SUPPORTING INFORMATION

Effect of Fluorotelomer Alcohol Chain Length on Aqueous Solubility and Sorption by Soils

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LC/MS/MS Analysis of Potential Anionic Metabolites

Chemicals. Perfluorobutanoic acids (PFBA, 99%), perfluorohexanoic acid (PFHA, 97.0%), perfluorooctanoic acid (PFOA, 97%) and perfluorodecanoic acid (PFDA, 95%) were obtained from Sigma-Aldrich, Fluka and Oakwood Products Inc. (West Columbia, SC), respectively. 8:2 fluorotelomer carboxylate (8:2 FTCA, 98%) and 8:2fluorotelomer α,β-unsaturated carboxylate (8:2 FTUCA, 98.4%) were provided by DuPont (Wilmington, DE).

LC/MS/MS Analysis. The LC/MS/MS consisted of a Shimadzu HPLC system (Shimadzu America, Columbia, MD) coupled to an Applied Biosystem API 3000 triple quadrupole tandem mass spectrometer (MDS Sciex, Toronto, Canada). The separation of FTOHs was conducted using a 2 mM aqueous ethanolamine/methanol mobile phase with either a Phenomenex Luna C8(2) column (2 mm × 100mm, 3 µm, Phenomenex USA, Torrance, CA) as described previously (*1*) or a Phenomenex Synergi Max-RP column (2 mm × 150mm, 4 µm). The multiple reaction monitoring (MRM) transitions were monitored for each analyte using collision energy of 10 eV. The transitions were 263>203, 223 for 4:2 FTOH, 363>303, 333 for 6:2 FTOH, 563>503, 523 for 10:2 FTOH and 663>603, 633 for 10:2 FTOH (*2*). The first pair of transition for each analyte was used for quantitation and secondary transition was for compound confirmation. The detection limits in soil extracts were 5 $\mu g\ L^{-1}$ for 4:2 FTOH, 1.0 $\mu g\ L^{-1}$ for 6:2 FTOH and 0.3 $\mu g\ L^{-1}$ for 10:2 FTOH using a 20-µL injection volume. Quantitation was performed using 1/x weighted calibration curves in multiple calibration curves. The calibration curves were generally in the range of 30 to 2000 µg/L for 4:2, 3 to 1000 for µg/L for 6:2 and 1 to 1000 µg/L for 10:2 FTOH at 20 µL injection volume. Calibration curves of less than 3 orders of magnitude but with higher minimal and maximum concentrations at 2 to 5 µL injection volumes were used for some highly concentrated samples, such as the soil extracts for 10:2 FTOH at ≤ 20% cosolvent. We routinely target injecting less than 20 ng mass and minimizing sample dilutions to reduce variation. The appropriateness of the internal standard selection and reduction of matrix effects using a graphitized activated carbon cleanup are discussed below.

The potential anionic metabolites from unintended transformation of 4:2, 6:2 and 10:2 FTOH were monitored with the same HPLC/MS/MS system with negative electrospray ionization. The two main anionic metabolites expected from each parent compound were the fluorotelomer carboxylates (FTCA, $F(CF_2)_nCH_2COOH$, where n = 4, 6 and 10) and the fluorotelomer unsaturated carbxylates (FTUCA, $F(CF_2)_{n-1}CF{=}CHCOOH$) based on the aerobic biotransformation pathway of 8:2 FTOH (*3*). The metabolites profile among FTOHs was expected to be similar except for differences in perfluorocarbon chain length noted in isolated rat hepatocytes (*4*). Liquid chromatography was performed with an Agilent Zorbax Rx-C8 column (5 mm × 150mm, 5 µm, Agilent Technologies, CA) using a gradient elution with 0.15% acetic acid/acetonitrile mobile phase at 0.4 mL min^{-1}. Initial acetonitrile was at 5%, ramped to 80% at 1.1 min, held for 5 min, and returned to 5% at 6 min. The LC/MS/MS conditions were tested with authentic standards of 8:2 FTCA and 8:2 FTUCA to achieve instrument detection limits of 0.003 ~ 0.01 ug L^{-1}. The transitions monitored were: decarbxylation reaction and loss of 2 HF for FTCAs (m/z 277>193 for 4:2 FTCA; m/z 377>293 for 6:2 FTCA; m/z 477>393 for 8:2 FTCA; m/z 577>493 for 10:2 FTCA) and decarboxylation reaction and loss of one HF for FTUCAs (m/z 257>193 for 4:2 FTUCA; m/z 357>293 for 6:2 FTUCA; m/z 457>393 for 8:2 FTUCA; m/z 557>493 for 10:2 FTUCA), according the transitions for 8:2 FTCA and 8:2 FTUCA (*3*).

Examination of Potential Matrix Effect for 6:2 FTOH and 10:2 FTOH. The appropriateness of using [1D, 1D, 2D, 2D-3^{13}C] 8:2 FTOH as an internal standard (IS) for 6:2 and 10:2 FTOHs and the effectiveness of matrix effect reduction by graphitized activated carbon cleanup were tested using soil 7CB2, which has the highest OC content among all the soils. Aliquots of 10 to 100 µL of 6:2 and 10:2 FTOHs stock solutions of varying concentrations were added into 990 to 900 µL aliquots of soil supernatants and soil extracts in 1.5 mL polyproprylene microtube containing ~ 25 mg Envi-Carb. Then IS was added to give a final concentration of ~250 µg L^{-1} and the samples were processed as described before (*1*). The soil supernatants and extracted were prepared under the same conditions as in actual sorption studies, and diluted with 50% acetonitrile before spiking. For 10:2 FTOH, 20/80 v/v acetone/water was the cosolvent/water solution selected for the test, and therefore, the soil supernatant matrix was approximately 10/40/50 v/v/v acetone/water/acetonitrile. The acetonitrile extracts of the soil were neutralized with HCl before adding FTOH. A parallel set of standard solutions in clean acetonitrile solvent was also prepared, but did not go through cleanup step. The standard curves generated from three different matrix solutions were presented in Figure S1.The nearly identical slopes showed that

[1D, 1D, 2D, 2D-3^{13}C] 8:2 FTOH performed well as IS for both 6:2 and 10:2 FTOHs no matter of matrix type in the concentration range up to three orders of magnitude. The most variation occurred at high concentrations, but the relative differences in slopes between three lines were smaller than 15%.

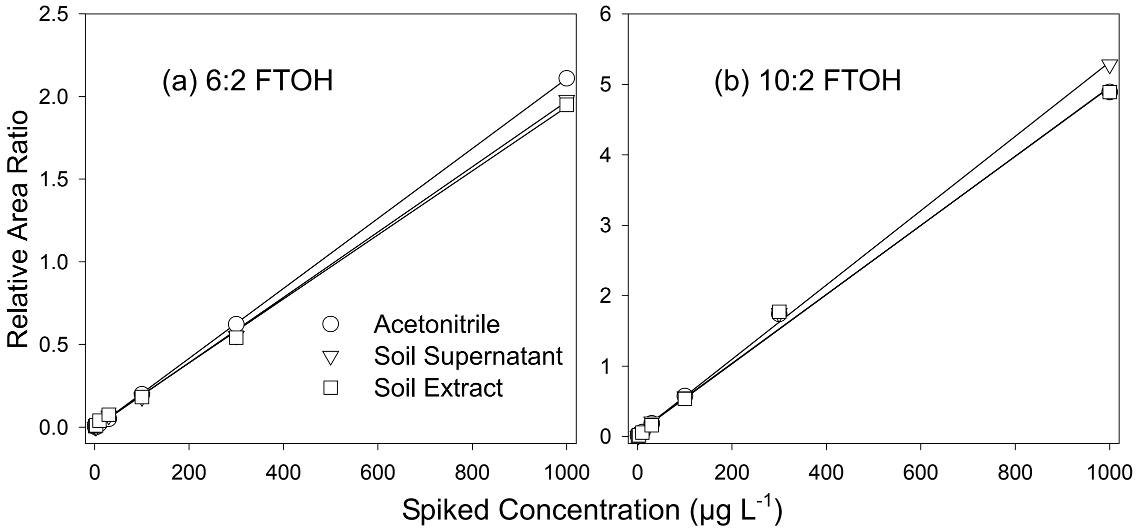

FIGURE S1. Comparison of the internal standard-normalized calibration curves for (a) 6:2 FTOH and (b) 10:2 FTOH, which were added to clean acetonitrile solvent, aqueous or 20/80 acetone/water soil supernatants for 6:2 FTOH and 10:2 FTOH, respectively, and acetonitrile soil extracts of soil 7CB under the same conditions as used for the sorption studies.

12:2 FTOH Aqueous Solubility Determination. The same experimental procedures used for 10:2 FTOH was tested for 12:2 FTOH, but we could not obtain a reliable value either in aqueous systems (f_c = 0) or with the cosolvency approach even with up to a 14-d equilibration time. The only relatively consistent value obtained was a solubility in 50% methanol of 33.6 mg L^{-1} (SE = 3.4, n = 7). There was great variation in all f_c < 0.5 solutions, but relative standard errors did decrease with increasing f_c, *e.g.*, 78% SE at f_c = 0 and 10.4% at f_c = 0.5. Aqueous measurements varied from 0.023 to 2.995 mg L^{-1} among 9 replicates, but these values were obviously too high; solubility of 12:2 FTOH should be proportionally less than 10:2 FTOH (0.011 mg L^{-1} as estimated from acetone cosolvent) based on chain length.

Conducting Mass Recovery of 10:2 FTOH in Blank Controls. The test containers (12-mL glass centrifuge tubes for 40% acetone systems, 40-mL glass centrifuge tubes for 20% and 30% acetone systems, and 125-mL glass round bottom bottles for the 10% acetone systems) were pre-filled nearly to capacity with the respective acetone/water (0.01 N $CaCl_2$), and 10:2 FTOH methanol stock added with a microliter syringe, followed by filling any headspace remaining with the matrix and closing tight with screw caps. The test containers were mixed and centrifuged in the same way and time as the companion soil slurries followed by sampling of the supernatant. After sampling, the solution was emptied and quickly rinsed with deionized water (3 mL for the 12-mL tube, 5 mL for 40-mL tube and 10 mL for 125-mL bottle). The water was discarded and acetonitrile of equal volume was added. The containers were vortexed for 15 s, and 1 mL of acetonitrile was transferred to autosampler vials and analyzed with LC/MS/MS. The concentrations and mass of 10:2 FTOH in the initial equilibrating solutions and acetonitrile wash are listed in Table S1.

TABLE S1. Mass Recovery of 10:2 FTOH Blank Controls in 10 ~ 40% Acetone Cosolvent

f_c	10:2 FTOH Spiked		% Average Mass Recovered ± SE (n = 3)		
	Mass (μg)	**Concentration (μg L^{-1})**	**Solution**	**Glass**	**Total**
10%	1.13	113	20.5 ± 1.1	10.6 ± 2.7	31.3 ± 3.0
	4.70	470	33.7 ± 8.7	17.7 ± 1.0	51.5 ± 7.8
	11.40	1140	34.0 ± 2.2 [a]	19.3 ± 2.8	55.1 ± 1.4 [a]
20%	0.29	28.6	82.8 ± 5.5	0.7 ± 0.2	83.5 ± 5.3
	10.70	1070	31.6 ± 4.7	26.5 ± 0.3	58.1 ± 4.4
	18.10	1810	20.8 ± 1.4	35.9 ± 3.1	56.7 ± 4.5
30%	0.29	28.6	83.6 ± 4.7	0.7 ± 0.2	75.4 ± 4.5
	10.70	1070	83.9 ± 1.1	0.4 ± 0.0	84.3 ± 1.1
	76.50	7650	51.4 ± 1.3	12.0 ± 3.1	63.4 ± 4.3
40%	0.11	10.8	85.3 ± 3.3	0.0 ± 0.0	85.3 ± 3.3
	3.02	302	88.5 ± 3.6	0.4 ± 0.1	88.9 ± 3.6
	16.90	1690	108.5 ± 5.3	0.5 ± 0.0	109.0 ± 5.3

[a] average of two replicates

Discussion on Low Mass Recovery for 4:2 and 6:2 FTOHs and Soil Sterilization. Several possible causes of low recovery such as photolysis, volatility, irreversible sorption, and biotransformation were considered. Photolysis was excluded because exposure to light during the experiments was minimal. Volatility was not considered a likely cause for substantial mass loss given the relatively low air/water partition coefficients for 6:2 FTOH (*5*), which is confirmed by close to a 100% recovery in blank controls (Table 1). Irreversible sorption was also not considered likely with sorption by soils being relatively weak for the shorter chained FTOHs. Ultimately biotransformation was confirmed as the primary cause for not only 6:2 FTOH, but also 4:2 FTOH with soil 7CB2. For both Drummer-6 and 7CB2 soils, quantifiable amounts of fluorotelomer saturated and unsaturated carboxylates of corresponding chain lengths typical of FTOH aerobic biotransformation (*3*) were measured in the supernatants and soil extracts (see Figure S2 in the Supporting Information). No metabolites were detected in the Oakville-24 soil for which close to 100% recovery was achieved. Bacterial contamination after irradiation was not likely given that isotherms for all three soils were measured at the same time and both Oakville-24 and the no soil blanks were free of biotransformation metabolites. No standards were available to confirm the 4:2 and 6:2 fluorotelomer saturated and unsaturated carboxylates; however, all the transitions typical of fluorotelomer acids were detected, suggesting positive identification of the compounds. Also these peaks were not observed in the no-soil FTOH controls or the soil blanks (Figure S2) supporting that the observed metabolites are from soil-induced transformation of the applied FTOHs (see the extended discussion in the Supporting information).

Although soils and the equilibrating solutions were ^{60}Co(γ)-irradiated and autoclaved, respectively, soils are known to harbor microorganisms or enzymes that are resistant to γ-irradiation. A review (*6*) on effects of γ-irradiation summarized that generally a 1 Mrad dosage can eliminate actinomyctes, fungi and invertebrates in most soils and 2 Mrad eliminates the majority of soil bacteria, but up to 7 Mrad may be needed to eliminate resistant microbes. Enzymes are not necessarily deactivated by γ-irradiation as reported in several studies (*6*). For example, Shih and Souza (*7*) reported that about 70% of phosphatase activity and almost all the urease activity were retained after 7.5 Mrad γ-irradiation. Likewise, it is apparent that either the enzymes or the microbes responsible for 4:2 and 6:2 FTOHs biotransformation in our study were not deactivated with a 3.2 Mrad γ-irradiation dosage.

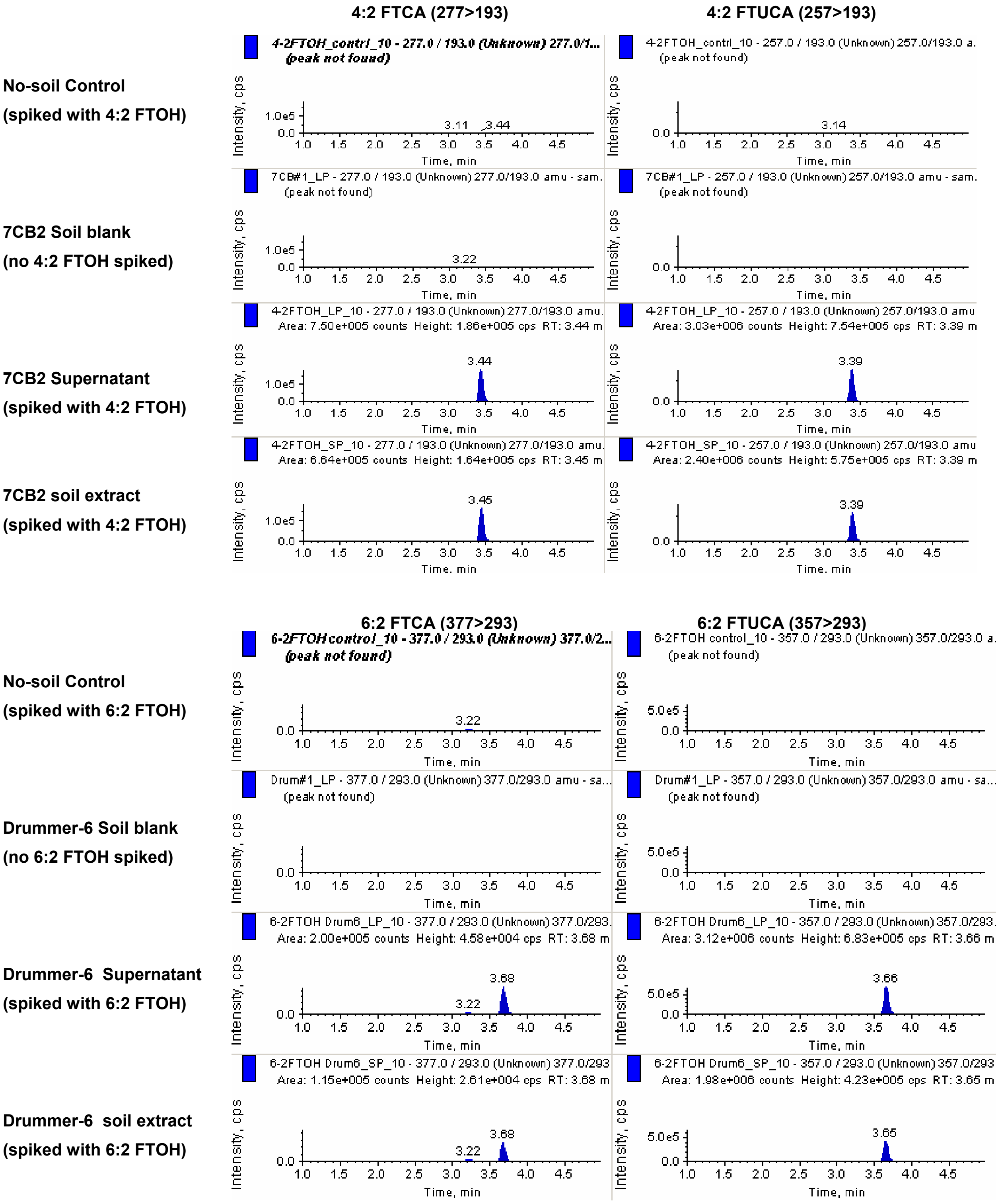

FIGURE S2. Chromatograms from analysis of the supernatants and soil extracts where biotransformation metabolites of 4:2 and 6:2 FTOH were observed after equilibration with γ-irradiated (accumulative dosage of 3.2 Mrad) soils in the sorption experiments.

Sample calculation of subcooled liquid aqueous solubility for 8:2 and 10:2 FTOHs

TABLE S2 Experimental values of normal melting points and entropies of melt (n = 3)

Compound	**Tm (°C)**	$\Delta_m S_i(T_m)$ **(J·mol^{-1}·K^{-1})**
8:2 FTOH	46.2 ±0.24	37.4 ±0.64
10:2 FTOH	92.7 ±0.32	55.9 ±0.89

(1) 8:2 FTOH

1a. Calculation of the hypothetical sub-cooled liquid solubility, S_{iw}^{sat} (*liquid*), of 8:2 FTOH using the measured value of entropy of fusion $\Delta_m S_i(T_m) = 37.4$ J mol^{-1} K^{-1} and the solid solubility, S_{iw}^{sat} (solid) = 0.224 mg L^{-1}, from the log-linear cosolvency extrapolation from acetone/water solutions:

$$S_{iw}^{sat}(liquid) = S_{iw}^{sat}(solid) \cdot \exp\left[\frac{\Delta_m S_i(T_m)}{R}\left(\frac{T_m}{T} - 1\right)\right]$$

$$= 0.224 \cdot \exp\left[\frac{37.4\,\text{J mol}^{-1}\,\text{K}^{-1}}{8.314\,\text{J mol}^{-1}\,\text{K}^{-1}}\left(\frac{46.2 + 273.15}{22.5 + 273.15} - 1\right)\right] = 0.321\,mg\,L^{-1}$$

1b. Using the following empirical relationship (*8*) to estimate entropy of fusion $\Delta_m S_i(T_m)$:

$$\Delta_m S_i(T_m) = 56.5 + 9.2\tau - 19.2\log\sigma \quad (\text{J mol}^{-1}\text{ K}^{-1})$$

Where T_m is the normal melting point of the solid, K^{-1},

τ is the effective number of torsional bonds and can be calculated by (*9*)

$$\tau = SP3 + 0.5SP2 + 0.5RING - 1$$

where SP3 and SP2 are the numbers of non-ring non-terminable *sp*^{3} and *sp*^{2}atoms and RING is the number of rings.

σ is the rotational symmetry number

For 8:2 FTOH: $\tau = 10 + 0 + 0 - 1 = 9$; $\sigma = 2$;

$$\Delta_m S_i(T_m) = 56.5 + 9.2(9) - 19.2\log 2 = 133.5\ \text{J mol}^{-1}\text{ K}^{-1}$$

$$S_{iw}^{sat}(liquid) = S_{iw}^{sat}(solid) \cdot \exp\left[\frac{\Delta_m S_i(T_m)}{R}\left(\frac{T_m}{T} - 1\right)\right]$$

$$= 0.224 \cdot \exp\left[\frac{133.5\,\mathrm{J\ mol^{-1}\ K^{-1}}}{8.314\ \mathrm{J\ mol^{-1}\ K^{-1}}}\left(\frac{46.2 + 273.15}{22.5 + 273.15} - 1\right)\right] = 0.812\ mgL^{-1}$$

(2) 10:2 FTOH

2a. Using experimental entropy of fusion $\Delta_m S_i(T_m) = 55.9\ \mathrm{J\ mol^{-1}\ K^{-1}}$ and S_{iw}^{sat} (solid) = 0.011 mg L^{-1} estimated from the log-linear cosolvency extrapolation from acetone/water solutions:

$$S_{iw}^{sat}(liquid) = S_{iw}^{sat}(solid) \cdot \exp\left[\frac{\Delta_m S_i(T_m)}{R}\left(\frac{T_m}{T} - 1\right)\right]$$

$$= 0.011 \cdot \exp\left[\frac{55.9\,\mathrm{J\ mol^{-1}\ K^{-1}}}{8.314\ \mathrm{J\ mol^{-1}\ K^{-1}}}\exp\left(\frac{92.7 + 273.15}{22.5 + 273.15} - 1\right)\right] = 0.054\ mgL^{-1}$$

2b. Using the empirical relationship to estimate entropy of fusion $\Delta_m S_i(T_m)$:

For 10:2 FTOH: $\tau = 12 + 0 + 0 - 1 = 11$; $\sigma = 2$;
$\Delta_m S_i(T_m) = 56.5 + 9.2(11) - 19.2 \log 2 = 151.9\ \mathrm{J\ mol^{-1}\ K^{-1}}$

$$S_{iw}^{sat}(liquid) = S_{iw}^{sat}(solid) \cdot \exp\left[\frac{\Delta_m S_i(T_m)}{R}\left(\frac{T_m}{T} - 1\right)\right]$$

$$= 0.011 \cdot \exp\left[\frac{151.9\,\mathrm{J\ mol^{-1}\ K^{-1}}}{8.314\ \mathrm{J\ mol^{-1}\ K^{-1}}}\left(\frac{92.7 + 273.15}{22.5 + 273.15} - 1\right)\right] = 0.842\ mgL^{-1}$$

TABLE S3. Freundlich and linear sorption model fits[a] to the measured isotherms (n = number of data points used for regression)

Chemical	***Soil***	***Cosolvent***	**n**	***Freundlich Isotherm Regression***				***Linear Isotherm Regression***	
				Log K_f [95% CI]	**K_f**	**N [95% CI]**	**R^2**	**Log K_d [95% CI]**	**R^2**
4:2 FTOH	7CB2	none	24	0.072 [-0.131, -0.012]	1.18	0.933 [0.844, 1.021]	0.956	0.701 [0.643, 0.760]	0.964
6:2 FTOH	7CB2	none	24	1.23 [1.19,1.27]	17.0	0.967 [0.921, 1.013]	0.989	17.0 [16.23, 17.8]	0.990
	Drummer6	none	24	0.780 [0.736, 0.825]	6.03	0.946 [0.899, 0.993]	0.987	6.09 [5.87, 6.31]	0.993
	Oakville24	none	12	0.300 [0.259, 0.341]	2.00	0.991 [0.894, 1.087]	0.981	1.94 [1.84, 2.03]	0.995
10:2 FTOH	7CB2	40%	12	0.177 [0.145, 0.209]	1.50	0.929 [0.866, 0.993]	0.991	1.47 [1.34, 1.60]	0.983
	7CB2	30%	24	1.59 [1.52, 1.66]	38.9	1.25 [1.15, 1.35]	0.966	33.9[31.3, 36.6]	0.969
	7CB2	24%	24	2.47 [2.29, 2.64]	295	1.26 [1.09,1.43]	0.916	199 [175, 223]	0.927
	7CB2	20%	6	3.52 [2.78, 4.25]	3311	1.53 [1.09, 1.97]	0.959	593 [408, 779]	0.931
	7CB2	17%	6	3.55 [2.72, 4.38]	3548	1.40 [0.918, 1.88]	0.942	893 [661, 1124]	0.952
	7CB2	10%	6	3.40 [2.11, 4.69]	2512	0.910 [0.227, 1.59]	0.774	3713 [3159, 4267]	0.983
	Drummer-6	40%	12	-0.048 [-0.099, 0.003]	0.895	1.12 [1.01, 1.23]	0.980	0.939 [0.843, 1.034]	0.977
	Drummer-6	30%	12	1.22 [1.06, 1.39]	16.6	1.10 [0.838, 1.36]	0.900	17.9 [13.9, 21.9]	0.898
	Drummer-6	24%	6	1.69 [0.981, 2.40]	49.0	1.00 [0.315, 1.68]	0.804	57.4 [31.9, 83.0]	0.870
	Drummer-6	20%	4	2.17 [0.307, 4.02]	148	0.918 [0, 2.09]	0.849	199 [147, 251]	0.980
	Drummer-6	10%	4	3.80 [0.758, 6.84]	6310	1.26 [0, 2.74]	0.872	2051 [1064, 3037]	0.936
	Oakville-24	40%	12	-0.444 [-0.496, -0.392]	0.360	0.919 [0.816, 1.02]	0.975	0.358 [0.316, 0.399]	0.970
	Oakville-24	30%	12	1.07 [0.988, 1.15]	11.7	2.20 [1.86, 2.14]	0.990	7.64 [6.28, 9.00]	0.933
	Oakville-24	24%	6	1.48 [0.897, 2.07]	30.2	1.17 [0.572, 1.78]	0.880	23.8 [18.4, 29.2]	0.962
	Oakville-24	20%	4	3.11 [2.64, 3.59]	1288	1.91 [1.58, 2.24]	0.997	88.9 [53.4, 124]	0.954

[a] Isotherm data were fit with the Freundlich sorption model, $C_s = K_f\, C_w^{N}$, where C_w (mg L^{-1}) and C_s, (mg kg^{-1}) are the solution and sorbed phase concentrations at equilibrium, K_f is the Freundlich adsorption coefficient ($mg^{1-N}\, L^{N} kg^{-1}$) and N (unitless) is a measure of isotherm nonlinearity; and the Linear sorption model $C_s = K_d\, C_w$ where K_d (L kg^{-1}) is the linear sorption coefficient.

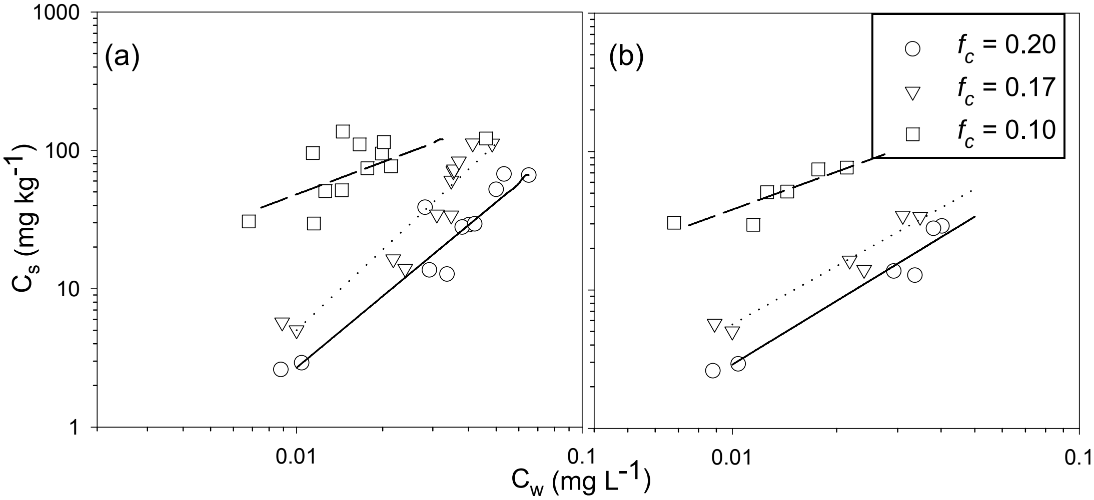

FIGURE S3. Sorption of 10:2 FTOH onto soil 7CB2 in acetone/water solutions at low acetone cosolvent volume fraction (*f_c*). The lines in (a) are Freundlich isotherm model fits to all the data points; and the lines in (b) Freundlich isotherm fits to C_w values (n = 6) resulting from the lowest three applied concentrations.

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