Supporting Information

Fluorotelomer Alcohol Biodegradation – Direct Evidence that Perfluorinated Carbon Chains Breakdown

Ning Wang[*1], Bogdan Szostek[2], Robert C. Buck[3], Patrick W. Folsom[1], Lisa M. Sulecki[1], Vladimir Capka[4], William R. Berti[1], and John T. Gannon[1]*

* Corresponding author phone: (302)366-6665; fax: (302)366-6602; email: ning.wang@usa.dupont.com

[1] DuPont Central Research and Development, Glasgow Business Community 301, PO Box 6101, Newark, DE 19714

[2] DuPont Haskell Laboratory for Health and Environmental Sciences, Newark, DE 19714

[3] DuPont Chemical Solutions Enterprise, Wilmington, DE 19803

[4] Present address: Tandem Labs, A Division of NWT Inc, Salt Lake City, UT 84124

Synthesis of Authentic Standards

$CF_3(CF_2)_6CH_2CH_2COOH$ [7-3 Acid], $CF_3(CF_2)_6CH(OH)CH_3$ [7-2 sFTOH] and $CF_3(CF_2)_6CH=CHCOOH$ [7-3 u Acid] were prepared and characterized by numerous analytical methods including NMR (^1H, ^{19}F) and Mass Spectrometry to verify their chemical structures. Their MS/MS spectra matched those of metabolites identified in the studies conducted thereby confirming these three substances as biodegradation products from 8-2 FTOH.

Identification of New Metabolites (LC/ARC Peaks 3, 6, 7)

In addition to verifying the chemical structures of previously unknown metabolites with authentic standards, the MS/MS spectral patterns were analyzed to elucidate the structures before authentic standards were available. We report here aspects of the mass spectra interpretation to benefit the reader of the manuscript and further support the metabolite chemical identification.

The identification of metabolites was conducted using negative ion electrospray ionization on an LC/Q-TOF MS system and applying chromatographic conditions matching those used on the LC-ARC system. For volatile metabolite(s), electron impact ionization GC/TOF and GC-MSD were used.

$CF_3(CF_2)_6{}^{14}CHOHCH_3$ (7-2 sFTOH). The identity of peak 7 was elucidated by LC/Q-TOF and GC/TOF and GC/MSD. The LC/Q-TOF runs indicated the presence of a chromatographic peak with m/z 475 at the retention time where the peak 7 was eluted. The daughter ion spectrum of m/z 475 indicated that this ion represents an acetate adduct of a neutral molecule (only acetate ion, m/z 59 was observed in the daughter ion spectrum). Therefore, m/z 475 represents the acetate adduct of ^{14}C-labeled 7-2 sFTOH

(Table 1). Similarly, a peak with m/z 473 was observed for this metabolite at the same retention time when study was done with non-labeled 8-2 FTOH. The GC/MS analysis of MTBE concentrated extracts from a large bottle experiment yielded a good quality EI spectrum for peak 7. Figure 3A presents the EI spectra of peak 7 obtained for the non-labeled metabolite on GC-TOF system. The accurate mass measurement of deprotonated molecular ion m/z 413 confirmed elemental composition of C9H4OF15 with –4.3-ppm error. The ion m/z 399 represents a loss of methyl group from neutral molecule of mass 414 and m/z 45 represents ion of elemental composition C2H5O. The presence of ion m/z 45 in the EI spectra is characteristic for secondary alcohols. The majority of other ions observed in the spectra are characteristic for fragmentation of fluorinated carbon chain molecules. This was confirmed by accurate mass measurement and resulting elemental compositions. Final structural confirmation of this new metabolite was achieved when EI spectra and retention times of a synthesized standard ($CF_3(CF_2)_6CHOHCH_3$) were matched with those of peak 7. Thus the structure of peak 7 was determined as $CF_3(CF_2)_6{}^{14}CHOHCH_3$ (7-2 sFTOH).

$CF_3(CF_2)_6{}^{14}CH$=CHCOOH (7-3 u acid). The identity of peak 3 was elucidated based on precursor ion spectra obtained for deprotonated molecular ion m/z 441 for ^{14}C-labeled metabolite and ion m/z 439 for non-labeled metabolite. Figure 3B presents a precursor ion spectrum of deprotonated molecular ion m/z 441 obtained with collision energy of 20 eV. The ion m/z 397 represents loss of 44 (CO_2) from deprotonated molecular ion, m/z 369 is a loss of 28 (^{14}CH=CH; loss of 26 for non-labeled metabolite) from ion m/z 397, and ion m/z 357 represents loss of two HF (2×20) from m/z 397. Ions m/z 169 and 219 represent fragments characteristic for fragmentation of fully fluorinated

carbon chain anions, such as obtained for PFOA after loss of CO_2 from deprotonated molecular ion. All these characteristics uniquely point to the structure of peak 3 as $CF_3(CF_2)_6{}^{14}CH{=}CHCOOH$ (7-3 u acid). Final structural confirmation of peak 3 was achieved when the daughter ion spectra and retention time of a synthesized standard ($CF_3(CF_2)_6CH{=}CHCOOH$) were matched with these of peak 3. Thus the structure of peak 3 was determined as $CF_3(CF_2)_6{}^{14}CH{=}CHCOOH$.

$CF_3(CF_2)_6{}^{14}CH{=}CHCONH_2$ (7-3 u amide). Similar to 7-3 u acid identification, the structure of peak 6 was elucidated based on the precursor ion spectra obtained for deprotonated molecular ion m/z 440 for ^{14}C labeled metabolite and ion m/z 438 for non-labeled metabolite. The even mass of deprotonated molecular ion indicates that the molecule contains an odd number of nitrogens. Figure 3C presents a precursor ion spectrum of deprotonated molecular ion m/z 440 obtained with collision energy of 20 eV. Ion m/z 70 for ^{14}C labeled metabolite (or m/z 68 for non-labeled metabolite) represents the breakage of the single bond at 3-rd carbon and formation of anion containing carbons 1 through 3 and neutral fluorocarbon with one hydrogen. Similarly, ion m/z 369 is explained by retention of the negative charge on the fluorinated part of the molecule (loss of neutral fragment of mass 71 from deprotonated molecular ion), while the single bond at the 3-rd carbon is broken. Ions m/z 169 and 219, analogous to metabolite 3 (peak 3), represent fragment ion obtained for fully fluorinated carbon chain anions. Thus the structure of peak 6 was determined as $CF_3(CF_2)_6{}^{14}CH{=}CHCONH_2$ (7-3 u amide). An authentic standard of peak 6 is not available to allow confirmation of proposed structure by comparison of spectral data and retention time with the standard.

References

(1) Wang, N.; Szostek, B.; Folsom, P. W.; Sulecki, L. M.; Capka, V.; Buck, R. C.; Berti, W. R.; Gannon, J. T. Aerobic biotransformation of ^{14}C-labeled 8-2 Telomer B Alcohol by activated sludge from a domestic sewage treatment plant. *Environ. Sci & Tech* **2005** *39*, 531-538.

Table S1. Experimental design for ^{14}C-8-2 FTOH [$CF_3(CF_2)_6{}^{14}CF_2CH_2CH_2OH$] Biodegradation in mixed bacterial culture[†].

Treatment	Replicate (n)	^{14}C-8-2 FTOH ($\mu g\ L^{-1}$)	Ethanol ($g\ L^{-1}$)	NaCN (mM)	Non-^{14}C 8-2 FTOH [$CF_3(CF_2)_6CF_2CH_2CH_2OH$] ($\mu g\ L^{-1}$)
Live test vessels	4	1049	0.1[‡]	0	0
Sterile control	4	986	0.1[‡]	0.5	0
Sample matrix blank	2	0	0.1[‡]	0	0
O_2 monitoring vessels	3	0	0.1[‡]	0	1000

[†]**:** Each sample bottle (glass serum bottle, 118-mL volume) contains 30 mL of bacterial growth medium consist of 1 g L^{-1} of yeast extract, KH_2PO_4 (0.70 g L^{-1}), $NaH_2PO_4{\cdot}H_2O$ (0.395g L^{-1}), KCl (0.5 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.5 g L^{-1}), $CaCl_2{\cdot}2H_2O$ (0.025 g L^{-1}), NaCl (1.0 g L^{-1}), $FeSO_4{\cdot}7H_2O$ (0.5 mg L^{-1}), $CuSO_4{\cdot}5H_2O$ (0.05 mg L^{-1}), $CoCl_2{\cdot}6H_2O$ (0.1 mg L^{-1}), $MnCl_2{\cdot}4H_2O$ (0.01 mg L^{-1}), $Na_2MoO_2{\cdot}2H_2O$ (0.025 mg L^{-1}), $C_6H_5Na_3O_7{\cdot}2H_20$ (Sodium citrate dihydrate tribasic; 1 mg L^{-1}), $NiCl_2{\cdot}6H_2O$ (0.1 mg L^{-1}), and $ZnCl_2$ (0.005 mg L^{-1}). The pH of the medium was adjusted to 7.2. [‡]: As a co-solvent.

Table S2. Experimental design for monitoring $^{14}CO_2$ and ^{14}C-organic volatiles in the headspace of sealed sample bottles and continuous air flow bottles containing activated sludge medium incubated with ^{14}C-8-2 FTOH [$CF_3(CF_2)_6{}^{14}CF_2CH_2CH_2OH$]†.

Treatment	Replicate (n)	^{14}C-8-2 FTOH ($\mu g\ L^{-1}$)	Ethanol ($g\ L^{-1}$)	Yeast extract ($g\ L^{-1}$)	Chloramphenicol ($g\ L^{-1}$)
Activated sludge	3	210	0.1§	0	0
Activated sludge plus yeast extract	3	122	0.1§	1.0	0
Activated sludge plus ethanol supplement‡	3	231	0.9	0	0
Sterile sludge	3	231	0.1§	0	0.2
Matrix blank	2	0	0.1§	0	0
Activated sludge plus continuous air flow (1.5 $L\ min^{-1}$) in headspace	3	52	0.1§	0	0
Sterile sludge plus continuous air flow (1.5 $L\ min^{-1}$) in headspace	3	52	0.1§	0	0.2

†**:** Each sealed sample bottle (glass serum bottle, 118-mL volume) contains 10 mL of activated or sterile sludge from a domestic sewage treatment plant and 20 mL of mineral medium consists of 8.5 mg L^{-1} of KH_2PO_4, 21.8 mg L^{-1} of K_2HPO_4, 33.4 mg L^{-1} of $Na_2HPO_4{\cdot}2H_2O$, 0.5 mg L^{-1} of NH_4Cl, 36.4 mg L^{-1} of $CaCl_2{\cdot}2H_2O$, 22.5 mg L^{-1} of $MgSO_4{\cdot}7H_2O$, and 0.25 mg L^{-1} of $FeCl_3{\cdot}6H_2O$. The pH was adjusted to 7.0. The bottles with continuous air flow contained 30 mL sludge without dilution with mineral medium. ‡: 0.4 g L^{-1} of ethanol was also added to the sample bottles periodically at each sampling time point. §: As a co-solvent.

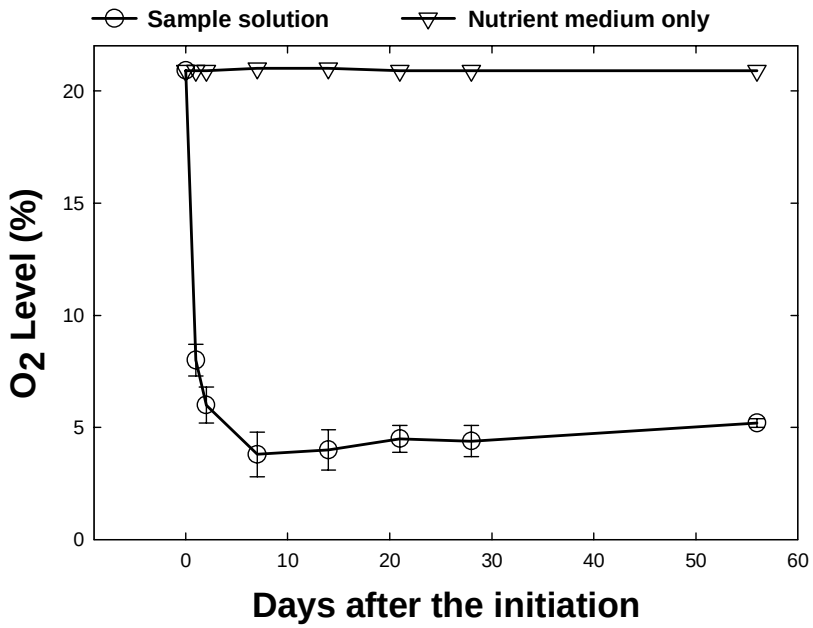

FIGURE S1. The oxygen concentration in the headspace of the sealed sample bottles containing mixed bacterial culture at different sampling time points.

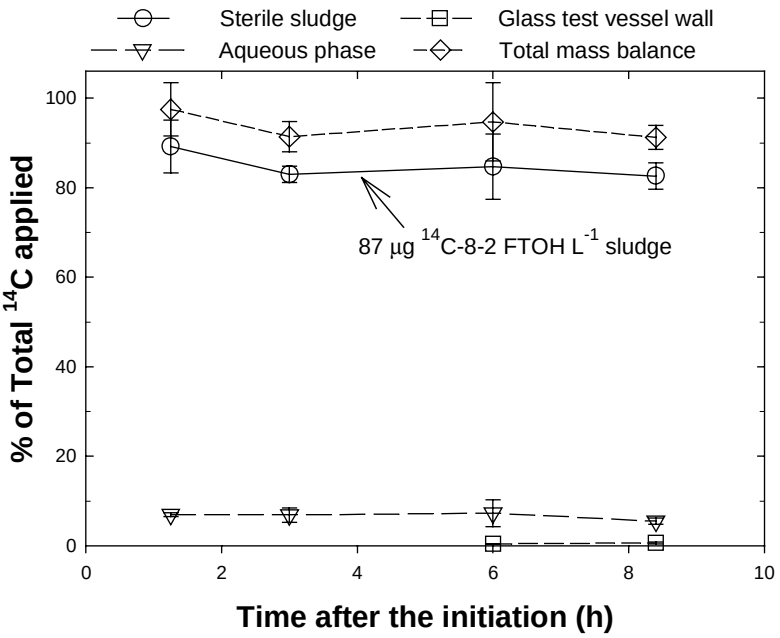

FIGURE S2. The adsorption of ^{14}C-8-2 FTOH to sterile sludge, which was collected and combined from two domestic sewage treatment plants. The experiment (n =3) was conducted based on US EPA study guideline: OPPTS 835.1110-Activated sludge sorption isotherm. US EPA document EPA-712-c-98-298, January, 1998.