



A Holding Time Evaluation of the Stability of “Forever Chemicals” in Wastewater

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Environment Testing
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EPA published a draft method 1633 that introduces the need for storing aqueous, solids, biosolids and tissue samples at freezing temperature $\leq -20^{\circ}\text{C}$ until sample preparation for maintaining and/or extending sample holding times. There has been some push-back and questioning as to the need for such a requirement for compounds considered “forever chemicals”.

The objective of this study was to understand the effects of storage temperature on the stability of 70 per- and polyfluorinated alkyl substances (PFAS) in two different kinds of water.

70 PFAS STUDIED – 11 groups

Perfluoroalkyl acids (PFAAs)

Short-chain perfluorocarboxylic acids (PFCAs)

PFPrA, PFBA, PFPeA,
PFHxA, PFHpA

Long-chain PFCAs
PFOA, PFNA, PFDA, PFDoA
PFTeDA, PFTrDA, PFuDA,
PFHxDA, PFODA

Short-chain perfluoro sulfonates (PFSAs)

PFBS, PFPeS

Long-chain (PFSAs)
PFHxS, PFHpS, PFOS
PFNS, PFDS, PFDoS

70 PFAS STUDIED – 11 groups

Precursors

Fluorotelomer sulfonic acids (FTSs)

4:2 FTS 8:2 FTS
6:2 FTS 10:2 FTS

Fluorotelomer carboxylic acids (FTCAs)

3:3 FTCA 7:3 FTCA
5:3 FTCA 8:2 FTCA
6:2 FTCA 10:2 FTCA

Fluorotelomer unsaturated carboxylic acids (FTUCAs)

6:2 FTUCA
8:2 FTUCA
10:2 FTUCA

Perfluoro sulfonamidoacetic acid (FOSAAs)

N-MeFOSAA, N-EtFOSAA

Perfluoro sulfonamide (FOSAs)

FOSA, N-MeFOSA, N-EtFOSA

Perfluoro sulfonamidoethanol (FOSAEs)

2-(N-ethylperfluoro-1-octanesulfonamido) ethanol
2-(N-methylperfluoro-1-octanesulfonamido) ethanol

70 PFAS STUDIED – 11 groups

Other polyfluoroacids

DONA	PFECA A	PFO ₃ DA
EVE Acid	PFECA B	PFO ₄ DA
Hydro-EVE Acid	PFECA F	PES
Hydrolyzed PSDA	PFECA G	PFP _r S
Hydro-PS Acid	PFECHS	PMPA
MTP	PFM _{OO} AA	PPF Acid
NVHOS	PFO ₂ H _x A	PS Acid
R-EVE	R-PSDA	R-PSDCA
TAF	Perfluoro(2-propoxypropanoic) acid	

EXPERIMENTAL DESIGN

Samples

1. Effluent wastewater
2. Lab spiked water

Storage conditions

1. Room temp (20°C)
2. Refrigerator temperature (1 to 6°C)
3. Freezing temperature (- 20°C)
4. Room temperature, pH 3

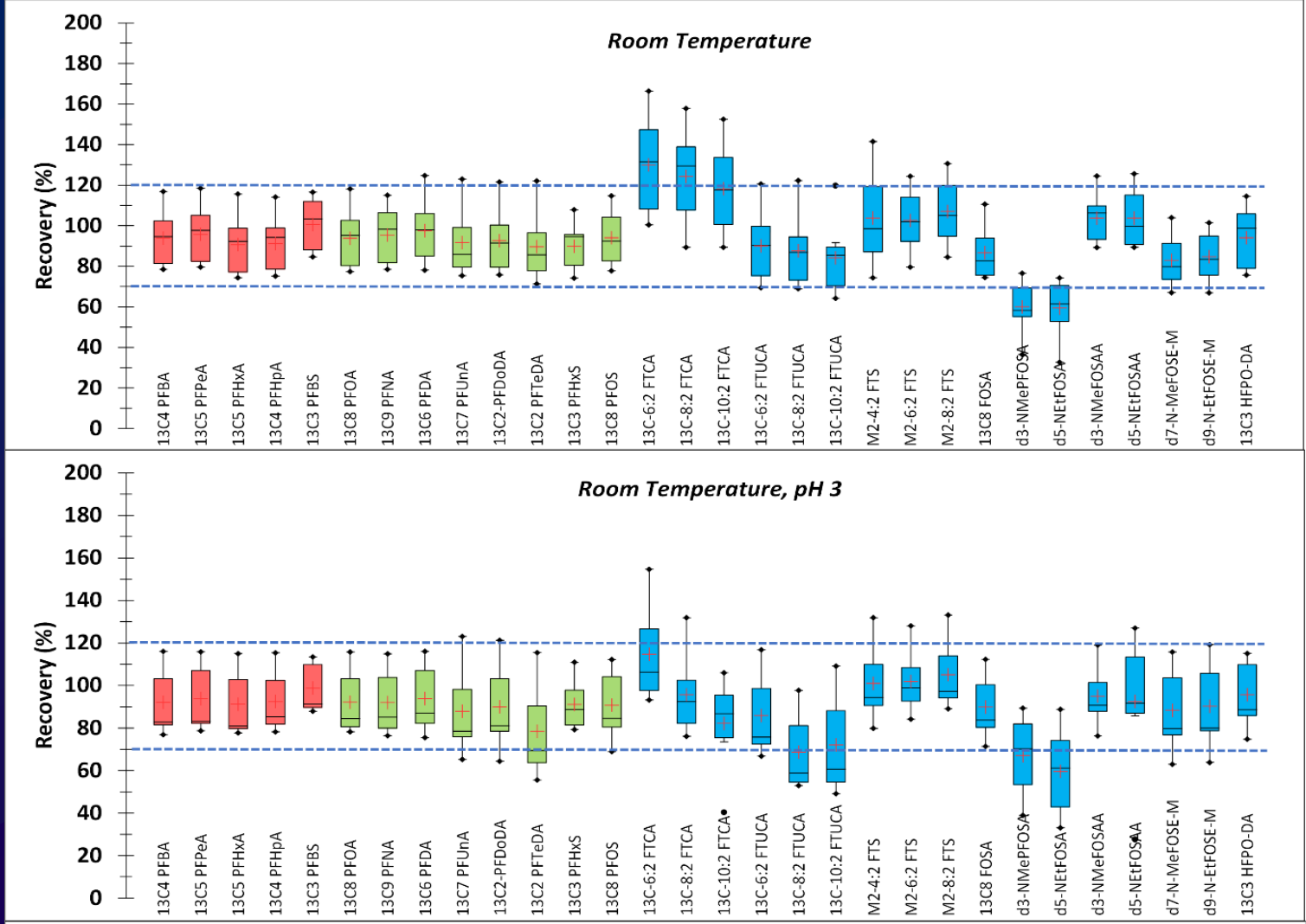
Extraction schedule

0, 1 , 3, 8, 15, 21 and 35th day
Extraction volume – 250 mL in duplicate
Solid phase extraction (SPE)

Analytical

LC-MS/MS – Sciex 5500 Triple Quad

Recoveries of Mass Labeled PFAS during Storage



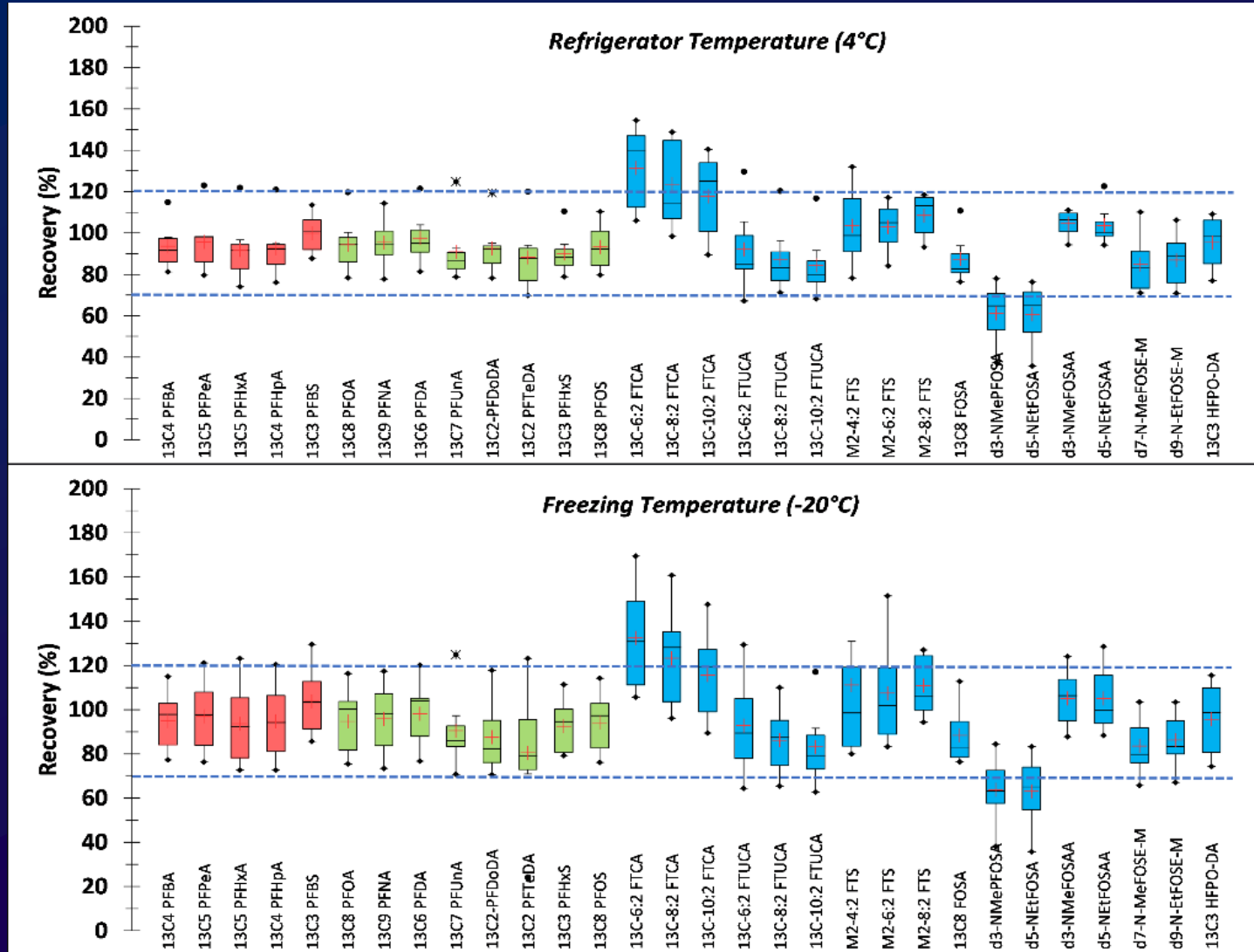
Spiked Lab Water – 25 ng/mL

- Recoveries for all the PFAS ranged between ~60 and 140%
- No difference among storage conditions

Dashed lines – recovery window (70 – 120%)

Red – Short-chain PFAAs
Green – Long-chain PFAAs
Blue – Precursors

Recoveries of Mass Labeled PFAS during Storage



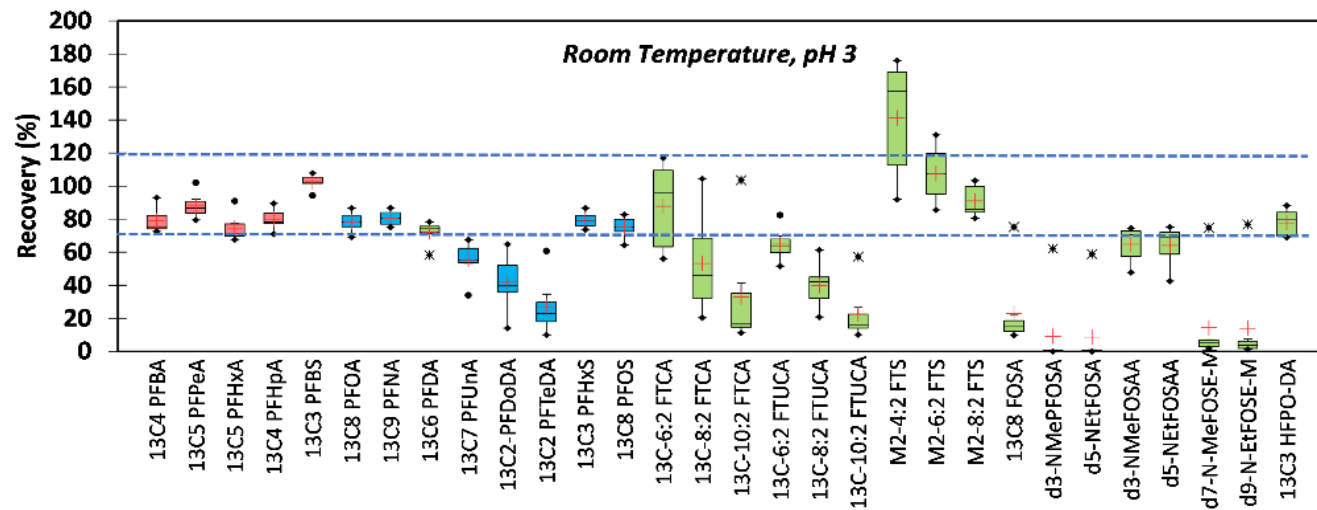
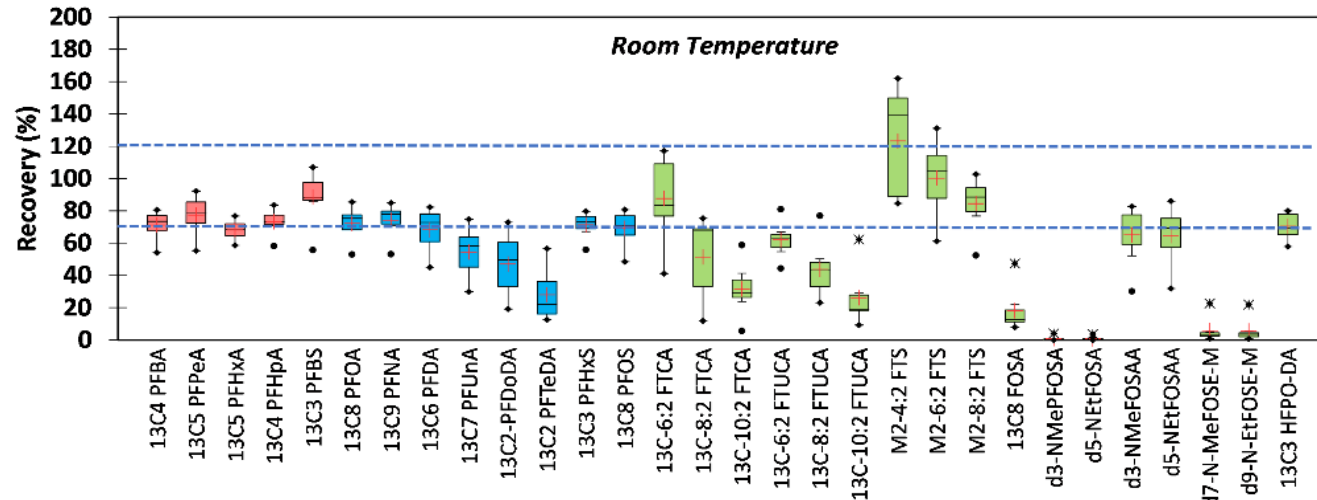
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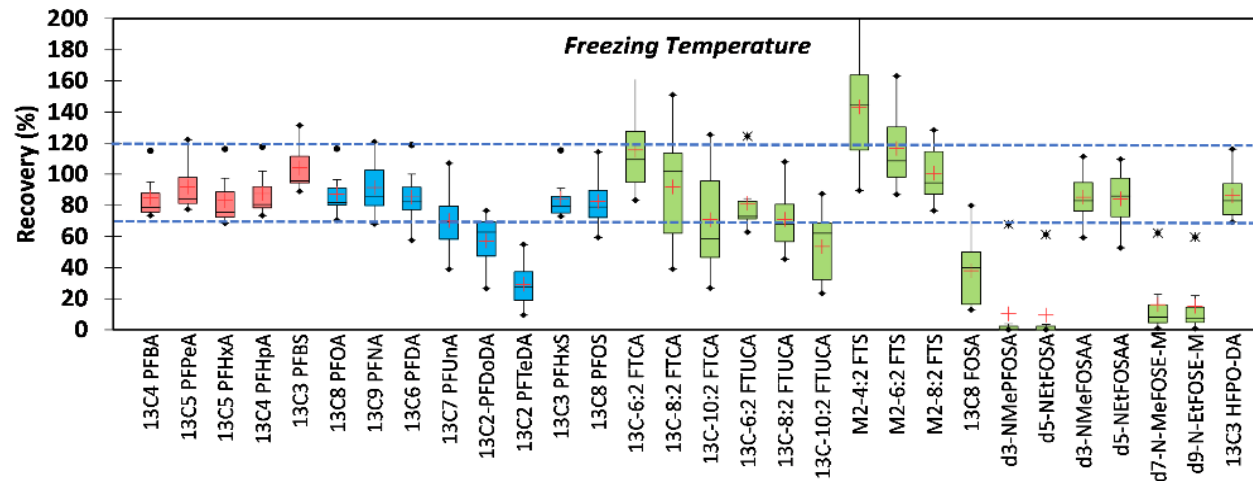
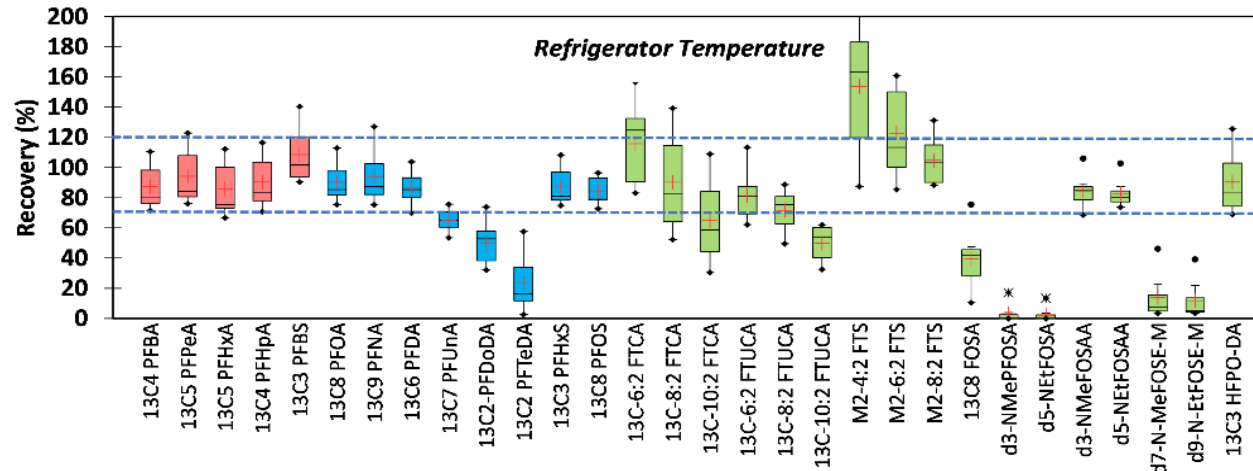
Spiked Effluent Wastewater – 25 ng/mL

- Perfluoroalkyl sulfonamides and sulfonamido ethanol showed very low recoveries of 4 and 22% on day 0.
- No significant difference among storage conditions

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Recoveries of Mass Labeled PFAS during Storage



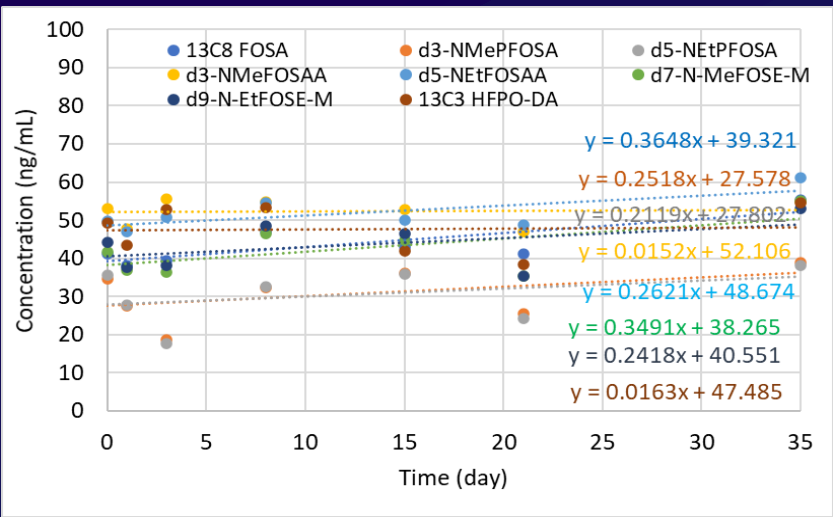
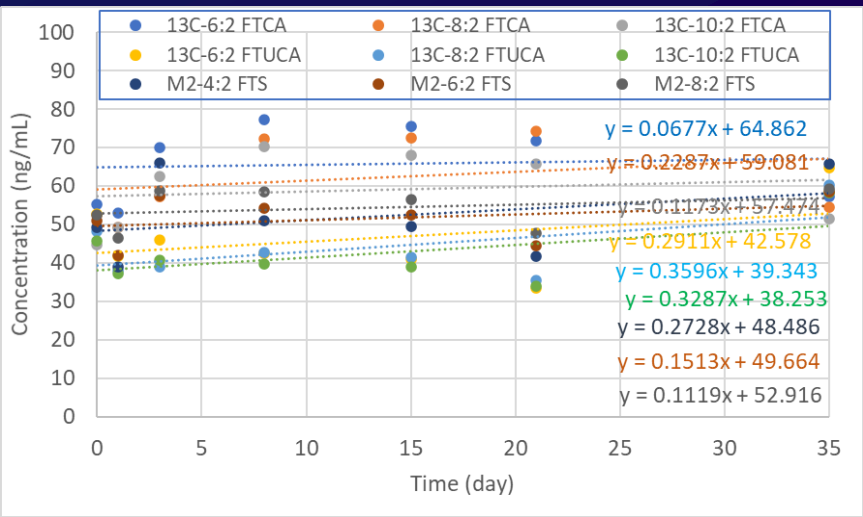
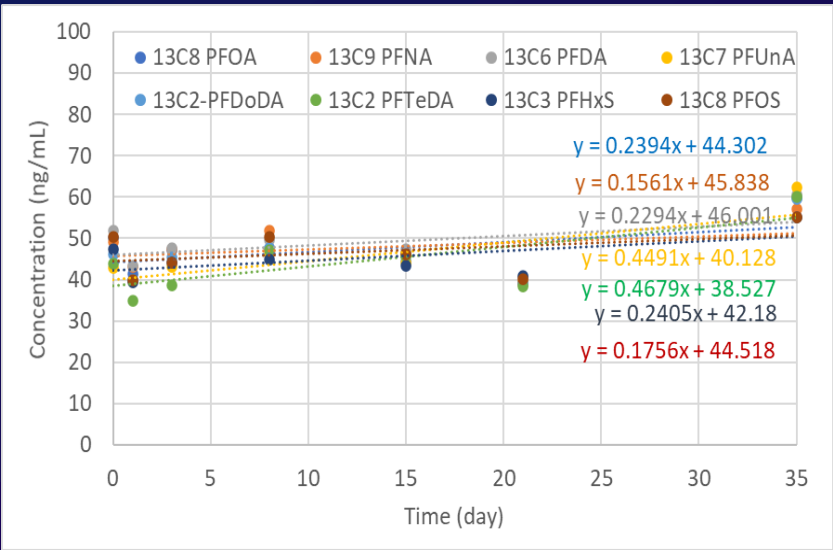
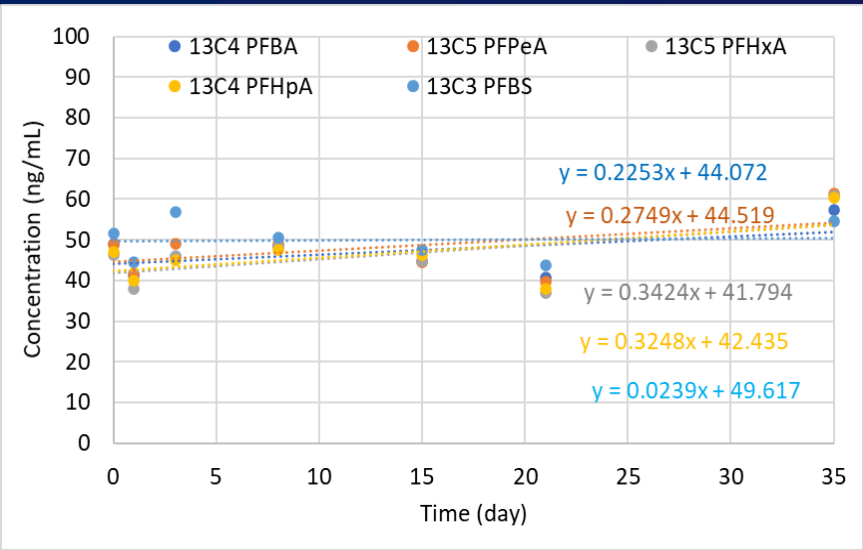
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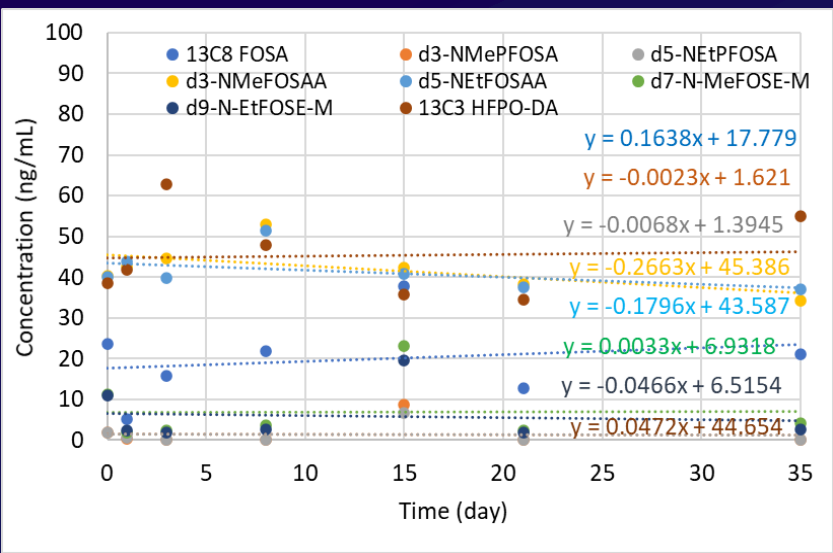
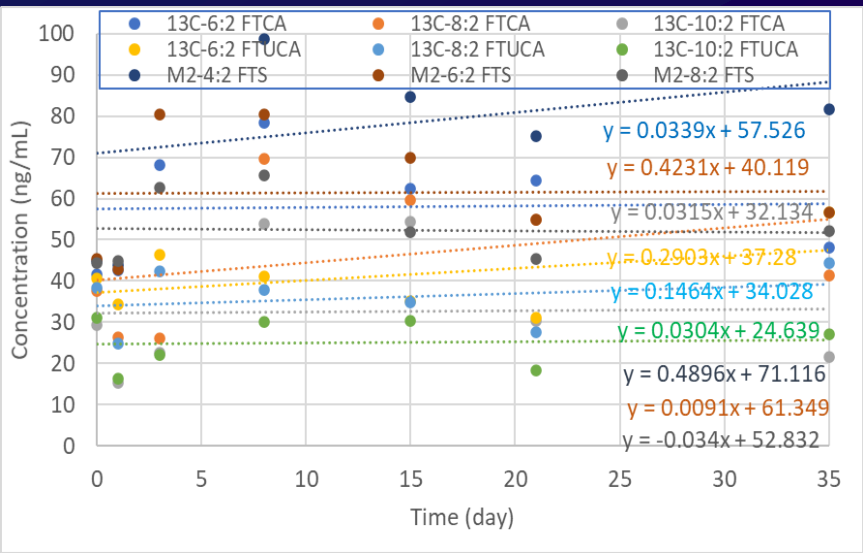
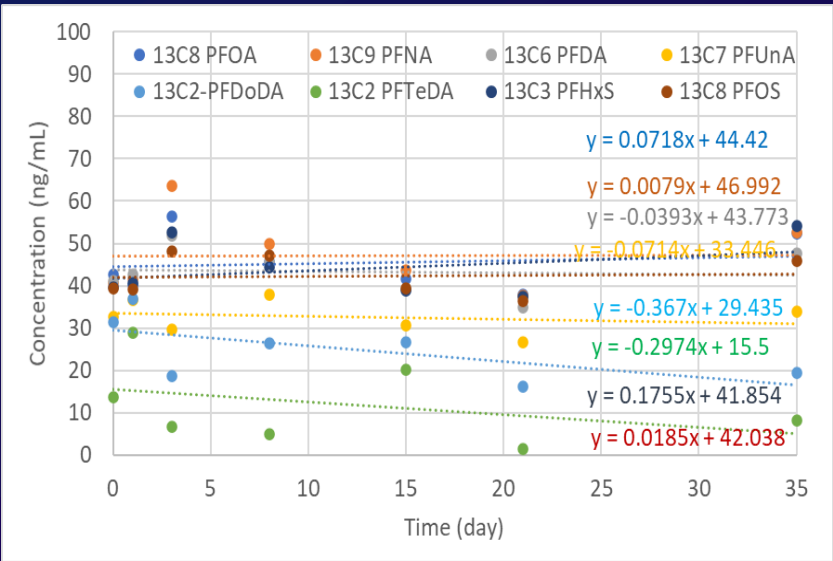
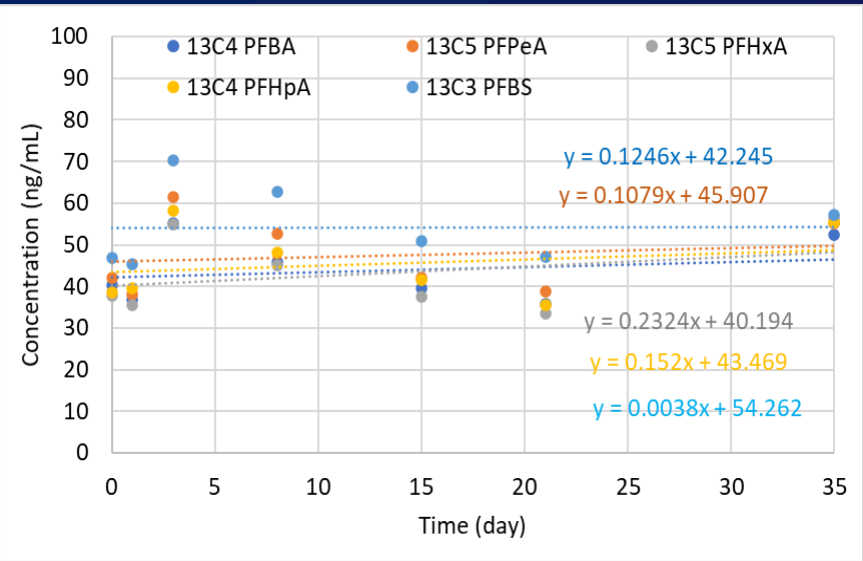
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Recoveries in Laboratory Water - Refrigeration



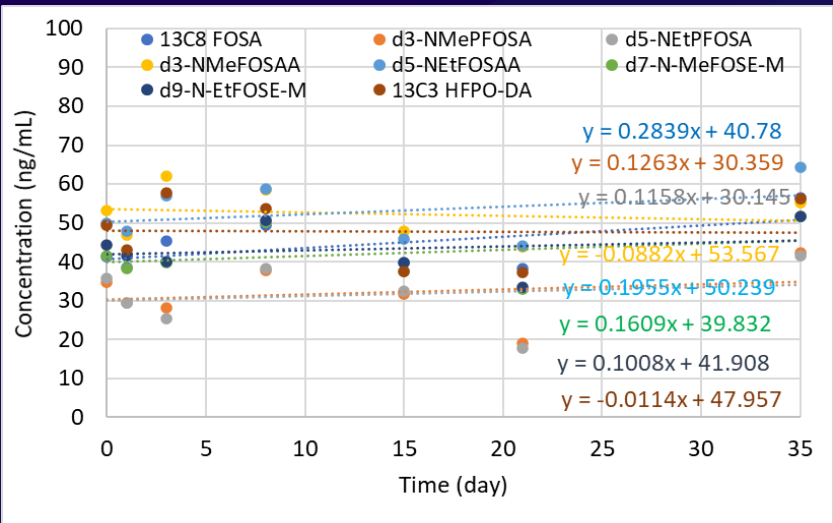
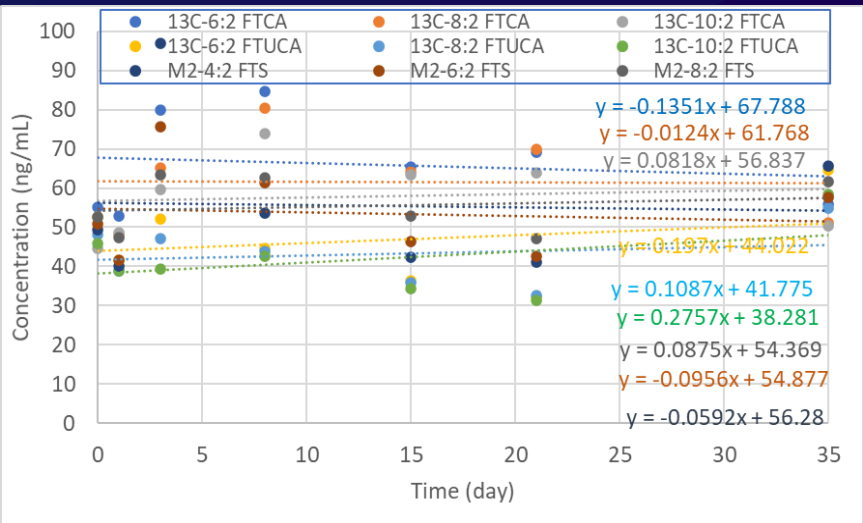
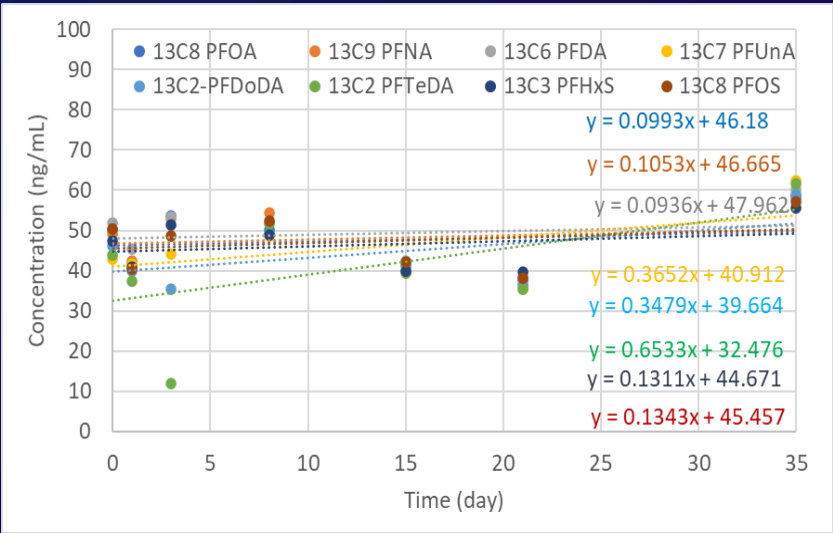
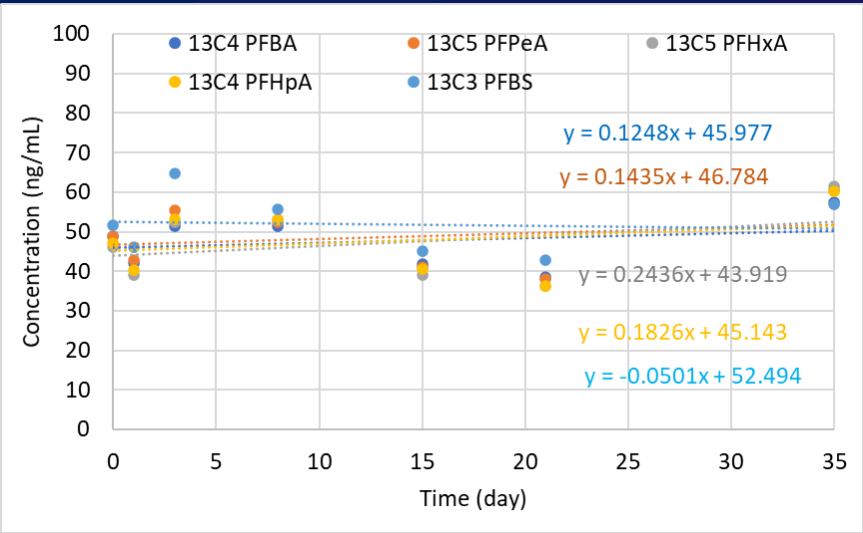
Time
dependent
concentration
profiles and
regression lines
of mass
labelled PFAS

Recoveries in Effluent Wastewater - Refrigeration



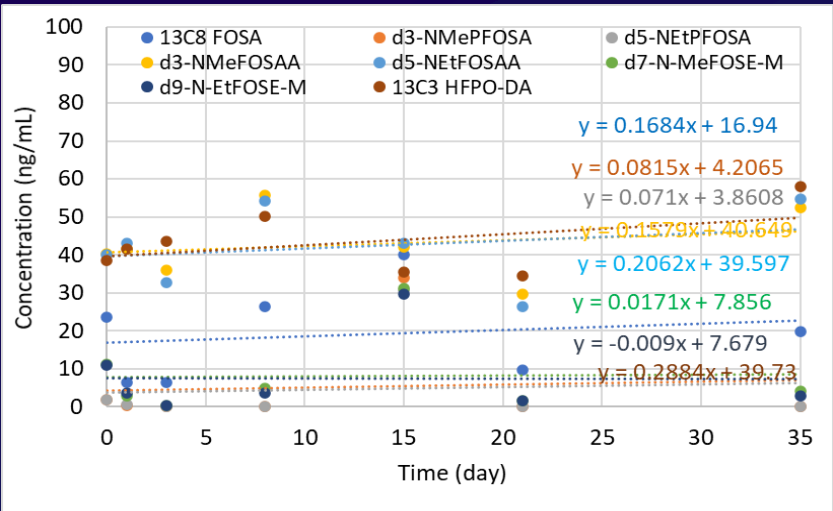
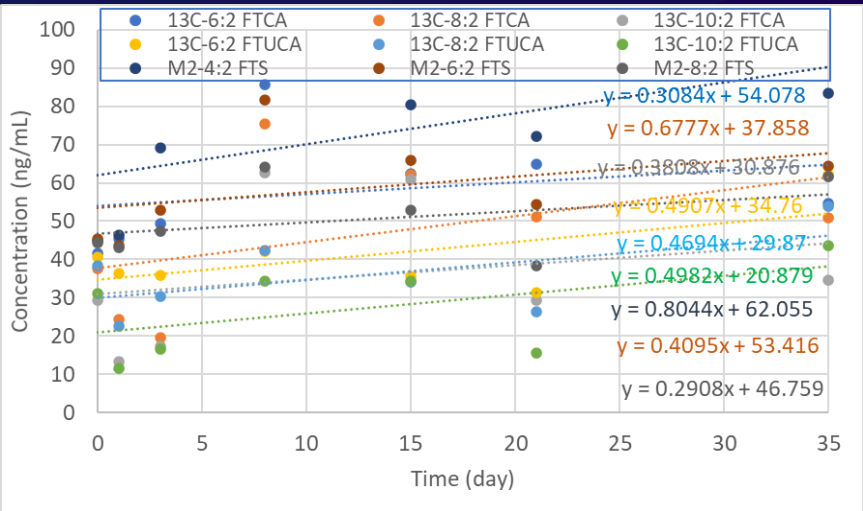
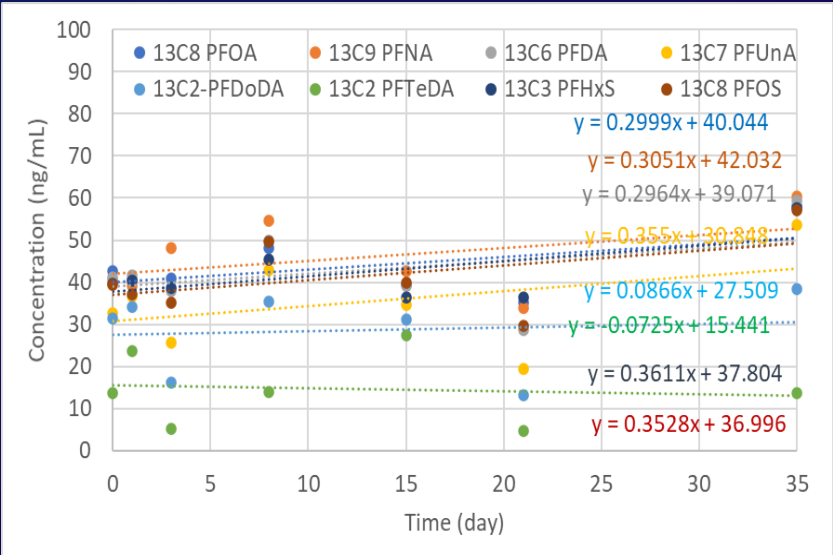
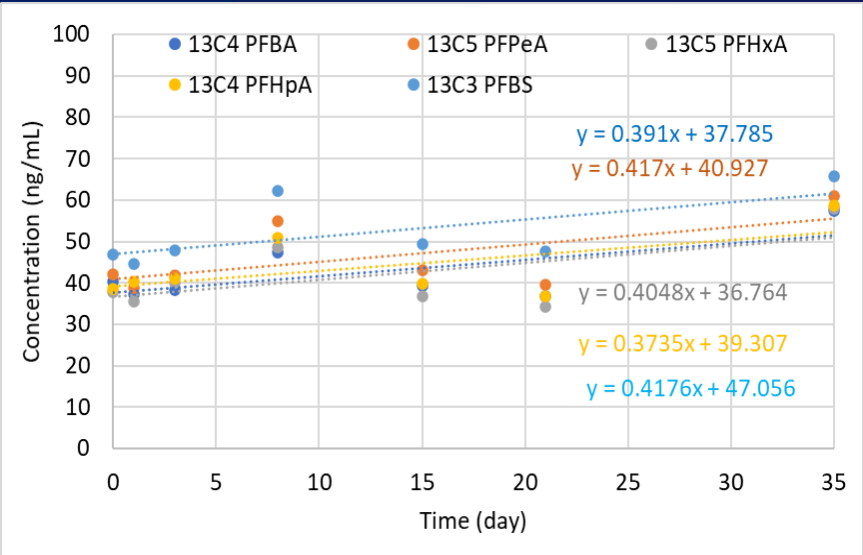
Time
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Recoveries in Laboratory Water – Frozen Storage



Time
dependent
concentration
profiles and
regression lines
of mass
labelled PFAS

Recoveries in Effluent Wastewater – Frozen Storage



Time
dependent
concentration
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Effect Of Storage Conditions On PFAS Stability

Spiked Lab Water

Regression coefficients (p-value)

	FTS	FTCAs	FOSAs	FOSAAs	FTUCAs	FOSAEs
Room Temperature	0.32 (0.53)	0.05 (0.95)	0.29 (0.64)	-0.39 (0.62)	0.08 (0.74)	-0.1 (0.62)
RT pH 3	0.14 (0.74)	-0.67 (0.30)	-0.31 (0.30)	-0.46 (0.14)	-0.07 (0.76)	-0.22 (0.39)
Refrigerator Temp	0.37 (0.44)	0.78 (0.25)	1.01 (0.06)	0.10 (0.77)	0.15 (0.56)	0.15 (0.60)
Freezer Temp	0.24 (0.62)	-0.12 (0.87)	0.19 (0.74)	-0.04 (0.89)	0.02 (0.93)	0.08 (0.75)
	Short-chain PFCAs	Long-chain PFCAs	Short-chain PFSA	Long-chain PFSA	Other polyfluoroacids	
Room Temperature	0.00 (0.99)	0.42 (0.76)	0.09 (0.77)	0.28 (0.74)	-0.05 (0.99)	
RT pH 3	0.00 (0.99)	-0.26 (0.84)	-0.05 (0.88)	-0.21 (0.84)	-0.13 (0.97)	
Refrigerator Temp	-0.02 (0.98)	0.75 (0.60)	0.08 (0.78)	0.25 (0.76)	0.18 (0.96)	
Freezer Temp	-0.18 (0.80)	0.1 (0.94)	0.04 (0.88)	0.22 (0.79)	-1.13 (0.75)	

None of the PFAS group showed increasing or decreasing trend of statistically significant ($p = 0.05$)

Effect Of Storage Conditions On PFAS Stability

Spiked Wastewater

Regression coefficients (p-value)

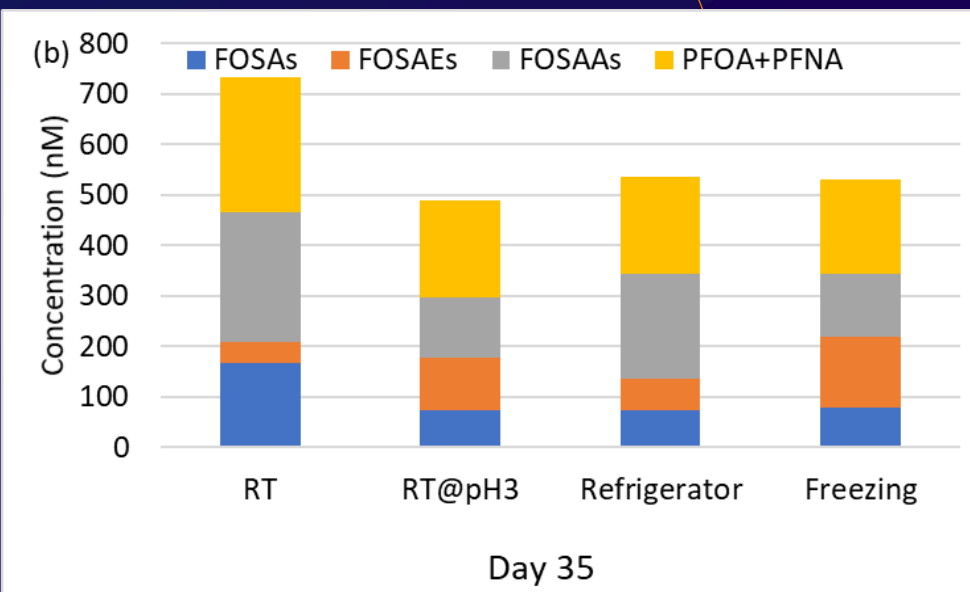
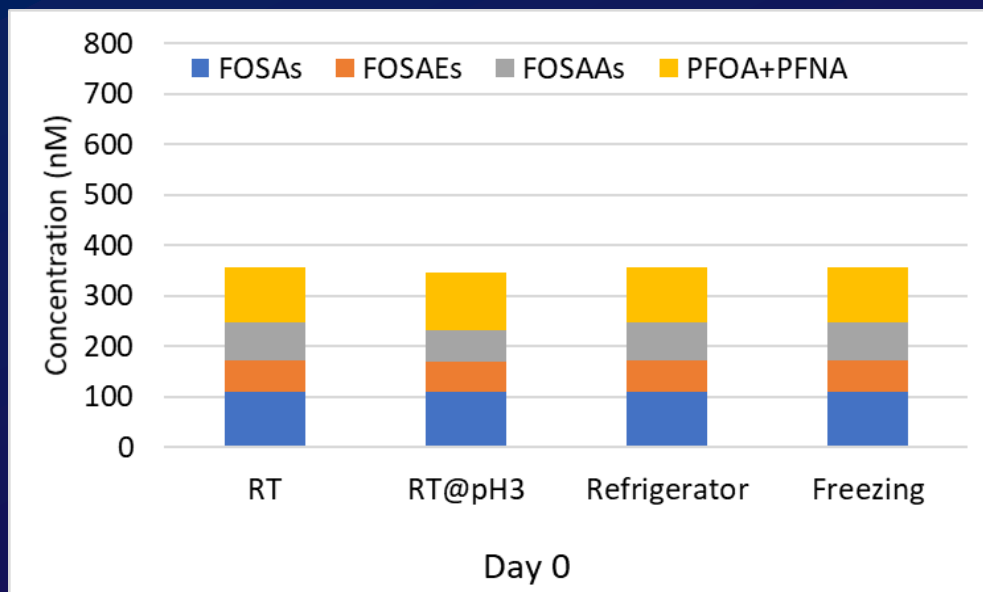
	FTS	FTCAs	FTUCAs	FOSAs	FOSAAs	FOSAEs
Room Temperature	1.3 (0.03)	-1.9 (0.06)	-1.87 (0.02)	0.98 (0.14)	3.06 (0.00)	-0.46 (0.21)
RT pH 2-3	1.6 (0.02)	-0.35 (0.7)	0.9 (0.03)	-0.4 (0.05)	0.8 (0.02)	0.55 (0.04)
Refrigerator Temp	1.34 (0.02)	0.59 (0.36)	1.08 (0.03)	-0.5 (0.09)	1.88 (0.00)	-0.02 (0.92)
Freezer Temp	1.51 (0.01)	2.51 (0.01)	1.6 (0.00)	-0.35 (0.6)	0.66 (0.00)	1.31 (0.02)
	Short-chain PFCAs	Long-chain PFCAs	Short-chain PFSA	Long-chain PFSA	Other polyfluoroacids	
Room Temperature	1.91 (0.03)	4.11 (0.00)	0.63 (0.01)	0.93 (0.14)	13.3 (0.01)	
RT pH 2-3	2.58 (0.02)	4.79 (0.00)	0.89 (0.01)	1.67 (0.06)	18.6 (0.01)	
Refrigerator Temp	2.17 (0.06)	4.34 (0.00)	0.85 (0.02)	1.17 (0.21)	16.07 (0.03)	
Freezer Temp	1.99 (0.05)	4.60 (0.00)	0.73 (0.02)	1.57 (0.08)	7.72 (0.22)	

Statistically significant values are shown in **bold**

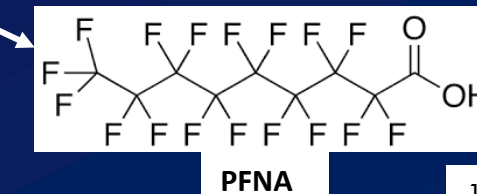
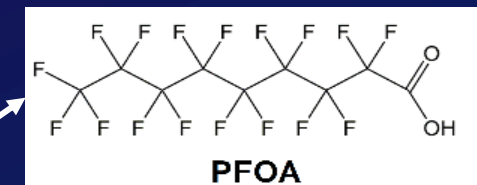
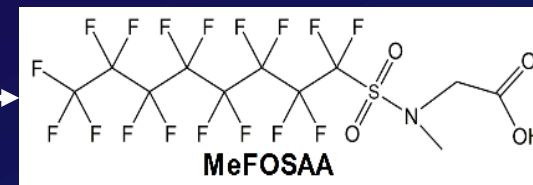
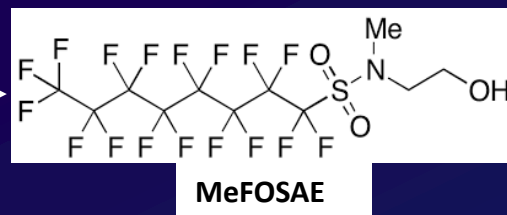
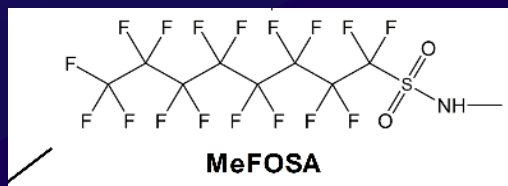
None of the studied storage condition was found appropriate for the stability of PFAS for effluent wastewater during storage period of 35 days

Mass balance and interconversion of PFAS

Spiked Wastewater



A range of 140–200% mass balance closure for interconversion of FOSAs, FOSAEs, FOSAA and PFOA+PFNA was achieved.



- None of the storage conditions (RT, RT@pH3, refrigeration and frozen storage temperature) provided an appropriate environment for maintaining the stability of all the PFAS in effluent wastewater studied over 35 days.
- Laboratory grade water showed little to no difference between the four storage conditions
- Room Temperature performed no worse (but also not better) than the frozen storage conditions

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QUESTIONS?

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Environment Testing
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