Results of Analyses of Sludge and Sludge-Applied Soils From the September 2008 Decatur, AL Reconnaissance Study

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Background

The Decatur, AL wastewater treatment plant (WWTP) processes waste and wastewater from various public and private sectors within and surrounding the local Decatur community. This includes the waste and wastewater from several industries that manufacture or use perfluorinated chemicals (PFCs). Processed Decatur WWTP sludge materials, along with municipal sludge materials shipped from New York City, NY, have been applied to designated agricultural fields near Decatur, AL for more than ten years. In 2007, EPA and the U.S. PFC industry initiated the PFOA (perfluorooctanoic acid) Stewardship Program (http://www.epa.gov/oppt/pfoa/pubs/pfoastewardship.htm), a program with the objective of eliminating PFOA emissions by 2015. While reviewing information submitted through this program, EPA's Office of Pollution Prevention and Toxics (OPPT) expressed concern that the field application of municipal WWTP sludge materials might present a major PFC exposure pathway for PFOA and other related PFCs. OPPT requested that EPA Region IV, supported by the Office of Research and Development's (ORD) National Exposure Research Laboratory (NERL), conduct a small scale reconnaissance screening level study in Decatur, AL to generate a limited dataset that could be used by the Agency to better understand the potential for environmental PFC exposures. This report provides a brief overview of the field sample collection activities and summarizes ORD/NERL's analytical results supporting this reconnaissance study.

Sample Collection and Analysis

In September 2007, Region IV scientists conducted a small scale reconnaissance study and collected a very limited number of environmental samples. The samples collected included: sludge samples from the Decatur WWTP and from bulk NYC sludge materials; and surface soil samples from fields where municipal sludge had been applied as well as surface soil samples in near-by fields where municipal sludge had not been applied. A primary set of samples was collected using methanol-rinsed, stainless-steel sampling equipment and then stored/shipped in NERL pre-cleaned (3x methanol rinse) HDPE containers, without preservatives. Additional samples were collected by the Region IV scientists and shipped to other organizations for their analyses. Special precautions were taken in the field to prevent inadvertent sample contamination during the sampling, storage and/or shipping processes. The primary set of samples was shipped to NERL's Ecosystems Research Division (ERD) for analysis using research methods developed and/or modified for this study.

ERD scientists analyzed nine soil samples for the targeted PFCs (Table 1). Table 1 includes the compound name, acronym, and ERD analytical method. The nine soil samples included:

- soil samples from four sludge-applied fields (sites 3, 6, 7, and 9)
- two duplicate soil samples taken in close proximity to the primary soil samples at sites 3 and 7
- two background soil samples taken from nearby fields where sludge materials had not been applied, and,
- one commercially-available Ottawa sand field blank sample (the sand blank was transported to the field, transferred to another container in the field, and returned to the laboratory). The Ottawa blanks have been shown to have low levels of PFCs.

The sludge-applied soil samples were analyzed for perfluorocarboxylic acids having carbon chain lengths ranging from C_3 to C_{14}; fluorotelomer carboxylic acids having carbon chain lengths of $C_{6:2}$, $C_{8:2}$and $C_{10:2}$; perfluorosulfonates having carbon chain lengths of C_4, and C_6-C_{10}; the 8:2 fluorotelomer acrylate; and fluorotelomer alcohols ranging in carbon chain lengths from $C_{6:2}$ to $C_{14:2}$. In addition to the soil samples, the Region IV scientists collected five sludge samples (two from Decatur and three from New York City). These five sludge samples were analyzed by the ERD scientists for the targeted perfluorocarboxylic acids (carbon chain lengths ranging from C_6 to C_{14}) and for perfluorooctane sulfonate (Table 1). In addition, a commercially purchased soil sample was also extracted and analyzed in the laboratory to characterize any potential bias resulting from the laboratory extraction and analysis processes. Copies of the ERD analytical SOPs and related materials are attached (Appendix A).

A second set of six sludge-applied soil samples (from the four primary sites plus duplicates as noted above) was obtained indirectly by NERL's Human Exposure and Atmospheric Sciences Division (HEASD) through a collaborative research program with a local university. The Region IV sampling team noted that these sludge-applied soil samples were collected in very close proximity to the primary sludge-applied soil samples provided to ERD. However, these samples are not duplicates of the primary samples analyzed by ERD. The HEASD scientists analyzed this second set of sludge-applied soil samples for selected perfluorocarboxylic acids and perfluorosulfonates using an independent research method developed previously for characterizing PFCs in soils and housedust. Since this second set of soil samples are neither extracts from homogenized primary samples nor true duplicates of the primary samples, the HEASD results can only provide confirmatory data for evaluating the efficacy of characterization of PFCs in this uniquely complex matrix. As such, this report focuses on the ERD methodologies as these are considered the primary samples.

It is important to note that the sampling and analysis of environmental soil and sludge samples for PFCs constitute novel analytical challenges as soil and sludge chemistry are very complex processes. In addition, the land application of sludge material likely results in a non-uniform distribution of sludge across the area, a factor that must be considered in the interpretation and use of the analytical results. Also, the Region IV and NERL scientists developed and/or modified readily available research methods, methods produced for other PFC-related research activities, over a very short time period to support the Region IV program objectives.

Sample Preparation and Analysis

The primary sludge-applied soil samples were sieved through a methanol-washed, stainless-

steel 2-mm sieve. The sieved soil samples were then extracted following an ERD Standard Operating Method (ERD SOP PMB 54.0) with modifications as described below. This method is unique in that it allows the recovery of the fluorotelomer alcohols as well as the other PFCs from each sample as opposed to splitting the sample, and analyzing one portion for alcohols and a separate portion for the other PFCs.

ERD SOP PMB 54.0 was originally developed for extracting perfluorocarboxylic acids (PFCAs) and fluorotelomer alcohols (FTOHs) from soil that contains small concentrations of fluorotelomer-based polymers. For this project, the material being extracted is a mixture of soil and sewage-treatment sludge. The SOP has not been tested for its PFCA and FTOH extraction efficacy with soil-sludge mixtures and, there is no known published method for the extraction of PFCAs and FTOHs from soil-sludge mixtures. Therefore, a pilot research effort was conducted to assess and improve the research methodology. First, the efficacy of employing the SOP was tested by extracting one sample with MTBE seven times in sequence and analyzing each extraction step to determine the number of extraction steps required to achieve satisfactory extraction efficacy. Based on these efforts, the SOP was modified and the remaining samples underwent four extraction steps in sequence as opposed to the standard three extraction steps. Secondly, to monitor extraction efficacy, instead of combining the MTBE extracts of each extraction step prior to analysis, each extract was analyzed individually to monitor the amounts of analytes liberated in each extraction step.

The sludge samples were extracted with a research developmental method. In summary, an aliquot of sludge was air-dried and pretreated with 1 M NaOH overnight; sonicated in methanol for 30 minutes; neutralized with HCl; shaken for 1 hour; centrifuged; extracted with methanol two more times; the extract blown down; and then treated with ion-pairing cleanup.

Analyses for the fluorotelomer alcohols in sludge-applied soils were performed on a gas chromatograph, mass spectrometer (GC/MS) operated in positive chemical ionization mode (ERD SOP PMB 54.0). The other PFCs were analyzed on a liquid chromatograph, tandem mass spectrometer (LC/MS/MS) with negative electro-spray ionization (ERD SOP PMB 52.0). All analytes were quantitated by isotopic dilution or internal standards.

Summary of Analytical Results

Since research grade methods were employed to support this screening level reconnaissance study, only those values calculated above the reporting limits are being provided. The procedures for calculating the reporting limits is provided in the Data Quality Control section below.

Table 2 summarizes the ERD analytical results for PFCAs for the soil samples. The highest concentrations of PFCAs measured in the sludge-applied soil samples were found in the C_6 through C_{12} chain lengths. PFOA (C_8) and perfluorodecanoic acid (C_{10}) were consistently higher than the other PFCAs in all the sludge-applied soil samples. The Site 3 yielded the highest levels (>2000 ng/g of soil or 2 parts per million (ppm) for both species) measured. Table 3 summarizes the other soil sample PFC analysis results. Perfluorooctane sulfonate (PFOS) was the only sulfonate detected above reporting limits, with the highest concentrations also measured at Site 3 (>1300 ng/g of soil or 1.3 ppm). None of the targeted fluorotelomer carboxylic acids were detected above the reporting limits. In addition, none of the targeted PFCs shown in Tables 2 or 3 were detected in either of the background soil samples or in the field sand blank above the reporting limits, suggesting no or very minimal background PFC levels. The blank sample results suggest the soil samples were not

inadvertently contaminated during the sampling, storage, and/or shipment processes.

A summary of the HEASD analytical results for the second set of similar soil samples is provided in Table 4. These data, generated via a different and independent technique, are very comparable to the ERD data. In general, the HEASD PFC levels are very similar, although slightly lower, to the ERD results discussed above and shown in Tables 2 and 3. Similar to the ERD results, the HEASD soil sample PFOA, perfluorodecanoic acid, and PFOS (in that order) levels are consistently higher (generally >50%) than the levels measured for the other targeted PFCs. The differences observed between the two laboratories' data most likely are associated with: 1) sample collection locations; 2) extraction methods; 3) analytical instrumentation and methodologies; 4) likely non-uniformity in the distribution of the sludge-applied materials; and 5) overall complexity in sampling, extracting, and analyzing soils and sludge materials.

ERD's fluorotelomer alcohol (FTOH) congener analysis results for the soil samples are summarized in Table 5. Reportable levels were observed for the 7:2 through 14:2 FTOH congeners, with the exception of the 13:2 FTOH congener. The 10:2 FTOH congener level for the Site 3 sample was the highest measured (>800 ng/g of soil or 0.8 ppm). As a general trend, the 10:2 FTOH congener concentrations were higher than the 8:2 and 12:2 FTOH congener levels. The other FTOH congeners were generally lower than the 8:2 and 12:2 FTOH congeners levels. The 6:2 and 13:2 FTOH congeners and 8:2 fluorotelomer acrylate were not detected at reportable levels. Only the 8:2 FTOH congeners were detected above the reportable level in either the background soil samples or in the sand blank.

The sludge sample analysis results are provided in Table 6. PFOS is the dominant species measured. PFOS concentrations for the Decatur sludge samples (~400 ng/g sludge or 0.4 ppm) were 3-5 times higher than the corresponding Decatur PFOA levels (highest ~120 ng/g of sludge or 0.1 ppm). Interestingly, the New City sludge sample PFOS:PFOA ratios (~3-6) were similar to the Decatur ratios. However, the Decatur PFOS and PFOA levels were more than five time higher than the PFOS and PFOA levels measured in the New York City sludge samples. Most of the other PFCs were detected at very low levels in the sludge samples for both cities, with small differences observed between the samples and locations. None of the targeted PFCs were detected at the reporting levels in the soil blank sample.

Data Quality Control

Although this was a small scale reconnaissance screening study employing newly developed or modified research methods, significant effort was taken to generate data that would help define the overall quality of the results being reported. Several types of field and laboratory quality control samples were prepared and analyzed by the ERD scientists, including:

- Instrument calibration and determination of extract and soil reporting limits
- Analysis of field and laboratory blank samples
- Analysis of spiked soil and sludge samples
- Repeated analysis of the same extract
- Analysis of multiple extracts from the same sample
- Analysis of duplicate sludge-applied soil samples
- Analysis of similar samples by a second NERL laboratory

Instrument calibration and determination of extract and soil reporting limits. The GC/MS and LC/MS/MS systems were operated as outlined in the attached SOPs and documentation. The systems performance was acceptable throughout the sample analysis period. Linearity over the concentration range of the standards was demonstrated for the targeted species by an $r > 0.99$ for all calibrations, all of which were performed with 1/x-weighted linear regression and the analyst's confirmation of the central tendency of the regression line at all standards levels. Table 7 provides the results of multiple standard analyses at one standard concentration that falls in the general range of some of the higher concentrations observed in the samples; the low values of the RMDs demonstrate satisfactory accuracy and precision. Tables 8A, 8B and 8C provide the extract and soil calculated reporting limits for the PFC analytes along with narrative explaining how these levels were calculated.

Analysis of field and laboratory blank samples. The sand blank sample concentrations of perfluorocarboxylic acids and sulfonates were below the minimum reporting limits (Tables 9A and 9B). With the exception of the 8:2 FTOH congener, the results of analysis of the sand blank for the other FTOH congeners were at or below the minimum reporting limit. These data suggest minimal, if any, inadvertent contamination of the soil samples either in through field sampling or bias through the laboratory process. No perfluorocarboxylic acids or sulfonates were detected above the minimum reporting limits in the commercially available soil sample analyzed with the field sludge samples (Table 9C), indicating no inadvertent bias due to the laboratory procedures.

Analysis of spiked soil and sludge samples. The sludge and sludge-applied soil samples were spiked with mass-labeled standards for both PFOA and 8:2 FTOH. The mass-labeled PFOA standard spiked in the sludge samples was recovered satisfactorily with average 90% recovery (Table 10A). The recovery of the mass-labeled 8:2 FTOH standard from the sludge-applied soils also was satisfactory (Table 10B). Whereas ERD's extraction method was shown to be effective for the soil-polymer mixes for which ERD SOP PMB 54.0 was developed, the recovery of the mass-labeled PFOA from the sludge-applied soils was low. This result is attributed to the fact that recovery was distributed over too many extraction steps for the small mass of recovery standard spiked into the samples. Regardless, in the absence of artifact-free recovery of the mass-labeled spike for PFOA, the efficacy of extraction and general accuracy of these values were validated by comparing ERD's results to those compiled by HEASD. Splits of these samples were extracted and analyzed by HEASD using methods developed independently. The confirmatory sample PFCA concentrations (Table 4) generally are about 50% to 80% of ERD's measured concentrations. These are considered to be internally consistent and supportive of the validity of both methods considering that: 1) ERD's extraction method, which is composed of five extraction steps performed in sequence, likely is more aggressive than the one-step extraction used by HEASD; 2) neither ERD's method nor HEASD's method was developed specifically for this complex mixture of matrices; and 3) the concentrations detected generally are relatively low, in the part-per-billion range in most cases.

Repeated analysis of the same extract. Multiple aliquots from each of the soil and sludge samples were independently extracted and analyzed for the PFCAs and FTOH congeners. Since this data is extensive, we are only showing the data for Site 3. Table 11A (columns 2-4) presents the PFCA results and Table 11B (columns 2-4) presents the FTOH results. These data demonstrate that there is limited variability imparted from repeated measures of one extract of a single sample.

Analysis of multiple extracts from the same sample. The statistics for the FTOHs (summing four sequential extractions each consisting of two repeated measures, in each of two independent

extractions) are summarized in Table 11B, columns 5-7. The precipitous increase in variation from statistics for a single extraction, suggests heterogeneity on a small scale relative to sample size.

Analysis of duplicate sludge applied soil samples. Tables 11A (columns 5-7) and 11B (columns 8-10) characterize the sources of variation for the PFCAs and the FTOHs in the Site 3 sample and the duplicate for the sludge-applied soil. There is little variability between the Site 3 sample and its duplicate for the PFCA analytes. The variability for the FTOHs between sample 3 and its duplicate qualitatively is on the same scale as that for variability for parallel extractions of a single sample, suggesting spatial heterogeneity within the scale of a sample is on the order as that for the distance in the field between a sample and its duplicate.

Analysis of similar samples by a second laboratory. As noted previous, confirmatory analysis of a similar set of sludge-applied soil samples was conducted by a second NERL laboratory using a different research extraction and analytical method. In general there is very good agreement between the two laboratory reports, especially when considering the differences in methods, difference in sample locations, likely non-uniform application of sludge across the field area, and complexity in sampling and analyzing soil samples for PFCs.

Conclusions

The analysis of the Region IV reconnaissance samples yielded relatively high concentrations of a wide variety of PFCs in the Decatur sludge-applied soil samples. PFOA, PFOS, decanoic acid, and the 8:2, 10:2 and 12:2 FTOH congeners were the highest PFCs measured. Virtually no PFCs were reported for the background or field blank samples. PFOS was the dominant species measured in the sludge samples. The extensive quality control data indicate that the analytical methods yielded data of exceptional precision and accuracy. The quality control data suggest that, after minor refinement, these methods could be used to support other studies examining PFC concentrations in soil and sludge samples.

The results of analysis on this limited number of samples suggest that the application of Decatur municipal sludge is the likely source of the PFC soil contamination, the primary Region IV hypothesis. It's also important to note that many PFCs were measured in the New York City sludge samples, and though at much lower levels, suggest the potential for PFC contamination in other areas across the U.S. However, based on the study scale and the lack of knowledge regarding the application processes, the generalization of the study results is limited.

There are other general observations that can be reported. Six FTOH compounds were detected and reported in this study that have not been reported previously in the published literature as being detected in field-collected samples as far as we know, and for which straight-chain standards have not been identified. These compounds include 7:2 sFTOH, 9:2 sFTOH, 11:2 sFTOH, 12:2 FTOH, 13:2 sFTOH and 14:2 FTOH. Branched-chain standards were available for two compounds, molecular weight 414 (7-methyl 6:2 FTOH) and 514 (9-methyl 8:2 FTOH), however, no standards were available for molecular weights 614, 664, 714, and 764. The retention times of the subject peaks in the extract with $[M+H]^+$ m/z values 415 and 515 eluted earlier than the 7-methyl 6:2 FTOH and 9-methyl 8:2 FTOH standards and this observation led us to suspect that the subject peaks represent other isomers of these compounds. Given this suspicion, we obtained a standard for the 7:2 sFTOH and determined that the suspected detection of the 7:2 sFTOH in our

field samples, indeed, eluted at the same time as this standard and with common m/z values. Although we did not obtain standards for the remaining five compounds, we regard these remaining five FTOHs as 'tentatively identified' based upon five independent modes of identification: 1) scan spectra that contained the expected $[M + H]^+$ ion for perfluorinated alcohols having molecular weights of 414, 514, 614, 664, 714, and 764; 2) scan spectra corresponding to loss of m/z = 38 from the $[M + H]^+$ ion for perfluorinated alcohols having molecular weights of 414, 514, 614, 664, 714, and 764; 3) elution times of these tentatively identified compounds were consistent with surrounding alcohols for which we did have standards; 4) when we derivitized the extracts with trimethylsilylimidazole (TMSI), the $[M+1]^+$ peaks exhibited the temporal elution shift that was expected; and 5) when we derivatized the extracts with TMSI the original peaks all disappeared quantitatively as was expected. Based on these five independent modes of identification, but in the absence of authentic standards, these compound identifications are considered to be tentative. Quantification of these tentatively identified
compounds, as well as the 7:2 sFTOH, was performed using the calibration curve for the nearest-preceding-eluting alcohol for which there was a standard.

Table 1: Compound names, acronyms & analytical methods

Compound	Acronym	Analytical Method
Perfluoropropanoic acid	C3	LC/MS/MS
Perfluorobutanoic acid	C4	LC/MS/MS
Perfluoropentanoic acid	C5	LC/MS/MS
Perfluorohexanoic acid	C6	LC/MS/MS
Perfluoroheptanoic acid	C7	LC/MS/MS
Perfluorooctanoic acid	C8	LC/MS/MS
Perfluorononanoic acid	C9	LC/MS/MS
Perfluorodecanoic acid	C10	LC/MS/MS
Perfluoroundecanoic acid	C11	LC/MS/MS
Perfluorododecanoic acid	C12	LC/MS/MS
Perfluorotridecanoic acid	C13	LC/MS/MS
Perfluorotetradecanoic acid	C14	LC/MS/MS
Perfluorobutane sulfonate	PFBS	LC/MS/MS
Perfluorohexane sulfonate	PFHxS	LC/MS/MS
Perfluoroheptane sulfonate	PFHpS	LC/MS/MS
Perfluorooctane sulfonate	PFOS	LC/MS/MS
6:2 Fluorotelomer carboxylic acid	6-2FTUCA	LC/MS/MS
8:2 Fluorotelomer carboxylic acid	8-2FTUCA	LC/MS/MS
10:2 Fluorotelomer carboxylic acid	10-2FTUCA	LC/MS/MS
6:2 Fluorotelomer alcohol	6:2 FTOH	GC/MS
7:2 sFluorotelomer alcohol	7:2 sFTOH	GC/MS
8:2 Fluorotelomer alcohol	8:2 FTOH	GC/MS
9:2 sFluorotelomer alcohol	9:2 sFTOH	GC/MS
10:2 Fluorotelomer alcohol	10:2 FTOH	GC/MS
11:2 sFluorotelomer alcohol	11:2 sFTOH	GC/MS
12:2 Fluorotelomer alcohol	12:2 FTOH	GC/MS
13:2 sFluorotelomer alcohol	13:2 sFTOH	GC/MS
14:2 Fluorotelomer alcohol	14:2 FTOH	GC/MS
8:2 Fluorotelomer acrylate	8:2 FTAcr	GC/MS
$^{13}C_8$-Perfluorooctanoic acid	M8C8	LC/MS/MS
$^{13}C_2$-8:2 Fluorotelomer alcohol	M8:2 FTOH	GC/MS

Table 2. Results of Analysis of Primary Soil Samples for Perfluorocarboxylic Acids (ng/g soil)

Site	Soil Sample Type	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14
3	**Field Sludge**	<870	<420	143	328	425	2531	649	2029	481	491	<100	<100
3'	**Field Sludge Duplicate**	<870	<420	<100	199	314	1895	464	1593	425	743	140	209
6	**Field Sludge**	<870	<420	<100	<100	139	818	221	760	219	328	<100	112
7	**Field Sludge**	<870	<420	<100	111	239	1340	428	1496	370	445	100	<100
7'	**Field Sludge Duplicate**	<870	<420	<100	<100	154	541	125	398	100	140	<100	<100
9	**Field Sludge**	<870	<420	<100	131	273	1598	484	2088	687	1240	245	345
10	**Background**	<870	<420	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
11	**Background**	<870	<420	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
Sand	**Blank**	<870	<420	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100

Table 3. Results of Analysis of Primary Soil Samples for Perfluorosulfonates and Fluorotelomer Carboxylic Acids (ng/g soil)

Site	Soil Sample Type	PFBS	PFHxS	PFHpS	PFOS	6-2 FTUCA	8-2 FTUCA	10-2 FTUCA
3	**Field Sludge**	<100	<100	<100	1296	<100	<100	<100
3'	**Field Sludge Duplicate**	<100	<100	<100	1409	<100	<100	<100
6	**Field Sludge**	<100	<100	<100	715	<100	<100	<100
7	**Field Sludge**	<100	<100	<100	979	<100	<100	<100
7'	**Field Sludge Duplicate**	<100	<100	<100	276	<100	<100	<100
9	**Field Sludge**	<100	<100	<100	972	<100	<100	<100
10	**Background**	<100	<100	<100	<100	<100	<100	<100
11	**Background**	<100	<100	<100	<100	<100	<100	<100
Sand	**Blank**	<100	<100	<100	<100	<100	<100	<100

Table 4. Results of Analysis of Confirmatory Soil Samples for Perfluorocarboxylic Acids and Sulfonates (ng/g oven dried soil)

Site	Soil Sample Type	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	PFBS	PFHS	PFOS	PFDS
3	**Field Sludge**	NA	NA	NA	132	316	2190	478	1174	395	275	88	70	<20	<20	707	32
3'	**Field Sludge Duplicate**	NA	NA	NA	73	162	1118	251	774	294	223	62	60	<20	<20	575	<20
6	**Field Sludge**	NA	NA	NA	35	86	511	160	420	175	173	42	55	<20	<20	276	<20
7	**Field Sludge**	NA	NA	NA	41	164	856	308	778	282	253	63	51	<20	<20	452	<20
7'	**Field Sludge Duplicate**	NA	NA	NA	40	104	408	105	341	106	124	39	29	<20	<20	145	<20
9	**Field Sludge**	NA	NA	NA	45	144	936	322	1159	533	502	170	188	<20	<20	412	23

NA = not analyzed for. Reporting Limit = 20 ng/g soil.

Table 5. Results of Analysis of Primary Soil Samples for Telomer Alcohols (ng/g soil)

Site	Soil Sample Type	6:2 FTOH	7:2 sFTOH	M8:2 FTOH	8:2 FTOH	8:2 FTAcr	9:2 sFTOH	10:2 FTOH	11:2 sFTOH	12:2 FTOH	13:2 sFTOH	14:2 FTOH	M8:2/8:2 Ratio
3	**Field Sludge**	<4	123	20	377	<4	70	817	26	376	<4	120	0.64
3'	**Field Sludge**	<4	54	9	430	<4	23	563	11	165	<4	65	0.36
6	**Field Sludge**	<4	117	29	83	<4	41	294	9	124	<4	50	1.13
7	**Field Sludge**	<4	27	29	24	<4	13	20	5	10	10	13	1.19
7'	**Field Sludge**	<4	16	47	15	<4	6	11	<4	5	<4	6	1.16
9	**Field Sludge**	<4	151	36	137	<4	56	227	16	75	4	50	1.13
10	**Background**	<4	<4	36	6	<4	<4	<4	<4	<4	<4	<4	1.25
11	**Background**	<4	<4	45	6	<4	<4	<4	<4	<4	<4	<4	1.19
Sand	**Blank**	<4	<4	43	5	<4	<4	<4	<4	<4	<4	<4	1.13

Table 6. Results of Analysis of Primary Sludge Samples (ng/g sludge)

Sample	Site	C6	C7	C8	C9	C10	C11	C12	C13	C14	PFOS
1	**Decatur**	9	5	59	6	24	23	3	<3	5	405
2	**Decatur**	18	10	128	7	44	23	20	10	4	418
3	**New York City**	7	<3	9	12	<6	7	3	7	10	77
4	**New York City**	5	<3	8	14	<6	<6	7	9	9	61
5	**New York City**	6	<3	20	15	<6	9	3	6	3	32
6	**Soil Blank**	<3	<3	<6	<6	<6	<6	<3	<3	<3	<3

Table 7. Results of Multiple Standard Analyses

Fraction Relative Mean Deviation (RMD) of Four Repeated Measures of the 238 pg/g Standard (1)					
Analyte	Measured Concentration (pg/g)			Fraction RMD (2)	
	Mean	Standard Deviation	Median	Mean	Median
C3	248	42	267	0.04	0.12
C4	248	8	249	0.04	0.05
C5	241	26	245	0.01	0.03
C6	229	25	222	0.04	0.07
C7	231	6	230	0.03	0.03
C8	235	22	235	0.01	0.01
MC8	233	16	231	0.02	0.03
C9	242	27	248	0.02	0.04
C10	241	3	242	0.01	0.02
C11	231	30	230	0.03	0.03
C12	235	22	240	0.01	0.01
C13	301	24	296	0.26	0.25
C14	288	44	296	0.21	0.24
PFBS	226	42	217	0.05	0.09
PFHxS	228	12	225	0.04	0.05
PFHpS	242	31	247	0.02	0.04
PFOS	229	53	230	0.04	0.03
6-2FTUCA	225	25	230	0.06	0.04
8-2FTUCA	225	55	224	0.06	0.06
10-2FTUCA	235	31	245	0.01	0.03

(1) Calibrations were performed with four repeated measures of each standard level using 1/x linear regression with resulting correlation of r>0.99 with as many as 13 levels (4 to 4800 pg/g) to as few as 10 levels (38 to 4800 pg/g); the lower level standards were dropped only when the central tendency of the regression deviated from the spread of the repeated standards measurements.

(2) Fraction RMD = |C-238|/238 where C is mean or median measured concentration in pg/g.

Table 8A. Reporting Limits for PFCAs in Sludge-Applied Soils

Compound	ERL (1) (pg/g solvent)	SRL (2) (ng/g soil)
C3	484	870
C4	231	420
C5	56	100
C6	56	100
C7	56	100
C8 (PFOA)	56	100
C9	56	100
C10	56	100
C11	56	100
C12	56	100
C13	56	100
C14	56	100
PFBS	56	100
PFHxS	56	100
PFHpS	56	100
PFOS	56	100
6-2FTUCA	56	100
8-2FTUCA	56	100
10-2FTUCA	56	100

Table 8B. Reporting Limits for FTOHs in Sludge-Applied Soils

Compound	ERL (1) (ng/mL solvent)	SRL (2) (ng/g soil)
6:2FTOH	1	4
7:2sFTOH	1	4
8:2FTOH	1	4
8:2FTAc	1	4
9:2sFTOH	1	4
10:2FTOH	1	4
11:2sFTOH	1	4
12:2FTOH	1	4
13:2sFTOH	1	4
14:2FTOH	1	4

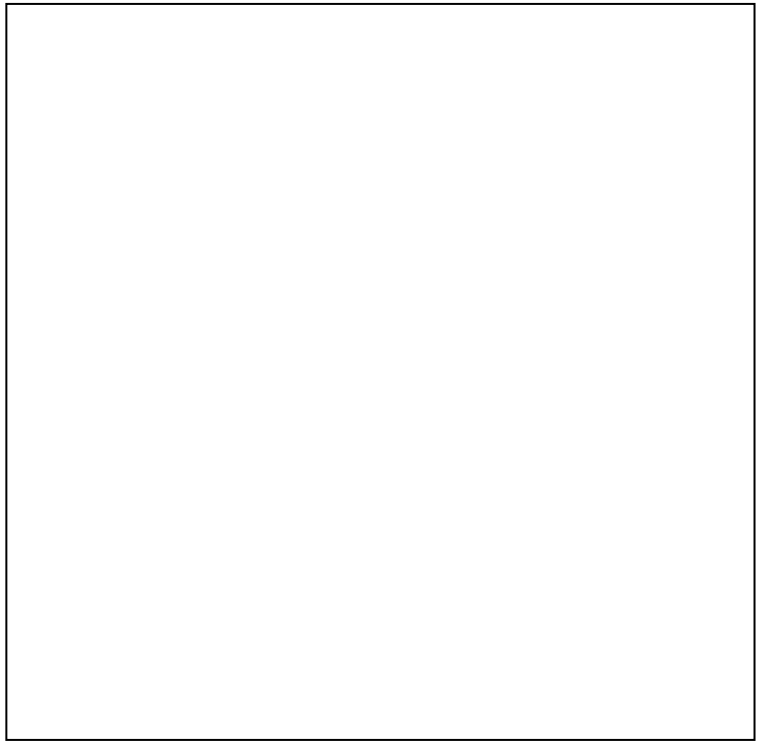

Table 8C. Reporting Limits for PFCAs in Sludge

Compound	ERL (1) (pg/g solvent)	SRL (2) (ng/g sludge)
C6	30	3
C7	30	3
C8 (PFOA)	60	6
C9	60	6
C10	60	6
C11	60	6
C12	30	3
C13	30	3
C14	30	3
PFOS	30	3

Table 9A. Analyses of Sand Blank for Accompanying Analyses of PFCAs in Sludge-Applied Soils [1]

Compound	Concentration (ng/g soil)
C3	<870
C4	<420
C4	<100
C5	<100
C6	<100
C7	<100
C8 (PFOA)	<100
C9	<100
C10	<100
C11	<100
C12	<100
C13	<100
C14	<100
PFBS	<100
PFHxS	<100
PFHpS	<100
PFOS	<100
6-2FTUCA	<100
8-2FTUCA	<100
10-2FTUCA	<100

[1] Reported here are concentrations for an extract of commercially purchased Ottawa Sand. A solvent process blank also was run, but is not tabulated here as all results were equal to, or lower than, the results for this Sand Blank.

Table 9B. Analyses of Sand Blank for Accompanying Analyses of FTOHs in Sludge-Applied Soils [1]

Compound	Concentration (ng/g soil)
6:2FTOH	None detected
7:2sFTOH	None detected
8:2FTOH	5
8:2FTAc	<4
9:2sFTOH	None detected
10:2FTOH	<4
11:2sFTOH	<4
12:2FTOH	<4
13:2sFTOH	None detected
14:2FTOH	None detected

[1] Reported here are concentrations for an extract of commercially purchased Ottawa Sand. A solvent process blank also was run, but is not tabulated here as all results were equal to, or lower than, the results for this Sand Blank.

Table 9C. Analyses of Soil Blank for Accompanying Analyses of PFCAs in Sludge-Applied Soils [1]

Compound	Concentration (ng/g soil)
C6	<1.6
C7	<1.6
C8 (PFOA)	<3.4
C9	<3.4
C10	<3.4
C11	<3.4
C12	<0.8
C13	<1.6
C14	<1.6
PFOS	<1.6

[1] Reported here are concentrations for an extract of commercially purchased Cowart Soil. Soil was used as a blank instead of sand to include complexities from organic material (OM) because OM constitutes the dominant matrix challenge in sludge. A solvent process blank also was run, but is not tabulated here as all results were equal to, or lower than, the results for this Soil Blank.

Table 10A. Recoveries of Mass-Labeled Spike for Analyses of PFOA in Sludge [1]

Sample	Fraction Recovery (Mean±1Std. Dev.)
1 (Decatur)	0.92±0.12
2 (Decatur)	0.96±0.05
12 (NYC)	0.76±0.10
13 (NYC)	0.86±0.20
14 (NYC)	1.00±0.01
Soil Blank	0.86±0.05

[1] Each ~0.5 g sludge sample was spiked with a nominal 1 ng of $^{13}C_4$-PFOA, the actual amount determined by weighing.

Table 10B. Recoveries of Mass-Labeled Spike for Analyses of 8-2 FTOH in Sludge-Applied Soils [1]

Sample	Fraction Recovery (Mean±1Std. Dev.)
3	0.64±0.63
3 Duplicate	0.36±0.01
4 (Subsurface at 3)	1.12±0.06
6	1.13±0.05
7	1.19±0.07
7 Duplicate	1.16±0.03
9	1.13±0.04
10	1.25±0.00
11	1.19±0.03
Sand Blank	1.13±0.00

[1] Each ~5 g soil sample was spiked with a nominal 150 ng of ($^{13}C_2$-$^{2}H_2$)8-2FTOH, the actual amount determined by weighing. Total recovery is the sum recovered from 4 MTBE extractions, each analyzed individually and summed.

Table 11A. Characterization of Sources of Variation for PFCA Analyses of Sludge-applied Soil Using the Site 3 Sample and its Duplicate

Characterization of Sources of Variation for Samples 3 & Duplicate of 3						
Analyte	Sample 3 Alone (1)			Sample 3 & Duplicate of 3 (2)		
	Mean	Standard Deviation	Coefficient of Variation	Mean	Standard Deviation	Coefficient of Variation
C5	143	7	0.05			
C6	328	16	0.05	263	6	0.02
C7	425	22	0.05	369	19	0.05
C8	2531	67	0.03	2213	150	0.07
C9	649	79	0.12	557	38	0.07
C10	2029	95	0.05	1811	32	0.02
C11	481	81	0.17	453	30	0.07
C12	491	72	0.15	617	36	0.06
PFOS	1296	117	0.09	1352	94	0.07

(1) Standard deviation and coefficient of variation account for two repeated measures in each of two independent extractions.

(2) Standard deviation and coefficient of variation account for variation between mean values for the Site 3 sample and the duplicate of 3 only, i.e., these statistics exclude variation arising from repeated measures and independent extractions for each sample.

Table 11B. Characterization of Sources of Variation for FTOH Analyses of Sludge-applied Soil using the Site 3 Sample and its Duplicate

Characterization of Sources of Variation for Samples 3 & Duplicate of 3									
Analyte	Sample 3, Extraction A Alone (1)			Sample 3, Extraction A & B (2)			Sample 3 & Duplicate of 3 (3)		
	Mean	Standard Deviation	Coefficient of Variation	Mean	Standard Deviation	Coefficient of Variation	Mean	Standard Deviation	Coefficient of Variation
7:2sFTOH	60	0.98	0.02	123	94	0.76	89	49	0.55
M8:2FTOH	12	0.24	0.02	20	11	0.57	15	8	0.52
8:2FTOH	372	3.90	0.01	377	35	0.09	403	38	0.09
9:2sFTOH	30	0.48	0.02	70	59	0.85	46	33	0.71
10:2FTOH	536	8.19	0.02	817	433	0.53	690	179	0.26
11:2sFTOH	13	0.21	0.02	26	19	0.73	18	11	0.59
12:2FTOH	215	2.88	0.01	376	246	0.65	271	149	0.55
14:2FTOH	80	0.56	0.01	120	64	0.53			

(1) Standard deviation and coefficient of variation account for variation from summation of four sequential extraction steps, two repeated of each extraction step.

(2) Standard deviation and coefficient of variation account for variation from summing four sequential extractions, each consisting of two repeated measures, in each of two independent extractions.

(3) Standard deviation and coefficient of variation account for variation between mean values for sample 3 and the duplicate of 3 only, i.e., these statistics exclude variation arising from repeated measures and independent extractions for each sample.

Analysis of Perfluorocarboxylic-Acid Extracts from Soil Samples

I. REAGENTS:

A. Polished NPW (Nanopure Water)

1. Use glassware system dedicated to water polishing.
2. Pass 2L nanopure water through a 60cc "Oasis HLB" cartridge (use the same cartridge no more than 3 times).
3. Store polished NPW in dedicated 1L containers.
4. After polishing, degas water by stoppering vacuum flask, drawing house vacuum on it, warming on hotplate at about 3 setting, and stirring with the dedicated glass stir bar for 45 min or more.
5. Add 1.5 mL glacial acetic acid to 2L of polished water

B. Fisher Optima Grade Acetonitrile Solution

1. Starting with a 4L bottle of Fisher Optima Grade acetonitrile (ACN), add 3 mL glacial acetic acid, and hand mix.

C. Seal Wash Solution

1. To polished water described above (*without* glacial acetic acid added), add Fisher Optima Grade ACN to 10% ACN by volume.

D. Strong Needle Wash

1. To polished water described above (*with* glacial acetic acid added), add Fisher Optima Grade ACN to 60% ACN by volume.

II. SAMPLE ANALYSIS

A. Generally the Waters liquid chromatograph tandem mass spectrometer (LC/MS/MS) will be operating & MassLynx will be open on the personal computer (PC). If this is so, go to B. Otherwise:

1. Assure that sample manager, binary solvent manager, mass spectrometer (MS), PC and monitor are powered up and awaiting instruction.
2. Hit MassLynx icon.
3. Assure that MS vacuum is on by: "Shortcut," "Instrument," "MS Tune." Collision Cell Pirani should ~9.34e-3 and Analyzer Penning should ~1.50e-5. If not, then "Options," "Pump On."
4. After vacuum stabilizes assure atmospheric-pressure ionization gas (API Gas) and collision gas (Col Gas) buttons in the MS Tune window are depressed.

5. Load a tune method, commonly "070613_tc_msms_negesi.ipr." If you need to develop your own method see Appendices for suggested parameterization.
6. Minimize the tune page.

B. Get the LC stabilized by:

1. "Shortcut," "Instrument," "Inlet Method."
2. Load an inlet method, commonly "070611PFCA35To90_05mlmin.w2200." If you need to develop your own method see Appendices for suggested parameterization.
3. Assure LC pump is operating as indicated by the spray button being depressed in this window.
4. Minimize the tune page.

C. If starting a new project do the following, else go to D

1. "File," "Project Wizard," "Yes."
2. Enter Project name, always with date first, yr-mo-da, ex. "070611name.w2200," enter description, "Next."
3. "Create using current project as template," "Finish."

D. Prepare sample list instructions by:

1. Open a sample list, "File," "Open." Resave this list under a new name following our naming system of 'yr, mon, day, short phrase describing run,' ex:071003MTBE2ndExtract.spl.
2. Populate this sample list as appropriate. Usually start with 5 to 20 blanks consisting of 60:40 ACN:H_2O, the chosen number based on recent instrument operation, samples recently run, and how low the analytes concentrations are expected to by in the upcoming run you are preparing. Number these initial samples consistent with naming scheme described below, but ending in 001a, 001,b, 001c, etc to designate that these are initial blanks and not interspersed with samples later in the run.
3. Name blanks, standards and samples according to: 'yr, mon, day, short phrase describing sample,' for example, '071003P1Extract-001' or '071003-0-075-001' for a 0.075 pg/:L standard.
4. Designate the same inlet and MS tune files as you already are stabilizing the instrument with. Also enter an MS file, commonly "070808_JW_Segmented.exp." If you need to develop your own method see Appendices for suggested parameterization.
5. Resave this sample list.

E. Perform the analyses:

1. Record in instrument log book the date, what you are running, inlet method, MS method, tune method, the pressure of 65:35 ACN:H_2O at q=0.5 mL/min, and deltaP for stabilized isocratic flow.
2. Hit "Start Run," the right-pointing button in the open window.
3. If you desire an informative screen appearance regarding instrument and run status during the sample run:
    a. Hit "Chromatogram" and within the chromatogram window assure the 'Globe,' 'Replace' and 'Stopwatch' buttons are in depressed position to set the

chromatogram to a live-analysis window. Size and maneuver this window to the lower right of the screen.

b. Open LC window, select "ACQUITY Additional Status" hit "Launch ACQUITY UPLC Console" button on right side of window.
c. On ACQUITY UPLC Console window, expand "ACQUITY UPLC System" menu by hitting "+." Hit "Binary Solvent Manager" option. Move this window to upper right of screen so that sample number on the sample list is visible to its left and the chromatogram is visible below this window.
d. Click on back window, the MassLynx sample list window, hit "Status."
e. Hit "Chromatogram" and "ACQUITY UPLC Console" on Status Bar so that these windows move to the front of the Sample List window.

F. Quantitate Results:

1. When sample run is complete, click on Sample List window to bring it to the front.
2. Hit "Shortcut," "QuanLynx," "Process Samples" and "OK" to quantitated.
3. Check and edit integrations as appropriate.
4. Save quantitation.

III. APPENDICES

Appendix A: Parameters we use for Inlet file for LC operation

Selected extracts also were analyzed on a Waters Quattro Premier XE tandem mass spectrometer interfaced with a Waters Acquity ultra-performance liquid chromatograph (UPLC). All system operations were controlled by Waters MassLynx 4.1 and QuanLynx 4.1. To conserve sample extracts while maintaining sensitivity, 20 µL was withdrawn from extracts maintained in the autosampler at 4 ^{o}C and introduced into a 50 µL loop using "partial loop with needle overfill" mode to a Waters BEH C_{18} trapping cartridge followed by a Waters BEH C_{18} analytical column, 100x2.1x2.1 (mm length x mm inside diameter x µm particle size), kept at 35 ^{o}C.

To minimize background for PFOA, we altered the UPLC plumbing by: 1) substituting in Peek tubing to carry the solvents; 2) by-passing the solvent degassers which are composed of perfluorinated compounds; 3) inserting a C_{18} trap column (100x2.1x3.5) at the down-gradient-most point in the water-eluent line, immediately above the solvent-mixing cell; and 4) injecting ~1000 blanks and sample extracts, operating 24/7 to cleanse the system from its as-delivered state. In addition, we polished our doubly deionized eluent water by passing it through Waters 35 cm^3 Oasis HLB extraction cartridges, then degassing manually by mild heating under vacuum and stirring with a glass stir bar, all in dedicated glassware.

The UPLC was operated using ACN and water eluents, both containing 0.075% volume-to-volume (v/v) glacial acetic acid. Pumping at a constant total flow rate of 0.5 mL/min, we started runs with an eluent of 65/35, v/v, ACN/water, then linearly ramping to 90/10 at 5 min, holding composition constant until 11 min, linearly ramping down to 65/35 at 11.1 min, from which time we held composition constant until the end of analysis at 13 min.

Appendix B: Parameters we use for MS and Tune files

Upon elution from the UPLC, extracts were introduced to the mass spectrometer in ESI(-) mode with the capillary potential set at -600 V, the method-default cone potential at -17 V, the extractor potential at -2 V and the RF lens potential at 0.3 V. The cone potential was altered to -14 V for C_6 and -15 V for C_7, C_8 and $^{13}C_4$-PFOA. The source temperature was maintained at 140 ^oC. The desolvation gas, from the Peak N_2 generator, was maintained at 140 ^oC and flowed at 800 L/hr. The cone gas, also supplied by the N_2 generator, was set to flow at 25 L/hr.

The low- and high-mass resolutions in the first quadrupole both were set to 13.0 (unitless ratio of direct to radio-frequency current voltages) and the ion energy was set to 0.7 eV. In the collision cell, the entrance was set to -3 V, the interior set to -16 V and the exit set to -1 V. The Ar collision gas (Airgas) was set to flow at 0.45 mL/m. The low- and high-mass resolutions in the third quadrupole both were set to 12.0 and the ion energy was set to 1.0 eV. The detector multiplier was set to -700 V.

Collision energy for the quantification ions was 13 eV for the C_6 and C_7 acids, and 15 eV for the C_8 through C_{10} acids. Collision energy for the confirmation ions was set to 24 eV for C_6 and 22 eV for C_7 through C_{10}.

To maximize dwell time, our Waters analytical method called for monitoring each of the homologues, C_6 through C_{10}, as independent functions; two transitions, the quantitation-ion and the confirmation-ion, on each of the C_6, C_7 and C_{10} functions, and three transitions, the quantitation-ion, confirmation-ion and mass-labeled-analyte, on C_8 and C_9. Setting dwell time to 150 ms for the two-transition channels and 100 ms for the three-transition channels, we achieved 20 to 30 scans per peak over typical peak-elution times of 0.2 m.

Appendix C: Parameters we use in quantitation

Selected-reaction monitoring (SRM) chromatograms were smoothed using a second-order Savitsky-Golay algorithm, two five-point smooths for the Waters, which we determined accentuated the signal without imparting negative effects of peak broadening or deterioration of the separation of closely proximate peaks. Signal peaks were delimited by the valleys immediately bounding a peak having an apex exceeding noise or, should they be present, contiguous peaks exceeding noise so long as linking valleys exceeded the apices defining the general amplitude of surrounding noise peaks as well.

Appendix D: Mass transitions to monitor

Compound	Formula	Acronym (short formula)	Quantitation Transition (m/z)	Confirmation Transition (m/z)
perfluoro-n-hexanoic acid	$CF_3(CF_2)_4COOH$	PFXA (C_6)	313→269	313→119
perfluoro-n-heptanoic acid	$CF_3(CF_2)_5COOH$	PFHA (C_7)	363→319	363→169
perfluoro-n-octanoic acid	$CF_3(CF_2)_6COOH$	PFOA (C_8)	413→369	413→169
perfluoro-n-nonanoic acid	$CF_3(CF_2)_7COOH$	PFNA (C_9)	463→419	463→169
perfluoro-n-decanoic acid	$CF_3(CF_2)_8COOH$	PFDA (C_{10})	513→469	513→169
perfluoro-n-[1,2,3,4-^{13}C]butanoic acid	$^{13}CF_3(^{13}CF_2)_2{}^{13}COOH$	$^{13}C_4$-PFBA	217→172	217→121
perfluoro-n-[1,2,3,4-^{13}C]octanoic acid	$^{13}CF_3(^{13}CF_2)_3(CF_2)_3COOH$	$^{13}C_4$-PFOA	417→372	417→169
perfluoro-n-[1,2,3,4,5-^{13}C]nonanoic acid	$^{13}CF_3(^{13}CF_2)_4(CF_2)_3COOH$	$^{13}C_5$-PFNA	468→423	468→169
Perfluoro-n-[1,2-^{13}C]decanoic acid	$^{13}CF_3(^{13}CF_2)(CF_2)_7COOH$	$^{13}C_2$-PFDA	515→470	515→169

Extraction of Soil-Polymer Microcosms
For FTOHs and PFCAs

I. REAGENTS:

A. Polished Nanopure Water (NPW)

6. To polish water, i.e., purge of PFCAs, use glassware system dedicated to water polishing.
7. Pass 2L 18MΩ (nanopure) water through a 60cc "Oasis HLB" cartridge (use the same cartridge no more than 3 times).
8. Store polished NPW in dedicated 1L containers.

B. Polished Tetrabutylammonium (TBA) Mix (Ion Pairing Reagent)

2. Prepare 0.50M Tetrabutylammonium Hydrogen Sulfate (TBAHS) in 18MΩ nanopure water.
3. Prepare 0.25M Na_2CO_3 in 18MΩ nanopure water.
4. Add 2.0 parts Na_2CO_3 solution to 1.0 part TBAHS solution, mixing slowly to avoid spillage due to CO_2 generation.
5. Place a 500mL Nalgene waste collection bottle in the reservoir of a Waters or comparable solid-phase extraction (SPE) vacuum system.
6. Mount a 60cc HLB cartridge on the port above the Nalgene bottle.
7. Flush with 50mL NPW and 50mL methanol, HPLC grade.
8. Replace the waste Nalgene bottle with a methanol-washed Nalgene bottle; and discard the waste.
9. Pass the TBA Mix in part I.B.3 through the cartridge until desired volume has been polished; cap and label polished TBA mix.
10. Flush cartridge with 50mL methanol (MeOH) per steps I.B.4 and I.B.6. Store in labelled Ziploc bag for further use in polishing this reagent mix only.

C. $^{13}C_5$-PFNA (M8C8) Extraction Recovery Spike Solution

2. Prepare from Cambridge Isotope Labs Certified Stock Solution in 60/40 (v/v) ACN/polished water to give a concentration of ~50 ng M8C8 per gram of solution.

D. $^{13}C_4$-PFHxA (MC6) Cleanup Recovery Spike Solution

2. Prepare from Wellington Certified Stock Solution in 60/40 (v/v) ACN/polished water to give a concentration of ~15 ng MC6 per gram of solution.

E. $^{13}C_4$-PFOA (MC8) Internal Standard Solution

1. Prepare from Wellington Certified Stock Solution in 60/40 (v/v) ACN/polished water to give a concentration of ~15 ng MC8 per gram of solution.

F. 2.0 M NaOH Solution and 2.0M HCl Solution

1. Prepare from concentrated stock solutions using polished NPW.

G. Methyl-Tert-Butyl Ether (MTBE)
Use Optima grade.

H. ($^{13}C_2$-$^{2}H_2$)8-2FTOH (m8-2FTOH) Extraction Recovery Internal Solution
Prepare from Wellington Certified Stock Solution in MTBE to give a concentration of 300 ng m8-2FTOH per gram (or per mL, just assure units consistency) of solution.

I. ($^{13}C_2$-$^{2}H_2$)10-2FTOH (m10-2FTOH) Matrix Internal Solution
Prepare from Wellington Certified Stock Solution in MTBE to give a concentration of 500 ng m10-2FTOH per gram (or per mL) of solution or similar.

J. ($^{13}C_2$-$^{2}H_2$)10-2FTOH (m10-2FTOH) Matrix Internal Solution
Prepare from I.I above in MTBE to give a concentration of 5 ng m10-2FTOH per gram (or per mL) of solution or similar.

II. SOIL SAMPLE EXTRACTION

A. Prepared 2mm Sieved Soil Sample.

7. Using all methanol-washed equipment, sieve using a 2mm stainless steel sieve, forcing soil as needed with large rubber stopper. Store sieved soil in methanol-washed 500mL Nalgene bottle. Label and mix on roller mill for 30 minutes.
8. Weigh three ~1-5 gram aliquots to tared weigh boats; vacuum dry over Drierite for 18 hours and weigh again.
9. Repeat step II.A.3 as needed until constant weight is obtained. Calculate percent moisture of soil.

B. Prepare Spiked Soil Samples for Extraction

5. Starting with microcosms consisting of 5g-dry weight equivalent of soil, chosen mass of fluorotelomer-based polymer and water in pre-weighed (tube and cap) MeOH-washed, 16-mL polypropylene copolymer (PPCO) centrifuge tubes with size-18 PPCO caps. Re-weigh and record weight in data table.
6. Add polished NPW to a total of 8.3 g of H_2O considering calculated water content of soil. Reweigh.
7. Add 100uL M8C8 spike solution to provide a loading of no more than 1 ng M8C8 per gram of dry soil. Vortex . Reweigh.
8. Add 1.7 mL m8-2FTOH recovery solution. Reweigh.

C. Extract FTOHs from Spiked Soil Samples

6. Place tubes on Barnstead/Thermolyne Rotisserie Shaker and rotate for 15 to 24 hrs.
7. Centrifuge in Sorvall at 17,500 rpm (or 37,000 G) and 18 to 22 ^{o}C for 30 min.
8. Freeze samples until water is iced, decant MTBE into a preweighed 12 mL vial. Reweigh vial with MTBE, and reserve for analysis for FTOHs and PFCAs.
9. Thaw soil samples, assure centrifuged pellet is disaggregated, add 2 mL of MTBE. Reweigh.
10. Place tubes on Barnstead/Thermolyne Rotisserie Shaker and rotate for 15 to 24 hrs.
11. Centrifuge in Sorvall at 17,500 rpm (or 37,000 G) and 18 to 22 ^{o}C for 30 min.
12. Freeze samples until water is iced, decant MTBE into a preweighed 12 mL vial. Reweigh vial with MTBE, and reserve for analysis for FTOHs and PFCAs.
13. Repeat steps II.C.4 through II.C.7 one more time.

14. For each of the three MTBE extracts, typical sample preparation is as follows:
    a. Into a preweighed autosampler vial with vial insert, autopipette 0.1 mL of MTBE. Reweigh. Reserve for possible analysis.
    b. Into a preweighed 12 mL vial, autopipette 0.1 mL of MTBE. Reweigh. Dilute to 1 mL with MTBE containing 5 ng/mL m10-2 FTOH as matrix internal standard. Reweigh. Analyze on GC/MS for FTOHs.
    c. Into a preweighed 12 mL vial, autopipette 0.1 mL 1:10 dilution of step II.C.9.b. Reweigh. Dilute to 1 mL with MTBE containing 5 ng/mL m10-2 FTOH as matrix internal standard. Reweigh. Analyze on GC/MS for FTOHs.
    d. For the balance of the MTBE in each 12 mL vial from steps II.C.3, 7 and 8, reweigh. Evaporate remainders of MTBE from to dryness in SPE assembly, using nylon filters and 5 psi vacuum. Reconstitute in 60:40 ACN:H_2O containing 0.1 ng/g $^{13}C_4$-PFOA. Reweigh. Analyze on LC/MS/MS for PFCAs.

D. Extract PFCAs from Excess Water of Spiked Soil Samples

1. Weigh soil-water microcosm from II.C.4, calculate water content and determine mass of water.
2. Decant into preweighed 12 mL vial sufficient water to achieve remaining water content of 40% to 100% relative to soil dry mass. Reweigh both the soil microcosm and the 12 mL vial.
3. Reserve water in 12 mL vial for contingency analysis. (In our experience this fraction contains negligible PFCAs.)

E. Extract PFCAs from Spiked Soil Samples

1. Weigh soil-water microcosm from II.C.4, calculate water content and determine mass of water.
2. Decant into preweighed 12 mL vial sufficient water to achieve remaining water content of 40% to 100% relative to soil dry mass. The actual amount determined by laboratory personnel based on sample appearance with the objective being to achieve saturation, but about minimally so.
3. Add ACN to yield a 60:40 by-volume solution of ACN:H_2O. Reweigh.
4. Add 200uL 2.0 M NaOH. Vortex. Reweigh.
5. Vortex until homogeneous appearance, sonicate for 60 min using ice to maintain lower bath temperature, transfer to Eberbach shaker table and shake on Low for 15 to 24 hrs.
6. Add 200uL 2.0 M HCl. Vortex. Reweigh.
7. Centrifuge in Sorvall at 17,500 rpm (or 37,000 G) and 18 to 22 ^{o}C for 30 min. Reweigh.
8. Decant liquid to 10mL syringe fitted with 0.45 μm nylon syringe filter.
9. Express liquid to labeled, preweighed 12mL glass vial. Reweigh.
10. Add 200uL 15 ng/g MC6 cleanup recovery standard. Reweigh.
    a. For a quick, rough check, a sample may be withdrawn at this point for LCMSMS analysis. If so, reweigh.
11. Evaporate to dryness in SPE assembly, using nylon filters and 5 psi vacuum.

F. Cleanup Extract using Ion Pairing

4. Add 4 mL TBA Mix to dried extract from II.C.11. Vortex. Reweigh.
5. Add 5 mL methyl-tert-butyl-ether (MTBE). Vortex. Reweigh.
6. Transfer MTBE to tared 12 mL glass vial. Reweigh.
7. Evaporate to dryness in SPE apparatus. Reweigh.
8. Reconstitute with 2mL 60/40 ACN/polished NPW containing 0.1 ng/g $^{13}C_4$-PFOA. Reweigh.

Analyze on LC/MS/MS