

Biotransformation of ^{14}C -Labeled Fluorotelomer Substances by Soil Microorganisms

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Introduction

- Growing public interest in the environmental fate of fluorinated chemicals due to the global presence of perfluorinated acids such as perfluorosulfonic acid (PFOS) and perfluorooctanoic acid (PFOA).
- 8-2 Telomer B Alcohol (8-2 TBA, $\text{CF}_3(\text{CF}_2)_6\text{CF}_2\text{CH}_2\text{CH}_2\text{OH}$) is a major raw material used in the manufacture of sales products (e.g. fluorosurfactants and fluoropolymers).
- The chain length, $(\text{CF}_2)_8$, is representative of the telomer intermediates used to manufacture sales products.

Materials and Methods

• **Soil:** Sassaparilla soil - pH = 5.5, OM = 2.9%, Sandy loam, Microbial Biomass = 154.4 μg carbon /g dry soil.

• **Study Material:** Custom synthesized ^{14}C -labeled 8-2 TBA ($\text{CF}_3(\text{CF}_2)_6^{14}\text{CF}_2\text{CH}_2\text{CH}_2\text{OH}$) at ~ 180 μg Kg⁻¹ soil.

• **Additional Carbon source:** ~ 50 mg ethanol (as cosolvent) Kg⁻¹ soil.

• **Experimental System:** Closed bottles (10 g soil plus substance solution in 127-mL bottles). The experimental duration is up to one year and the O_2 content and the respiration rate of the soil were monitored during the study.

• **Extraction:** 1st: CH_3CN (acetonitrile) at room T for several days; 2nd: 90% CH_3CN + 20 mM NaOH at 50 °C overnight. The 1st and 2nd extract solutions were analyzed separately by LC/ARC.

• **Analysis:** LC/ARC (On-Line Chromatograph/Accurate Radioisotope Counting) and quadrupole time of flight mass spectrometry (Q-TOF-MS) for ^{14}C -metabolite quantification and identification, and LC/MS/MS for PFHA [$\text{CF}_3(\text{CF}_2)_n\text{COOH}$] quantification.

• **^{14}C -volatile trapping:** 10 mL of headspace gas were passed through Two C_{18} cartridges (0.6 g each) mounted in tandem to trap organic volatiles and the cartridges were eluted with 5 mL of CH_3CN .

• **$^{14}\text{CO}_2$ trapping:** The headspace gas after passing through two C_{18} cartridges was trapped in 5 mL of 1 N of NaOH.

Physical Properties and Purity of the Test Substance – $\text{CF}_3(\text{CF}_2)_6^{14}\text{CF}_2\text{CH}_2\text{CH}_2\text{OH}$

"A Difficult-to-Test Substance"

- Low solubility
 - ~ 150 $\mu\text{g/L}$
- Vapor pressure
 - 3 Pa at 21 °C
- Adsorption to surfaces

A Chromatogram of HPLC Characterization of Radiochemical Purity of the Test Substance

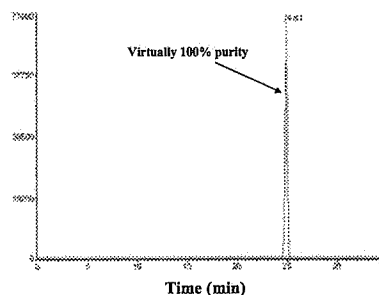


Figure 1

TABLE 1. LC/ARC peak number, acronym, structure, and molecular weight of the metabolites of ^{14}C -8-2 TBA biodegradation

Metabolite	LC/ARC Peak Number	Acronym Peak/Definitive structure determinations are in Progress	Structure	Molecular Mass
Unknown	1			
Unknown	2			
$\text{CF}_3(\text{CF}_2)_6^{14}\text{COOH}$ (PFOA) and an unknown	3			
$\text{CF}_3(\text{CF}_2)_6^{14}\text{CF}=\text{CFCH}_2\text{COOH}$	4	8-2 unsaturated acid		460
$\text{CF}_3(\text{CF}_2)_6^{14}\text{CH}_2\text{CH}_2\text{COOH}$	5	2H, 2H, 3H, 3H-PFDA		444
Unknown	6			
$\text{CF}_3(\text{CF}_2)_6^{14}\text{CF}_2\text{CH}_2\text{CH}_2\text{OH}$ (Parent)	7	8-2 TBA		466
$\text{CF}_3(\text{CF}_2)_6^{14}\text{CF}_2\text{CH}_2\text{CHO}$ and/or $\text{CF}_3(\text{CF}_2)_6^{14}\text{CF}=\text{CH}_2$	8	8-2 FTAL and/or 8-2 FT Olefin		
$\text{CF}_3(\text{CF}_2)_6^{14}\text{COOH}$		PFHA		314

Study Objectives

To answer the following questions:

- Is 8-2 TBA readily transformable in soil?
- What are the potential metabolites of 8-2 TBA biotransformation?
- What are the likely pathways for 8-2 TBA biotransformation?
- Are alternative biotransformation pathways available to metabolize 8-2 TBA beyond PFOA?
- Does microbial α -oxidation involve in 8-2 TBA transformation to form perfluorononanoic acid [$\text{CF}_3(\text{CF}_2)_8^{14}\text{CF}_2\text{COOH}$; PFNA]?
- Can 8-2 TBA and potential metabolites be strongly adsorbed to the soil and thereby not bioavailable?

Results and Discussion – Figure 2

- [3- ^{14}C]-8-2 TBA (peak 7) is transformed to other metabolites.
 - Over 90% of ^{14}C -8-2 TBA was converted at day 14, the majority of the metabolites observed were not volatile.
- PFOA, an unknown metabolite (overlapping peak 3) and 2H,2H,3H,3H-PFDA (peak 5) are the major metabolites at day 84.
- 8-2 unsaturated acid (peak 4) was formed from day 1-2, but was converted after day 7.
- Peak 6 is major metabolite from day 1-7, but is not present after day 28.
- Two unknown ^{14}C -metabolites (peaks 1&2), with molecular weight less than PFOA (retention time < PFOA), were observed during the study.
 - They may be possible intermediates for PFHA formation.
- Peak 8 was formed from day 1-7, but was not present after day 7.
 - Transient intermediate(s) accounting for small percentage of the mass balance.
- No $\text{CF}_3(\text{CF}_2)_6^{14}\text{CF}_2\text{COOH}$ (PFNA) was detected.
 - Microbial α -oxidation of ^{14}C -8-2 TBA was not observed.



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Results and Discussion – Figure 2

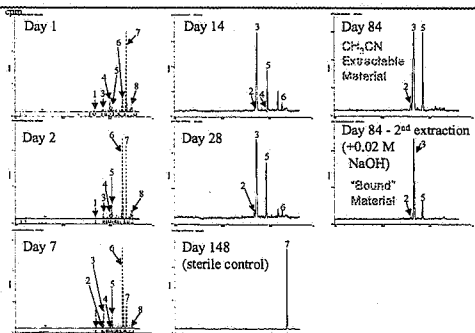


Figure 2
LC/ARC Chromatograms
of ^{14}C -parent and metabolites

Results and Discussion – Figure 4

Minimal Alcohol Volatility

- The volatility of ^{14}C -8-2 TBA once applied to the soil was minimal.
 - Only ~2% of ^{14}C was detected in the headspace over 45 days.
 - No additional ^{14}C -containing organic volatiles were detected after day 24.
- Strong adsorption to the soil and rapid conversion to other non-volatile metabolites significantly decrease the potential for alcohol release to air from soil.

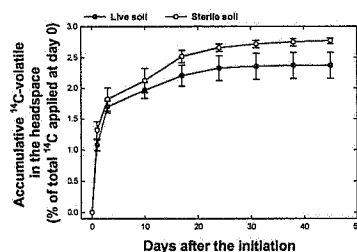


Figure 4

Results and Discussion

Substantial "Sequestration" Observed

- ^{14}C -parent and metabolites are *strongly adsorbed* to the live soil, probably to the soil organic matters, within 7 days.
 - After day 7, about 50% of the ^{14}C -parent and metabolites became unrecoverable by conventional solvent extraction methods
 - (CH_3CN , ethanol, acetone, THF, or MTBE at 50°C ; CH_3CN plus 1 M NaCl, CH_3CN plus 1 M HCl).
 - Only 0.5% triethylamine and 0.020-1 M NaOH plus CH_3CN at 50°C overnight gave a good recovery for samples between days 7-28.
 - After day 28, even 20 mM NaOH plus CH_3CN at 50°C overnight can only gave a partial recovery of the ^{14}C from the soil.
 - Cobalt-60-sterilized, but not autoclaved soil, resulted in near complete recovery (even at day 84) of ^{14}C -parent and fluorinated carboxylic acids spiked (dosed) into the soil at day 0.
 - ($\text{CF}_3(\text{CF}_2)_6^{14}\text{CF}_2\text{CH}_2\text{CH}_2\text{OH}$, $\text{CF}_3(\text{CF}_2)_6\text{CF}_2\text{CH}_2\text{COOH}$, $\text{CF}_3(\text{CF}_2)_5\text{CF}=\text{CFCH}_2\text{COOH}$, PFOA, and PFHA)

Results and Discussion – Figure 5

Perfluorohexanoic Acid Formation = Fluorocarbon Chain "Degradation"

- PFHA formation is significant.
 - At day 56, it accounted for about 14% of the mass balance.
 - The third and fourth perfluorinated carbons have been defluorinated and mineralized.
 - This shows that alternative metabolic pathways are available to metabolize 8-2 TBA beyond PFOA.

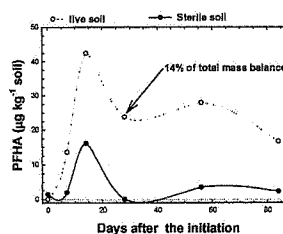


Figure 5

Results and Discussion – Figure 3

$^{14}\text{CO}_2$ Release: Evidence of Fluorocarbon Chain "Degradation"

- 2% $^{14}\text{CO}_2$ was detected in the headspace of the live soil sample bottles.
- Indicates that the fluorinated C_8 -carbon has been de-fluorinated and mineralized.
- The loss of ^{14}C -labeling should result in metabolites with fluorocarbon chain lengths less than 8.

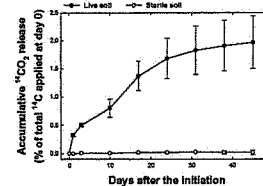
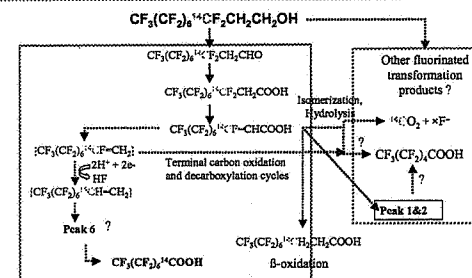


Figure 3

Possible Biotransformation Pathways in Soil



Conclusions

- [3- ^{14}C] - 8-2 TBA was rapidly transformed.
- PFOA, an unknown metabolite and 2H,2H,3H,3H-PFDA are the prevalent metabolites at day 84.
 - A substantial amount are strongly sorbed and not easily extracted.
- No PFNA was observed.
- Minimal 8-2 TBA volatility was observed.
- Acid Metabolites identified are not expected to have potential for release to air and long-range atmospheric transport.
- Significant $^{14}\text{CO}_2$ evolution and PFHA were observed.
 - Indicate that *biotransformation pathways* are available which metabolize multiple $-\text{CF}_2-$ groups resulting in metabolites with less than eight fluorinated carbons.



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