1 **Aerobic Biodegradation of Toluene-2,4-di(8:2 fluorotelomer urethane) and Hexamethylene-1,6-**
2 **di(8:2 fluorotelomer urethane) Monomers in Soils**

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17 **Running Title:** Aerobic soil biotransformation of di-8:2 fluorotelomer urethanes
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Abstract. Aerobic soil biodegradation of toluene-2,4-di(8:2 fluorotelomer urethane) (FTU) and hexamethylene-1,6-di(8:2 fluorotelomer urethane) (HMU) in a forest soil and FTU in an agricultural silty clay loam soil was monitored for up to 6 months. Fluorotelomer alcohols were measured in head-space and parent monomers and all metabolites in soil extracts. Negligible degradation of FTU biodegradation occurred in the agricultural soil with $94 \pm 15\%$ recovered at day 180. However, in the forest soil, both FTU and HMU degradation was evident with significant losses of 24% (117 d) and 27% (180 day), respectively, and concomitant increases in the terminal metabolite, perfluorooctanoic acid (PFOA) concentrations were well above what could result from residual 8:2 FTOH. Kinetic modeling estimated half-lives for FTU (aromatic backbone) and HMU (aliphatic backbone) in the forest soil to be 3-5 months and 14.4-17.4 months, respectively. The addition of a structurally similar non-fluorinated FTU analog, toluene-2,4-dicarbamic acid diethyl ester (TDAEE) enhanced production of terminal end products from 8:2 FTOH degradation. However, there was no clear evidence that TDAEE enhanced cleavage of the urethane bond, thus TDAEE appeared to just serve as an additional carbon source. TDAEE's half-life was ~ one week. A second addition of TDAEE appeared to retard subsequent degradation of FTU exemplifying the microbial dynamics and diversity impacting degradation of polyfluoroalkyl substances. Enhanced degradation of HMU was observed upon re-aeration indicating oxygen may have been limiting during some periods although degradation of intermediate metabolites to terminal metabolites was still occurring, albeit at slower rates.

Keywords: PFOA, perfluoroalkyl substances, fluorotelomer urethane monomers, urethane cleavage, biotransformation

Introduction

Fluorotelomer (FT) polymer production was estimated around 20 million pounds in 2006 (USEPA, 2009). Degradation of FT-based compounds is a potential source of perfluorinated carboxylic acids (PFCAs) such as perfluorooctanoic acid (PFOA) in the environment (USEPA 2009; Buck et al., 2011). Global detection of PFOA in different environmental matrices (Zareitalabad et al., 2013), human blood, (Kato et al., 2011) wild life (Houde, et al., 2011) has led to many regulatory initiatives to phase out the use of perfluoroalkyl chain lengths greater than C6 (USEPA, 2015a) and investigate their sources to the environment. Direct emissions of these chemicals has been dramatically reduced in recent years (Ritter, 2010; EPA 2015b) raising the importance of potential contributions from indirect sources of fluoroalkyl substances used in the manufacturing process and present as residuals in final commercial products. Estimates by Wang et al. (2014) reflect substantial increases (factor of ≥ 3) in the relative amounts of total PFCAs from indirect sources between 1951-2002 and 2003-2015.

FT monomers and FT intermediates are present as residuals in the final polymer products including well recognized indirect sources including fluorotelomer alcohols (FTOHs) and fluorotelomer iodides (FTIs). FTOHs are intermediates used to synthesize a variety of FT monomers by linking them to hydrocarbon backbones with ester, ether or urethane linkages. Monomers are further polymerized to specialty polymers and surfactants offering stain repellent properties (Rao and Baker, 1994). Residual monomers in the final polymers and surfactants are not uncommon with the relative amounts dependent on the efficiency of the polymerization and cleanup processes (Russell et al., 2008, Washington et al. 2014). Current regulatory allowance requires residual monomers to be no more than 2 wt% (Washington et al., 2014). Monomers may be released during manufacturing, biodegradation of polymeric materials or from the use and

disposal of consumer products with polymer coatings containing residuals (USEPA, 2009). Especially, given relatively short half-lives for monomers of a few days to a few weeks (Dasu et al., 2012 & 2013, Royer et al., 2014, Liu and Avendaño, 2013; Butt et al., 2014), these residuals could potentially be as steady source of PFOA to the environment prior to, or even without, polymer breakdown. Recently airborne FT acrylate and FT methacrylate monomers were detected in Japan (Oono, et al., 2008a, 2008b) and 8:2 FT acrylate monomer was detected in some of soils where industrial-impacted municipal sludges were applied in Decatur, AL (Yoo et al., 2010).

Fluorotelomer based urethane polymers are commonly used in nylon carpeting, washable fabrics such as table cloths, uniforms, and the like (Kirchner, 1995). In aerobic soil biodegradation studies, Russell et al. (2010) estimated the geomean effective half-lives of 28-241 years for a fluorotelomer urethane polymer (MW ~ 3500 Daltons); however, the monomers expected to degrade much faster. To assess the inherent biotransformation potential of well-characterized urethane monomers with structural backbones (aromatic versus aliphatic), toluene-2,4-di(8:2 fluorotelomer urethane) (FTU) and hexamethylene-1,6-di(8:2 fluorotelomer urethane) (HMU) (Fig. 1) were synthesized and evaluated for their stability in soil under aerobic conditions. These have been used in the production of the fluorotelomer urethane polymers, thus potentially present as residuals in commercial products. Cleavage of the urethane linkage in FTU was hypothesized to be slower than HMU due to the close proximity of the two urethane moieties present on the toluene backbone at ortho and para positions, thus potentially hindering enzyme access (Kearney, 1965; Kaufman et al., 1967). Therefore, the potential for analog induced biodegradation of FTU was also assessed using a structurally similar non-fluorinated compound, toluene-2,4-dicarbamic acid diethyl ester (TDAEE) (Fig. 1). It was hypothesized that if microbes tried to metabolize the non-fluorinated urethane monomer as a growth substrate, the production of en-

zymes for cleaving the urethane bond would be enhanced and then through co-metabolism, cleavage of the urethane linkage in the fluorinated urethane monomer would also be enhanced. Co-metabolic degradation of organic pollutants by both bacteria and fungi are well documented in the literature (Fritsche et al., 2005). For example, co-metabolic transformation of trichloroethylene occurs by a methane monooxygenase enzyme released by methanotrophic bacteria to oxidize methane, and co-metabolic transformation of 3,4-dichlorophenol occurs by the mold *Penicillium frequentans* growing on phenol (Fritsche et al., 2005).

1. Materials

2.1. Chemicals.

Hexamethylene-1,6-di(8:2 fluorotelomer urethane) (HMU) and toluene-2,4-di(8:2 fluorotelomer urethane) (FTU) were synthesized in-house following published methods (Roy, 1958; Kirchner, 1995). Briefly, FTU and HMU were synthesized using 8:2 FTOH for both and 2,4-toluene diisocyanate (Fig S-1) and 1,6-hexamethylene diisocyanate (Fig. S-2). Additional synthesis details are provided in the Supplemental Information (SI). Synthesized monomers were scanned using LC/MS/MS to look for mono- substituted perfluoroethyl chains of which there were no prominent peaks for either monomer (scan spectra shown in Fig. SI3). Non-fluorinated 2,4-toluenedicarbamic acid diethyl ester (TDAEE) was purchased from Sigma-Aldrich. Details on metabolites, solvents and cartridges and their sources are summarized in the SI.

2.2. Soil

Aerobic biotransformation of FTU was measured in an agricultural soil (Raub-40) for 6 months and in forest soil (FRST-44) for ~4 months. HMU biotransformation was measured in FRST-44 soil for 6 months. Raub-40 is a silty clay loam soil collected immediately below the

vegetated zone of a turf grass area from the Purdue Agronomy Farm (West Lafayette, IN) in June 2009. FRST-44 is an acidic forest soil collected immediately below the vegetated zone under the tree canopy of forested area in Cherry Lane (West Lafayette, IN) in July (2010). Soil details are provided in the SI. The gravimetric moisture content for this soil at field capacity (corresponds to a -0.03 MPa matrix potential) was used as the initial soil moisture level in all experiments (24% for Raub-40 and 32% for the forest soil). The soil mass throughout the text refers to the oven-dry mass at 105 °C unless otherwise stated. Both the FTU degradation studies were conducted with the Raub-40 and FRST-44 soils soon after collection whereas the HMU study was done on FRST-44 soil after 3 months of cold storage (4 °C).

2. Methods

3.1. Soil Microcosm Setup

Microcosms consisted of 125-mL crimped amber bottles with ~10 g soil in each vessel with airtight closures (butyl rubber stoppers) to allow for monitoring volatile analytes both in the soil and the microcosm headspace. FTU aerobic biotransformation was assessed in both the FRST-44 and Raub-40 soils. An autoclave-sterilized soil control was (Dasu et al., 2012) included for the FTU study in Raub-40. HMU aerobic degradation was assessed only in FRST-44. To investigate the potential for analog enhanced biotransformation of FTU, a structurally similar non-fluorinated analog, toluene-2,4-dicarbamic acid diethyl ester (TDAEE) was added to the FRST-44 soil microcosms containing FTU on day 52 and 74 - post FTU addition. Re-aeration of soil microcosms occurred for FTU/Raub-40 soil on day 106, FTU/FRST-44 during the TDAEE additions on day 52 and day 74, and HMU/FRST-44 on day 104.

Live soils were pre-incubated for 6 d at field capacity prior to FT addition. FT was added to each soil microcosm via 50 mg of FT-coated talc powder ($\sim 20 \mu\text{g}$ FTU or HMU mg^{-1} talc) and then mixed manually using sterile spatulas resulting in a soil concentration of $\sim 100 \mu\text{g}$ FTU or HMU g^{-1} soil. The small amount of talc added (0.5 % of the soil weight) was assumed to exert no influence on soil properties. The talc carrier allows for the even distribution of sparingly water soluble compounds (Khan and Lee, 2009; Dasu et al., 2012 and 2013). Monomer-coated talc was prepared by mixing talc powder with an acetone solution or tetrahydrofuran (THF) containing FTU or HMU respectively, followed by evaporation of solvents. TDAEE dissolved in acetone (50 μL) was amended to soil resulting in a soil concentration of $\sim 50 \mu\text{g}$ TDAEE g^{-1} soil. All incubations were conducted under static conditions in the dark at $22 \text{ }^{\circ}\text{C} \pm 2$. Aerobic biotransformation studies of FTU were monitored for 4-6 months whereas biotransformation of HMU was monitored for 6 months. The residual amounts of 8:2 FTOH in FTU used in Raub-40 study was $0.56 \pm 0.03 \text{ mol\%}$ and in FRST-44 soil study was $0.31 \pm 0.02 \text{ mol\%}$ (about half of what was observed in FTU in Raub-40 study), respectively. To reduce the amount of volatile residuals, nitrogen was blown overnight through the FTU coated talc used for the FRST-44 study, hence resulted in lower residuals compared to the FTU used for the Raub-40 study. The residual 8:2 FTOH in HMU was $1.18 \pm 0.09 \text{ mol\%}$.

3.2. Sampling and sample treatment

Triplicate soil microcosms were destructively sampled at each sampling time. Prior to extracting the soils, headspace (10 mL) was drawn using a gastight syringe through two in series 600-mg C_{18} dry solid phase extraction (SPE) cartridges followed by elution of the cartridges with 5 mL acetonitrile. Headspace of the soil microcosms was analyzed for volatile 8:2 FTOH and 7:2 sFTOH.

Selected soil extraction methods were used as described in Dasu et al. (2012 and 2013). The initial extraction was done using ethyl acetate (EA) in the FTU studies or tetrahydrofuran (THF) in the HMU studies at 1:2 or 1:3 soil:solvent ratio for ~18 hr for analysis of parent, FTOHs, acid metabolites and unknown metabolites, followed by two sequential extractions using 20 mL 90/10 v/v acetonitrile (ACN)/water (200 mmol NaOH) at 45 °C for ~18 h to recover any remaining acid metabolites and 7:2 sFTOH. One exception was that only one subsequent base-acetonitrile extraction was performed in the FTU Raub-40 study. Each extraction was followed by neutralization with equal moles of HCl after solid-liquid separation via centrifugation (700 g for 30 min). All soil extracts were cleaned with graphitized carbon adsorbent (ENVI-Carb) prior to analysis to minimize matrix effects (Liu et al., 2007) and stored at ≤ -10 °C in the dark.

3.3. Chemical analysis

Both EA and THF extracts of FTU and HMU respectively were diluted with 1:9 v/v (EA or THF)/ACN and all ACN extracts were analyzed for FTU and HMU using the same HPLC/MS/MS but operated in the positive atmospheric pressure chemical ionization (APCI) mode with multiple reaction monitoring (details in the SI). Diluted EA/THF extracts were analyzed for 8:2 FTOH, 7:2s FTOH, acid metabolites and TDAEE analog. Undiluted ACN-base extracts were analyzed for 7:2s FTOH and acid metabolites. 8:2 FTOH was not targeted for analyses in the ACN-base extracts given the base induced hydrolysis that has been observed with other FT-monomer esters during the extraction process (Dasu et al 2010). Headspace SPE eluents and the septa extracts were analyzed for 8:2 FTOH and 7:2s FTOHs. All the FTOHs and acid metabolites were analyzed using a high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) operated in the negative electrospray ionization mode (details in

the SI). The list of fluorinated analytes monitored and additional method and quality assurance details are provided in the SI. TDAEE was analyzed using HPLC with UV detector (details in the SI). An internal standard mix (50 μ L) of [1,1,2,2-D₄; 3-¹³C] 8:2 FTOH (for the FTOHs) and [1,2-¹³C₂] PFOA (for the acid degradation products) was added to sample extracts immediately before analysis. Internal standard corrected external calibration curves were used to quantify fluorinated analytes. Precursor to product transitions, limits of quantitation and detection, and extraction recoveries using additional mass-labeled perfluorinated acids are summarized in the SI (Tables SI-1 through SI-4). Recoveries ranged from 77 to 109% for the various metabolites (Table SI-3). Extraction recovery of 8:2 FTOH amended to soil via the talc powder using EA from similar soils was previously determined to be 99.2 ± 1 wt% (Dasu et al. 2010). Extraction recovery of FTU using EA and HMU using THF from soil amended via talc powder was 93 ± 2 wt% and 84 ± 14 wt%, respectively. Concentrations reported for the degradation studies are not corrected for estimated recoveries.

3.4. Modeling

To better quantify apparent degradation of HMU and FTU as well as estimate half-lives, the experimental data were fitted with the Kinetic Graphic User Interface (KinGUI) v2.1, which is an R package *mkim* for performing kinetic evaluations and designed specifically for determining kinetic parameters from environmental fate studies (Boesten et al., 2006). The iteratively reweighted least squares (IRLS) algorithm was selected for simulation optimization. Biotransformation from one parent compound to its degradation product(s) was assumed to follow single 1st order kinetics (SFO) or a bi-exponential model (referred to as double 1st order kinetics in parallel, DFOP) with all subsequent reactions with metabolites to follow SFO kinetics. All reactions were considered irreversible with PFOA, PFHpA, and PFHxA considered the terminal end prod-

ucts following the paths shown in **Fig. S1-4**. A sink was allowed to exist for each transformation step (exemplified in **Fig. S-5**). The sink represents bound residues and unidentified or unquantified metabolites/pathways (see SI for additional details).

3. Results and Discussion

4.1 Biotransformation of FTU in an agricultural turf grass soil (Raub-40)

Little to no biotransformation of 8:2 FTU was observed in both autoclave-sterilized and live Raub-40 agricultural soil with $99 \pm 10\%$ and $94 \pm 15\%$, respectively, recovered at the end of the study (Fig. 2A). CO_2 concentrations monitored in the headspace show that the live microcosms were microbially active and that the autoclaved-sterilized soils had minimal activity (Table SI-5). Residual FTOH degraded in the live soils with parallel generation of PFOA (Fig. 2B). The FTU concentrations over time between live and autoclaved systems are not statistically different; however, the 8:2 FTOH concentrations on sampling days 5, 10, and 20 are relatively constant (not statistically different from each other), thus do not reflect the rate of decrease before 10 days and after 40 days suggesting that some 8:2 FTOH may have also been generated. No other metabolites were observed above quantifiable levels ($<\text{LOQ}$). Assuming PFOA was produced from only residual 8:2 FTOH ($0.56 \pm 0.03 \text{ mol}\%$), PFOA concentrations on day 180 reflect a conversion efficiency between 70% and 145% ($91 \pm 41 \text{ mol}\%$) (Fig. 2B). The 8:2 FTOH conversion to PFOA on day 180 as well as the previous sampling point (day 96) are high compared to the maximum values of 40% previously observed (Wang et al., 2009) indicating higher conversion efficiency at these low concentrations or that some FTU did indeed degrade. Nevertheless, FTU degradation in this agricultural soil during the 180-d period was minimal.

4.2 Biotransformation of 8:2 FTU and HMU in FRST-44 soil

Forest soils are known to be enriched in fungal enzymes (Anderson, et al., 1975) and these fungal enzymes may be more effective at breaking urethane linkages (Owen et al., 1996). Therefore, a forest soil was selected for subsequent studies to assess biodegradability potential of both the FTU aromatic urethane and an aliphatic urethane HMU (Fig. 1). Concentrations of FTU (Fig. 3A) up to 14 days and HMU (Fig. 3C) up to 104 days did not appear to be decreasing significantly given the standard error between triplicates. At 104 days in the HMU study, the remaining set of triplicate microcosms were re-aerated after which HMU levels statistically decreased to 73 ± 6 mol% by day 190. The impact of re-aeration suggests that oxygen consumption in the forest soil microcosms depleted much quicker than anticipated from previous studies with similar soils and may have slowed HMU degradation over time. In the FTU/FRST-44 microcosms, FTU levels decreased significantly after 14 days with $76 \pm 8\%$ of FTU recovered at day 52 immediately prior to adding FTU's non-fluorinated analog TDAEE (Fig. 1) targeted to enhance FTU degradation. All remaining FTU/FRST-44 microcosms were also re-aerated at this time.

While terminal PFCA metabolites (e.g., PFHxA, PFHxA, and PFOA) were observed, the fluorotelomer acid intermediates such as 8:2 FTUCA, 8:2 FTCA and 7:3 FTCA, which were observed in previous 8:2 FTOH degradation studies (reviewed by Liu and Avendaño, 2013; Butt et al., 2014), were below the limits of quantitation (LOQ) in both 8:2 FTU and 8:2 HMU FRST-44 biotransformation studies (Fig. 3B & 3D). 7:2s FTOH, an immediate precursor of PFOA was only above LOQs in the HMU biotransformation study with quantifiable amounts measured at day 104 (0.06 ± 0.02 mol%) (Fig. 3D).

4.3 Estimating FTU and HMU half-lives ($t_{1/2}$) in the Forest Soil (FRST-44)

Given the production patterns of FTOH and the levels of POFA produced in the forest soil (FRST-44), clearly FTU and HMU were degrading during the timeframe of the study although studies were not conducted long enough to observe a half-life ($t_{1/2}$) directly. Therefore, $t_{1/2}$ values for FTU and HMU were estimated with a modeling approach facilitated by using KinGUIi v2.1. For FTU, applying either a simple 1st-order or the bi-exponential model for FTU degradation across the entire incubation period resulted in $t_{1/2}$ values of 126 d and 148 d, respectively, although fits appeared equally good (Table SI-6, Fig. SI-6, and Fig. SI-7). For HMU, the generation and subsequent degradation of 8:2 FTOH when using averages could only be fit well by applying the bi-exponential model (Figure SI-8); however, when fitting all individual data points, both 1st-order and bi-exponential rate models gave similar fits with estimated $t_{1/2}$ value of 521 and 432 days, respectively (Table SI-7, Fig. SI-9, and Fig. SI-10). Regardless, contradictory to what we originally hypothesized, FTU's estimated $t_{1/2}$ is much shorter than HMU's $t_{1/2}$, which is consistent with the large production of PFOA (Fig. 4A). The overall $t_{1/2}$ value estimated for 8:2 FTOH in the FTU microcosm averaged both before and after TDAEE addition is ~ 0.2 d versus ≥ 3.8 d in the HMU microcosm, which corresponds to the lower observed production of PFOA. In previous biotransformation studies with 8:2 FTOH and monomers that serve as precursors to 8:2 FTOH, 8:2 FTOH values have typically been a few days or less, but not longer than a week (Dasu et al., 2012; Liu and Avendaño, 2013; Royer et al., 2014). The reasons for the apparent persistence of 8:2 FTOH in the soils amended with HMU is unclear. The studies were conducted simultaneously with soil from the same field sample, amended similarly, and the monomers were washed several times to remove residual synthesis reactants.

4.4 Effect of additions of the non-fluorinated analogue TDAEE

Based on the agricultural soil data where little to no degradation of FTU was observed and no apparent decrease of FTU in FRTS-44 for the first 2 weeks, it was decided to add TDAEE immediately after the next sampling time (day 52). Later analyses of day 52 samples revealed that FTU had degraded substantially between the two sampling periods (~ 2 and 7 weeks) and prior to TDAEE addition (Fig. 3A). Although added TDAEE degraded rapidly (Fig. SI-11) with an estimated half-life of 7.3 d (insert in Fig. SI-11), similar increases in FTU degradation was not apparent. TDAEE was added again on day 72, but the FTU recovered between 62-117 days did not change statistically and averaged 68 ± 7 mol%, thus TDAEE additions did not appear to enhance FTU degradation. FTU's $t_{1/2}$ values estimated from only the data collected prior to pre-TDAEE additions are shorter (90 d vs > 120 d) (Table SI-6, Fig. SI-12) also suggesting FTU degradation was not enhanced with TDAEE additions. Slow or lack of FTU degradation compared to TDAEE may be due to sorption-limited access to the FTU ester linkages or additional steric hindrances caused by the perfluoroalkyl chains. However, the generation of PFOA increased substantially with TDAEE additions to a rate similar to the initial rate of PFOA generation from residual 8:2 FTOH. In a parallel study, where only TDAEE was amended to the FRST-44 soil with more frequent sampling times, TDAEE degradation was first-order ($r^2 = 0.997$) with half-life of 5.4 days (Fig. SI-11), which is similar to what was estimated within the FTU-amended soil microcosms (Fig. SI-11).

4.5 Trends in 8:2 FTOH and PFOA from 8:2 FTU and HMU

8:2 FTOH is the first metabolite expected with the cleavage of the urethane bond and PFOA being the primary terminal metabolite of 8:2 FTOH degradation (Fig. SI-3). A more care-

ful look at changes in 8:2 FTOH levels and subsequent generation of the terminal acid metabolite PFOA for all three degradation studies (Fig. 4) offers additional evidence with regards to the likelihood of monomer degradation.

FTU loss was not statistically significant (Fig. 2A), 8:2 FTOH levels did not statistically increase but levels did not decline consistently as would be expected if no 8:2 FTOH generation occurred (Fig. 2B and 4B), and average PFOA levels (91 ± 41 mol% relative to residual FTOH) exceeded than what was expected to form from only residual 8:2 FTOH. In FRST-44, 73% FTU still remained at the end of the study (117 d), and as in Raub-40, increases in 8:2 FTOH levels were not observed (Fig. 4B). However, PFOA generation far exceeded (269 ± 1 mol% relative to residual FTOH) what could result from residual 8:2 FTOH even with 1:1 mole conversion (Fig. 4A), thus clearly reflecting 8:2 FTOH generation from the cleavage of FTU urethane linkage. TDAEE additions clearly enhanced PFOA generation, likely due to enhanced microbial activity with the additional carbon source. For HMU in FRST-44, 76% still remained at the end of the study (180 d); however, 8:2 FTOH concentrations increased (Fig. 3D and 4B) and PFOA generation again was higher (78 ± 2 mol% relative to residual FTOH, Fig. 4A) than expected if only from residual 8:2 FTOH. Given the substantial increase in 7:2s FTOH (Fig. 3D), immediate precursor to PFOA, in the last 2 sampling days (~22 mol% from day 103 to day 180) and subsequent increase in PFOA, HMU degradation is evident and would likely have continued with adequate oxygen and nutrients.

5. Summary and Implications

FTU and HMU degradation clearly occurred in the forest soil and possibly some in the agricultural soil with the production of PFOA above levels expected or even possible from only

residual 8:2 FTOH. Biodegradation of the urethane linkage to the aromatic backbone (FTU) compared to the aliphatic backbone (HMU) was faster, but both were slow relative to other monomers studied to date with estimated half-lives of 3 to 5 months and 14 to 17 months, respectively. The greater activity observed in the forest soil may be due to fungal enzyme activity playing the larger role in urethane bond cleavage. The addition of TDAEE (the non-fluorinated FTU analog) clearly increased soil microbial activity with enhanced production of terminal end products from 8:2 FTOH degradation, but there was no clear evidence that TDAEE enhanced the microbes responsible for urethane bond cleavage. In fact a second addition of TDAEE appeared to retard subsequent degradation of FTU (Fig. 3A). Slow or lack of FTU degradation in different soils, therefore, may be due to the right enzymes not being produced, and if these enzymes originate from fungi, enhanced bacterial activity could lead to decreased fungal activity and an increased consumption of fungal enzymes (de Boer et al., 2005). Also the dynamics observed in these studies further indicates the role of microbial diversity, or the need thereof, in the breakdown of these substances to PFOA. These urethane monomers as well as the FT stearate and citrate monomers (Dasu et al., 2012 and 2013), which are present as residuals in commercial products, are much more easily biodegraded than their corresponding polymeric substance (Russell et al., 2008, 2010; Washington et al., 2009, 2015). Efforts to reduce residuals in commercial products have been underway the past few years (USEPA, 2009; 2015b); however, given longevity of some products and existing disposal sites, monomer degradation will clearly serve as an indirect source of PFCAs into the environment for the next few decades.

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Appendix A. Supporting Information

Additional details on analytical and extraction efficiency methods, limits of quantitation and detection, supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/#####>.

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Toluene-2,4-di(8:2 fluorotelomer urethane) (FTU)

Aerobic Biotransformation

Hexamethylene-1,6-di(8:2 fluorotelomer urethane) (HMU)

Fig. 1. Chemical structures of Hexamethylene-1,6-di(8:2 fluorotelomer urethane) (HMU), Toluene-2,4-di(8:2 fluorotelomer urethane) (FTU) and non-fluorinated Toluene-2,4-dicarbamic acid diethyl ester (TDAEE). The double slash marks highlight the bond that will be broken if ester hydrolysis occurs.

Fig. 2. (A) Mol% FTU relative to FTU at t=0 and (B) Mol % 8:2 FTOH and PFOA relative to residual 8:2 FTOH at t=0 recovered in the live and autoclave-sterilized Raub-40 agricultural soil. Asterisk indicates re-aeration at that sampling point.

Fig. 3. Loss of parent and degradation products recovered from the biotransformation of 8:2 FTU and 8:2 HMU in FRST-44 live soil over time: (A) mol % 8:2 FTU and TDAEE remaining and (B) mol % metabolites recovered in the FTU study; and (C) mol % 8:2 HMU remaining and (D) mol % metabolites recovered in HMU study. Note differences in the y-axis scales. Asterisk indicates re-aeration at that sampling point.

Fig. 4. Mol% PFOA (left) and 8:2 FTOH (right) relative to residual 8:2 FTOH at t=0 in FTU studies with FRST-44 and Raub-40 soils and in HMU studies with FRST-44 soils.

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