Study Title

ACCELERATED BIODEGRADATION OF 8-2 TELOMER B ALCOHOL – A PRELIMINARY SCREENING STUDY

Test Guideline

This study uses the concept of OECD Guideline for Testing of Chemicals; Section 3: Inherent Biodegradability (1992).

Author

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Study Completion Date

20 March 2003

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CERTIFICATION OF AUTHENTICITY

ACCELERATED BIODEGRADATION OF 8-2 TELOMER B ALCOHOL – A PRELIMINARY SCREENING STUDY

We, the undersigned, declare that the work described in this report was performed under our supervision, and that this report provides an accurate record of the procedures and results.

Report by:

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Study Initiation Date:
14-July-2002

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1.0 SUMMARY

Rationale of the Study

This test generates environmental fate information potentially relevant to the persistence of the test substance. When a chemical enters the environment, biodegradation is one of the major routes that determines the environmental fate of the chemical. The biodegradation of 8-2 Telomer B Alcohol (8-2 TBA, CAS# 678-39-7) may be slow, because only 2 hydrocarbons of the molecule may be readily accessible for microbial metabolism, the rest of the 8 perfluorinated carbons are expected to be difficult to be metabolized by microorganisms. Hypothetically, microorganisms that have been pre-exposed to the test chemical may adapt to have a higher metabolic capacity to transform and potentially defluorinate 8-2 TBA under favorable growth conditions. A test with pre-adapted bacterial culture under favorable growth conditions may give a clearer indication of the biotransformation potential for a given test chemical. Such a test may provide optimal conditions for potential microbial metabolism of 8-2 TBA. For example, if a chemical is not metabolized under such optimized conditions, it is less likely to be metabolized/biodegraded in the environment. The test system is also useful to rapidly identify potential biotransformation products and this will enable use of authentic standards to facilitate identification and quantification of transformation products in the subsequent definitive biodegradability studies.

Test System:

The primary biotransformation potential of the test substance 8-2 TBA in bacterial growth medium plus bacterial culture that has pre-grown in 50-100 mg/L of 8-2 TBA was determined. The bacterial culture originated from an industrial waste water treatment facility. The test system consisted of individually crimped test vessels (glass serum bottles). The test was conducted at room temperature (~24°C). The 8-2 TBA stock solution (made in ethanol) was introduced into the bacterial growth medium (Table A-8) with the bacterial inoculum and was kept in closed bottles in the dark at room temperature. Periodically (days 0, 14, and 28), sample bottles were sacrificed for extraction and analysis of the test chemical, fluorinated acid metabolites, and fluoride (F^- ion).

Findings:

The primary biotransformation of 8-2 TBA was rapid (>70% at day 14 and near 100% at day 28) compared with the abiotic control. Significant defluorination of 8-2 TBA was observed during the test. Four of the potential biotransformation products were monitored for and were identified through tandem mass spectrometry analysis and comparison with analytical standards: 1) 2-perfluorooctyl ethanoic acid (2-PFOEA, $F(CF_2)_8CH_2COOH$, CAS# 27854-31-5); 2) 2H-hexadecafluoro-2-decenoic acid (2H-HDF-2-DA, $F(CF_2)_7CF{=}CHCOOH$, CAS# 161094-76-4); 3) perfluorooctanoic acid (PFOA,

$F(CF_2)_7COOH$, CAS# 335-67-1); and 4) perfluorohexanoic acid (PFHA, $F(CF_2)_5COOH$, CAS# 307-24-4). Other potential transformation products that formed during the test were not determined.

Conclusions:

Under the test conditions, 8-2 TBA is being rapidly transformed to fluorinated acids and other unidentified transformation products. Although PFOA is one of the identified metabolites, it accounted for <2% of mass balance. PFOA apparently may be further metabolized to form PFHA and may not be an ultimate (stable) metabolite under the test conditions.

2.0 GENERAL STUDY INFORMATION

Study Objectives

- Determine the biotransformation potential of 8-2 TBA by monitoring its concentration changes during a biodegradation test with optimized conditions
- Determine the degree of defluorination of 8-2 TBA
- Identify potential metabolites such as fluorinated acids during the test
- Assess whether perfluorinated acids can be further metabolized
- Determine possible biodegradation pathways of 8-2 TBA
- Confirm expectation that mass balance will be difficult to achieve in cold studies due to potential abiotic removal mechanisms – volatility and adsorption.

Test System Justification

The test system is modified from OECD 302 guidelines and was requested by the submitter.

Study Personnel

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- Haskell Laboratory for Health and Environmental Sciences

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Study Execution Dates

Experimental Start Date:	19-Jul-2002
Experimental Completion Date:	19-Sep-2002
Study Completion Date:	20-March-2003

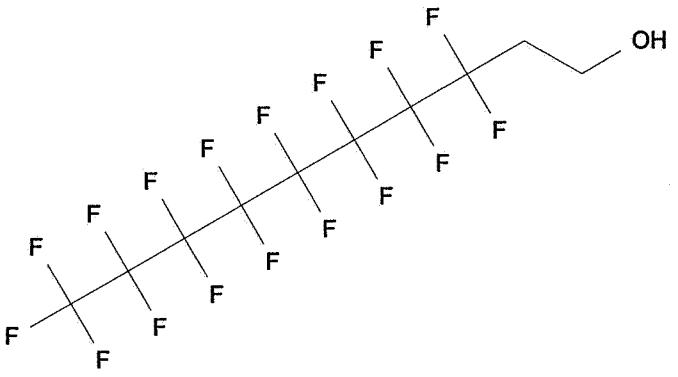
3.0 MATERIALS AND METHODS

3.1 Test System

3.1.1 Test Substance

Name:	8-2 Telomer B Alcohol
Synonym:	(Perfluorooctyl)ethanol, 8-2 TBA
Active substance(s)	8-2 Telomer B Alcohol, 99%
CAS Name:	1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10- heptadecafluoro-
Molecular weight	464.12 g mole^{-1}
CAS Number(s):	678-39-7

Structure:



Lot Number:	P.00/001
EMSE Sample Number:	E93386-80
Concentration of a.s., nominal:	99%
Concentration of a.s., analyzed:	99.2%
Major impurity	0.8% as $C_7F_{15}CF{=}CHCH_2OH$ (Figure A-7)
Certificate of Analysis Date:	13-Sept-2001
Date Received:	26-Mar-2002
Solubility at 25°C.:	~140 µg L^{-1}
Vapor pressure:	0.023 mm Hg
Stability:	Stable at ambient room temperature
Appearance/Color:	White solid
Storage Conditions:	Room temperature; keep tightly closed
Safety Precautions:	Wear lab coat, protective gloves, and safety glasses

3.1.2 Reference Substance

None

3.1.3 Preparation of Bacterial Growth Medium

The 20% yeast extract and different mineral stock solutions were prepared and were autoclaved. The bacterial growth medium was prepared by dilution of the different stock solutions (see Table A-8 for details of the medium components). The growth medium was sterile filtered into Nalgene 1 liter filter units and was stored at room temperature.

3.1.4 Test vessel Coating with 8-2 TBA solution

On 15-Jul-2002, Glass serum bottles (120 mL volume) were filled with 100 mL of sterile water and 10 µL of 8-2 TBA stock solution (3 mg/mL in ethanol) was injected into each of the bottles for a final concentration of 300 µg/L. The bottles were sealed and incubated for 3 days at room temperature with about 250 RPM shaking. The bottles were rinsed with sterile deionized water and capped for the test the next day.

3.1.5 *Adapted bacterial culture preparation*

Approximately 500 mL of industrial waste sludge from an industrial waste water treatment facility was collected from aeriation tanks on 13-June-2002 and were sent to the test lab. Upon arriving at the test lab the same day, the sludge was assigned an ID number E93386-87. The sludge was mixed by briefly shaking the jug to suspend the microorganisms. After settling the sludge for approximately 15 min to remove coarse matters, about 2 mL aliquot of the sludge was transferred to a 250 mL sterile cell culture flask that contained about 50 mg/L of 8-2 TBA in 100 mL bacterial growth medium. The bacterial culture was maintained at room temperature by periodically transferring about 0.5 mL of the culture into another 50 mL polypropylene tube that contained about 5 mL of the bacterial growth medium plus 50-100 mg/L of 8-2 TBA. The culture was mixed by constant shaking at about 210 RPM. The day before the initiation of the test (18-Jul-2002), 0.5 mL of the bacterial culture was transferred into a 250 mL flask that already contained 100 mL of the bacterial growth medium plus 600 µg/L of 8-2 TBA and incubated overnight at room temperature. The cell culture was centrifuged and the pellet was redissolved in the growth medium after discarding the supernatant. This wash step was repeated once. The washed bacterial culture will serve as inoculum for the test. Ten mL of the washed bacterial culture was transferred with a plastic pipette to a 20 mL scintillation vila and was autoclaved. The autoclaved culture was referred to as killed bacterial culture and will be used for abiotic control treatment.

3.1.6 *Activated Sludge Collection*

Approximately 4 liters of activated sludge was collected from the City of Wilmington (DE) Municipal Waste Treatment Facility (POTW) - Aeriation Basin # 2 on 16 September 2002. Upon arriving at the test lab, the sludge was assigned an ID number E93386-100. The sludge was mixed by briefly shaking the jug to suspend the microorganisms. After settling the sludge for approximately 15 min to remove coarse matters, the upper aqueous phase of the sludge was filtered through a nylon net with a pore size of 85 µm. The filtrate was inoculated into the bacterial growth medium and incubated overnight. This bacterial culture was used as inoculum in a separate experiment to conduct spike recovery of four specific fluorinated acid standards, which were quantified in the study. These spike recoveries are assumed to be also representative of fluorinated acids recovery from the adapted baterial culture.

3.1.7 *Test Units*

Test vessels were 120 mL borosilicate glass serum bottles with pre-cleaned aluminum-lined crimp caps. The pre-cleaning was done by rinsing the aluminum foil and septa with methanol once and then with sterile deionized water three times.

3.2 *Test Conduct*

Five types of experimental treatments were conducted. The pre-cleaning was done by rinsing the aluminum foil and septa with methanol once and then with sterile deionized water three times.

3.2.1 *Treatment 1 – 600 µg/L of 8-2 TBA in Bacterial Growth Medium plus the Bacterial Culture Inoculum in Coated Bottles*

For a total of 15 coated glass serum bottles (5 replicates × 3 sampling time points), 29.34 mL of of the growth medium, 0.663 mL of the washed bacterial culture, and 6 µL of 8-2 TBA stock solution (3 mg/mL in ethanol) were added to each of the glass serum bottles. The bottles were crimped with pre-cleaned aluminum foil and PTFE/silicone septa.

3.2.2 *Treatment 2 – 600 µg/L of 8-2 TBA in Bacterial Growth Medium plus Killed Bacterial Culture in Coated Bottles*

For a total of 9 coated glass serum bottles (3 replicates × 3 sampling time points), 29.04 mL of the growth medium, 0.663 mL of the killed bacterial culture, 0.3 mL of 0.2 M NaCN, and 6 µL of 8-2 TBA stock solution (3 mg/mL in ethanol) were added to each of the glass serum bottles. The bottles were crimped with pre-cleaned aluminum foil and PTFE/silicone septa.

This treatment served as an abiotic control.

3.2.3 *Treatment 3 – Growth Medium plus Bacterial Culture Inoculum in Coated Bottles for 8-2 TBA Spike Recovery*

For a total of 9 coated glass serum bottles (3 replicates × 3 sampling time points), 29.34 mL of the growth medium and 0.663 mL of the washed bacterial culture was added to each of the glass serum bottles. The bottles were crimped with pre-cleaned aluminum foil and PTFE/silicone septa.

At each sampling time points (Day 0, 14, and 28), 3 bottles were spiked (dosed) with 6 µL of 8-2 TBA stock solution (3 mg/mL in ethanol) for a final concentration of 600 µg/L. The bottles were shaken at 200-300 RPM for approximately 30 min before sample collection and sample extraction.

3.2.4 *Treatment 4 – Growth Medium plus Bacterial Culture Inoculum in Coated Bottles*

For a total of 9 coated glass serum bottles (3 replicates × 3 sampling time points), 29.34 mL of the growth medium and 0.663 mL of the washed bacterial culture was added to each of the glass serum bottles. The bottles were crimped with pre-cleaned aluminum foil and PTFE/silicone septa. This treatment was used to provide an indication whether 8-2 TBA that has been adsorbed to the glass wall of the test vessels was available for biotransformation.

3.2.5 *Treatment 5 – Growth Medium plus Bacterial Culture Inoculum in non-Coated Bottles*

For a total of 9 non-coated glass serum bottles (3 replicates × 3 sampling time points), 29.34 mL of the growth medium and 0.663 mL of the washed bacterial culture was added to each of the glass serum bottles. The bottles were crimped with pre-cleaned aluminum foil and PTFE/silicone septa. This treatment will serve as sample matrix control for quantification of fluorinated acid metabolites by LC/MS/MS.

3.2.6 *Spike Recovery of Fluorinated Acids from Sample Matrix*

On 17 September 2002, a separate experiment was conducted to determine the spike recovery of fluorinated acids from bacterial culture sample matrix. Each of the sample bottles was filled with 30 mL of bacterial growth medium plus the bacterial inoculum that had been prepared the day before (***Section 3.1.6***). For day 2 samples, the bottles were crimped with pre-cleaned aluminum foil and PTFE/silicone septa and were kept shaken at

~250 RPM at room temperature to allow the bacteria to grow. For day 0 samples, 3 bottles were spiked with fluorinated acid mix solution (made from individual stock solution in ethanol) to a final concentration of 200 µg/L for each acid. The four acids used are: 1) 2-perfluorooctyl ethanoic acid (2-PFOEA; CAS# 27854-31-5, DuPont,); 2) 2H-hexadecafluoro-2-decenoic acid (2H-HDF-2-DA; CAS# 161094-76-4, DuPont,); 3) perfluorooctanoic acid (PFOA; CAS# 335-67-1, 97%, Oakwood Products); and 4) perfluorohexanoic acid (PFHA; CAS # 307-24-4, +98%, TCI America). Another 3 bottles were not spiked to serve as sample matrix control. The bottles were shaken at 200-300 RPM for approximately 30 min before sample collection and sample extraction. At day 2 sampling time point (19 September 2002), 3 crimped bottles were spiked with fluorinated acid mix solution (made from individual stock solution in ethanol) to a final concentration of 200 µg/L for each acid. Another 3 bottles were not spiked to serve as sample matrix control. The bottles were shaken at 200-300 RPM for approximately 30 min before sample collection and sample extraction.

3.2.7 *Test Conditions and Sampling*

3.2.7.1 *Sample Incubation*

The crimped glass serum bottles were incubated with 200-300 RPM of shaking at room temperature in the dark. The room temperature of the test lab was monitored and recorded throughout the course of the study.

3.2.7.2 *Sampling Interval*

5 crimped serum bottles from *Treatment 1 and 3* bottles from the rest of treatments were sampled for extraction of 8-2 TBA, fluoride, and fluorinated acids at day 0 (19-Jul-2002), day 14 (2-Aug-2002), and day 28 (16-Aug-2002).

3.2.7.3 *Sample Storage*

Analytical samples were stored at –10 to –20°C.

3.3 ***Sample Extraction and Analysis***

3.3.1 *Sample Collection and Extraction*

3.3.1.1 *Sample Collection*

At days 0, 14, and 28, crimped sample bottles were removed from the shaker and the bottles were turned upside down. A total of 10 mL of the test medium from each of the bottles were withdrawn with a 10-mL polypropylene syringe and was injected into a 15 mL polypropylene tube that contained 100 µL of 5 N sodium hydroxide for fluoride extraction.

3.3.1.2 *Sample Extraction*

Fluoride extraction: The 15 mL polypropylene tubes containing the 10 mL test medium plus NaOH were incubated at room temperature for 3 - 4 h with 250 - 300 RPM of shaking. Then 83.3 µL of 6N H_2SO_4 was added to each of the tubes to neutralize the test medium. The sample tubes were stored at approximately –20°C for fluoride analysis.

8-2 TBA and fluorinated acid extraction: After withdrawing a 10 mL of test medium for fluoride analysis, 0.34 mL of 6 N H_2SO_4 and 30 mL of chilled MTBE were injected into the sample bottles. After MTBE and H_2SO_4 were injected, the sample bottles were shaken (250 - 300 RPM) at room temperature for approximately 2 h. After settling the MTBE phase,

the crimped sample bottles were decapped and the MTBE phase from each of the sample bottles was transferred to 50 mL polypropylene centrifuge tubes with a glass transfer pipette. The MTBE phase was centrifuged at approximately 2000 RPM for 10 min. A 20 mL aliquot of the MTBE phase from each of the centrifuge tubes was transferred with a glass pipette to a 20 mL glass scintillation vial with a foil-lined cap and the scintillation vials were stored at ~-20°C. From each of the glass scintillation vials, 2 mL aliquot of the MTBE phase was transferred with a glass pipette to a GC vial and was sealed with aluminum-lined crimp cap for GC/MS quantification of 8-2 TBA. From each of the glass scintillation vials, 6 mL aliquot of the MTBE phase was sequentially dried in a GC vial under N_2 and was redissolved in 1.5 mL of methanol for fluorinated acid analysis.

3.3.1.3 Temperature Measurements

The temperature of the test system was monitored and recorded throughout the course of the study.

3.3.2 Analytical Methods for Test Substance and Products

3.3.2.1 Analysis of Test Substance

8-2 TBA Analysis:

Analytical standards:

The 1H,1H, 2H, 2H-perfluorodecan-1-ol (8-2 TBA, CAS# 678-39-7, 97.6%, Oakwood Products, West Columbia, SC) was used as the analytical standard. The 1H, 1H, 2H, 2H-perfluoro-9-methyldecan-1-ol (C-11 Iso; CAS# 31200-98-3, 98%, Oakwood Products,) was used as an internal standard for day 0 samples and the 1D, 1D, 2D, 2D, 3-^{13}C-heptadecafluoro decanol (M+5) (D-8-2 TBA, DuPont,) was used as the internal standard for day 14 and day 28 sample analysis. Stock solutions (1000 mg/L) of the analytical standard and the internal standards were prepared in methanol and refrigerated. The calibration standards were freshly prepared for each calibration in MTBE by dilution of the freshly made 50-mg/L stocks in methanol. Typically, the calibration standards were made in the range of 25-1000 µg/L of 8-2 TBA. Constant level of internal standard was used: approximately 300 µg/L of C-11 Iso for day 0 samples and D-8-2 TBA for day 14 and day 28 samples. For day 0 samples, the calibration curves were constructed using the ratio of the peak area for ion m/z 95 (8-2 TBA) and peak area for ion m/z 95 (C-11 Iso) and the ratio of the concentrations of 8-2 TBA and internal standard. An example of a calibration curve was given in Figure A-2 for quantification of samples TA50-1 to TA 50-26. A chromatograph of sample separation (Sample TA50-3) was given in Fugure A-3. For day 14 and day 28 samples, the calibration curves were constructed using the ratio of the peak area for ion m/z 31 (8-2 TBA) and peak area for ion m/z 33 (D-8-2 TBA) and the ratio of the concentrations of 8-2 TBA and the internal standard. An example of a calibration curve was given in Figure A-4 for quantification of samples TA51-1 to TA 51-17 and TA52-1 to TA 52-17. A chromatograph of sample separation (Sample TA52-3) was given in Fugure A-5.

Quantification of 8-2 TBA:

A 0.5 mL aliquot of the MTBE phase from the GC vials (***Section 3.3.1.2***) was placed in a glass GC vial (1.7 mL volume), 3 – 6 µL of 50 µg/mL of D-8-2 TBA internal standard was added to the vial using a GC syringe, the vial was capped and subjected to analysis. Each sample was analyzed twice by a GC/MS instrument according to the following conditions:

GC/MS system:		HP 6890 Plus GC (Agilent), HP 5973 Mass Selective Detector (Agilent), MPS2-MultiPurposeSampler (Gerstal)
Column:		DB-5MS, 30 m x 0.25 mm, 1 µm film (Agilent)
Temp. ramp:	Initial:	80°C for 2 min
		20°C/min to 120°C
		50°C/min to 300°C and hold for 3 min
Flow rate:		1.0 mL/min; He; constant flow mode
Split:		5:1
Inlet temp.:		250°C
Injection volume:		2 µL
MSD transfer line temp.:		280°C
Ionization:		EI, 70 eV
SIM ions monitored:		m/z: 31, 95, 131; dwell time: 50 ms for each ion (day 0 samples)
		m/z: 31, 33, 95,98; dwell time: 25 ms for each ion (days 14 and 28 samples)

Retention time:

8-2 Telomer B Alcohol (C8-2A):	4.97 min
Internal standard (D-8-2 TBA):	4.93 - 4.95 min
Internal Standard (C-11 Iso)	5.42 min

Fluorinated acid Analysis:

Analytical standards:

The perfluorohexanoic acid (PFHA; CAS # 307-24-4, +98%, TCI America), perfluorooctanoic acid (PFOA; CAS# 335-67-1, 97%, Oakwood Products), 2H-hexadecafluoro-2-decenoic acid (2H-HDF-2-DA; CAS# 161094-76-4, DuPont), and 2-perfluorooctyl ethanoic acid (2-PFOEA; CAS# 27854-31-5, DuPont) were used as standards for analysis of fluorinated acids in test samples. The analytical standard stock solutions for each of the above acids were prepared at approx. 200 µg/mL concentration by dissolving appropriate amount of the acids in methanol. A single, mixed analytical stock solution at approx. 200 µg/L for each acid was prepared by dilution of each of the above primary stock solutions with methanol in a single volumetric flask. An aliquot of the mixed acid stock solution (~200 µg/L) and an aliquot of a negative control sample matrix (*Treatment 5*) were diluted together in a single vial to produce approx. 30 µg/L concentration of each acid in 10x diluted sample matrix in methanol. A separate 30 µg/L solution was prepared with a day 0 negative control (Sample TA 50-15) and a day 28 negative control (Sample TA 52-15). These two solutions were designated as 30 µg/L standards and a pure methanol was designated as a 0 µg/L standard. For fluorinated acid spike recovery experiment (***Section 3.2.6***), a calibration standard curve was constructed in the range of 10 – 50 µg/L for quantification of individual fluorinated acid in the acid-spiked samples.

Sample Analysis:

Aliquots of all samples were diluted 10x with methanol before the analysis. In addition, aliquots of all samples were transferred to HPLC vials for direct analysis without dilution. All 10x diluted and all undiluted samples were analyzed by liquid chromatography-tandem mass spectrometry (LC/MS/MS). A set of standards (see above) was run at the beginning and at the end of the analysis sequence. Quantitation was done using a single two-point calibration curve for each acid constructed using standards from the beginning and the end of the analysis sequence. For maximal accuracy of results, the results for undiluted samples for PFHA, PFOA, and 2-PFOEA were considered final. For 2H-HDF-2-DA, the results from 10x diluted samples were considered final. This was done to ensure that the peak area data values of analytes in samples were as close as possible to those of 30 µg/L standards. For fluorinated acid spike recovery experiment (***Section 3.2.6***), a calibration standard curve was constructed in the range of 10 – 50 µg/L for quantification of individual fluorinated acid in the acid-spiked samples. Chromatographs of samples TA52-3 and TA52-6, , 30 µg/L of standard acid mix made in sample matrix TA50-15, sample matrix TA52-16, and a methanol solvent blank were given in Figure A-6. Each sample was analyzed twice according to the following conditions:

HPLC instrument:	Waters 2795 HPLC
MS instrument:	Micromass Quattro Micro
HPLC Column:	Phenomenex® Luna C18 (2), 20 mm x 2.0 mm, 3 µm particle size
Mobile Phase:	A: 2 mM ammonium acetate in deionized water (18.2 MΩ cm) C: methanol purge solvent: deionized water (18.2 MΩ cm) needle wash solvent: isopropanol

Mobile Phase Gradient:

Time (min)	% A	% C	Flow Rate (mL/min)
0	90	10	0.3
0.5	90	10	0.3
1.5	0	100	0.3
4.5	0	100	0.3
4.6	90	10	0.4
6.5	90	10	0.3

Flow Direction Switching:	0 to1.0 min – flow to waste to 4.5 min – flow to MS 4.5 to 6.5 min – flow to waste
Column Temperature:	35°C
Injection Volume:	10 µL
Ionization mode:	electrospray ionization in negative mode
Capillary voltage:	3.50 kV

Ion source temperature:	140°C
Desolvation temperature:	350°C
Desolvation Gas Flow:	N_2, 500 l/hr
Cone Gas Flow:	N_2, 100 l/hr
Quadrupole Resolution:	Q1 – low and high mass resolution 10
	Q2 – low and high mass resolution 15
Collision Gas, Pressure:	argon, 3.5 x 10^{-3} mbar
Data acquisition mode:	multiple reaction monitoring (0-4.5 min), inter-channel delay 0.05 sec

	Reaction	Dwell Time (sec)	Cone Voltage (V)	Collision Energy (eV)
PFHA	313 > 269	0.1	15	15
PFOA	413 > 369	0.1	15	15
2H-HDF-2-DA	457 > 393	0.1	15	15
2-PFOEA	477 > 393	0.1	20	20

Fluoride Analysis:

Analytical standard:

Certified standard of 100 mg/L of fluoride in water (Thermo Orion) was used for standard calibration. The calibration standards normally were made in the range of 5 – 100 µg/L by dilution of the 100 mg/L of fluoride standard with TISABII® (total ionic strength adjustment buffer II; Thermo Orion) and deionized water from Barnstead E-Pure system (Megohm-cm = 16 - 18). Half strength TISABII® (one part of TISABII® plus one part of deionized water) was used for the dilution. A 10 mL aliquot of each of the standard solution was used for fluoride analysis to construct a standard curve for quantification of fluoride in samples.

Quantification of Fluoride:

The 10 mL test medium that was treated with NaOH and H_2SO_4 (***Section 3.3.1.2***) from each of the 15 mL polypropylene tubes was thawed at room temperature and was centrifuged. Five milliliter from each of the tubes was transferred to a 50 mL polypropylene tube that contained 5 mL of TISABII® solution. After mixing, the medium was analyzed for fluoride using a 710 A Plus pH/ISE meter (Thermo Orion, serial # 066814) and a Ionplus fluoride selective electrode (Thermo Orion, model 96-09, lot # GX1). After filling the reference chamber with reference electrode filling solution (Thermo Orion), the electrode was inserted into each of the sample medium and fluoride standard solution and the conductivity in millivolts was recorded after 5 min of incubation. Each sample was measured 1 - 2 times. A standard curve was generated by plotting the standard fluoride concentration in LOG scale versus millivolts (Figure A-1) for quantification of fluoride.

4.0 RESULTS AND DISCUSSION

4.1 Under the test conditions, 8-2 TBA was rapidly transformed (Figure 1 and Table A-1).

- Spike recovery of 8-2 TBA from the test medium averaged 123 ± 8 % at day 0, 120 ± 6.0% at day 14, and 144 ± 2% at day 28 (Table A-2), indicating that MTBE extraction method obtained a good recovery of 8-2 TBA from the test medium.
- Spike recovery of fluorinated acids for day 0 samples was moderate, ranged 62-76% (Table B-1, B-2, B-3, and B-4). Spike recovery of 2-PFOEA and 2H-HDF-2-DA was poor for day 2 samples (***Section 3.2.6***, full-grown bacterial culture), averaged about 36% and 34%, respectively; spike recovery of PFOA and PFHA was moderate, averaged about 81% and 66%, respectively (Table B-1, B-2, B-3, and B-4). This indicated that full grown bacterial culture matrix may cause significant underestimation of 2-PFOEA and 2H-HDF-2-DA in day 14 and day 28 samples as well.
- At day 14, >70% of 8-2 TBA has been biotransformed in test vessels (***Treatment 1***) compared with abiotic control vessels (***Treatment 2***).
- At day 28, no 8-2 TBA was detected in test vessels. The loss of parent chemical was due to a combination of biotransformation and abiotic removal.
- In the abiotic controls, the concentration of 8-2 TBA decreased continuously. The loss is possibly caused by volatilization during the incubation or by incomplete extraction due to adsorption to the glass walls of the test vessels and/or the sludge inoculum.
- The 8-2 TBA that adsorbed to the walls of the test vessels during the coating was fully accessible for metabolism, with more than 90% of biotransformation at day 14 and 99% at day 28 (Table A-1).

4.2 Under the test conditions, significant defluorination occurred during the test, suggesting that the adapted microorganisms were able to metabolize 8-2 TBA to fluorinated acids and other metabolites.

- The fluoride concentration in test vessels increased from ~10 µg/L at day 0 to 64 µg/L at day 14, and 70 µg/L at day 28, while there was no increase for the abiotic controls (Table A-3).

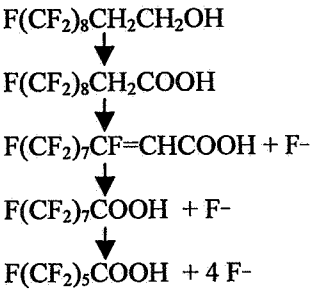
4.3 Four fluorinated acid metabolites (biotransformation products) have been identified from the test system and their mass balance contributions at day 28 are roughly estimated based in Table 1 and Table A-1:

- The $F(CF_2)_7CF{=}CHCOOH$ (2H-HDF-2-DA) is the most abundant metabolite and accounted for at least 10% of total mass present.
- $F(CF_2)_8CH_2COOH$ (2-PFOEA) is the second abundant metabolite, accounted for ~2% of total mass present.
- $F(CF_2)_7COOH$ (PFOA) accounted for <2% of total mass present.
- $F(CF_2)_5COOH$ (PFHA) accounted for ~0.4% of total mass present.
- The formation of PFHA is not due to impurity of the test chemical, since 6-2 Telomer B Alcohol [$F(CF_2)_6CH_2CH_2OH$, CAS# 647-42-7], which could lead to PFHA formation, was not detected in the test chemical (Figure A-7). The concentration of

PFHA increased significantly from day 0 to day 28 in test vessels, while only background level of PFHA was present in the abiotic controls during the test.

- Defluorination of PFOA may lead to the formation of PFHA during the test.

4.4 Based on the metabolites identified, the following is a potential pathway for 8-2 TBA biotransformation (a simplified pathway which does not include all potential biotransformation products):



A notable feature of this pathway is that PFOA may be defluorinated (metabolized) to form PFHA.

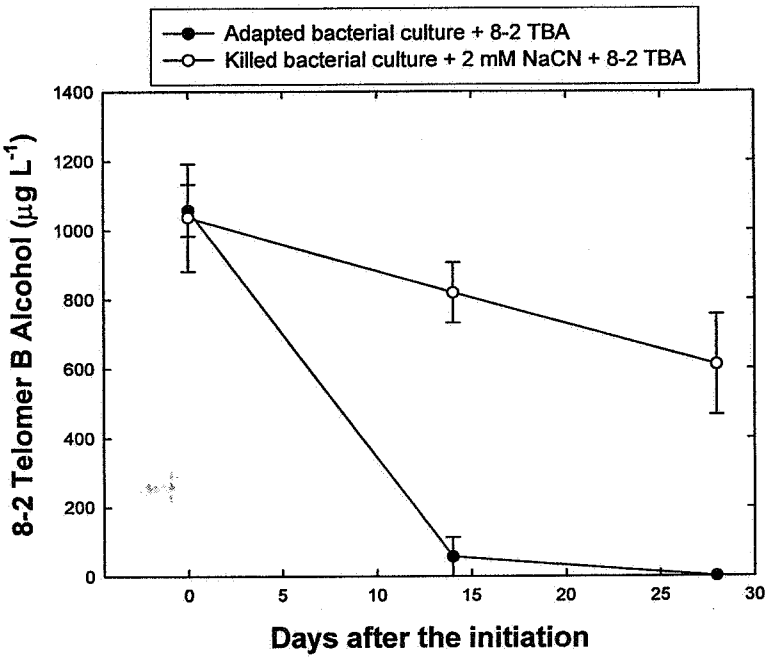
5.0 CONCLUSIONS

Under the test conditions, 8-2 TBA is being rapidly transformed to fluorinated acids and other unidentified transformation products. Although PFOA is one of the identified metabolites, it accounted for <2% of mass balance. PFOA apparently may be further metabolized to form PFHA and may not be an ultimate (stable) metabolite under the test conditions.

6.0 REFERENCE

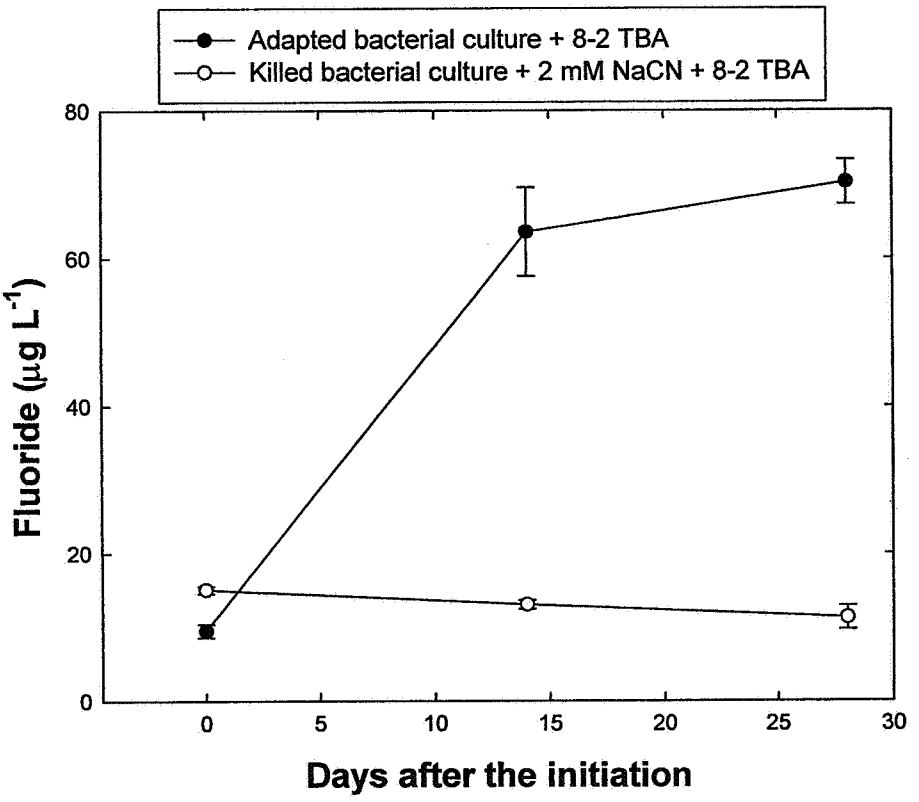
6.1 OECD Guideline for Testing of Chemicals, 302B: Zahn-Wellens/EMPA Test (1992); 302C: Inherent Biodegradability: Modified MITI Test (II) (1981).

FIGURE 1
8-2 TELOMER B ALCOHOL (8-2 TBA) CONCENTRATION DURING THE 28-DAY TEST*



* The test was initiated on 19 July 2002 and finished on 16 August 2002. The graph is derived from the original data of Table A-1.

FIGURE 2
FLUORIDE CONCENTRATION DURING THE 28-DAY TEST*



* The test was initiated on 19 July 2002 and finished on 16 August 2002. The graph is derived from the original data of Table A-3.

TABLE 1:
ESTIMATED CONCENTRATIONS† OF FLUORINATED ACID METABOLITES AT DAY 0 (19-JUL-2002) AND DAY 28 (16-AUG-2002).

Type of Treatments		2-PFOEA	2H-HDF-2-DA	PFOA	PFHA
			$\mu g\ L^{-1}$		
Treatment 1 - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	Day 0	ND¶	ND	ND	0.2
	Day 28	20	147	12	4.2
Treatment 2 - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	Day 0	ND	ND	ND	0.05
	Day 28	ND	ND	ND	0.08

* This table is derived from the original data of Table A-4, A-5, A-6, A-7, B-1, B-2, B-3, and B-4.

† Estimated concentration, $C_e = C_f/S_r$ where:
C_f = final concentration of individual fluorinated acid metabolites at day 0 or 28
S_r = % Spike recovery of the individual fluorinated acids.

Due to the low spike recovery of 2-PFOEA and 2H-HDF-2-DA and moderate spike recovery of PFOA and PFHA, the % spike recovery value was used for correcting possible underestimation of fluorinated acids in test samples.

¶ ND: Not detected above sample matrix background level

TABLE 2:
DAILY TEMPERATURE READINGS IN THE LAB WHERE THE TEST WAS CONDUCTED. THE TEMPERATURE WAS RECORDED BY A CALIBRATED DICKSON RECORDER (MODEL THDX, SERIAL # 0118247)

Time	Date	Temperature
Day		*°C*
0	19-Jul-2002	24.0
1	20-Jul-2002	23.5
2	21-Jul-2002	23.5
3	22-Jul-2002	23.75
4	23-Jul-2002	24.0
5	24-Jul-2002	23.75
6	25-Jul-2002	25.50
7	26-Jul-2002	23.75
8	27-Jul-2002	23.5
9	28-Jul-2002	24.0
10	29-Jul-2002	24.50
11	30-Jul-2002	24.0
12	31-Jul-2002	24.5
13	1-Aug-2002	24.5
14	2-Aug-2002	24.0
15	3-Aug-2002	24.0
16	4-Aug-2002	23.75
17	5-Aug-2002	24.25
18	6-Aug-2002	24.0
19	7-Aug-2002	23.75
20	8-Aug-2002	24.0
21	9-Aug-2002	24.0
22	10-Aug-2002	23.5
23	11-Aug-2002	23.75
24	12-Aug-2002	24.25
25	13-Aug-2002	24.25
26	14-Aug-2002	24.25
27	15-Aug-2002	24.5
28	16-Aug-2002	25.0
Average, n = 20		**24.1**
Standard Deviation		**0.5**

APPENDIX A:

TABLE A-1.
ANALYTICAL RESULTS OF 8-2 TBA CONCENTRATION AT DAY 0 (19-JUL-2002), DAY 14 (2-AUG-2002), AND DAY 28 (16-AUG-2002)

No	Type of Treatment	Time	Rep	8-2 TBA Analytical concentration	8-2 TBA Analytical Average	8-2 TBA final concentration‡
		day		*µg L^{-1}*	*µg L^{-1}*	*µg L^{-1}*
TA50-1	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	0	1	637	645	968
			1	652		
TA50-2		0	2	694	694	1041
			2	693		
TA50-3		0	3	699	690	1035
			3	681		
TA50-4		0	4	769	781	1172
			4	792		
TA50-5		0	5	710	717	1076
			5	724		
Average ± Standard Deviation † (n =5)						**1058 ± 75**
TA50-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	0	1	757	749	1124
			1	740		
TA 50-7		0	2	801	797	1196
			2	793		
TA50-8		0	3	592	599	899
			3	606		
Average ± Standard Deviation (n =3)						**1073 ± 155**
TA50-12	***Treatment 4*** - Adapted bacterial culture in Growth medium in coated serum bottle	0	1	139	147	221
			1	154		
TA50-13		0	2	194	189	284
			2	184		
TA50-14		0	3	125	124	186
			3	123		
Average ± Standard Deviation (n = 3)						**230 ± 50**
TA51-1	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	14	1	13.0	13.8	20.7
			1	14.6		
TA51-2		14	2	12.6	13.0	19.5
			2	13.3		
TA51-3		14	3	83.2	81.3	122
			3	79.4		
TA51-4		14	4	3.65	3.37	5.1
			4	3.08		
TA51-5		14	5	79.5	75.9	114
			5	72.3		
Average ± Standard Deviation (n = 5)						**56.3 ± 57.8**

TABLE A-1 (CONTINUED)

No	Type of Treatment	Time	Rep	8-2 TBA Analytical concentration	8-2 TBA Analytical Average	8-2 TBA final concentration‡
		day		*µg L^{-1}*	*µg L^{-1}*	*µg L^{-1}*
TA51-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	14	1	499	501	752
			1	502		
TA51-7		14	2	607	611	917
			2	615		
TA51-8		14	3	521	526	789
			3	531		
Average ± Standard Deviation (n = 3)						**819 ± 87**
TA51-12	***Treatment 4*** - Adapted bacterial culture in Growth medium in coated serum bottle	14	1	8.14	6.34	9.51
			1	4.53		
TA51-13		14	2	12.5	11.2	16.8
			2	9.92		
TA51-14		14	3	7.05	8.04	12.1
			3	9.03		
Average ± Standard Deviation (n = 3)						**12.8 ± 3.7**
TA52-1	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	28	1	0	0	0
			1	0		
TA52-2		28	2	0	0	0
			2	0		
TA52-3		28	3	0	0	0
			3	0		
TA52-4		28	4	0	0	0
			4	0		
TA52-5		28	5	0	0	0
			5	0		
Average ± Standard Deviation (n =5)						**0**
TA52-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	28	1	388	388	582
			1	388		
TA52-7		28	2	321	322	483
			2	322		
TA52-8		28	3	500	512	768
			3	523		
Average ± Standard Deviation (n =5)						**611 ± 145**

TABLE A-1 (CONTINUED)

No	Type of Treatment	Time	Rep	8-2 TBA Analytical concentration	8-2 TBA Analytical Average	8-2 TBA final concentration‡
		day		*μg L⁻¹*	*μg L⁻¹*	*μg L⁻¹*
TA52-12	***Treatment 4*** - Adapted bacterial culture in Growth medium in coated serum bottle	28	1	0	6.34	0
			1	0		
TA52-13		28	2	6.36	5.16	7.7
			2	3.96		
TA52-14		28	3	0.65	1.01	1.5
			3	1.37		
Average ± Standard Deviation						**3.1 ± 4.1**

† Standard Deviation was calculated using the Final concentration of 8-2 TBA.

‡ Final concentration of 8-2 TBA, $C_f = C_a \times (V_{MTBE}/V_t)$ where:

C_a = 8-2 TBA analytical average value,

V_{MTBE} = 30 mL (MTBE used to extract the test medium),

V_t = 20 mL (Test medium used fro extraction).

TABLE A-2.
ANALYTICAL RESULTS OF SPIKE RECOVERY OF 8-2 TBA FROM THE SAMPLE MATRIX (TREATMENT 3, ADAPTED BACTERIAL CULTURE PLUS GROWTH MEDIUM IN COATED GLASS SERUM BOTTLES) AT DAY 0 (19-JUL-2002), DAY 14 (2-AUG-2002), AND DAY 28 (16-AUG-2002)

No	Time	Rep	8-2 TBA Analytical Concentration	8-2 TBA Analytical Average	Final 8-2 TBA Concentration†	% of Spike Recovery‡
	day		*μg L⁻¹*	*μg L⁻¹*	*μg L⁻¹*	
TA50-9	0	1	638	628	942	119
		1	617			
TA50-10	0	2	624	626	939	118
		2	627			
TA50-11	0	3	678	683	1025	133
		3	688			
Average ± Standard Deviation						**123 ± 8**
TA51-9	14	1	459	460	690	113
		1	461			
TA51-10	14	2	502	506	759	124
		2	509			
TA51-11	14	3	502	496	744	122
		3	489			
Average ± Standard Deviation						**120 ± 6**
TA52-9	28	1	586	568	852	142
		1	549			
TA52-10	28	2	572	580	870	145
		2	588			
TA52-11	28	3	594	578	867	144.0
		3	562			
Average ± Standard Deviation						**144 ± 2**

† Final 8-2 TBA concentration, $C_f = C_a (V_{MTBE}/V_t)$ where:

C_a = 8-2 TBA analytical average

V_{MTBE} = 30 mL (MTBE used to extract the test medium)

V_t = 20 mL (Test medium used fro extraction)

‡ % of spike recovery, $R = [(C_f-C_c)/C_s] \times 100$, where:

C_f = Final 8-2 TBA concentration in the spiked bottles

C_c = Final 8-2 TBA concentration in the coated bottles without adding or spiking with 8-2 TBA stock solution (Treatment 4)

C_s = Concentration of 8-2 TBA spiked into the sample bottles (600 μg/L; Treatment 3)

TABLE A-3.
ANALYTICAL RESULTS OF FLUORIDE CONCENTRATION AT DAY 0 (19-JUL-2002), DAY 14 (2-AUG-2002), AND DAY 28 (16-AUG-2002)

No	Type of Treatment	Time	Rep	Fluoride Analytical Concentration	Fluoride Analytical Average	Final Fluoride concentration‡
		day		*µg L^{-1}*	*µg L^{-1}*	*µg L^{-1}*
TA50-1	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	0	1	5.3	5.4	10.8
			1	5.4		
TA50-2		0	2	4.5	4.7	9.4
			2	4.8		
TA50-3		0	3	4.4	4.3	8.6
			3	4.2		
TA50-4		0	4	4.4	4.3	8.6
			4	4.1		
TA50-5		0	5	4.1	5.0	10.0
			5	5.9		
Average ± Standard Deviation (n = 5)†						**9.5 ± 0.9**
TA50-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	0	1	7.3	7.3	14.6
			1			
TA 50-7		0	2	7.8	7.8	15.6
			2			
TA50-8		0	3	7.6	7.6	15.2
			3			
Average ± Standard Deviation (n = 3)						**15.1 ± 0.5**
TA51-1	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	14	1	34.4	34.4	68.8
			1			
TA51-2		14	2	33.5	33.5	67.0
			2			
TA51-3		14	3	31.6	31.6	63.2
			3			
TA51-4		14	4	26.8	26.8	53.6
			4			
TA51-5		14	5	32.8	32.8	65.6
			5			
Average ± Standard Deviation (n = 5)						**63.6 ± 6.0**
TA51-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	14	1	6.4	6.4	12.8
			1			
TA 51-7		14	2	6.3	6.3	12.6
			2			
TA51-8		14	3	6.9	6.9	13.8
Average ± Standard Deviation (n = 3)						**13.1 ± 0.6**

TABLE A-3 (CONTINUED)

No	Type of Treatment	Time	Rep	Fluoride Analytical Concentration	Fluoride Analytical Average	Final Fluoride concentration‡
		day		*µg L⁻¹*	*µg L⁻¹*	*µg L⁻¹*
TA52-1	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	28	1	36.0	36.8	73.6
			1	37.6		
TA52-2		28	2	34.3	34.3	68.6
			2	34.3		
TA52-3		28	3	34.3	34.2	68.4
			3	34.0		
TA52-4		28	4	36.1	36.8	73.6
			4	37.4		
TA52-5		28	5	33.5	33.7	67.4
			5	34.0		
Average ± Standard Deviation (n = 5)						**70.3 ± 3.0**
TA52-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	28	1	6.5	6.5	13.0
			1			
TA 52-7		28	2	5.4	5.4	10.8
			2			
TA52-8		28	3	5.0	5.0	10.0
			3			
Average ± Standard Deviation (n = 3)						**11.3 ± 1.6**

† Standard Deviation was calculated using the final fluoride concentration.

‡ Final fluoride concentration, $C_f = C_a \times [(V_i+V_t)/V_t]$ where:

C_a = Fluoride analytical average value,

V_i = 5 mL (TISABII® buffer added to the test medium for fluoride measurement),

V_t = 5 mL (Test medium used for fluoride measurement).

TABLE A-4.
ANALYTICAL RESULTS OF FLUORINATED ACID METABOLITE 2-PERFLUOROOCTYL ETHANOIC ACID (2-PFOEA) AT DAY 0 (19-JUL-2002), AND DAY 28 (16-AUG-2002)

No	Type of Treatment	Time	Rep	2-PFOEA Analytical concentration	2-PFOEA Analytical Average	Final 2-PFOEA concentration‡
		day		*μg L⁻¹*	*μg L⁻¹*	*μg L⁻¹*
TA50-2	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 μg/L of 8-2 TBA in coated serum bottle	0	1	ND	ND	ND†
			1	ND		
TA50-3		0	2	ND	ND	ND
			2	ND		
Average (n =2)						**ND**
TA50-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 μg/L of 8-2 TBA in coated serum bottle	0	1	ND	ND	ND
			1	ND		
TA 50-7		0	2	ND	ND	ND
		0	2	ND		
Average (n = 2)						**ND**
TA52-2	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 μg/L of 8-2 TBA in coated serum bottle	28	1	15.4	15.4	5.8
			1	15.3		
TA52-3		28	2	22.1	22.4	8.4
			2	22.6		
Average (n =2)						**7.1**
TA52-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 μg/L of 8-2 TBA in coated serum bottle	28	1	ND	ND	ND
			1	ND		
TA 52-7		28	2	ND	ND	ND
			2	ND		
Average (n = 2)						**ND**

† ND: Not detected above sample matrix background level.

‡ Final 2-PFOEA concentration, $C_f = C_a \times [(V_{MTBE}/V_t)/(V_s/V_m)]$ where:

C_a = 2-PFOEA analytical average value,

V_{MTBE} = 30 mL (MTBE used to extract the test medium),

V_t = 20 mL (Test medium used for extraction),

V_s = 6 mL (MTBE phase dried for metabolite analysis),

V_m = 1.5 mL (Methanol used to redissolve the dried MTBE phase for metabolite analysis).

TABLE A-5.
ANALYTICAL RESULTS OF FLUORINATED ACID METABOLITE 2H-HEXADECAFLUORO-2-DECENOIC ACID (2H-HDF-2-DA) AT DAY 0 (19-JUL-2002), AND DAY 28 (16-AUG-2002)

No	Type of Treatment	Time	Rep	2H-HDF-2-DA Analytical concentration	2H-HDF-2-DA Analytical Average	Final 2H-HDF-2-DA concentration‡
		day		*µg L⁻¹*	*µg L⁻¹*	*µg L⁻¹*
TA50-2	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	0	1	ND	ND	ND†
			1	ND		
TA50-3		0	2	ND	ND	ND
			2	ND		
Average (n =2)						**ND**
TA50-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	0	1	ND	ND	ND
			1	ND		
TA 50-7		0	2	ND	ND	ND
		0	2	ND		
Average (n = 2)						**ND**
TA52-2	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	28	1	117	114	42.8
			1	111		
TA52-3		28	2	156	152	57.0
			2	147		
Average (n =2)						**50**
TA52-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	28	1	ND	ND	ND
			1	ND		
TA 52-7		28	2	ND	ND	ND
			2	ND		
Average (n = 2)						**ND**

† ND: Not detected above sample matrix background level

‡ Final 2H-HDF-2-DA concentration, $C_f = C_a \times [(V_{MTBE}/V_t)/(V_s/V_m)]$ where:

C_a = 2H-HDF-2-DA analytical average value,

V_{MTBE} = 30 mL (MTBE used to extract the test medium),

V_t = 20 mL (Test medium used for extraction),

V_s = 6 mL (MTBE phase dried for the metabolite analysis),

V_m = 1.5 mL (Methanol used to redissolve the dried MTBE phase for metabolite analysis).

TABLE A-6.
ANALYTICAL RESULTS OF FLUORINATED ACID METABOLITE PERFLUOROOCTANOIC ACID (PFOA) AT DAY 0 (19-JUL-2002), AND DAY 28 (16-AUG-2002)

No	Type of Treatment	Time	Rep	PFOA Analytical concentration	PFOA Analytical Average	Final PFOA concentration‡
		day		*µg L⁻¹*	*µg L⁻¹*	*µg L⁻¹*
TA50-2	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	0	1	ND	ND	ND†
			1	ND		
TA50-3		0	2	ND	ND	ND
			2	ND		
Average (n =2)						**ND**
TA50-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	0	1	ND	ND	ND
			1	ND		
TA 50-7		0	2	ND	ND	ND
		0	2	ND		
Average (n = 2)						**ND**
TA52-2	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	28	1	27.5	27.5	10.3
			1	27.5		
TA52-3		28	2	25.1	25.5	9.6
			2	25.8		
Average (n =2)						**10.0**
TA52-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	28	1	ND	ND	ND
			1	ND		
TA 52-7		28	2	ND	ND	ND
			2	ND		
Average (n = 2)						**ND**

† ND: Not detected above sample matrix background level.

‡ Final PFOA concentration, $C_f = C_a \times [(V_{MTBE}/V_t)/(V_s/V_m)]$ where:

C_a = PFOA analytical average value,

V_{MTBE} = 30 mL (MTBE used to extract the test medium),

V_t = 20 mL (Test medium used for extraction),

V_s = 6 mL (MTBE phase dried for metabolite analysis),

V_m = 1.5 mL (Methanol used to redissolve the dried MTBE phase for metabolite analysis).

TABLE A-7.
ANALYTICAL RESULTS OF FLUORINATED ACID METABOLITE PERFLUOROHEXANOIC ACID (PFHA) AT DAY 0 (19-JUL-2002), AND DAY 28 (16-AUG-2002)

No	Type of Treatment	Time	Rep	PFHA Analytical concentration	PFHA Analytical Average	Final PFHA concentration‡
		day		*µg L⁻¹*	*µg L⁻¹*	*µg L⁻¹*
TA50-2	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	0	1	0.6	0.5	0.2†
			1	0.4		
TA50-3		0	2	ND	ND	ND
			2	ND		
Average (n =2)						**0.1**
TA50-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	0	1	0.2	0.1	0.04
			1	ND		
TA 50-7		0	2	0.09	0.06	0.02
		0	2	0.03		
Average (n = 2)						**0.03**
TA52-2	***Treatment 1*** - Adapted bacterial culture in growth medium plus 600 µg/L of 8-2 TBA in coated serum bottle	28	1	4.6	4.8	1.8
			1	5.0		
TA52-3		28	2	9.4	10.1	3.8
			2	10.8		
Average (n =2)						**2.8**
TA52-6	***Treatment 2*** - Killed bacterial culture in growth medium plus 2mM KCN and 600 µg/L of 8-2 TBA in coated serum bottle	28	1	ND	0.2	0.08
			1	0.4		
TA 52-7		28	2	0.1	0.05	0.02
			2	ND		
Average (n = 2)						**0.05**

† ND: Not detected above sample matrix background level.

‡ Final PFHA concentration, $C_f = C_a \times [(V_{MTBE}/V_t)/(V_s/V_m)]$ where:

C_a = PFHA analytical average value

V_{MTBE} = 30 mL (MTBE used to extract the test medium)

V_t = 20 mL (Test medium used for extraction)

V_s = 6 mL (MTBE phase dried for metabolite analysis)

V_m = 1.5 mL (Methanol used to redissolve the dried MTBE phase for metabolite analysis).

TABLE A-8.
COMPONENTS OF BACTERIAL GROWTH MEDIUM USED FOR THE TEST.

	Final Concentration
KH_2PO_4	0.70 g/l
$NaH_2PO_4*H_2O$	0.395g/l
KCl	0.5 g/l
$MgSO_4*7H_2O$	0.5 g/l
$CaCL_2*2H_2O$	0.025 g/l
NaCl	1.0 g/l
$FeSO_4*7H_2O$	0.5 mg/L
$CuSO_4*5H_2O$	0.05 mg/L
$CoCl_2*6H_2O$	0.1 mg/L
$MnCl_2*4H_2O$	0.01 mg/L
$Na_2MoO_2*2H_2O$	0.025 mg/L
$C_6H_5Na_3O_7*2H_20$ (Sodium citrate dihydrate tribasic)	1 mg/L
$NiCl_2*6H_2O$	0.1 mg/L
$ZnCl_2$	0.005 mg/L
Yeast Extract	2g/L
PH = 7.2	

FIGURE A-1
A FLUORIDE STANDARD CALIBRATION CURVE

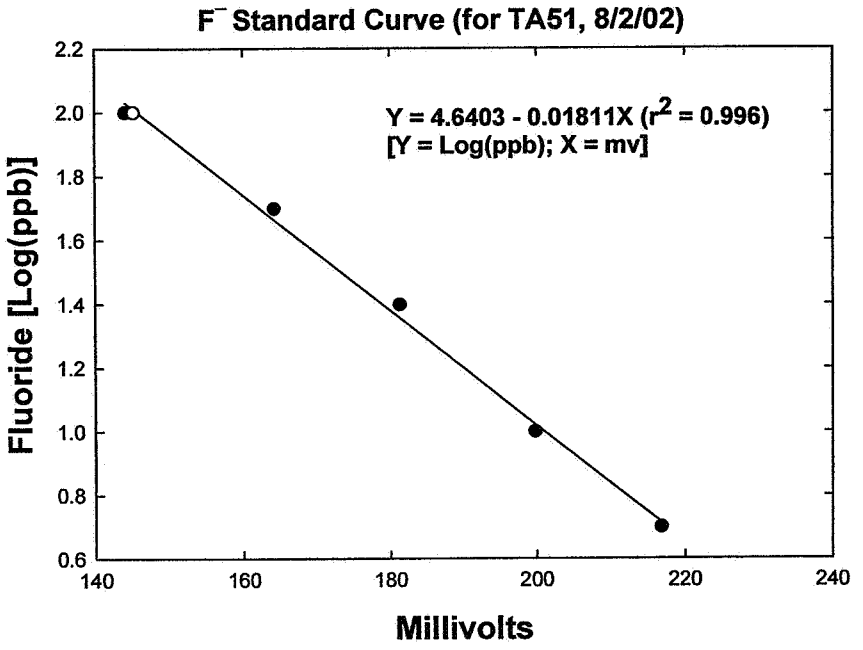
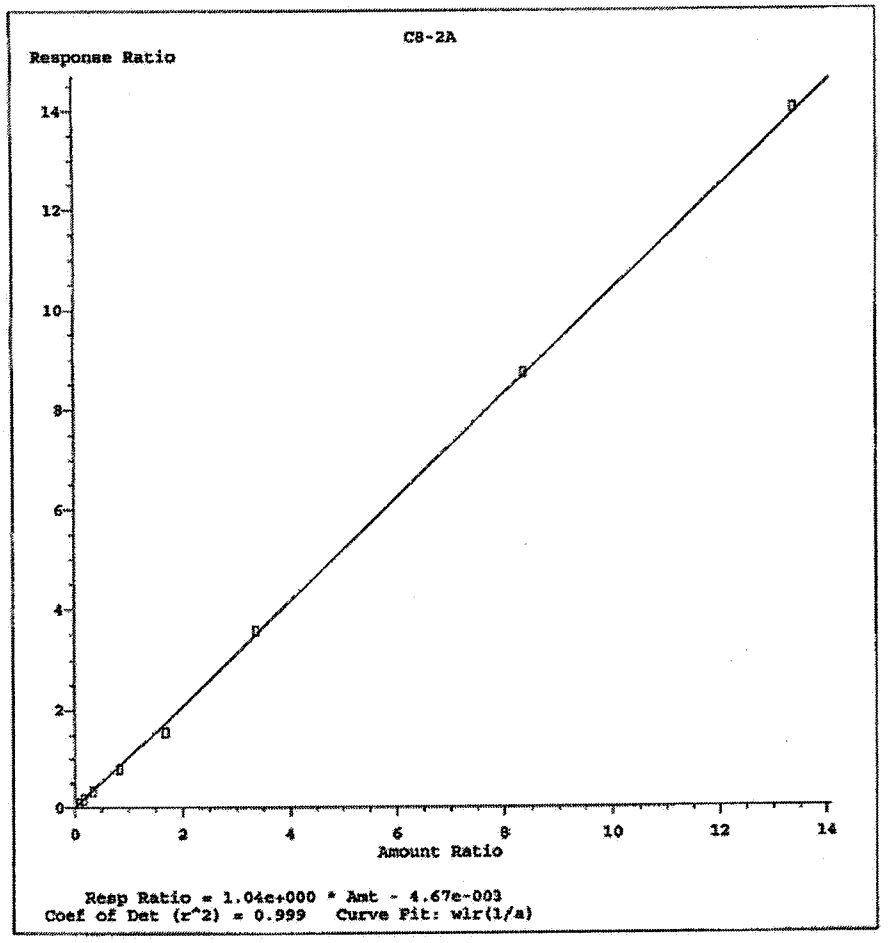
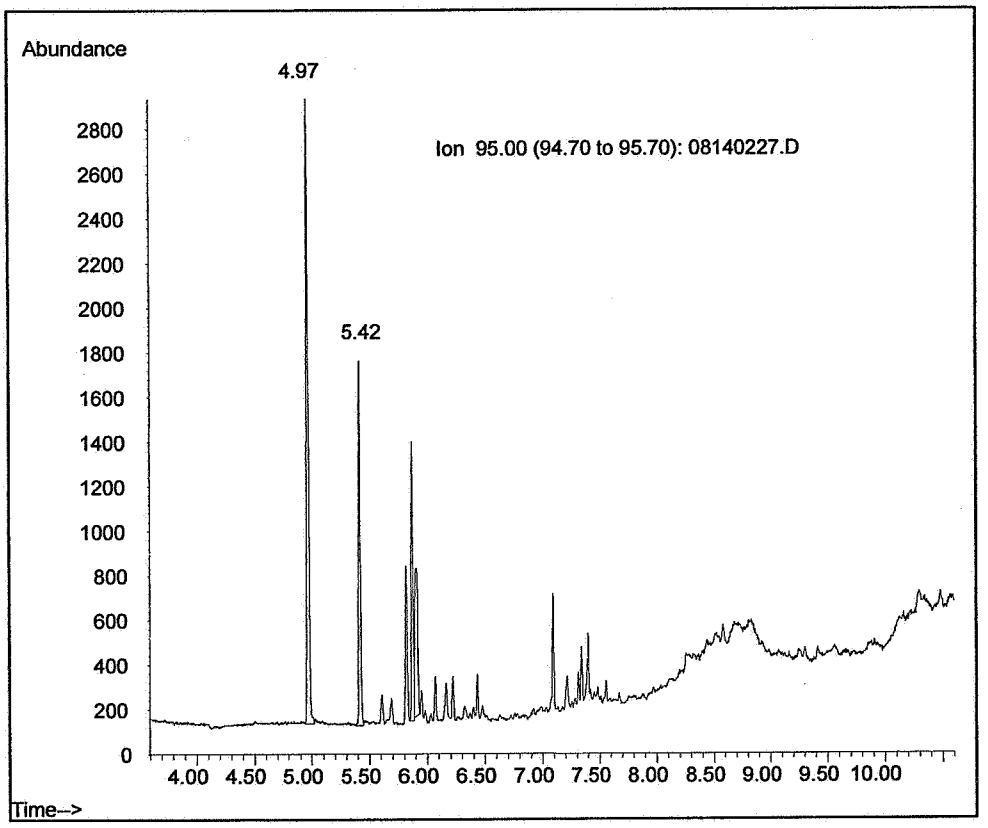


FIGURE A-2
A CALIBRATION CURVE USED FOR 8-2 TBA QUANTIFICATION OF SAMPLES TA 50-1 TO TA 50-26



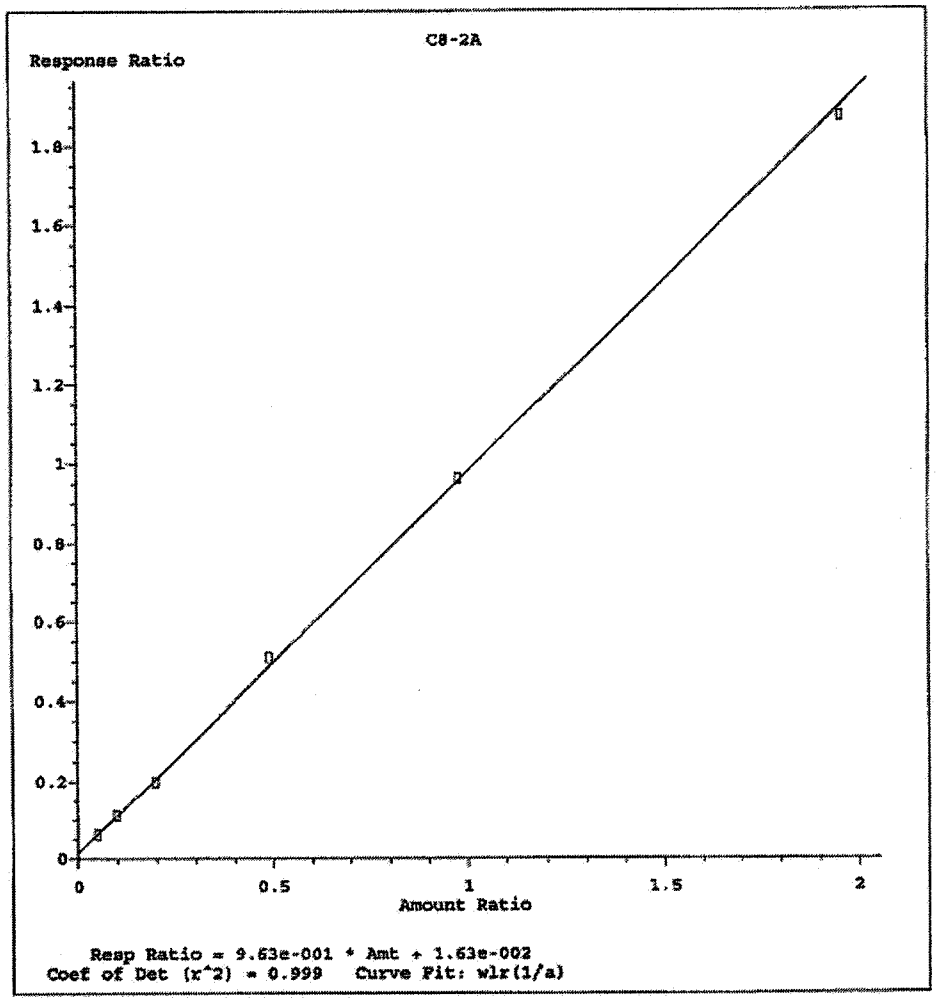
Calibration curve used for samples TA 50 – 1:26. The concentration of the internal standard: 300 µg/L of C11-iso; the range of concentrations of 8-2 TBA: 25.1 – 4020 µg/L.

FIGURE A-3
A CHROMATOGRAPH OF SAMPLE TA50-3 USED FOR 8-2 TBA ANALYSIS



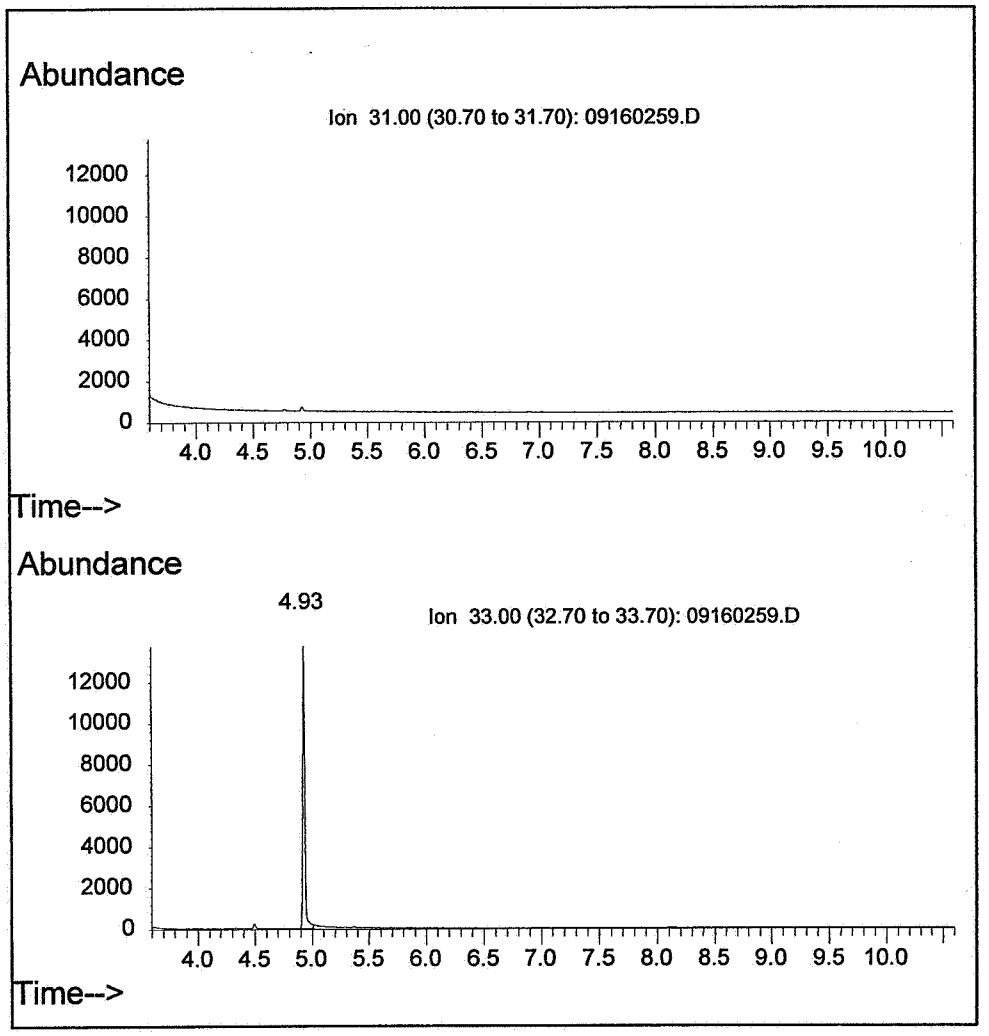
Sample TA50-3. Quantification was done using peak area of ion m/z 95; retention time 4.97 min = 8-2 TBA; retention time 5.42 min = C11-iso.

FIGURE A-4
A CALIBRATION CURVE USED FOR 8-2 TBA QUANTIFICATION OF SAMPLES TA 51-1 TO TA51-17 AND TA 52-1 TO TA52-17



Calibration curve used for samples TA 51 – 1:17 and TA 52-1:17. The concentration of the internal standard: 514 µg/L of D-8-2 TBA; the range of concentrations of 8-2 TBA: 25.1 –1005 µg/L.

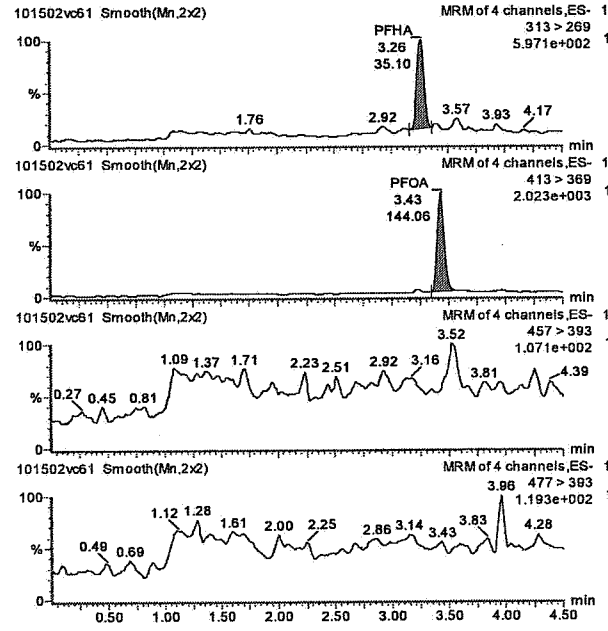
FIGURE A-5
A CHROMATOGRAPH OF SAMPLE TA 52-3 USED FOR 8-2 TBA ANALYSIS



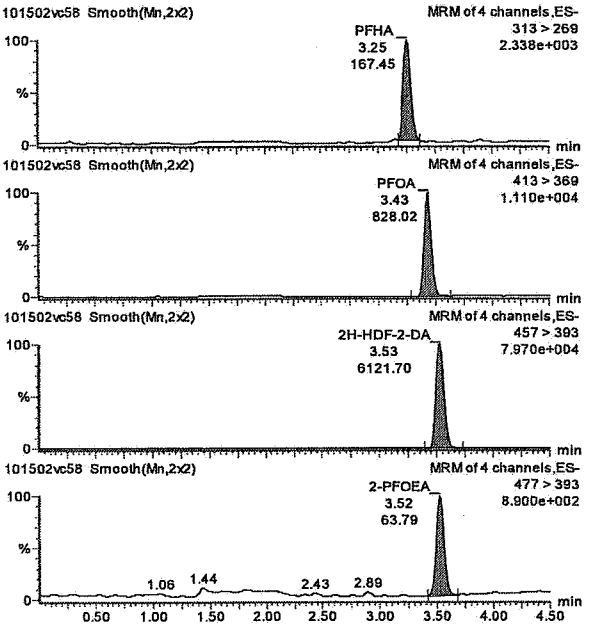
Sample TA52-3; Ion 31, retention time 4.95 min = 8-2 TBA; Ion 33, RT 4.93 min = D-8-2 TBA (internal standard).

FIGURE A-6 CHROMATOGRAPHS OF SAMPLES TA52-3 AND TA52-6, 30 µG L^{-1} OF STANDARD ACID MIX MADE IN SAMPLE MATRIX TA50-15, SAMPLE MATRIX TA52-16, AND A METHANOL SOLVENT BLANK.

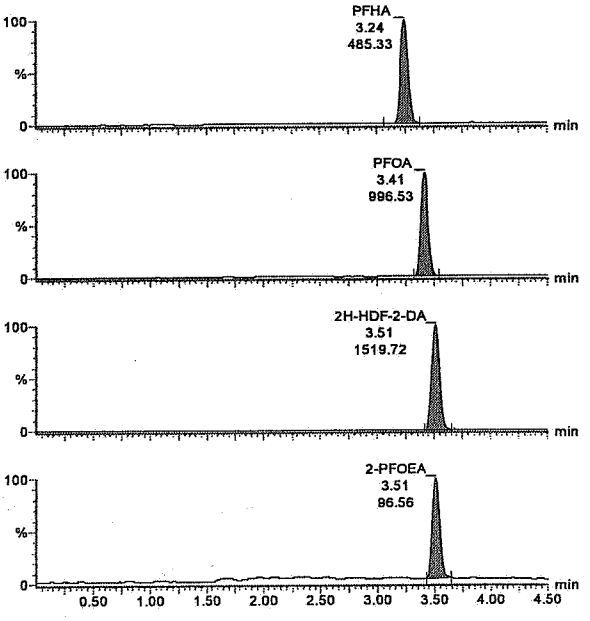
TA 52-6 (Abiotic control, Treatment 2)



TA 52-3 (Test sample, Treatment 1)



Standard: TA50-15 (Sample matrix, *Treatment 5*) + 30 µg L^{-1} acids



TA 52-16 (Sample matrix, *Treatment 5*)

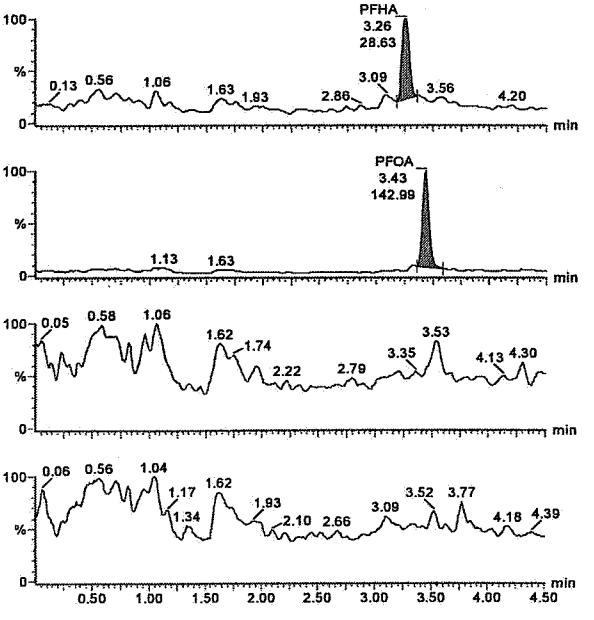


FIGURE A-6 CHROMATOGRAPHS OF SAMPLES TA52-3 AND TA52-6, 30 µG L^{-1} OF STANDARD ACID MIX MADE IN SAMPLE MATRIX TA50-15, SAMPLE MATRIX TA52-16, AND A METHANOL SOLVENT BLANK – CONTINUED FROM PAGE 42.

Methanol solvent blank

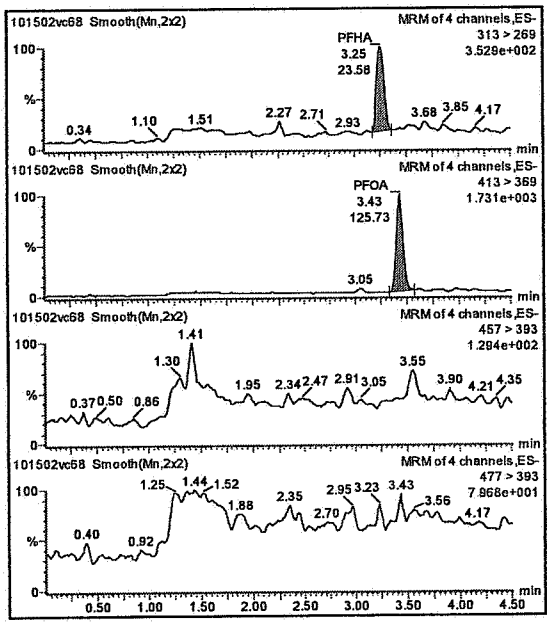
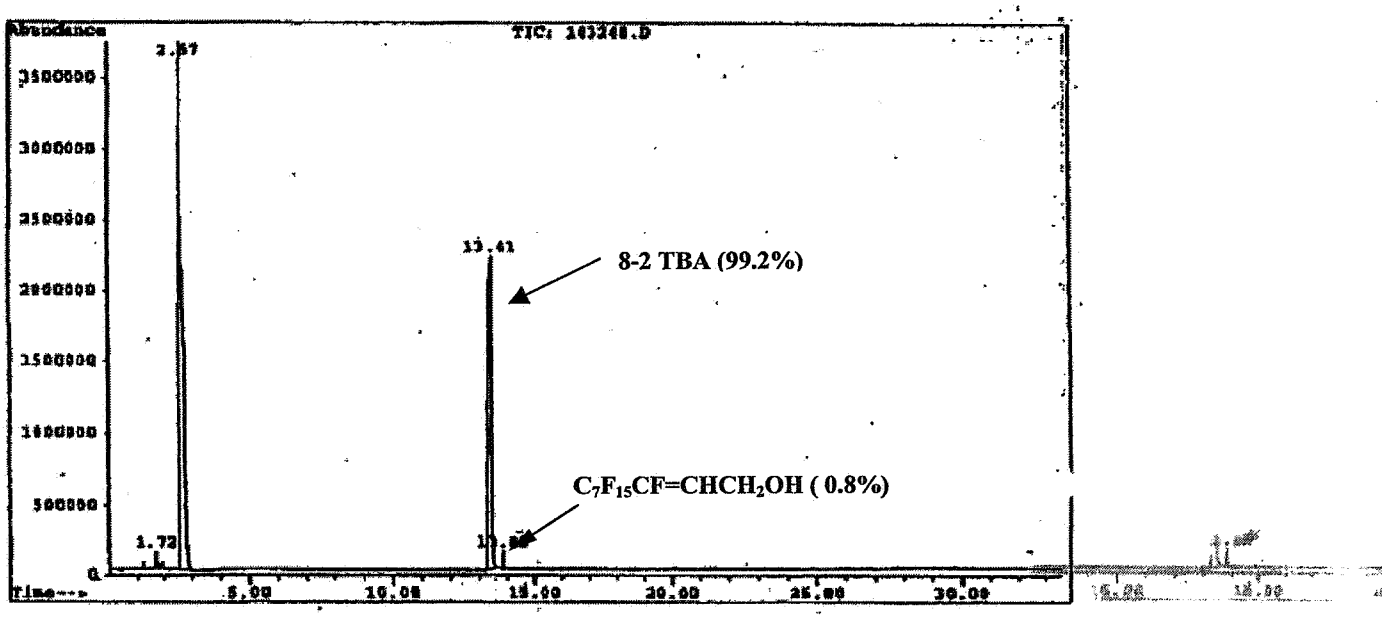


FIGURE A-7 A GC/MS CHROMATOGRAPH OF MATERIAL CHARACTERIZATION OF 8-2 TBA TEST SUBSTANCE USED IN THIS STUDY



APPENDIX B:

TABLE B-1.
ANALYTICAL RESULTS OF SPIKE RECOVERY OF 2-PERFLUOROOCTYL ETHANOIC ACID (2-PFOEA) AT DAY 0 (17-SEP-2002), AND DAY 2 (19-SEP-2002).

No	Time	Rep	2-PFOEA Analytical concentration	2-PFOEA Analytical Average	Final 2-PFOEA concentration‡	% of Spike Recovery§
	day		*µg L^{-1}*	*µg L^{-1}*	*µg L^{-1}*	
TA86-4	0	1	113	104	156	78
		1	95.7			
TA86-5	0	2	99.0	98.9	148	74
		2	98.7			
TA86-6	0	3	94.7	101	152	76
		3	108			
Average ± Standard Deviation† (n = 3)						**76 ± 2**
TA87-4	2	1	45.0	44.3	66.5	33
		1	43.5			
TA87-5	2	2	44.8	44.4	66.6	33
		2	44.0			
TA 87-6	2	3	54.6	56.1	84.2	42
		3	57.6			
Average ± Standard Deviation (n = 3)						**36 ± 5**

† Standard Deviation was calculated using the final 2-PFOEA concentration.

‡ Final 2-PFOEA concentration, $C_f = C_a \times (V_{MTBE}/V_t)$ where:

C_a = 2-PFOEA analytical average value,

V_{MTBE} = 30 mL (MTBE used to extract the test medium),

V_t = 20 mL (Test medium used for extraction).

§ % of spike recovery, $R = (C_f/C_s) \times 100$, where:

C_f = Final 2-PFOEA concentration in the spiked bottles,

C_s = Concentration of 2-PFOEA spiked into the sample bottles (200 µg/L).

TABLE B-2.
ANALYTICAL RESULTS OF SPIKE RECOVERY OF 2H-HEXADECAFLUORO-2-DECENOIC ACID (2H-HDF-2-DA) AT DAY 0 (17-SEP-2002), AND DAY 2 (19-SEP-2002)

No	Time	Rep	2H-HDF-DA Analytical concentration	2H-HDF-DA Analytical Average	Final 2H-HDF-DA concentration‡	% of Spike Recovery§
	day		*µg L^{-1}*	*µg L^{-1}*	*µg L^{-1}*	
TA86-4	0	1	91.2	90.4	136	68
		1	89.5			
TA86-5	0	2	88.0	88.1	132	66
		2	88.1			
TA86-6	0	3	93.5	94.3	141	71
		3	95.0			
Average ± Standard Deviation† (n = 3)						**68 ± 3**
TA87-4	2	1	38.1	39.6	59.4	30
		1	41.1			
TA87-5	2	2	41.3	41.0	61.5	31
		2	40.6			
TA 87-6	2	3	54.6	55.1	82.7	41
		3	55.5			
Average ± Standard Deviation (n = 3)						**34 ± 6**

† Standard Deviation was calculated using the final 2H-HDF-2-DA concentration.

‡ Final 2H-HDF-2-DA concentration, $C_f = C_a \times (V_{MTBE}/V_t)$ where:

C_a = 2H-HDF-2-DA analytical average value,

V_{MTBE} = 30 mL (MTBE used to extract the test medium),

V_t = 20 mL (Test medium used for extraction).

§ % of spike recovery, $R = (C_f/C_s) \times 100$, where:

C_f = Final 2H-HDF-2-DA concentration in the spiked bottles,

C_s = Concentration of 2H-HDF-2-DA spiked into the sample bottles (200 µg/L).

TABLE B-3.
ANALYTICAL RESULTS OF SPIKE RECOVERY OF PERFLUOROOCTANOIC ACID (PFOA) AT DAY 0 (17-SEP-2002), AND DAY 2 (19-SEP-2002)

No	Time	Rep	PFOA Analytical concentration	PFOA Analytical Average	Final PFOA concentration‡	% of Spike Recovery§
	day		*µg L⁻¹*	*µg L⁻¹*	*µg L⁻¹*	
TA86-4	0	1	88.2	88.5	133	67
		1	88.7			
TA86-5	0	2	89.4	90	135	68
		2	90.6			
TA86-6	0	3	94.3	94.8	142	71
		3	95.3			
Average ± Standard Deviation† (n = 3)						**69 ± 2**
TA87-4	2	1	98.0	100	150	75
		1	102			
TA87-5	2	2	95.9	99.5	149	75
		2	103			
TA 87-6	2	3	122	123	185	93
		3	124			
Average ± Standard Deviation (n = 3)						**81 ± 10**

† Standard Deviation was calculated using the final 2-PFOA concentration.

‡ Final PFOA concentration, $C_f = C_a \times (V_{MTBE}/V_t)$ where:

C_a = PFOA analytical average value,

V_{MTBE} = 30 mL (MTBE used to extract the test medium),

V_t = 20 mL (Test medium used for extraction).

§ % of spike recovery, $R = (C_f/C_s) \times 100$, where:

C_f = Final PFOA concentration in the spiked bottles,

C_s = Concentration of PFOA spiked into the sample bottles (200 µg/L).

TABLE B-4.
ANALYTICAL RESULTS OF SPIKE RECOVERY OF PERFLUOROHEXANOIC ACID (PFHA) AT DAY 0 (17-SEP-2002), AND DAY 2 (19-SEP-2002)

No	Time	Rep	PFHA Analytical concentration	PFHA Analytical Average	Final PFHA concentration‡	% of Spike Recovery§
	day		*μg L^{-1}*	*μg L^{-1}*	*μg L^{-1}*	
TA86-4	0	1	78.8	79.2	119	60
		1	79.5			
TA86-5	0	2	82.1	81.4	122	61
		2	80.7			
TA86-6	0	3	82.7	86.2	129	65
		3	89.7			
Average ± Standard Deviation† (n = 3)						**62 ± 3**
TA87-4	2	1	80.4	79.9	120	60
		1	79.3			
TA87-5	2	2	84.8	84.8	127	64
		2	84.7			
TA 87-6	2	3	100	98.7	148	74
		3	97.4			
Average ± Standard Deviation (n = 3)						**66 ± 7**

† Standard Deviation was calculated using the final 2-PFHA concentration.

‡ Final PFHA concentration, $C_f = C_a \times (V_{MTBE}/V_t)$ where:

C_a = PFHA analytical average value,

V_{MTBE} = 30 mL (MTBE used to extract the test medium),

V_t = 20 mL (Test medium used for extraction).

§ % of spike recovery, $R = (C_f/C_s) \times 100$, where:

C_f = Final PFHA concentration in the spiked bottles,

C_s = Concentration of PFHA spiked into the sample bottles (200 μg/L).