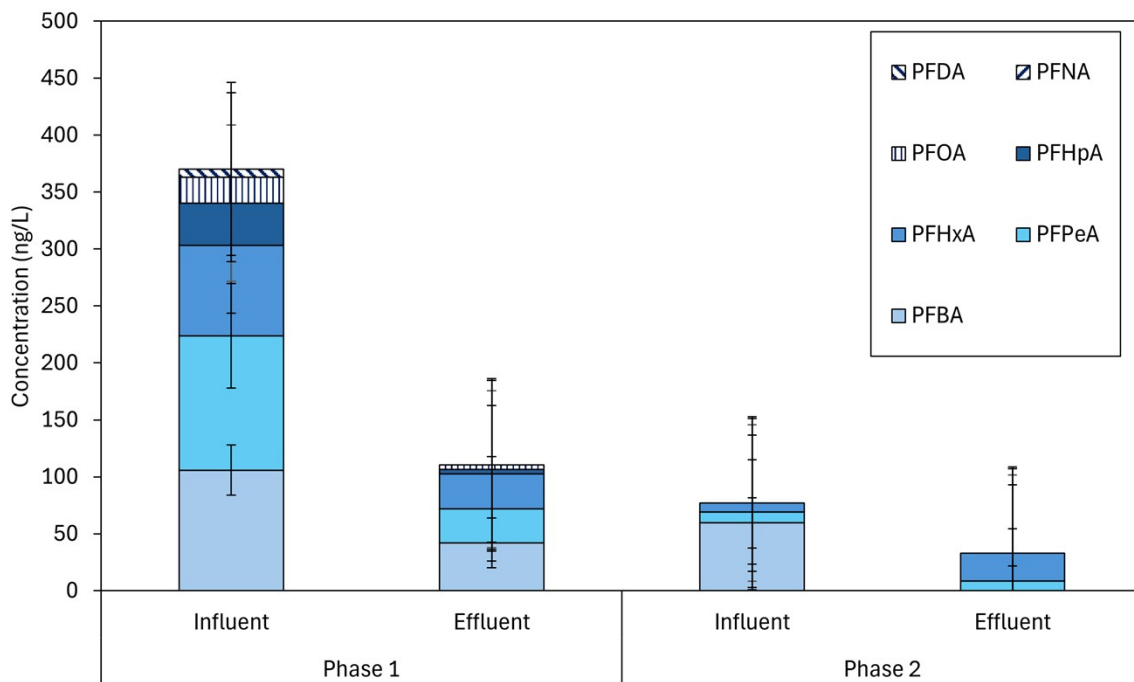
**Figure S3.** Target PFAS in WWTP D. Mean of triplicate samples; error bars represent standard errors.



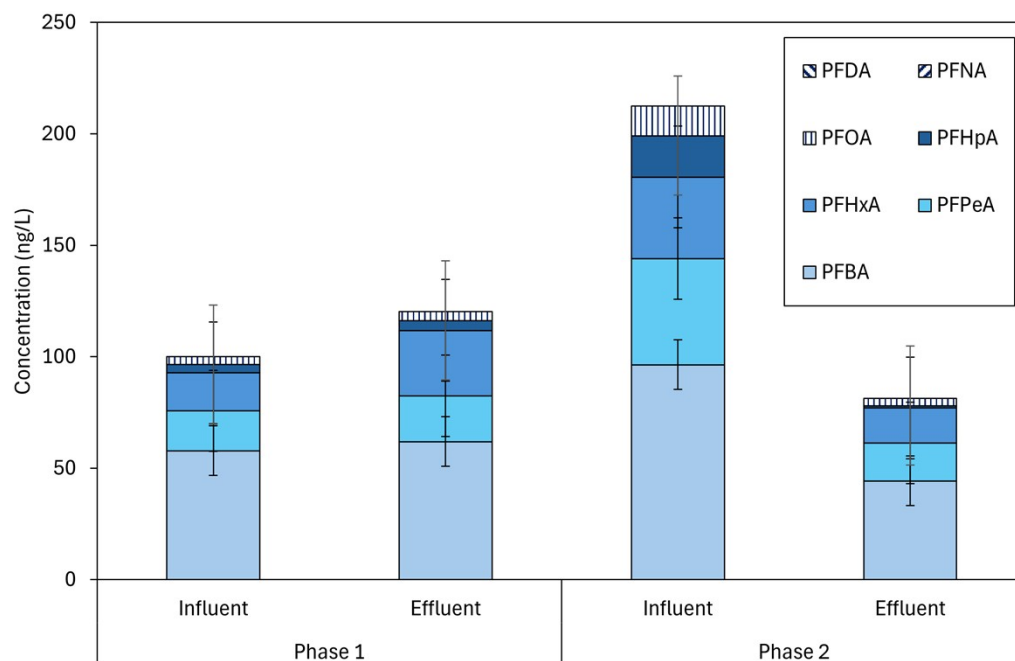
**Figure S4.** Target PFAS in WWTP E. Mean of triplicate samples; error bars represent standard errors.



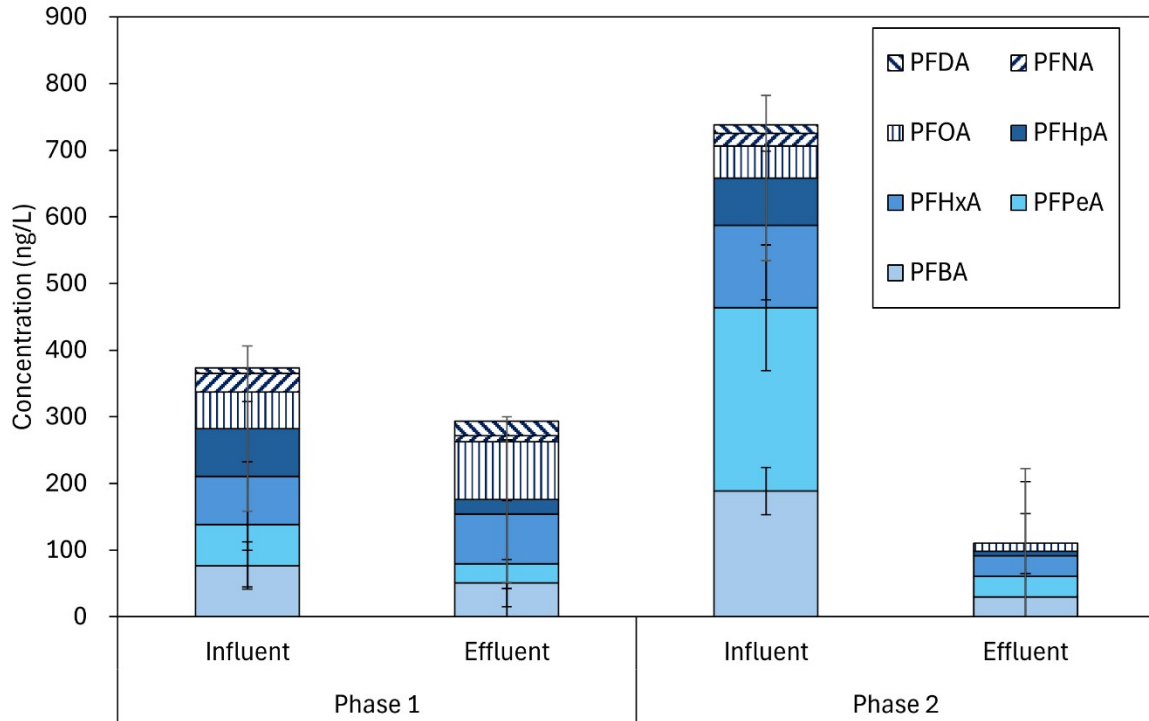
**Figure S5.** Target PFAS in WWTP F. Mean of triplicate samples; error bars represent standard errors.



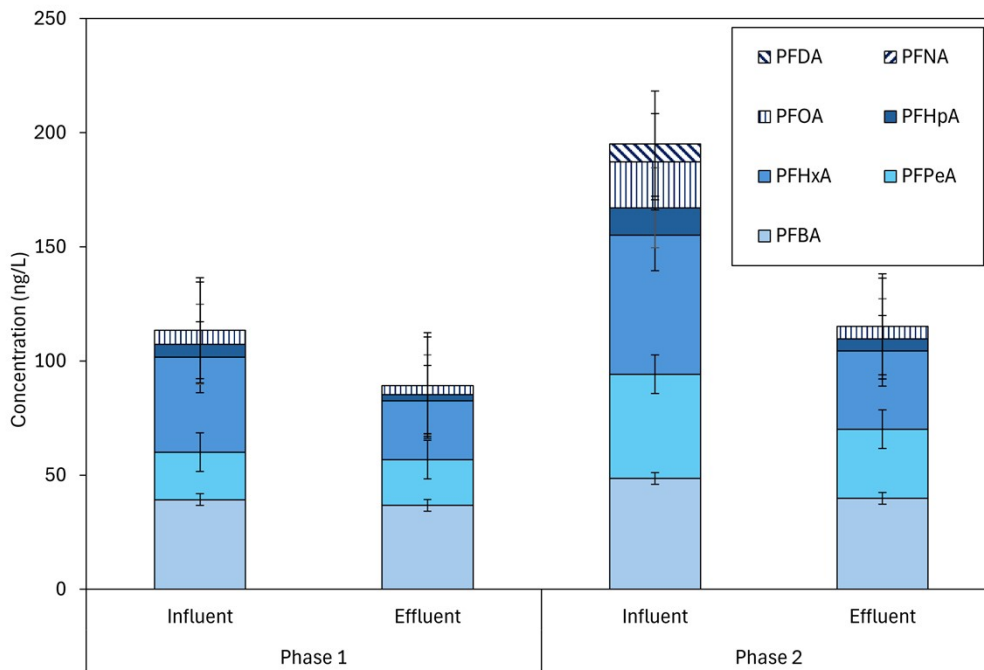
**Figure S6.** Distribution of PFAS Post-TOP Assay in WWTP A. Mean of triplicate samples; error bars represent standard errors.



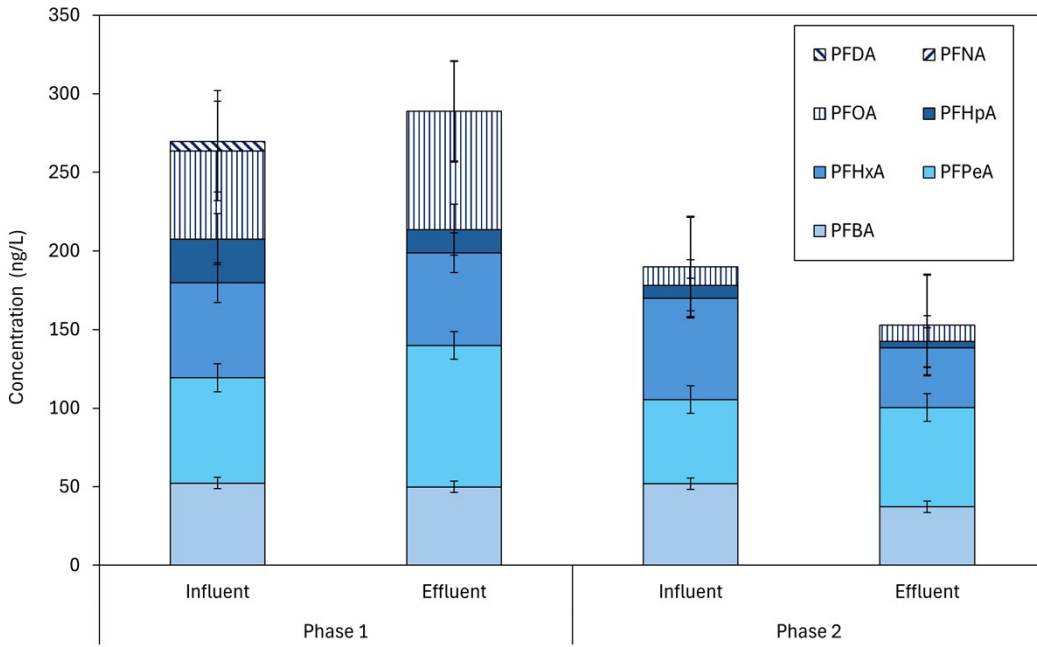
**Figure S7.** Distribution of PFAS Post-TOP Assay in WWTP B. Mean of triplicate samples; error bars represent standard errors.



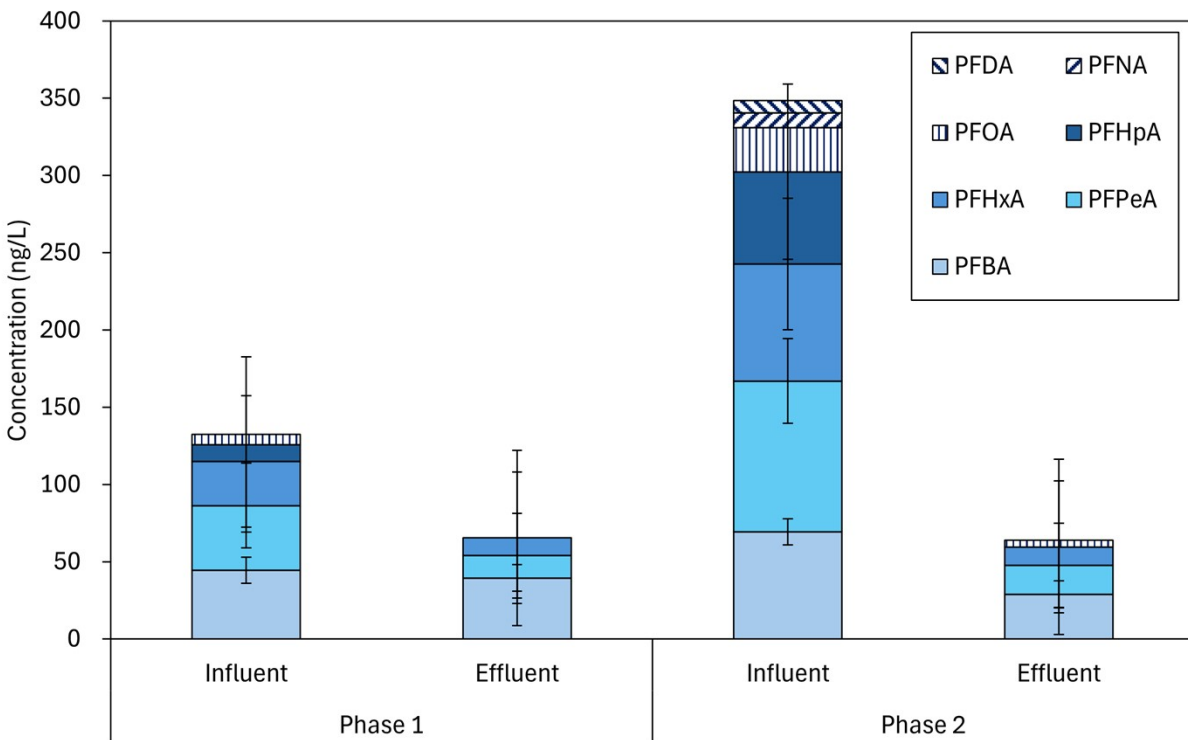
**Figure S8.** Distribution of PFAS Post-TOP Assay in WWTP C. Mean of triplicate samples; error bars represent standard errors.



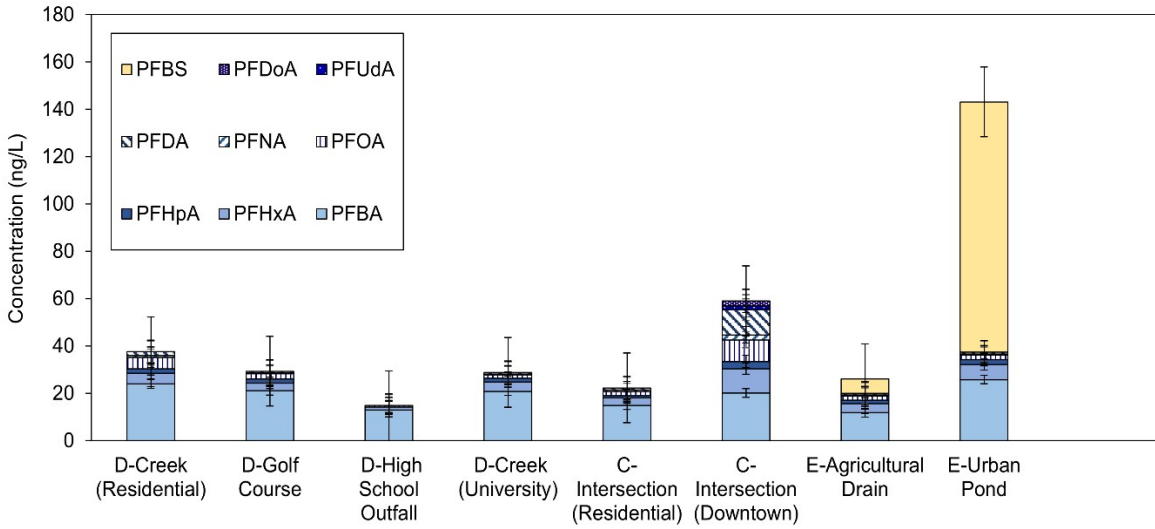
**Figure S9.** Distribution of PFAS Post-TOP Assay in WWTP D. Mean of triplicate samples; error bars represent standard errors.



**Figure S10.** Distribution of PFAS Post-TOP Assay in WWTP E. Mean of triplicate samples; error bars represent standard errors.

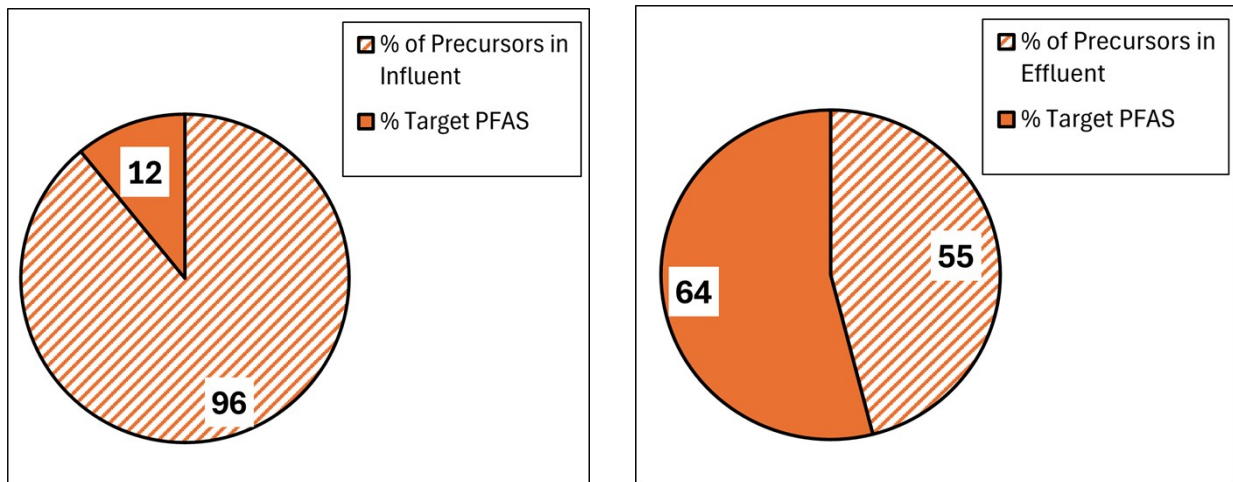


**Figure S11.** Distribution of PFAS Post-TOP Assay in WWTP F. Mean of triplicate samples; error bars represent standard errors.



**Figure S12.** Distribution of PFAS Post-TOP Assay in Stormwater Samples. Mean of triplicate samples; error bars represent standard errors.

### Results: Precursor PFAS Fractions for Phase 1



**Figure S13.** WWTP A influent (left) and effluent (right)

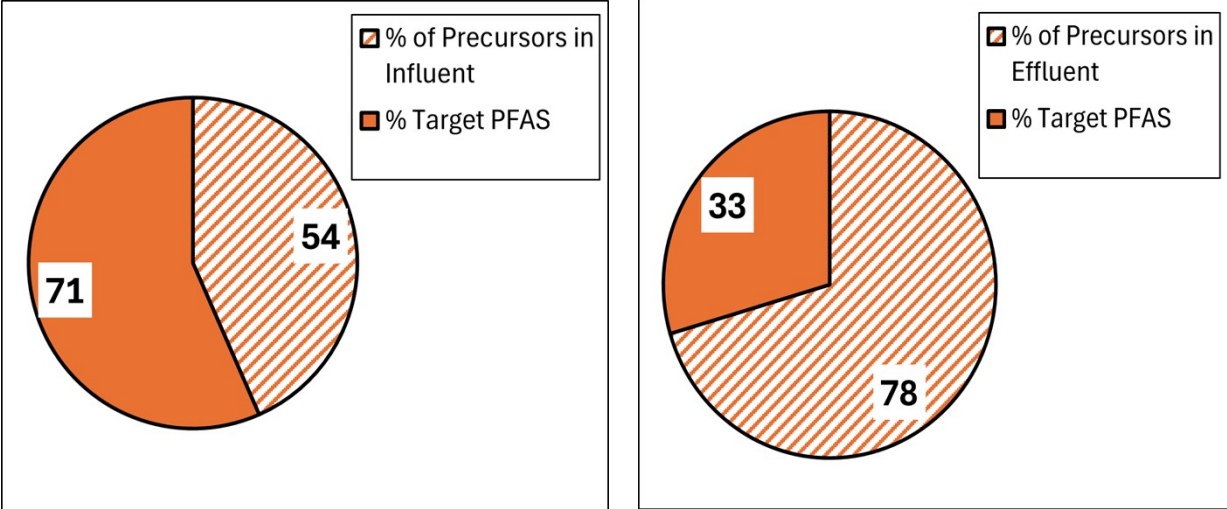


Figure S14. WWTP B (left) and effluent (right)

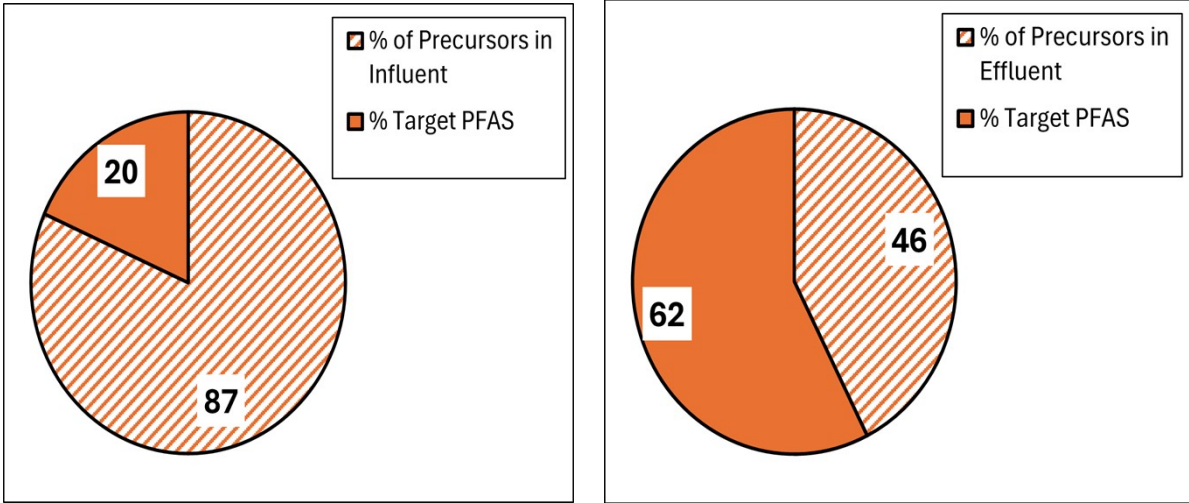


Figure S15. WWTP C influent (left) and effluent (right)

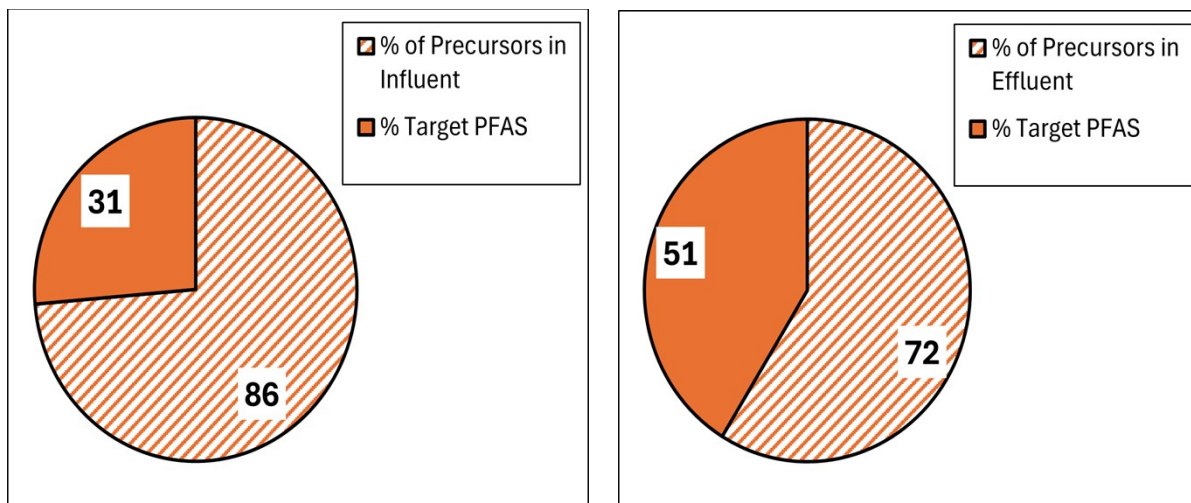


Figure S16. WWTP D influent (left) and effluent (right)

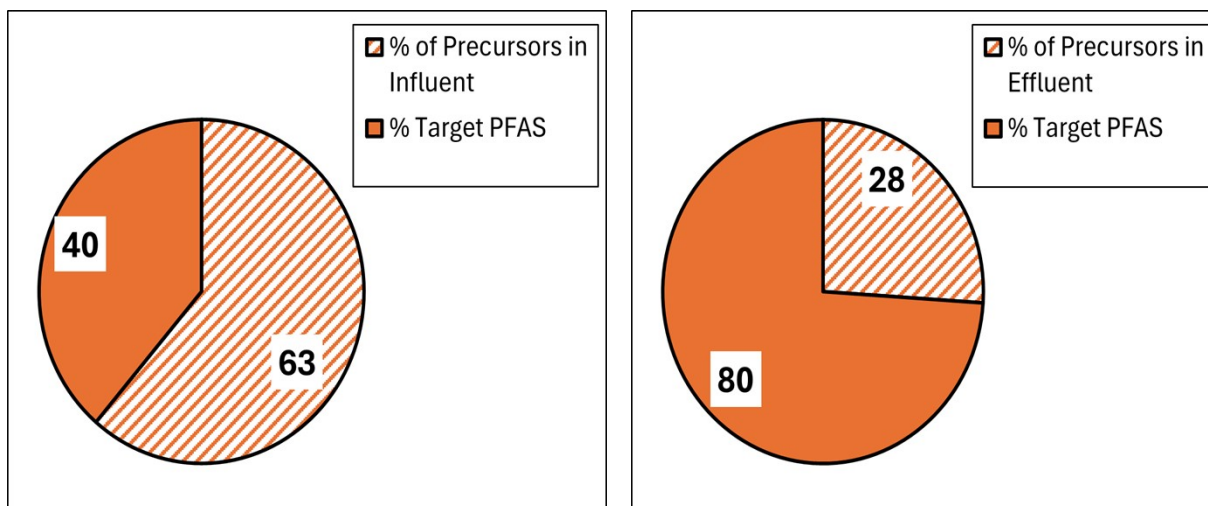


Figure S17. WWTP E influent (left) and effluent (right)

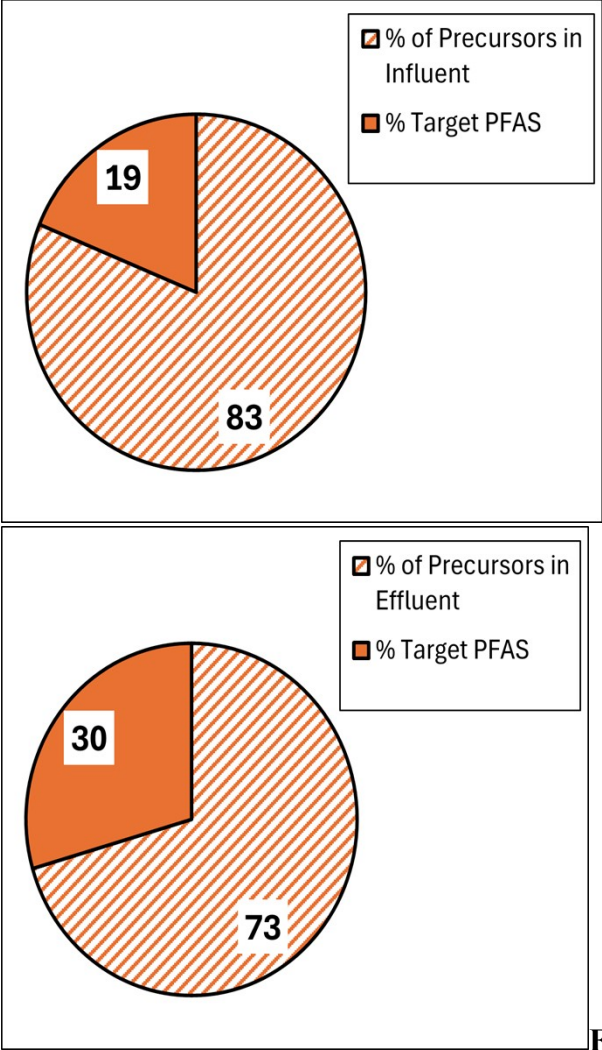


Figure S18. WWTP F influent (left) and effluent

(right)

Results: Precursor PFAS fractions for Phase 2

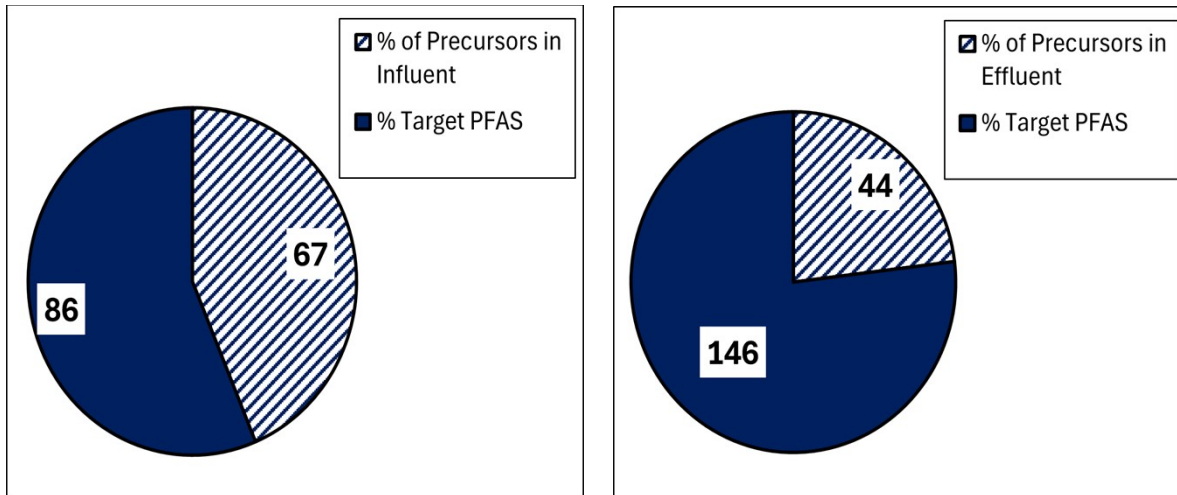


Figure S19. WWTP A influent (left) and effluent (right)

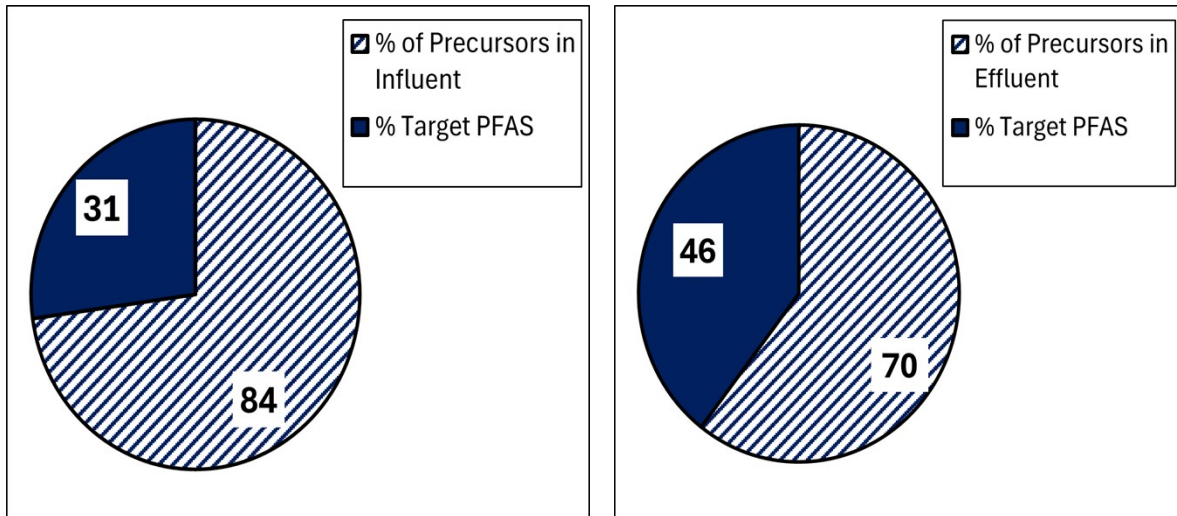
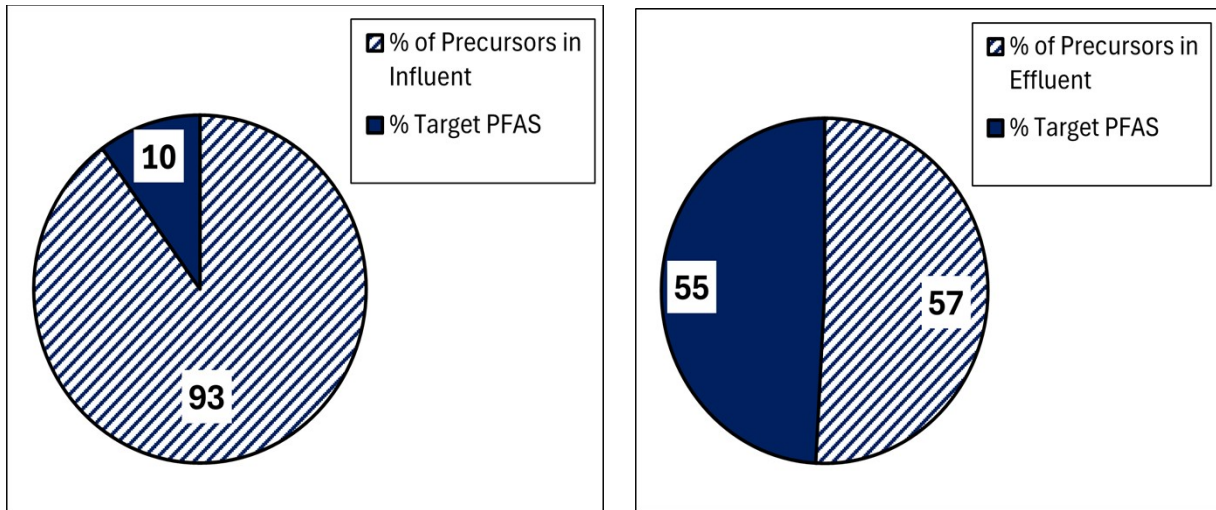
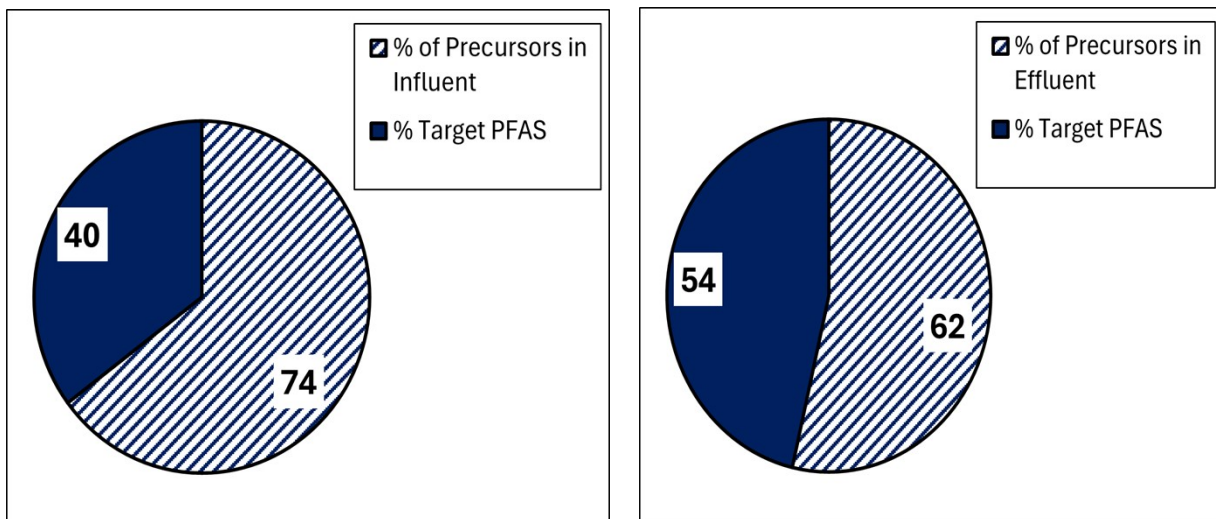


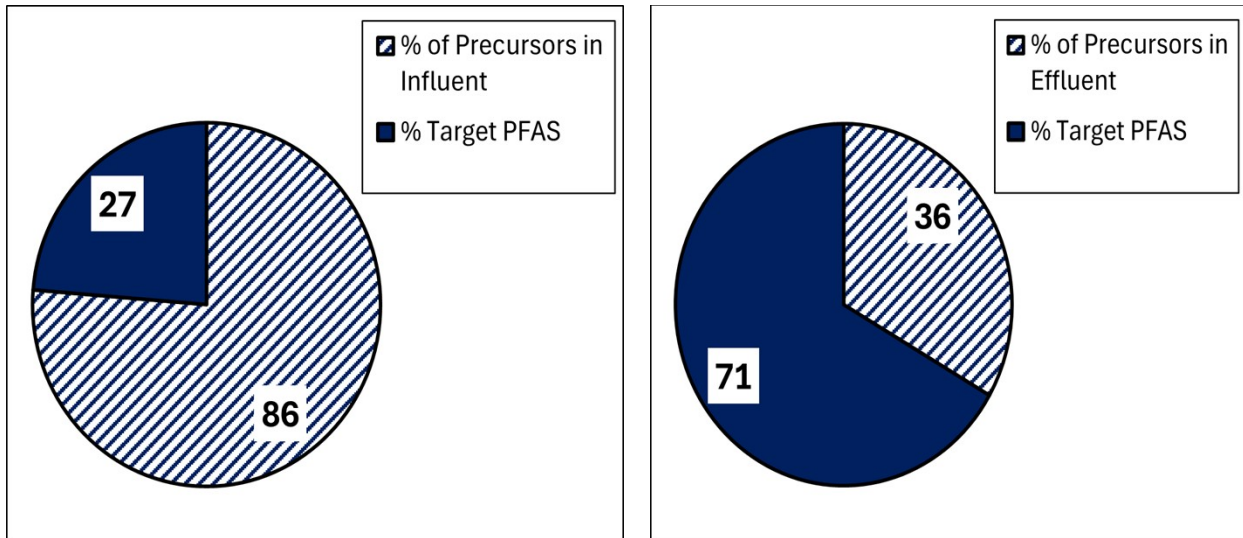
Figure S20. WWTP B influent (left) and effluent (right)



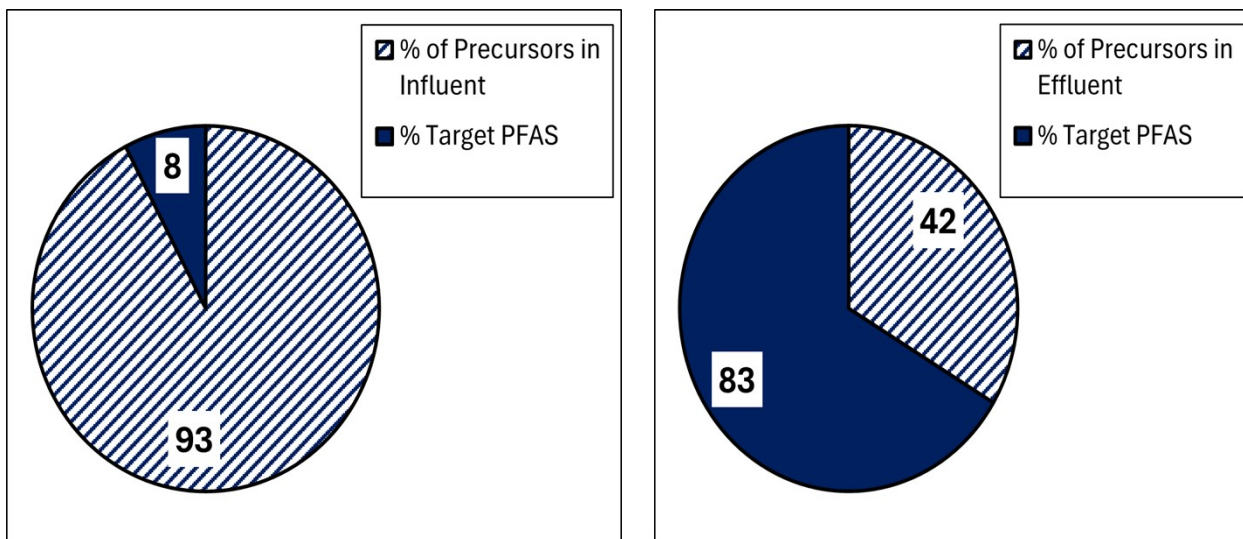
**Figure S21.** WWTP C influent (left) and effluent (right)



**Figure S22.** WWTP D influent (left) and effluent (right)



**Figure S23.** WWTP E influent (left) and effluent (right)



**Figure S24.** WWTP F influent (left) and effluent (right)

## References

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<https://www.weather.gov/wrh/climate?wfo=dmx> (accessed 2024-03-07).
- (2) Cappiella, K.; Brown, K. Impervious Cover and Land Use in the Chesapeake Bay Watershed. *Watershed Protection Techniques* **2001**, 3 (4), 0\_1.
- (3) Center for Watershed Protection; 8391 Main Street; Ellicott City, MD 21043. *New York State Stormwater Management Design Manual*.  
<https://extapps.dec.ny.gov/fs/docs/pdf/stormwaterdesignmanual2015.pdf>.
- (4) *The Simple Method to Calculate Urban Stormwater Loads*.  
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