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GOOD LABORATORY PRACTICES COMPLIANCE

This study was performed in compliance with the FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160 of the Code of Federal Regulations.

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Quality Assurance Unit Statement

Study Title: S-2200: Anaerobic Soil Metabolism Study

Protocol_Number: V-09-37166

This Study has been inspected for compliance with EPA FIFRA Good Laboratory Practice (GLP) Standards (40 CFR Part 160, October 16, 1989).

Quality Assurance inspections of the study were conducted and findings from the inspections were reported to the Study Director and to Management on the following dates:

Audit Type	Audit Date	Date Reported To Study Director	Date Reported To Mgmt
DOSING	12/1/2009	12/2/2009	12/3/2009
SAMPLING, WATER/ SOIL MEASUREMENTS, EXTRACTION, CONCENTRATION	3/10/2010	3/16/2010	3/17/2010
RAW DATA - STANDARDS	8/17/2011	10/4/2011	10/6/2011
RAW DATA	5/1/2012	7/5/2012	7/30/2012
HOBO TEMPERATURE DATA	8/29/2012	7/5/2012	7/30/2012
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STUDY REPORT	2/11/2013	2/25/2013	3/1/2013
STUDY REPORT	9/3/2013	9/4/2013	9/4/2013

Approved: _____ Date: 9-18-2013

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STUDY IDENTIFICATION

Study Title: S-2200: Anaerobic Soil Metabolism Study

Valent Project No.: VP-37166

Valent Protocol No.: V-09-37166

Study Location:
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Test Materials: [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB), [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB), and [phenoxy-^{14}C]S-2167 (*S*-2200 *R*-isomer, RP)

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RECORD RETENTION

Raw Data Storage Location: Upon completion of the study, the final report and all original raw data will be will be archived at Valent Technical Center, 6560 Trinity Court, Dublin, CA. Facility maintenance records and equipment logs, etc. will be archived at Valent Technical Center, 6560 Trinity Court, Dublin, CA. Samples of the test and reference substances are archived at Valent Technical Center, 6560 Trinity Court, Dublin, CA.

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SUMMARY

During agricultural use, circumstances may occur where S-2200 is applied to a terrestrial soil that may become waterlogged and in which anaerobic conditions may develop. The biotransformation of S-2200 was studied in a terrestrial soil from Baptistown, NJ (USDA soil classification loam, pH 6.9, organic matter 2.0%) under aerobic and then anaerobic conditions for 363-364 days at 20 ± 2 °C in the dark to assess this environmental dissipation route. The test soil (Penn series) was the same soil used in an S-2200 aerobic soil metabolism study. The study was conducted using three different radiolabeled compounds of S-2200 - [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB), [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB), and [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP). S-2200 is a racemic mixture of the two isomers, S-2167 and S-2354. The target concentration was 8.96 ppm on a dry soil basis, equivalent to a field application rate of 2,240 g a.i./ha (2.0 lb a.i./acre). The experiments were conducted in accordance with the U.S. EPA Pesticide Assessment Guidelines, Environmental Fate Data Requirement, 40 CFR 158.290 OCSPP 835.4200 Anaerobic Soil Metabolism, and in compliance with the U.S. Environmental Protection Agency Good Laboratory Practice (GLP) Standards as specified in 40 CFR Part 160. The studies were conducted with guidance from OECD Guidelines for the Testing of Chemicals, Guideline 307, Aerobic and Anaerobic Transformation in Soil.

Aerobic test systems (50 g of dry weight soil contained in an open beaker) at approximately 60% of the maximum water holding capacity (WHC) were dosed at ca. 8.95, 8.94, and 8.95 ppm with RB, SB, and RP, respectively. The test systems were incubated in chambers equipped with NaOH traps for the collection of evolved $^{14}CO_2$. Analysis at 14 or 15 DAT indicated that S-2200 would not degrade through a single half-life prior to 30 days, therefore the individual samples were quantitatively transferred to closed biometer flasks at 30 DAT (RB) or 34 DAT (SB and RP). Dried ground alfalfa was added to each biometer at a rate of 0.1% of the soil dry weight to speed the formation of anaerobic conditions and to insure the survival of the microbial anaerobes. The soil was then flooded to commence the anaerobic phase. The anaerobic test system consisted of custom biometer flasks equipped with side-arm traps containing NaOH for the collection of evolved $^{14}CO_2$. The RB samples were analyzed at 0, 14, 30, 37, 44, 63, 86, 154, 259, and 364 days after dosing (aerobic phase – 0 to 30 DAT; anaerobic phase – 30 to 364 DAT). The SB and RP samples were analyzed at 0, 15, 34, 41, 48, 64, 90, 153, 266, and 363 days after dosing (aerobic phase – 0 to 34 DAT; anaerobic phase – 34 to 363 DAT). The aerobic phase samples were extracted twice with a neutral organic solution to yield extract #1 (9:1 acetone:water) and then extracted twice with a weakly acidic organic solution to yield extract #2 (5:1 acetone:0.1M HCl). The anaerobic phase samples were analyzed for evolved $^{14}CH_4$ by oxidation of a sample of the biometer flask headspace gas. The physical parameters of the anaerobic systems (oxygen concentration, redox and pH) were measured and the water separated from the soil. Activity in the water phase was either analyzed by HPLC directly or after partition into an organic phase. The remaining soil was extracted twice with a neutral organic solution to yield extract #1 (9:1 acetone:water or 9:1 methanol:water) and then extracted twice with a acidic organic solution to yield extract #2 (5:1 acetone:0.1M HCl or 9:1 methanol:0.5M HCl or 5:1 methanol:0.5M HCl). An additional dismembrator extraction with 5:1 methanol:0.5M HCl was

performed on representative post extraction soils (PES) at 30, 41, and 363 DAT (all samples contained bound residues between 5 and 12% of the applied radioactivity). Finally, the remaining PES was fractionated into humin, humic acids and fulvic acids. S-2200 residues in the water, soil extracts, dismembrator extracts, and the fulvic acid organosoluble fraction were analyzed by HPLC with a radioflow detector or fraction collector/LSC (co-chromatography). Identification was performed by 2D-TLC with a radio-PhosphorImager detector (co-chromatography).

The average material balance for the study was 102.6 ± 1.4%, 103.4 ± 1.8%, and 103.1 ± 1.7% of the applied radioactivity (AR), respectively for RB, SB and RP, respectively. The cumulative production of ^{14}C-volatile activity ($^{14}CO_2$ and $^{14}CH_4$) was minimal, reaching 3%, 7%, and 10% of the AR at 363/364 DAT for RB, SB, and RP, respectively. In each case the majority of $^{14}CO_2$ production occurred during the aerobic phase. Extractable radioactive residues in soil decreased from an average of ca. 100% of the AR at day 0, to 98.5, 94.6, and 86.9% of the AR at the end of the aerobic phase for the RB, SB and RP labels, respectively. Extractability continued to decrease during the anaerobic phase to 71.2, 67.5, and 65.1 % of the AR for the RB, SB and RP labels, respectively by the last sampling interval. Radioactive residues were 18, 17, and 13% of the AR in the water at 37/41 DAT (7 days into the anaerobic phase) and increased for the duration of the study to 23, 21, and 17% of the AR by the last sampling point for RB, SB, and RP, respectively. S-2200 residues were rapidly incorporated into the PES (post extraction soil) during the aerobic phase, rising to 5-12% of the AR at 30/34 DAT, and then decreased during the anaerobic phase to 4, 6, and 10% of the AR by the last sampling interval for RB, SB, and RP, respectively. Details of the aerobic and anaerobic phases are presented below.

Aerobic phase (RB: 0–30 DAT, SB and RP: 0-34 DAT). Analysis of the soil extracts revealed that S-2200 declined to 73-77% of the AR by the end of the aerobic phase (34/30 DAT), with a aerobic half-life estimated at 78, 120, and 79 days for the RB, SB, and RP labels, respectively. The principal degradation routes were oxidation of the methyl groups on the phenoxy ring to form 2-COOH-S-2200 and 5-COOH-S-2200 and cleavage of the ether linkage to form De-Xy-S-2200 which oxidized to Dx-CA-S-2200 (all < 8% of the AR at 30/34 DAT). 2D-TLC analyses of the neutral and acidic soil extracts for all three labels confirmed the degradates. No extractable degradate exceeded 10% of applied radioactivity during the aerobic phase (highest individual unknown – 2.1% of AR).

Radioactive residues were rapidly incorporated into the PES during the aerobic phase. PES residues in the RB label peaked at 5% of the AR at 30 DAT. Sequential extractions of the 30 DAT RB PES were attempted to release identifiable degradates. Dismembrator extraction released 1.5% of the AR. The remaining bound residues at 30 DAT were fractionated into humin (1.5% of AR), humic acid (0.9% of AR) and fulvic acid fractions (1.2% of AR). HPLC analysis of the dismembrator and fulvic acid organic fractions identified small amounts of S-2200 degradates and S-2200 (<1% of AR).

Anaerobic phase (S-2200 *R*-isomers, RB: 30–364 DAT, RP: 34-363 DAT). The early intervals of the anaerobic phase (37/41, 44/48, 63/64, 86/90, and 154/153 DAT) can be described as anoxic (low oxygen, redox transitioning to negative values). By 154/153 DAT, the systems had more anaerobic character and from 259/266 DAT to the end of the study (363/364 DAT), anaerobic test conditions (low oxygen and strongly negative redox values) were maintained. S-2200 degraded very slowly during the anaerobic phase, declining to an average of 71 and 68% of the AR in the water phase plus soil extracts by the last DAT for RB and RP, respectively. The anaerobic half-lives for S-2200 in the RB and RP systems were estimated to be > 1 year. As with the *R*-isomer of S-2200, the concentrations of the degradates, 2-COOH-S-2200, 5-COOH-S-2200 and DX-CA-S-2200 did not change significantly under anaerobic conditions. 2-COOH-S-2200 concentrations peaked at 6% of the AR for RB (44 DAT) and 6% for RP (90 DAT) in the water plus soil extracts and stayed at similar levels for the balance of the study. Concentrations of 5-COOH-S-2200 peaked at 9% of the AR for RB (63 DAT) and 8% for RP (64 DAT) and decreased only slightly by the end of the study. Dx-CA-S-2200 concentrations rose slightly during the anaerobic phase and peaked at 5% of the AR (RB, 364 DAT). De-Xy-S-2200 concentrations peaked at 3% of the AR (RB, 44 DAT) and declined to 0% by the end of the study. 2D-TLC analyses confirmed 2-COOH-S-2200, 5-COOH-S-2200 and Dx-CA-S-2200 in the water and soil. Chiral HPLC analysis confirmed that the S-2200 *R*-isomer did not isomerize during the incubation. No extractable degradate (identified or unknown) exceeded 10% of applied radioactivity during the anaerobic phase (highest individual unknown – 2.1% of AR).

Radioactive residues were rapidly incorporated into the RP PES during the aerobic phase, peaking at 12% of the AR at 41 DAT. PES residues in the RP label decreased to 10% by 363 DAT. Sequential extractions of the 41 and 363 DAT RP PES were attempted to release identifiable degradates. Similar amounts of activity were released at both intervals. Dismembrator extraction released 0.9 and 1.2% of the AR. The remaining bound residues were fractionated into humin (3.6 and 3.9% of AR), humic acid (2.7 and 2.8% of AR) and fulvic acid fractions (2.1 and 3.2% of AR). HPLC analysis of the dismembrator and fulvic acid organic fraction identified small amounts of S-2200 degradates and S-2200 (<1% of AR).

Anaerobic phase (S-2200 *S*-isomer, SB: 34-363 DAT). The transition from aerobic to anoxic to anaerobic conditions in the SB system was similar as described above for the RB and RP systems. S-2200 degraded very slowly during the anaerobic phase. The S-2200 *S*-isomer degraded slightly faster than the S-2200 *R*-isomers (RB and RP), declining to an average of 66% of the AR in the water phase plus soil extracts by 363 DAT. The anaerobic half-life for S-2200 in the SB study was estimated to be > 1 year. As with the *S*-isomer of S-2200, the concentrations of 2-COOH-S-2200, 5-COOH-S-2200 and DX-CA-S-2200 in water and soil did not change significantly under anaerobic conditions. 2-COOH-S-2200 concentrations peaked at 5% of the AR (90 DAT) in the water plus soil extracts and remained at similar levels for the balance of the study. Concentrations of 5-COOH-S-2200 peaked at 7% of the AR (64 DAT) and decreased slightly by the end of the study. Dx-CA-S-2200 concentrations rose slightly during the anaerobic phase and peaked at 6% of the AR (266 DAT). De-Xy-S-2200 concentrations peaked at 3% of the AR (48 DAT) and declined to 0% by the end of the study. 2D-TLC analyses

confirmed Dx-CA-S-2200, 2-COOH-S-2200 and 5-COOH-S-2200 in the water and soil. Chiral HPLC analysis confirmed that the S-2200 *S*-isomer did not isomerize during the incubation. No extractable degradate (identified or unknown) exceeded 10% of applied radioactivity during the anaerobic phase (highest individual unknown – 3.6% of AR).

While radioactive residues were rapidly incorporated into the PES during the aerobic phase, the SB PES residues peaked at 7% of the AR at 41 DAT. Sequential extractions of the 41 DAT SB PES were attempted to release identifiable degradates. Dismembrator extraction released 1.4% of the AR. The remaining bound residues were fractionated into humin (2.2% of AR), humic acid (1.3% of AR) and fulvic acid fractions (1.6% of AR). HPLC analysis of the dismembrator and fulvic acid organic fraction identified small amounts of S-2200 degradates and S-2200 (<1% of AR).

Demethylation of S-2200 to form MCBX was observed in the benzyl label of the S-isomer at the end of the study (3% of the AR at 363 DAT), while MCBX formation in the *R*-isomer studies remained low (<1% of AR). HPLC and 2D-TLC analysis confirmed the formation of MCBX.

The pattern of S-2200 decline and formation of degradates in the aerobic and anaerobic phases of the anaerobic soil study was similar to S-2200 aerobic soil and anaerobic aquatic studies.

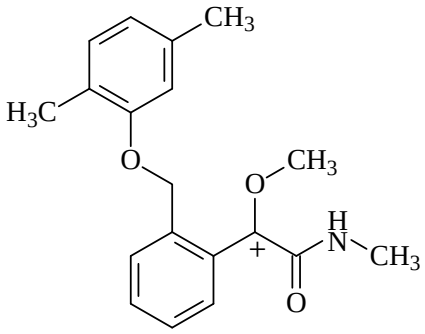
1. INTRODUCTION

S-2200 is being developed as a fungicide and soil can be exposed to S-2200 as a result of its use. This study was conducted to quantitatively evaluate the degradation of S-2200 [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer), [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer), and [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer) in a water/soil system under aerobic followed by anaerobic conditions with respect to rates, DT_{50}, and degradates. The study was conducted following Environmental Fate Data Requirement, 40 CFR 158.290, OCSPP 835.4200 Anaerobic Soil Metabolism. The study was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice Standards (40 CFR Part 160). A copy of the protocol, amendments and deviations can be found in Appendix A.

2. MATERIALS

2.1 Test Substance General Information

Code names:	V-10190, S-2200
Common Name:	Not Available
Isomer Code Names:	S-2167 (S-2200 *R*-isomer) S-2354 (S-2200 *S*-isomer)
Chemical Name:	(*R*,*S*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide (IUPAC) 2-[(2,5-dimethylphenoxy)methyl]-α-methoxy-*N*-methyl-benzeneacetamide (CAS)
CAS Number:	173662-97-0
Molecular Weight:	313.39
Molecular Formula:	$C_{19}H_{23}NO_3$ (non-radiolabeled)
Hydrolysis:	> 1 year half-lives at pH 4, 7 and 9
Chiral location (+):	



Additional physicochemical properties of technical grade S-2200 are given below.

Parameter	Properties/Value		
Physical State: (Reference 1)	Powdery solid		
Odor: (Reference 1)	No odor		
Melting Point/Range: (Reference 1)	102 °C		
Relative Density: (Reference 1)	1.24 g/mL at 20.6 °C		
Water Solubility (OECD # 105 - Flask Method): (Reference 2)	15.8 mg/L (at 20 °C)		
Solvent Solubility (at 20°C) (racemic) (Reference 3)	Hexane 1.46 g/L Dichloromethane 522 g/L Acetone 275 g/L Methanol 169 g/L Ethyl Acetate 158 g/L n-Octanol 31.8 g/L Toluene 114 g/L		
Vapor Pressure (Reference 4)	3.36×10^{-8} Pa at 20 °C (racemic)		
Dissociation constant (Reference 1)	No dissociation activity at pH		
Octanol-Water Partition Coefficient (Reference 5)	Log P_{ow} = 3.51 at 25±1°C		
UV/Visible Absorption Spectrum (pH 2 – 10) (Reference 1)	Solution	λmax (nm)	Molar absorptivity, (ε) $(mol/L)^{-1}cm^{-1}$
	Methanol	273	2140
	Acidic	273	1920
	Basic	273	1880
	Neutral	273	1740

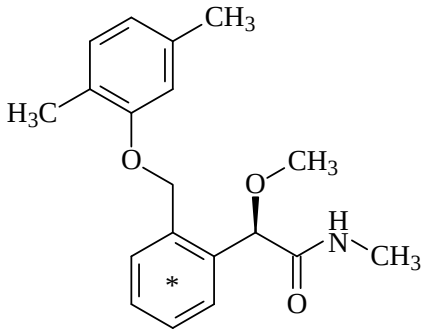
2.1.2 Radiolabeled Test Substance

Three test substances were used in the study (Appendix D), S-2200 [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer), [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer), and [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer). Identical experiments were conducted using each radiolabeled test material. The radiochemical purity of the test substances were determined prior to application using High Performance Liquid Chromatography (HPLC). Test substances were stored in a freezer (F14, ca. –5 ºC) when not in use.

(i) [Benzyl-^{14}C]S-2167 (S-2200 *R*-isomer)

Chemical Name: (*R*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide (IUPAC)
Lot No.: RIS2008-010 (radioactive)
Source: Sumitomo Chemical Co., Ltd.
Valent Inventory No.: 401
Specific Activity: 4.22 GBq/mmol, 114 mCi/mmol, 807,900 dpm/µg

Radiolabeled Position (*): Uniformly radiolabeled in the benzyl ring

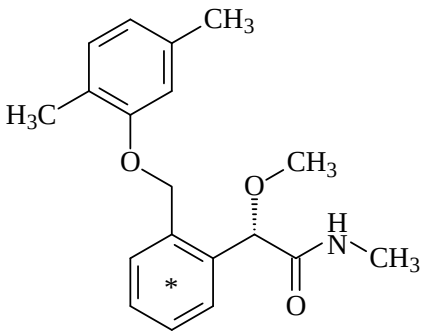


Purity: The radiochemical purity was determined to be 98.91% prior to dosing.

(ii) [Benzyl-^{14}C]S-2354 (S-2200 *S*-isomer)

Chemical Name: (*S*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide (IUPAC)
Lot No.: RIS2008-009 (radioactive)
Source: Sumitomo Chemical Co., Ltd.
Valent Inventory No.: 400
Specific Activity: 4.22 GBq/mmol, 114 mCi/mmol, 807,900 dpm/µg

Radiolabeled Position (*): Uniformly radiolabeled in the benzyl ring

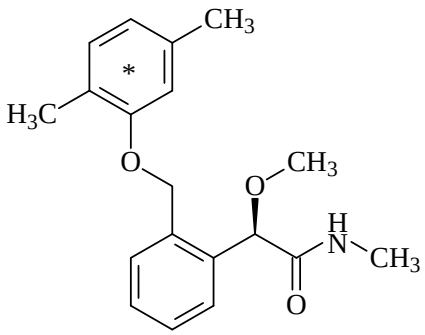


Purity: The radiochemical purity was determined to be 98.62% prior to dosing.

(iii) [Phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer)

Chemical Name:	(*R*)-2-methoxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide (IUPAC)
Lot No.:	RIS2009-002 (radioactive)
Source:	Sumitomo Chemical Co., Ltd.
Valent Inventory No.:	404
Specific Activity:	4.44 GBq/mmol, 120 mCi/mmol, 850,100 dpm/µg

Radiolabeled Position (*): Uniformly radiolabeled in the phenoxy ring



Purity: The radiochemical purity was determined to be 97.91% prior to dosing.

2.2 Control and Reference Substances

Control Substance. [UL-^{14}C]-*D*-glucose is readily degraded by aerobic and anaerobic microbes (UL = uniformly labeled). Therefore, glucose degradation can be used to verify the presence of viable microbial populations. [UL-^{14}C]-*D*-glucose was used as a control substance to verify methanogenic activity in the test system (strictest anaerobic conditions obtained). The glucose was stored in freezer F14 (-5 ºC) when not in use.

Chemical Name:	[UL-^{14}C]-*D*-glucose
Batch/Lot No.	3615373
Valent Inventory No.	408
Specific activity:	319 mCi/mmole, and the chemical purity is $\geq$ 97% (stated)
Molecular wt.:	180.2 (non-radioactive)
Source:	Perkin Elmer

Reference Substances. The following analytical reference standards were used in this study. Sumitomo Chemical Company, Ltd. supplied the reference standards. The following reference substances were used in this study:

Reference Standard	Lot No.	Purity (%)[1]	Expiration[1] Date
De-Xy-S-2200	AS 2264a	99.9	7/26/11
DX-CA-S-2200	AS 2269a	100	7/28/11
2,5-Dimethylphenol (DMP)	MKAA2399	99.8	11/11/2011
2-COOH-S-2200	AS 2268a	99.0	7/28/11
5-COOH-S-2200	AS 2267a	99.6	7/28/11
MCBX *R*-isomer	AS 2265a	98.9	8/1/13
MCBX *S*-isomer	AS 2266a	93.4	8/1/13
MCBX (racemic)	AS 2270a	95.2	7/11/13
S-2200 *R*-isomer	AS 2262a	99.8	7/20/13
S-2200 *S*-isomer	AS 2263a	99.4	7/20/13
S-2200 (racemic)	AS 2261a	99.6	7/8/11

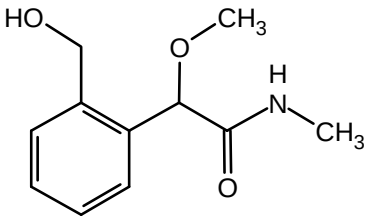
NA = Not applicable (commercial standard obtained from Sigma-Aldrich, specification date Jan 2009)
1) Most recent purities and expiration dates.

Standards were prepared in acetonitrile or acetonitrile/water solutions for use in HPLC and TLC analysis (Table 1). The mixed standards for the [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer) and [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer) studies contained De-Xy-S-2200, DX-CA-S-2200, 2-COOH-S-2200, 5-COOH-S-2200, MCBX and S-2200. The mixed standards for the [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer) study contained DMP, 2-COOH-S-2200, 5-COOH-S-2200, MCBX and S-2200. See Appendix B for Certificates of Quality for the reference substances. The standards and standard solutions were stored frozen when not in use. See below for structures.

Chemical structures of the reference substances used in this study were as follows:

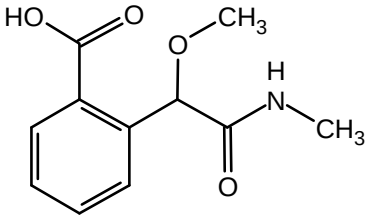
Designation: De-Xy-S-2200
Chemical Name: (*RS*)-2-(2-hydroxymethylphenyl)-2-methoxy-*N*-methylacetamide

Structure:



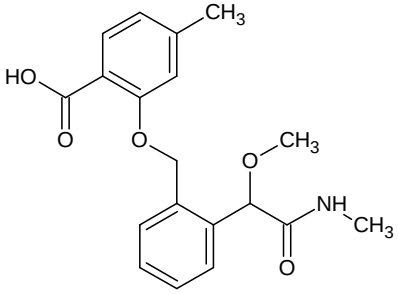
Designation: DX-CA-S-2200
Chemical Name: (*RS*)-2-(*N*-methylcarbamoyl-methoxymethyl)benzoic acid

Structure:



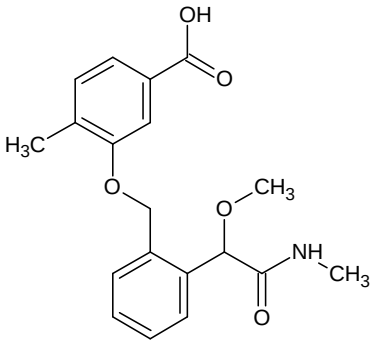
Designation: 2-COOH-S-2200
Chemical Name: (*RS*)-2-{2-[1-methoxy-1-(*N*-methylcarbamoyl)methyl]benzyloxy}-4-methylbenzoic acid

Structure:



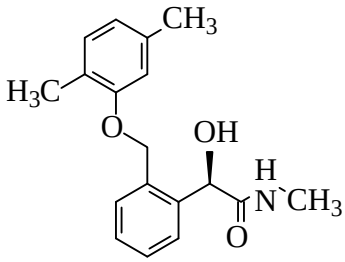
Designation: 5-COOH-S-2200
Chemical Name: (*RS*)-3-{2-[1-methoxy-1-(*N*-methylcarbamoyl)methyl]benzyloxy}-4-methylbenzoic acid

Structure:



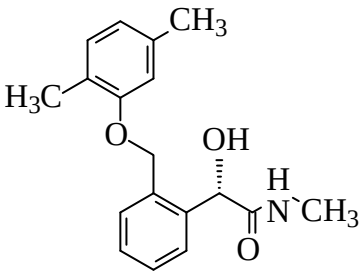
Designation: MCBX, *R*-isomer
Chemical Name: (*R*)-2-hydroxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide

Structure:



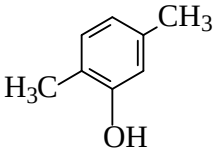
Designation: MCBX, *S*-isomer
Chemical Name: (*S*)-2-hydroxy-*N*-methyl-2-[α-(2,5-xylyloxy)-*o*-tolyl]acetamide

Structure:



Designation: DMP
IUPAC Name: 2,5-dimethylphenol

Structure:



2.3 Reagents and Chemicals

Solvents and reagents used in this study are listed below with their commercial sources and grades.

Acetone (Fisher, Optima; EM Science, Omnisolv)
Acetic acid (J.T. Baker, Instra-Analyzed, Aldrich)
Acetonitrile (Fisher, Optima; EM Science, Omnisolv)
Barium chloride dihydrate (Aldrich, ACS reagent)
Carbon 14 Cocktail (R.J. Harvey Instrument Corp.)
Chloroform (Sigma, reagent grade)
Ethanol (EM Science, Omnisolv)
Ethyl acetate (EM Science, Omnisolv)
Formic acid (95%, Sigma)
Hydrochloric acid (J.T. Baker, Instra-Analyzed, Aldrich)
Methanol (Fisher, Optima; EM Science, Omnisolv)

Sodium hydroxide (Aldrich, reagent grade)
Toluene (EM Science, Omnisolv)
Trifluoroacetic acid (Sigma, reagent grade)
Ultima Gold LSC cocktail (Packard Instrument Company)
Water (ASTM Type II, Ricca Chemical company)
Water (HPLC grade, EM Science, Omnisolv)

2.4 Instrumentation

A list of the equipment used in the study is given below.

Balance (Mettler model PM 4000, S/N H28153 #B10)
Balance (Mettler model Toledo, S/N 1113461365 #B23)
Balance (Mettler model AE160 S/N E17218 #VA-1)
Centrifuge (Eppendorf model 5415C S/N 58833)
Centrifuge (Sorvall model RT6000 with model A500 fixed-angle rotor, S/N BT 126255)
Centrifuge (Sorvall model RC26 Plus with a model SH-4 rotor, S/N 9900956)
Centrifuge (Sorvall model Evolution with model SH-3000 swinging bucket rotor)
Dissolved oxygen electrode, Orion Research, model 97-08 electrode
Dissolved oxygen electrode, OakTon, model DO 6 Acorn series
Evaporation unit (Pierce, Reactivap, Model 18780)
Fraction collector (ISCO model Foxy 200)
Freezers, F8 (Lab-Line Instruments, Inc.), F12 (Baxter Scientific Products, Cryofridge), F14 (Jewett, frost-free) and F16 (Fisher Scientific, Isotemp)
Heating module (Pierce, ReactiTherm, Model 18870, S/N 548961141642)
HPLC (Hewlett-Packard model HP 1100, HPLC #38, with a Raytest model Ramona 2000 radioflow detector, solid scintillant flow cell 250 µL LiGl)
Incubator I16 (Precision Scientific)
Incubator I1 (Bally Case & Cooler, Inc.)
Liquid Scintillation Counter (Packard Tri-Carb 2100TR, S/N 414244)
Liquid Scintillation Counter (Perkin Elmer Tri-Carb 2900TR, S/N 433678)
Orbital shaker (IKA-Werke, model KS501)
Oxidizers (Harvey, Model OX-500 or OX-700)
pH meter, Corning, Model 455, SN-32199
Radio TLC scanner - PhosphorImager (Molecular Dynamics, model PSI-Pentium, S/N 75104)
Radio TLC scanner - Storm 820 (Amersham Biosciences, S/N 003601)
Redox electrode, Wissenschaftlich Technische Werkstatten, model SenTixORP [Combination electrode containing a platinum reference electrode. The measured potentials (which are the values in the tables in the report) can be compared to the standard hydrogen potential using the following equation (all in mV).

U_h (standard hydrogen potential) = $U_{Measured}$ (measured ORP from the platinum electrode) + U_{Ref} (voltage of the reference system relative to the standard hydrogen electrode) U_{Ref} values at the study measurement temperatures (~25-30 °C) are +207 to + 203 mV.]

Shaker, reciprocal (Eberbach, model 6000, S/N 029702)
Sonic Dismembrator, Fisher Scientific, model 500, probe 102C (CE)
Survey meters (Ludlum model 3, S/N 67089, 57075, & 145115)
Transilluminator for TLC (Model VWR TW-26, #95-0209-01)
Ultrasonic bath (VWR Scientific, model 150T, and P250T)
UV cabinet for TLC (*UVP, Inc*. model CC-60)
Water bath (Buchi model B-461, S/N 1143944 & 1147480)

3. EXPERIMENTAL DESIGN

The test system simulates the circumstances where the test substance is applied to a terrestrial soil that becomes waterlogged and in which anaerobic conditions develop. Initially, the individual soil samples (Treatments A-G as described in Section 3.3) were incubated in an aerobic test system as described in Sections 3.1 and 3.2. Analysis at 14 or 15 DAT indicated that S-2200 would not have degraded through a single half-life during the maximum period of aerobic incubation, therefore the individual samples were quantitatively transferred to closed biometer flasks at 30 or 34 DAT. The soil samples were waterlogged, the systems purged with nitrogen and anaerobic conditions allowed to develop as described in Sections 3.1 and 3.2. Samples were analyzed during the 30/34 day aerobic phase and the 329/334 day anaerobic phase (total study 363/364 days). Details of the experimental design are presented in the table below and discussed in the following sections.

Parameter		**Experimental details**
Duration of the test:		30/34 days aerobic, 329/334 days anaerobic (total study 363/364 days)
Soil condition: (Air dried/fresh)		Fresh
Soil: (g/replicate)		50 g (dry weight)
Target test concentrations:		8.96 ppm (µg per g of dry soil) Based on use rate of 2.0 lb a.i./acre
Experimental conditions:	Incubation conditions	Incubated in the dark at 20 ± 2 ^{o}C
	Moisture content Moisture maintenance method	50 ± 10% of the water holding capacity Weigh sample to verify moisture levels and add required water
Any indication of the test material adsorbing to the walls of the test apparatus:		No
No. of Replication:	Controls, if used	2
	Treatments	2
Aerobic test apparatus:		50 g of soil (dry) incubated in a 100 mL glass beaker with metal spatula. Incubated in closed, continuous flow, negative pressure incubation apparatus
Traps for CO_2:		CO_2: 2 traps of 50 mL 2.0 M NaOH in petri dishes inside apparatus, 2 traps each 50 mL 2.0 N NaOH outside apparatus.
Anaerobic Test apparatus:		50 g of soil (dry) plus 95 mL of HPLC water incubated in a closed custom glass biometer.
Identity and concentration of co-solvent:		Acetonitrile neat
Test material application	volume of test solution used for treatment	250 µL
	application method	applied on surface with syringe then 5 minutes of homogeneous mixing
	co-solvent	evaporated

3.1 Test System

The test system consisted of a loam soil that was recently collected from an agricultural area, top 3 inch layer (Penn series, Baptistown, NJ). The test soil was the same (collected at the same time and place) as the soil used in the S-2200 aerobic soil metabolism study (Study number VTC-09-37114). Prior to use, the soil was stored in the dark in accordance with ISO 10381-6 (1993), Soil Quality – Sampling – Part 6. The soil was thoroughly mixed and passed through a 2-mm mesh sieve with a minimum of air-drying. Soil characterization, according to GLP, was performed by AGVISE Laboratories Inc. (Northwood, ND, Appendix C). Details of the soil analysis are presented in Table 2. The soil was classified as a loam according to the USDA classification system for soil. The pH of the collected soil was 6.9. The percentage of organic matter in the soil was 2.0%, and the Cation Exchange Capacity (CEC) was 8.7 meq/100 g. The description of soil sampling and storage details are listed below. The Valent soil inventory designation is VTC-7-09A.

Description	Details
Geographic location	Baptistown, NJ; GPS N 40° 32 min, W 74° 39 min
Pesticide use history at the collection site	Not available. Site was a terrestrial soil where pesticides would normally not be applied. Same soil as used in aerobic soil metabolism.
Collection procedures for soil:	Collected with a shovel.
Sampling depth for soil:	Top 0-3 inches of soil.
Storage conditions:	Stored at ambient temperature from the date of collection (9/24/09) until arrival at Valent (9/30/09). Stored at ambient temperature at Valent through 2 mm sieving (10/2/09) and aerobic test system preparation (11/3/09).
Storage length:	34 days prior to aerobic test system preparation.
Preparation of soil samples:	Upon receipt, the soil was sieved through a 2 mm sieve.

The soil moisture (16.4%) and Water Holding Capacity (WHC, 50.5%) were determined before use. The target amount of dry soil was 50 g. Adjusting for soil moisture and WHC, 50 g of dry soil at 60% WHC was equivalent to 65.2 g. Samples were adjusted to 60% WHC and were maintained at 50 ± 10% of the WHC throughout the aerobic phase. In practice, the chambers contained petri dishes containing water that kept the samples at approximately 60% WHC during the incubations. Following moisture adjustment, and prior to treatment with the test substance, all soil samples for this study were acclimated in

the aerobic test system (see Section 3.2) for 28 or 41 days prior to dosing. Soil microbial biomass was determined on control samples around dosing and at the end of incubation.

3.2 Incubation Apparatus

Aerobic test system: A schematic drawing of the incubation apparatus is presented in Figure 1. The incubation apparatus consists of an airtight chamber with air inlet and outlet valves. Soil samples (50 g dry weight equivalent to a soil wet weight of 65.16 g, 60% water holding capacity) were placed inside the chamber in open individual 100 mL glass beakers. The soil moisture was adjusted periodically to 50 ± 10% of the water holding capacity during the aerobic incubation (moisture was adjusted by adding HPLC grade water as needed). Two open Petri dishes, each containing 50 mL of 2.0 M NaOH solution were placed inside the chamber to capture evolved $^{14}CO_2$. An open Petri dish full of water was also placed inside the chamber to maintain high air humidity. Chamber air was continuously evacuated through the chamber air outlet, which was connected to a vacuum source, at a steady rate of approximately 10 mL per minute. The chamber inlet air was bubbled through a water trap to humidify the air before entering the chamber. The continuous airflow was used to maintain aerobic conditions as well as to evacuate any radiolabeled volatiles into the outlet traps. The evacuated air passed through two consecutive NaOH traps to capture any $^{14}CO_2$ present in the chamber's air. Before opening the chamber for each sampling, airflow was increased for a length of time sufficient to evacuate any lingering volatile degradation products through the traps. The incubation apparatus was incubated in the dark at 20 ± 2 °C.

Anaerobic test system: A schematic of the individual airtight biometer flasks is presented in Figure 2. After the aerobic phase, each individual soil was quantitatively transferred (with 95 mL of N_2 purged HPLC water) into each of the biometer flasks (total water equals ca. 110 mL = water in the soil (15.2 g) + HPLC water added (95 mL)), this gave a waterlogged soil with ca. a 2 cm water layer. Dried ground alfalfa was added to each biometer at a rate of 0.1% (0.05 g) of the soil dry weight to speed the formation of anaerobic conditions and to insure the survival of the microbial anaerobes. The $^{14}CO_2$ side-arm trap was charged with 25 mL of 2 M NaOH solution (N_2 purged). The biometers were assembled and to facilitate the formation of anaerobic conditions in the test system, the biometer flasks were sparged with a stream of nitrogen for approximately 0.25 – 0.5 hour. Following sparging, the flasks were sealed and incubated in the dark at 20 ± 2 °C. The average total volume of several biometers were measured to permit the calculation of an average headspace volume and the amount of evolved $^{14}CH_4$ based on the oxidation of a known volume of the headspace gas.

Headspace (mL) = [flask volume (mL)] - [soil-water mixture (mL) + NaOH (mL)]

3.3 Treatments

All treatments were applied to identical glass beakers containing a mixture of 50 g soil (dry weight) with the soil moisture maintained to 50 ± 10% of the water holding capacity. Test and control substances were added to glass beakers or biometers containing the test system as follows:

Treatment A: Untreated Controls. Samples were used to measure the microbial biomass. Control samples were analyzed around the time of test substance application (i.e., aerobic phase time zero). Other samples were analyzed at the end of sampling (i.e., at the last anaerobic phase sampling interval).

Treatment B: Organic solvent controls. Samples were used to measure the effect of the administered solvent on microbial biomass at the end of sampling (i.e., at the last anaerobic phase sampling interval).

Treatment C: [UL-^{14}C]-*D*-glucose controls. This treatment was used to verify methanogenic microbial activity in the anaerobic phase. Several biometers were dosed with [UL-^{14}C]-*D*-glucose after the establishment of anaerobic conditions (as indicated by low O_2 and negative redox potentials, 10/21/10) and analyzed 21 days later (11/11/10).

Treatment D: Non-radiolabeled S-2200 (samples treated with non-radiolabeled S-2200 (AS 2261a)) were dosed at 8.98 ppm (449 µg/50 g dry weight soil). Samples were used to measure the effect of non-radiolabeled S-2200 and solvent on microbial biomass at the end of sampling (i.e., at the last anaerobic phase sampling interval).

Treatment E: [Benzyl-^{14}C]S-2167 (S-2200 *R*-isomer). Consists of the test substance [benzyl-^{14}C]S-2167 dosed at 8.95 ppm (448 µg/50 g dry weight soil). This treatment was used to determine the test substance dissipation rate during the aerobic and anaerobic phases, and its [^{14}C]-metabolite formation. Volatile production of $^{14}CO_2$ was evaluated during the aerobic phase. Volatile production of $^{14}CH_4$ and $^{14}CO_2$ was evaluated during the anaerobic phase.

Treatment F: [Benzyl-^{14}C]S-2354 (S-2200 *S*-isomer). Consists of the test substance [benzyl-^{14}C]S-2354 dosed at 8.94 ppm (447 µg/50 g dry weight soil). This treatment was used to determine the test substance dissipation rate during the aerobic and anaerobic phases, and its [^{14}C]-metabolite formation. Volatile production of $^{14}CO_2$ was evaluated during the aerobic phase. Volatile production of $^{14}CH_4$ and $^{14}CO_2$ was evaluated during the anaerobic phase.

Treatment G: [Phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer). Consists of the test substance [phenoxy-^{14}C]S-2167 dosed at 8.95 ppm (448 µg/50 g dry weight soil). This treatment was used to determine the test substance dissipation rate during the aerobic and anaerobic phases, and its [^{14}C]-metabolite formation. Volatile production of $^{14}CO_2$ was evaluated during the aerobic phase. Volatile production of $^{14}CH_4$ and $^{14}CO_2$ was evaluated during the anaerobic phase.

3.4 Test Substance Application Rate

The target application rate of the test substance to soil was 8.96 ppm based on a dry soil basis, equivalent to a field application rate of 2,240 g a.i./ha (equivalent to the total of 4 applications at 0.5 lb a.i./acre per application). This agricultural use rate (2.0 lb a.i./A) is approximately equivalent to 8.96 µg/g applied to one acre of soil 2.5 centimeters deep (bulk density, 1.0 g/cm^3). Beakers containing 50 g (dry weight) of soil were dosed with about 448 µg of ^{14}C-S-2200. Aliquots of the dosing solutions were taken for LSC and radiochemical analysis by HPLC. Dosing solutions were made to deliver about 23,000,000 to 26,000,000 dpm to each test sample in 250 µL.

3.5 Control Substances Application Rate

Since the purpose of the glucose control substance was to verify microbial activity (methanogenesis if possible) under conditions similar to those of the test substance, the control substance was applied at approximately the same molecular rate as the test substance. This rate was calculated as follows:

S-2200 MW	313.39	g per mole
S-2200 per sample (µg)	447	µg

Therefore, a molar equivalent of [UL-^{14}C]-*D*-glucose per sample was calculated as follows: 447 µg-S-2200/313.39 µg/µmole x 180.2 µg-glucose/µmole = 257 µg-glucose. The [UL-^{14}C]-*D*-glucose was isotopically diluted to 46,697 dpm/µg with cold glucose (giving a 257 µg-glucose dose rate equal to approximately 12,000,000 dpm/biometer). Based on aliquots of the dosing solution taken before and after dosing, the amount of radioactivity dosed per biometer flask was 12,010,500 dpm.

3.6 Test Substance and Control Substance Application

Test Substance: After aerobic acclimation (28 days for SB and RP, 41 days for RB), the test substances were added to the aerobic test systems at the concentrations specified in sections 3.3 and 3.4. The test substances were delivered in 250 µL of acetonitrile and applied drop wise to the soil surface with a syringe and the soil mixed with a metal spatula for 5 minutes. [Separate experiments determined that this mixing regimen gave a

homogeneous sample with a CV of 2.0% (Three 0.7 g subsamples were analyzed from four dosed samples (each 50g dry weight))]. The dosed samples were placed in 4 separate incubation chambers (Treatments A-D; Treatment E; Treatment F; and Treatment G).

Control Substance (*D*-glucose): When anaerobic conditions were established, the control substance was added to several untreated biometers at the concentration specified in section 3.5 (dosed 10/21/10, corresponding to 311 days into the RB incubation). The control substance was delivered in 300 µL of nitrogen-purged water anaerobically through the biometer syringe valve on top of the soil/water compartment to the surface of the water (Figure 2). The biometers were incubated in the dark at 20 ± 2 °C and analyzed 21 days later (11/11/10).

4. Methods

4.1 Preparation of [^{14}C]S-2200 Dosing Solutions

Three test substances were used in the study S-2200 [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer), [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer), and [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer). Identical experiments were conducted using each radiolabeled test material. The dosing solutions were prepared by isotopically diluting radiolabeled S-2200 with cold S-2200 (*R*-isomer with RB and RP, *S*-isomer with SB). The final dosing solutions were made up in acetonitrile and had specific activities of: S-2200 ([benzyl-^{14}C]S-2167 (S-2200 *R*-isomer) = 57,151 dpm/µg; [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer) = 51,415 dpm/µg; [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer) = 51,839 dpm/µg. Based on aliquots of the dosing solution taken before and after dosing, the amount of radioactivity per biometer flask was 25,568,700 dpm for [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, 401G), 22,976,400 dpm for [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, 400D), and 23,203,500 dpm for [phenoxy-^{14}C]S-2167 (S-2200 R-isomer, 404D). The three test materials were dosed at ca. 8.95, 8.94, and 8.95 ppm to the RB, SB, and RP test systems, respectively. The radiochemical purity of the dosing solutions at dosing were determined to be 98.91%, 98.62%, 97.91% for the RB, SB, and RP test systems, respectively (Appendix D). Chiral HPLC analysis determined that the assignments of chirality of the test substances were correct (100% *R*-isomer or 100% *S*-isomer depending on the test material).

4.2 Sampling, Sample Processing and Analysis

A sample, as used in this study, consisted of one glass beaker (aerobic soil and NaOH traps) or one biometer flask (anaerobic soil/water mixture, NaOH trap, and headspace gases). Two samples were removed for analysis according to the following schedule: RB samples were analyzed at 0, 14, 30, 37, 44, 63, 86, 154, 259, and 364 days after dosing (aerobic phase – 0 to 30 DAT; anaerobic phase – 30 to 364 DAT); SB and RP labels were analyzed at 0, 15, 34, 41, 48, 64, 90, 153, 266, and 363 days after dosing (aerobic phase – 0

to 34 DAT; anaerobic phase – 34 to 363 DAT). Aerobic samples were measured for $^{14}CO_2$ production and then extracted with organic solvents. Anaerobic samples were measured for $^{14}CH_4$ and $^{14}CO_2$ production, dissolved oxygen concentration, redox potential, and pH in that order at the time of analysis. Samples were analyzed immediately after removal from the incubator and were not stored prior to any of the physical-chemical property or chemical analyses. Summaries of the aerobic and anaerobic sample processing procedures can be found in Figures 3 and 4.

4.2.1 Aerobic Test System - NaOH Traps

NaOH traps for the RB system was removed for analysis and were replaced with fresh traps at the 14 and 30 DAT sampling intervals. NaOH traps for the SB and RP systems were removed for analysis and were replaced with fresh traps at the 15 and 34 DAT sampling intervals. The contents of each NaOH trap was measured and three aliquots of each trap were removed and counted by LSC. Radioactivity present in each trap was determined and its content of [^{14}C] radioactivity was calculated. The confirmed amount of measured $^{14}CO_2$ was normalized to the number of samples present in the incubation chamber for the corresponding sampling period. The cumulative amount of $^{14}CO_2$ evolved at each interval was calculated as described in section 5.1.1.

4.2.2 Aerobic Test System - Extraction of Radiolabeled Compounds in Soil Using Organic Solvents

At each sampling interval, duplicate soil samples were extracted and analyzed as described below. The aerobic phase samples were extracted with a neutral organic solution to yield extract #1 (9:1 acetone:water) (0, 14/15 and 30/34 DAT intervals) and extracted with a weakly acidic organic solution to yield extract #2 (5:1 acetone:0.1M HCl) (14/15 and 30/34 DAT intervals).

Neutral Extract: The soil sample was quantitatively transferred out of the beaker into separate pre-weighed centrifuge bottle. The beaker was rinsed with 150 mL of 9:1 acetone:water (v:v), which was added to the centrifuge bottle. The centrifuge bottle was shaken for 20 minutes (reciprocal shaker) and the phases separated by centrifugation (10 minutes at 7,500 RPM). The supernatant was decanted into a graduated cylinder. Another 150 mL of 9:1 acetone:water (v:v) was added to the centrifuge bottle and the sample was shaken for 20 minutes (reciprocal shaker) to rinse the soil. The phases were separated by centrifugation (10 minutes at 7,500 RPM) and the 2nd extraction added to the supernatant in the graduated cylinder (Neutral extract).

Acidic extract: The remaining soil pellet was re-suspended in with 150 mL of 5:1 acetone: 0.1 M HCl (v:v) and the sample shaken 20 minutes (reciprocal shaker) and

the phases separated by centrifugation (10 minutes at 7,500 RPM). The supernatant decanted into the graduated cylinder. Another 150 mL of 5:1 acetone: 0.1 M HCl (v:v) was added to the centrifuge bottle and the sample was shaken for 20 minutes (reciprocal shaker). The phases were separated by centrifugation (10 minutes at 7,500 RPM) and the second extract was added to the supernatant in the graduated cylinder. Another 100 mL of acetone was added to the centrifuge bottle and the sample was shaken for 20 minutes (reciprocal shaker) to rinse the soil. The phases were separated by centrifugation (10 minutes at 7,500 RPM) and the rinse was added to the supernatants in the graduated cylinder (Acidic extract).

The volume of each extract was measured and 3 aliquots were removed from each extract for LSC. The neutral extract was transferred to a separate round bottom flask and rotary evaporated (at about 35 °C) to near dryness. The remaining residue was dissolved in methanol with sonication and the solution was transferred into a small vial. The final volumes were measured and an aliquot was removed for LSC. The acidic extract was transferred to a separate round bottom flask and rotary evaporated (at about 35 °C) to remove the acetone (aqueous fraction remains). The aqueous fraction was partitioned twice with 150 mL of ethyl acetate (1 minute shake). The volumes of the combined organic and remaining aqueous fractions were measured and aliquots taken for LSC. The ethyl acetate fraction was transferred to a separate round bottom flask and rotary evaporated (at about 35 °C) to near dryness. The residue was dissolved in methanol with sonication and the solution was transferred into a small vial. The final volumes were measured and an aliquot was removed for LSC. The extracts were analyzed by HPLC-RAM and/or TLC-autoradiography.

The post extraction soil (PES) was weighed and sub-samples were combusted using a sample oxidizer and its content of radioactivity was determined. A PES samples from the 30 DAT RB sampling interval was further extracted using the extraction and fractionation procedures described in section 4.2.9.

4.2.3 Anaerobic Test System -Headspace Sampling and Analysis ($^{14}CH_4$ Quantification)

The biometer flask was removed from the incubator and a graduated Gastight™ syringe (50 mL) was used to withdraw a headspace sample through the sampling valve. The headspace sample was immediately injected into a Harvey Biological Oxidizer and the radioactivity was quantified using LSC. Total radioactivity in each biometer headspace was calculated based on the radioactivity in the oxidized sample and the total headspace volume of the flask. Measured radioactivity (dpm) was converted to percent of applied radioactivity and ppm S-2200 equivalents.

4.2.4 Anaerobic Test System - Verification of Anaerobic Conditions

Anaerobic conditions for each sample were verified and documented by measuring dissolved oxygen and redox potential. Following headspace sampling, the biometer flask was opened and a dissolved oxygen electrode was inserted into the water phase of the soil/water mixture and the dissolved oxygen reading was recorded. Following dissolved oxygen measurement, a redox electrode was placed into the soil phase of the soil/water mixture and the reading of electrical potential was recorded. A combination pH electrode was used to measure the pH of the water and soil, separately.

4.2.5 Anaerobic Test System - NaOH Traps

The volume of each NaOH solution in the side-arm trap was measured and three aliquots removed for LSC. Radioactivity present in each trap was determined and evolved $^{14}CO_2$ was calculated as a percentage of applied radioactivity and ppm S-2200 equivalents.

4.2.6 Anaerobic Test System - Separation of Water and Soil Phases

The water from each biometer was quantitatively transferred with a pipet into pre-weighed 250 mL centrifuge bottle. The bottle was capped and centrifuged at 7,500 RPM for 10 min. The water phase was carefully decanted into a graduated cylinder and its volume was recorded. Water and Soil phases were analyzed separately.

4.2.7 Anaerobic Test System - Water Analysis

The water phase was transferred to a separate round bottom flask and the concentration of radioactivity present in the water phase was determined by LSC.

7 day anaerobic intervals (study intervals 37/41 DAT): The activity in the water phase was partition with 150 mL of ethyl acetate. The neutral organic phase was removed and the remaining aqueous phase acidified with 3 mL of 1 M HCl. The acidified aqueous phase was partition with 150 mL of ethyl acetate. The neutral and acidic organic phases were combined, the volume measured and aliquots taken for LSC. The combined organic phase was concentrated by rotary evaporation (bath temperature 35 °C) to dryness and the residues dissolved in methanol. The volume was measured and an aliquot taken for LSC analysis. The water was analyzed by HPLC-RAM and/or TLC-autoradiography.

14 to 329/334 day anaerobic intervals (study intervals 44/48 to 363/364 DAT): The activity in the water phase was concentrated by rotary evaporation (bath

temperature 35 °C) to dryness and the residues dissolved in methanol or methanol - water. The volume was measured and an aliquot taken for LSC analysis. The water was analyzed by HPLC-RAM and/or TLC-autoradiography.

4.2.8 Anaerobic Test System - Soil Analysis

The soil remaining in the biometer was transferred (with solvent) into the pre-weighed 250 mL centrifuge bottle that the water phase was decanted out of (see section 4.2.6), and was extracted several times. The remaining soil was extracted twice with a neutral organic solution to yield extract #1 (9:1 acetone:water or 9:1 methanol:water (v:v) and then extracted twice with a acidic organic solution to yield extract #2 (5:1 acetone:0.1M HCl (v:v), 9:1 methanol:0.5M HCl (v:v) or 5:1 methanol:0.5M HCl (v:v).

Neutral extract: The soil was transferred out of the biometer with 150 mL of 9:1 acetone:water (v:v) (37/41 DAT) or 9:1 methanol:water (v:v) (44/48 to 363/364 DAT) into the pre-weighed 250 mL centrifuge bottle and the sample shaken for 20 minutes on a reciprocal shaker. The phases were separated by centrifugation for 10 minutes at 7,500 RPM. The sample was transferred to a graduated cylinder through a funnel containing glass wool. The extraction was repeated, the extracts pooled (Neutral extract), volumes measured and aliquots taken for LSC.

The Neutral soil extracts were transferred into round bottom flasks and concentrated to dryness using rotary evaporation under vacuum and at ca. 35 °C. The residues were dissolved in methanol with sonication and the solution was transferred into a small vial. Extracts were clarified by centrifugation as needed. The concentrated extract volumes were measured and their radioactivity determined by LSC. The extracts were analyzed by HPLC-RAM and/or TLC-autoradiography.

Acidic extract: 150 mL of 5:1 acetone:0.1M HCl (37 DAT) or 9:1 methanol:0.5M HCl (41, 48 DAT) or 5:1 methanol:0.5M HCl (44, 63/64 to 363/364 DAT) was added to the remaining soil and the sample shaken for 20 minutes on a reciprocal shaker. Note: The polarity and acidity of the extraction solvent was increased with time to increase the effectiveness of the acidic extraction. The phases were separated by centrifugation for 10 minutes at 7,500 RPM. The sample was transferred to a graduated cylinder thru a funnel containing glass wool. The extraction was repeated and the extracts pooled. A 100 mL of methanol was added to the centrifuge bottle and the sample was shaken for 20 minutes (reciprocal shaker) to rinse the soil. The phases were separated by centrifugation (10 minutes at 7,500 RPM) and the rinse was added to the supernatants in the graduated cylinder (Acidic extract), volumes measured and aliquots taken for LSC.

The Acidic soil extracts were transferred into round bottom flasks and concentrated to the aqueous phase using rotary evaporation under vacuum and at ca. 35 °C. The aqueous residue was transferred to a separatory funnel and partitioned twice with 150 mL of ethyl acetate. The volumes of the aqueous and organic layers were measured and aliquots taken for LSC. The organic layer was rotary evaporated to near dryness at ca. 35 °C. The residues were dissolved in methanol with sonication and the solution was transferred into a small vial. Extracts were clarified by centrifugation as needed. The concentrated extract volumes were measured and their radioactivity determined by LSC. The extracts were analyzed by HPLC-RAM and/or TLC-autoradiography.

The weight of the post-extraction soil (PES) was recorded. Three sub-samples of PES were weighed and combusted in a sample oxidizer to determine the soil-bound radioactivity as described in section 4.4. The non-extractable radioactivity remaining in the PES is termed as soil-bound radioactivity. PES samples from the 30 (RB), 41 (SB and RP) and 363 DAT (RP) sampling intervals were further extracted using the extraction and fractionation procedures described in section 4.2.9.

A final rinse of the biometer was done with 30 mL of ethanol/dichloromethane (1/1) to determine residual radioactivity adsorbed to the glass. Aliquots of the biometer rinse (BR) were taken for LSC.

4.2.9 Aerobic and Anaerobic Test Systems - Fractionation of Soil-Bound Radioactivity

An additional dismembrator extraction with 5:1 methanol:0.5M HCl was performed on representative post extraction soils (PES) at 30, 41, and 363 DAT (samples containing bound residues between 5 and 12% of the applied radioactivity). Finally, the remaining PES was fractionated into humin, humic acids and fulvic acids. S-2200 residues in the water, soil extracts, dismembrator extracts, and the fulvic acid organosoluble fraction were analyzed by HPLC with a radioflow detector or fraction collector/LSC (co-chromatography). The fractionation procedures are summarized in a schematic diagram in Figure 5.

Dismembrator Extraction - The radioactivity remaining in soil following extraction (Neutral and Acidic extract, see sections 4.2.2 and 4.2.8) was termed as soil-bound residues or post extraction soil (PES). The PES was subjected to harsher extraction (Dismembrator extraction). A 30 DAT PES (RB label), 41 DAT PES (RP and SB labels) and a 363 DAT PES (RP label) were subjected to dismembrator extraction.

The Dismembrator extraction was conducted for 15 minutes using 150 mL of 5:1

methanol:0.5 M HCl, (v:v). The sample (in a heavy walled beaker) was placed in a water bath and the temperature monitored. The Dismembrator amplitude was set to 50% with a pulse on and off cycle of 10 seconds. The soil and extract were transferred back to the original centrifuge tube and the phases were separated by centrifugation for 10 minutes at 7,500 RPM. The supernatant was transferred to a graduated cylinder, volumes measured and aliquots taken for LSC. The extracts were transferred to separate round bottom flask and rotary evaporated (30 - 35 °C) to near dryness. The remaining residue was dissolved in methanol. The concentrated extract volumes were measured and their radioactivity determined by LSC. The extracts were analyzed by HPLC-RAM and/or TLC-autoradiography.

The post Dismembrator extraction PES were further characterized using the base fractionation method described below.

Humic, Humin and Fulvic Acid Fractionation - The radioactivity remaining in the PES was fractionated into humin, humic acids, and fulvic acids associated radioactive residues. The PES was shaken with 150 mL of 0.5 M NaOH for 16 hours on a reciprocating shaker. The soil debris pellet and aqueous supernatant were separated after centrifugation for 20 minutes at approximately 7,500 RPM. The soil debris pellet was rinsed with 30 mL of water (10 minute shake), centrifuged as before and the water added to the NaOH solution. The NaOH solution contains the humic acids and fulvic acids associated radioactivity and the soil debris represent the humin fraction. The humin portion was weighed and sub-samples were analyzed by combustion/LSC. The radioactivity present in the NaOH extract was further fractionated into fulvic acid and humic acid associated radioactivity by acidification to pH ~1 (using concentrated HCl) to precipitate the humic acids (approximately 1 hour to precipitate the humic acids). Following centrifugation at ~7,500 RPM for 20 minutes, the soluble fulvic materials and the precipitated humic substances were separated. The supernatant, which contains the fulvic acids associated radioactivity was measured and it's content of radioactivity determined by LSC. The precipitated fraction (humic acids) was weighed and sub-samples were analyzed by combustion/LSC. The fulvic acid fraction was partitioned once with a 2X volume of ethyl acetate. The ethyl acetate and aqueous fractions were separated, and the associated radioactivity was measured and its content of radioactivity was determined by LSC. The aqueous fraction contains the fulvic acid associated activity. The fulvic acid organosoluble activity was transferred to a round bottom flask and rotary evaporated (at 25 - 35 °C) to dryness. The residue was dissolved in methanol and the solution was transferred into an HPLC vial for analysis.

4.2.10 $BaCl_2$ Precipitation of NaOH Trap Solution

The $BaCl_2$ assay was used to characterize the nature of radioactivity present in the

NaOH traps. Any evolved CO_2 was trapped in the NaOH solution as Na_2CO_3. The added Ba^{2+} ions replace the Na^+ atoms in Na_2CO_3 producing an insoluble $BaCO_3$ precipitate. Any radioactivity that remained in solution was assumed to come from dissolved ^{14}C materials other than $^{14}CO_2$. A 10 mL aliquot of the NaOH trap contents was transferred into a 50 mL centrifuge tube and an equal volume of saturated $BaCl_2$ solution was added. The mixture was vortexed and kept refrigerated for a minimum of 1 hour. The tube was centrifuged at 4700 × *g* for 10 minutes and the supernatant decanted and its volume measured. Three aliquots of the supernatant were counted using LSC and the detected radioactivity was calculated as a percentage of the radioactivity present in the original NaOH sample.

4.3 Liquid Scintillation Counting (LSC)

To quantify radioactivity in liquid replicates, aliquot(s) of known volume were transferred to 20-mL borosilicate glass scintillation vials, and 15 mL of ULTIMAGOLD™ scintillation cocktail was added. Alternatively, aliquot(s) of known volume were transferred to 7-mL borosilicate glass scintillation vials, and 5 mL of ULTIMAGOLD™ scintillation cocktail was added. After mixing thoroughly, the scintillation vials were counted for a minimum of 5 minutes using a liquid scintillation counter. Radioactivity (dpm) was determined by the instrument from a quench curve generated for the scintillation cocktail using an external quench standard. Background replicates contained scintillation cocktail only, or cocktail mixed with the solvent. All LSC measurements were corrected for instrument background.

4.4 Combustion Analysis (Sample Oxidation)

Total radioactivity in post-extraction soil (PES) or humin and humic acid fractions was determined by combustion in a Harvey Biological Oxidizer. Three sub-samples of PES were placed in combustion cups and the soil net wet weight was recorded. The sub-samples were then combusted using a 4-minute combustion cycle. The resulting $^{14}CO_2$ was trapped in Harvey Carbon 14 Cocktail, which was automatically dispensed by the oxidizer into a 20-mL glass scintillation vial. The samples were counted for a minimum of 5 minutes using LSC and a quench curve generated for the combustion cocktail. Oxidizer efficiency was checked before each combustion session using a standard of known radioactivity. Sample combustion was only conducted when oxidizer efficiency was ≥ 95%. The radioactivity per g of oxidized sample was calculated for each sub-sample, and then the mean radioactivity per g of soil from the 3 sub-samples was used to calculate the total radioactivity in the PES sample.

4.5 High Performance Liquid Chromatography (HPLC)

HPLC was used to determine radiochemical purity of the ^{14}C test materials, to analyze standard and test solutions, to identify S-2200 and its degradates based on retention time correspondence to standards, to quantitate the percentage distribution of radioactivity between eluted peaks. HPLC was performed on Instruments LC-38 or LC-39.

LC-38 - Hewlett Packard HP1100 system equipped with a solvent degasser, autosampler, column oven heater, and UV detector. Radioactivity in the HPLC effluent was detected using a radioflow-through detector (also known as radioactivity monitor (HPLC-RAM)) equipped with a lithium glass solid scintillant flow cell (250 µL). Percent distribution of radioactivity in the sample extract was calculated by peak area integration using system software. Electronic signals generated by the detectors on LC-38 were processed using a Raytest software program operating on a personal computer. Each chromatogram was carefully examined and manually integrated. The software then calculated the retention time, area integration, and percent area integration of each peak.

LC-39 - Hewlett Packard HP1100 system equipped with a solvent degasser, autosampler, column oven heater, and UV detector. Radioactivity in the HPLC effluent was detected using a radioflow-through detector (also known as radioactivity monitor (HPLC-RAM)) equipped with a lithium glass solid scintillant flow cell (200 µL). Percent distribution of radioactivity in the sample extract was calculated by peak area integration using system software or IN/US System Win-Flow™ Chromatography Software, version 1.2g). Each chromatogram was carefully examined and manually integrated. The software then calculated the retention time, area integration, and percent area integration of each peak.

The order in which the detectors were connected was: UV detector followed by the radioflow detector. The HPLC column, eluting solvents, gradients, and run parameters were as follows:

4.5.1 HPLC Methods

HPLC Method #1 (Reverse phase analysis): The test substance radiochemical purities and the definitive samples were analyzed using this method on LC-38 or LC-39. The typical retention times of the reference standards are given in Table 1 and example chromatograms can be seen in Appendix E, Appendix F and Appendix G. The HPLC separation of significant degradates was carried out using this method. The column effluent from each HPLC run was collected and representative samples were analyzed (by LSC) for calculation of recovery of injected radioactivity.

Column: Synergi Hydro-RP 4µ 80Å, 4.6 mm x 250 mm equipped with a 3 x 4 mm C18 Security Guard™ cartridge precolumn
Solvents: Solvent A, 0.05% trifluoroacetic acid in water
Solvent C Methanol
Solvent D, Acetonitrile
Flow rate: 1.0 mL/min.
Temperature: nominal 25 °C
Detection: UV at 230 nm
Radioflow in counts

Gradient#1:

Time, min.	%A	%C	%D
0	100	0	0
4	85	0	15
18	85	0	15
23	45	20	35
46	24	38	38
50	0	50	50
53	0	50	50
54	100	0	0
65	100	0	0

(data acquisition ends at 55 minutes)

Post-time: 0 min.

Gradient#2:	Time, min.	%A	%C	%D
	0	100	0	0
	4	85	0	15
	16	85	0	15
	23	60	15	25
	30	35	30	35
	46	24	38	38
	50	0	50	50
	53	0	50	50
	54	100	0	0
	65	100	0	0
	(data acquisition ends at 55 minutes)			

Post-time: 0 min.

Radioactivity recoveries (dpm recovered divided by dpm injected) for the HPLC analyses were conducted on representative test samples throughout the study. The methods gave acceptable HPLC recoveries (average RB - 103.4%, SB - 105.7%, RP - 102.7%).

HPLC Method #2 (Chiral analysis): The distribution of the S-2200 *R* and *S* isomers and MCBX *R* and *S* isomers for the RB, SB and RP exposures were analyzed using this method on LC-38 or LC-39. Various gradients were used to maximize R and *S* isomer separation. The typical retention times of the reference standards and example chromatograms can be seen in Appendices E, F and G.

Column:	Phenomenex LUX 5µ Amylose-2, 4.6 mm (80Å) x 150 mm equipped with a 3 x 4 mm LUX cartridge precolumn
Solvents:	Solvent A, water Solvent B, acetonitrile
Flow rate:	1.0 mL/min.
Temperature:	nominal 25 °C
Detection:	UV at 230 nm Radioflow in counts

Gradient #1:	Time, min.	%A	%B
	0	65	35
	23	65	35
	25	60	40
	60	60	40

Gradient #2:	Time, min.	%A	%B
	1	65	35
	20	65	35
	22	60	40
	60	60	40

Gradient #3:	Time, min.	%A	%B
	0	65	35
	20	65	35
	22	60	40
	55	60	40
	56	35	65
	61	35	65
	62	65	35
	75	65	35

Gradient #4:	Time, min.	%A	%B
	0	65	35
	20	65	35
	22	60	40
	61	60	40
	62	65	35
	75	65	35

4.6 Thin-Layer Chromatography (TLC)

Radioactive peak identification (confirmation) was accomplished using coincident R_f values of non-radiolabeled reference substances and radioactivity (2D TLC). TLC was performed using pre-coated glass-backed plates coated with silica gel 60 containing a fluorescent (254 nm) indicator (20 x 20 cm, 250 or 500µm thickness; EM Sciences). Samples and standards were applied to the plates and the plates were placed into solvent-equipped rectangular glass developing tanks fitted with cellulose vapor saturation pads and glass lids. Several solvent systems were used to develop the plates: CMT (chloroform - methanol - trifluoroacetic acid 90:10:0.1 (v/v/v)) and TEA (toluene – ethyl acetate – acetic acid, 5:7:1 (v/v/v)). After development, TLC plates were removed from the developing tank and the solvent allowed to evaporate in a fume hood. Radioactive markers were placed on the developed plate to indicate the location of the origin and solvent front. Short wavelength ultraviolet (UV) light was used to visualize standards while radiographic phosphor imaging was used to locate radioactivity on the TLC plate. Typical R_f values for the reference standards in the TLC systems are given in Table 1 and example chromatograms can be seen in Appendix H.

Radioactivity on TLC plates was detected by autoradiography. Air-dried TLC plates were wrapped in Mylar (1.5 μm, Persuasions Marketing Specialists) and placed into a cassette containing a low energy storage phosphor screen (20 x 25 cm). Exposure times varied from several hours to several days, depending on the amount of radioactivity present on the TLC plate. Following exposure, the phosphor screen was scanned in a Storm 820 PhosphorImager. The percent distribution of radioactivity in each chromatographic area was determined by the ImageQuant software program (version 4.2, Molecular Dynamics) or ImageQuant software program (ImageQuant TL, Version 2005).

5. CALCULATIONS

Copies of representative individual sample spreadsheets containing calculations described in the following section are presented in Appendix I, Appendix J and Appendix K.

5.1 Quantification of Volatile Radioactive Products

5.1.1 $^{14}CO_2$ Captured in NaOH Traps

Aerobic Test System: The amount of radioactivity captured in the NaOH traps between application (zero time) and the time of a sampling interval represents the cumulative $^{14}CO_2$ produced per incubation chamber during that interval. Because each incubation chamber has two internal NaOH and two external NaOH traps, the total amount of evolved $^{14}CO_2$ during a specified sampling interval is calculated as follows:

= *dpm in internal traps + dpm in external traps*

Radioactivity (dpm) in each trap is calculated as follows:

= [dpm per counted aliquot x (trap volume in mL ÷ counted aliquot volume)]

Since the aerobic incubation chamber, at any sampling interval, contains a known number of identical samples, the amount of $^{14}CO_2$ produced from a single soil sample is:

$$= \frac{\textit{Total } ^{14}CO_2 \textit{ activity (dpm) in all NaOH traps for a sampling interval}}{\textit{Number of soil samples present in the chamber for that sampling interval}}$$

The amount of $^{14}CO_2$ produced from a single soil sample as % of applied:

$$^{14}CO_2 \text{ (\% of applied)} = \frac{^{14}CO_2 \text{ produced from each soil sample (dpm)}}{\text{Total applied per sample (dpm)}} \text{ X 100}$$

The cumulative amount of $^{14}CO_2$ produced from a single soil sample between 0 DAT and 30/34 DAT is the sum of $^{14}CO_2$ produced per sample at each sampling interval between 0 DAT and 30/34 DAT.

Cumulative amount of $^{14}CO_2$ produced from a single soil sample between 0 DAT and 30/34 DAT expressed as % of applied:

$$^{14}CO_2 \text{ as \% of applied.} = \frac{\text{Cumulative } ^{14}CO_2 \text{ produced from one soil sample (dpm)}}{\text{Total applied per sample (dpm)}} \text{ X 100}$$

Anaerobic Test System: The volume of each biometer NaOH trap was measured and three aliquots (0.5 or 1.0 mL each) from each trap were counted using LSC. The amount of $^{14}CO_2$ produced from each soil replicate was normalized to the dry weight of the soil and the test substance specific activity to produce the ppm equivalents of [^{14}C]S-2200 mineralized to $^{14}CO_2$.

$$^{14}CO_2 \text{ produced per sample} = \frac{dpm}{0.5 \text{ or } 1.0 \text{ mL}} \text{ x (NaOH volume in mL)}$$

Where 0.5 or 1.0 mL is the volume of the aliquot counted using LSC

$$^{14}CO_2 \text{ as \% of applied} = \frac{^{14}CO_2 \text{ produced per sample (dpm)}}{\text{Applied radioactivity per sample (dpm)}} \text{ X 100}$$

$^{14}CO_2$ *as ppm (in S-2200 equivalents) =*

$$\frac{^{14}CO_2 \text{ produced per sample (dpm)}}{\text{Specific activity } (dpm/\mu g) \text{ x soil dry wt. } (g)}$$

5.1.2 $^{14}CH_4$ Present in the Biometer Flask Headspace

A graduated 50-mL Gastight™ syringe equipped with a needle was used to withdraw a headspace sample through the sampling valve. The headspace sample was injected into a Harvey Biological Oxidizer and the radioactivity

was quantified using LSC. Total radioactivity in each biometer headspace was calculated based on the radioactivity in the oxidized sample and the total headspace volume of the flask. Measured radioactivity (dpm) was converted to percent of applied [^{14}C]S-2200 and ppm of S-2200 equivalents.

$^{14}CH_4$ in a sample (biometer flask):

$$= \frac{dpm}{aliquot\ volume} \times headspace\ volume\ in\ biometer\ flask\ (mL)$$

Evolved $^{14}CH_4$ as % of applied radioactivity:

$$= \frac{Evolved\ ^{14}CH_4\ (dpm)}{Applied\ radioactivity\ per\ sample\ (dpm)} \times 100$$

Evolved $^{14}CH_4$ as ppm (in S-2200 equivalents)

$$= \frac{^{14}CH_4\ produced\ per\ sample\ (dpm)}{Specific\ activity\ (dpm/\mu g) \times sample\ dry\ wt.\ (g)}$$

5.2 Total Radioactive Residue (TRR) in Post-Extraction Soil (PES) as Determined by Combustion Analysis

To determine the TRR in the PES fractions, 3 sub-samples were combusted. The radioactive residues of a sample (dpm/g) were calculated by dividing the radioactivity in each combusted sub-sample (dpm) by its weight (g). The radioactive residues (dpm/g) for the 3 sub-samples was averaged to obtain the mean radioactive residue value (dpm/g). Total radioactivity in the soil sample was calculated by multiplying the soil wet weight (g) by the mean TRR value (dpm per g of wet PES). Total radioactivity in soil was corrected for oxidizer efficiency (O.E.) by dividing the calculated dpm by oxidizer efficiency. Total radioactive residue in the soil sample was expressed as a percentage (%) of the applied [^{14}C]S-2200. Also, the concentration (ppm) was determined based on [^{14}C]S-2200 specific activity and on soil sample dry weight.

$$O.E.\ (oxidizer\ efficiency) = \frac{Mean\ dpm\ of\ triplicate\ standard\ (combusted)}{Mean\ dpm\ of\ triplicate\ standards\ (not\ combusted)}$$

$$TRR\ by\ combustion\ (dpm/g) = \frac{Sub\text{-}sample\ dpm}{Sub\text{-}sample\ wt.\ (g)}$$

TRR (dpm/sample) = TRR by combustion (dpm/g) x Total sample wt (g).

TRR (% of Applied) = [TRR (dpm/sample) ÷ Applied (dpm)] x 100 ÷ O.E.

5.3 Recovery of Radioactivity from the HPLC Column

An aliquot of the representative sample (concentrated water or soil phase extracts) was counted using LSC to determine the radioactivity injected (analyzed by HPLC). The HPLC eluent was collected for the length of the run and an aliquot was counted using LSC. Total radioactivity content in the eluent was calculated. Recovery of radioactivity from the HPLC column was calculated as follows:

$$Recovery\ (\%) = \frac{Radioactivity\ in\ eluent\ (dpm)}{Radioactivity\ injected\ (dpm)} \times 100$$

5.4 Characterization of Radioactivity in Water and Soil Extracts

Concentrated water (section 4.2.7), soil (neutral and acidic extracts, sections 4.2.2 and 4.2.8) and PES extractions (dismembrator, fulvic acid organosoluble activity, section 4.2.9) were analyzed by HPLC and/or 2D TLC to identify and quantify [^{14}C]S-2200 and its [^{14}C]labeled metabolites. The primary radioactive peak identification was accomplished using coincident retention time of non-radiolabeled reference substances and radioactive peaks (HPLC analysis). Radioactivity was detected using an in-line radioactivity detector or fraction collection and LSC while non-labeled standards were detected using a UV detector at 230 nm. Radioactive peak area was integrated using radioflow software and the integration was corrected for instrument background. Results for each peak were reported as percentage of total radioactivity present in all quantifiable peaks. Values were reported as percentage of applied and as ppm of [^{14}C]S-2200 equivalents based on total radioactivity present in the sample and [^{14}C]S-2200 specific activity.

$$Individual\ Peak\ area\ (\%) = \frac{Area\ of\ a\ individual\ peak}{Areas\ of\ all\ quantified\ peaks} \times 100$$

$$[^{14}C]\ in\ peak\ (dpm) = \frac{Individual\ Peak\ area\ (\%) \times [^{14}C]\ in\ sample\ (dpm)}{100}$$

$$[^{14}C]\ in\ peak\ (\%\ of\ Applied) = \{ [^{14}C]\ in\ peak\ (dpm) \div Applied\ (dpm)\} \times 100$$

$$\textit{Peak ppm (in S-2200 equivalents)} = \frac{[^{14}C]\ \textit{in peak (dpm)}}{\textit{Specific activity (dpm/}\mu g\textit{)} \times \textit{sample dry wt. (g)}}$$

5.5 Total Sample Mass Balance

Sample mass balance was calculated by accounting for total radioactivity recovered from each sample. The recovered radioactivity was compared to the total radioactivity applied to the sample.

Total radioactivity recovered from analyzed samples is calculated as the sum of the following components:

- Radioactivity in the NaOH traps
- Radioactivity in headspace (anaerobic only)
- Radioactivity in soil extracts
- Radioactivity in water phase (anaerobic only)
- Radioactivity in PES
- Radioactivity in the Beaker or Biometer rinse (BR)

Example: ^{14}C recovered from anaerobic sample = Radioactivity in the NaOH traps + Radioactivity in headspace + Radioactivity in soil extracts + Radioactivity in water phase + Radioactivity in PES + Radioactivity in the Biometer rinse

$$\textit{Sample mass balance (\%)} = \frac{^{14}C\ \textit{recovered from sample}}{^{14}C\ \textit{applied to sample}} \times 100$$

5.6 Statistical Analysis

Statistical analyses included mean (average), standard deviation, and goodness of fit (r^2) of regression analysis.

5.7 Detection Limits

Radioactivity in samples was quantitated by combustion and/or LSC analysis and when appropriate, the results were expressed as [^{14}C]S-2200 equivalents. The Detection Limit, L_D, and the Minimum Quantifiable Limit, L_Q, values for each set of samples were determined by the following equations (Reference 6):

$$L_D\,(dpm) = \frac{2.71 + 4.65\left(u_B\right)^{1/2}}{C_E \times C_t}$$

$$L_Q(dpm) = \frac{50(1+\left[1 + \frac{u_B}{12.5}\right]^{1/2}}{C_E \times C_t}$$

Where u_B is the total background count (cpm x C_t), C_t is the counting time in minutes, and C_E is the counting efficiency. For LSC, typical background was 20 cpm based on 5-minute counts with an average counting efficiency of 93%. For these values,

$$L_D(dpm) = \frac{2.71 + 4.65(20 \times 5)^{1/2}}{0.93 \times 5} = 11\ dpm$$

$$L_Q(dpm) = \frac{50\left(1 + \left[1 + \frac{20 \times 5}{12.5}\right]^{1/2}\right)}{0.93 \times 5} = 43\ dpm$$

Based on the above example, an aliquot having a determined value below 11 dpm (net) does not contain detectable radioactivity. If the determined value is equal to or greater than 11 dpm but less than 43 dpm, then the radioactivity is detected but not quantifiable. If the value is equal to or greater than 43 dpm (net), the radioactivity is both detected and quantifiable.

5.8 Half-life and Degradation Rate Constant Calculations

The dissipation DT_{50} for S-2200 was calculated for the aerobic phase (0 - 30/34 DAT, 3 data points) and the anaerobic soil phase (30/34 - 329/334 DAT, 8 data points). The anaerobic soil degradation phase, while an anaerobic aquatic system, cannot be considered equivalent to a pure anaerobic aquatic system due to the anoxic lag phase (conversion from aerobic to anaerobic). The calculations were based on the assumption that each degradation phase followed single first-order (SFO) kinetics,

$C = C_O e^{-kt}$ Eq. 1

where C is the concentration at time t, C_O is the concentration at time (t) = 0, and k is the degradation rate constant. The DT_{50} is achieved when C/C_O=0.5 and Eq. 2 reduces to

$DT_{50} = 0.693/k.$ Eq. 3

The DT_{90} is achieved when C/C_O=0.1 and Eq. 2 reduces to

$DT_{90} = 2.303/k$ Eq. 4

The rate constant, k, is obtained by plotting the (LN) concentration versus time after treatment using commercially available software (Excel).

6. RESULTS AND DISCUSSION

Physical and biological characteristics of the test system are discussed based on soil and water analysis as well as the results of the control substance metabolism in the test system under anaerobic aquatic conditions.

The results of the aerobic soil metabolism of [^{14}C]S-2200 are presented by grouping the radioactive degradation products into three groups:

-radioactivity mineralized to $^{14}CO_2$,
-radioactive products extractable from soil, and
-soil-bound radioactivity.

The results of the anaerobic soil metabolism of [^{14}C]S-2200 are presented by grouping the radioactive degradation products into four groups:

-radioactivity mineralized to $^{14}CO_2$, and $^{14}CH_4$,
-radioactive products present in water,
-radioactive products extractable from soil, and
-soil-bound radioactivity.

Degradation half-lives were calculated and a degradation pathway was proposed to describe the fate of [^{14}C]S-2200 under aerobic followed by anaerobic soil conditions.

Reported values are expressed as percentage (%) of applied [^{14}C]S-2200, and as concentration (ppm in S-2200 equivalents dosed to 50 g of dry weight soil). Summary table values and graphed values are based on the arithmetic mean of two samples.

6.1 Characteristics of the Test System

6.1.1 Physical Characteristics of the Test System

Detailed soil analysis information are presented in Table 2 and in Appendix C. The terrestrial soil was classified as a loam according to the USDA classification system for soil. The percentage of organic matter in the soil was 2.0%, and the Cation Exchange Capacity (CEC) was 8.7 meq/100g of soil. Detailed physical characteristics of each test system are presented in Table 3, Table 4 and Table 5. The pH of the soil at the beginning of the study was 6.9 (1:1, w/v, soil/water). The pH of the soil and water at the beginning of the anaerobic phase (7 days into the anaerobic phase, 37 DAT (RB label) or 41 DAT (SB and RP labels) averaged 6.4

(soil) and 6.7 (water). In the anaerobic phase, the soil pH averaged 7.6 (RB), 7.4 (SB) and 7.5 (RP). In the anaerobic phase, the water pH averaged 7.9 (RB), 7.7 (SB) and 7.8 (RP).

6.1.2 Aerobic Conditions in the Test System

On 12/2/09 (one day after dosing the SB and RP aerobic systems), several untreated controls (samples 1-3, 10-12) were pooled and sent to Agvise for microbial analysis. The microbial analyses showed these values: Actinomycetes (9,380,000 and 12,700,000 CFU/g of dry wt. soil with a 68 - 69 hour incubation); Fungi (22,100 and 24,400 CFU/g of dry wt. soil with a 69 hour incubation); Bacteria (2,910,000 and 8,850,000 CFU/g of dry wt. soil with a 69 - 70 hour incubation); Microbial Biomass Carbon 160.2 and 181.8 µg/g dry basis. On 12/14/09 (two days after dosing the RB aerobic systems), several untreated controls (samples 98-100) were pooled and sent to Agvise for microbial analysis. The microbial analyses showed these values: Actinomycetes (4,540,000 CFU/g of dry wt. soil with 94 hour incubation); Fungi (31,100 CFU/g of dry wt. soil with 94 hour incubation); Bacteria (4,960,000 CFU/g of dry wt. soil with 94 hour incubation); Microbial Biomass Carbon 447.9 µg/g dry basis. The starting microbial biomass values are higher than the recommended minimum value (1% of the organic carbon content). The Penn soil had 2.0% organic matter (1.2% organic carbon content). The equivalent 1% organic carbon value is 116 µg/g.

6.1.3 Anaerobic Conditions in the Test System

The aerobic test systems were transferred to closed biometers and flooded with water at 30 (RB label) and 34 DAT (SB and RP labels). Saturation of the pore space between soil particles decreases the diffusion of oxygen into the soil from the atmosphere. As a result, soils that are saturated with water for many days typically become anaerobic. Their free oxygen disappears and they show a decline in oxidation-reduction (redox) potential (Eh). The depletion of oxygen was accelerated by purging the biometer systems with N_2, making the systems anoxic (low oxygen concentration ca. 0.1 ppm, positive redox value) and by adding alfalfa. Bioavailable carbon is important in wetlands as an energy source, since it drives the development of anaerobic and reducing conditions. Since temperature (20 ± 2 °C in the dark) and bioavailable carbon (biometers amended with alfalfa) were not limiting conditions in the biometers, the microbes depleted the remaining oxygen that was trapped in the pores or dissolved in the soil solution of the saturated soil. After the soil oxygen becomes depleted, a succession of anaerobic microorganisms will use compounds in redox reactions (including manganese, iron, and sulfate), making the system progressively more reduced.

The development of anaerobic conditions was verified by measurements of dissolved oxygen and oxidation-reduction potential in each sample at the time of its analysis Table 3, Table 4 and Table 5. At 44 and 48 DAT (14 days into the anaerobic phase) the dissolved oxygen readings were low (average 0.14 ppm), while the redox potential was positive (average: +185 mV), indicating that the systems were anoxic. By ca. 260 DAT (ca. 230 days into the anaerobic phase) the dissolved oxygen readings were even lower (average was 0.08 ppm), while the redox potential had turned negative (average: -188 mV), indicating that the systems were anaerobic. Dissolved oxygen concentrations remained low and the redox potential remained negative for the remainder of the study. These measurements indicate that the system became strongly anaerobic (Reference 7). The samples exhibited physical symptoms of an anaerobic system, such as soil graying and gas production/cracking in the soil throughout the anaerobic phase.

Controls were incubated in separate chambers at the same time as the SB and RP labels. At 363 DAT (11/29/10), an untreated control (Treatment A), a solvent treated control (Treatment B) and a cold S-2200 sample (Treatment D) were confirmed as being anaerobic (redox potentials, -217 to -229 mV). The samples were analyzed by Springborn Laboratories for anaerobic microbial content. The microbial values were 9.6 x 10^5, 7.7 x 10^5 and 1.1 x 10^6 Cfu/mL, respectively, indicating no significant difference between the untreated control, solvent control and cold S-2200 treatments. These values were cited by the Laboratory as being typical for microbial soil populations under anaerobic conditions. (Appendix L).

Controls were incubated in separate chambers at the same time as the RB label. At 368 DAT (12/17/10, the last interval), an untreated control (Treatment A) was confirmed as being anaerobic (redox potential, -235mV). The sample was analyzed by Springborn Laboratories for anaerobic microbial content. The microbial value was 7.1 x 10^5 anaerobic colonies/gram, respectively, a value the Laboratory cited as being typical for microbial soil populations under anaerobic conditions. (Appendix L).

6.1.4 Biological Viability of the Anaerobic Test System

a. Anaerobic Transformation of Control Substance [UL-^{14}C]-*D*-glucose

D-glucose anaerobic transformation to methane (CH_4) and carbon dioxide (CO_2) is often used to gauge the activity of methanogenic microorganisms and microbial viability in anaerobic systems. Therefore, [UL-^{14}C]-*D*-glucose was used as a control substance to verify the presence and viability of anaerobes and methanogenic microorganisms in the test system. Untreated control samples were dosed on 10/21/10. At 21 days after dosing the dissolved O_2 and redox potential results (0.03 ppm and -164 mV)

indicated the systems were anaerobic. Twenty one days after treatment, an average of 21.7% and 6.8% of the applied [UL-^{14}C]-*D*-glucose was evolved as $^{14}CO_2$ and $^{14}CH_4$, respectively (Table 6), respectively. Soil-bound radioactivity was 30.6% of applied and 33.4% of applied radioactivity was found in the water phase. The water and soil fractions contain [UL-^{14}C]-*D*-glucose-derived radioactivity that was assimilated into the cells or cellular products of the anaerobes in the system as well as sequestered $^{14}CO_2$. The results of the control substance metabolism indicate that the test system used for the study contained active anaerobic microorganisms, and a population of the strictest anaerobes (methanogens).

6.2 Mass Balance and Distribution of Applied Radioactivity

The following is a summary of the applied radioactivity distribution during the **[benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB)** aerobic and anaerobic soil phases. The average mass balance was 102.6% during the study. Individual sample mass balances ranged from 100.1% to 105.5% during the study.

Aerobic Phase (0 – 30 DAT): The majority of the applied radioactivity (AR) was extractable at the end of the aerobic phase (99% of the AR at 30 DAT) and only a small portion was bound (5.0% of AR) or converted to $^{14}CO_2$ (1.8% of AR). The 0, 14 and 30 DAT soils were extracted with neutral and acidic extract solutions and the residual soil-bound ^{14}C-residues (PES) were determined by oxidation. Detailed mass balance data, presented as a percent of the applied radioactivity or ppm, can be found in Table 7 and Table 8. Graphical presentations of the mass balance data can be found in Figure 6.

Soil Phase: The soil phase (extractable plus soil-bound radioactivity) was 102% of the applied radioactivity at day zero and 105% at 30 DAT. This activity was characterized as:

Extractable radioactivity: Neutral extraction extracted 101% of the applied radioactivity at time zero and only 81% at 30 DAT. The radioactivity became progressively more difficult to extract with the neutral extract and the acidic extract was required to attain a quantitative extraction.

Soil-bound radioactivity: The soil-bound activity was minor at time zero, 1.5% of AR. As the extractability decreased the amount of soil-bound activity increased slightly, reaching 5% of the AR by 30 DAT. The 30 DAT PES (the highest PES value for this label) was subjected to more rigorous extraction conditions (acidic dismembrator released 1.5% of the AR) followed by base extractions to release additional activity into humin (1.5% of AR), humic acid (0.9% of AR) and fulvic acid (1.2% of AR) fractions.

Evolved $^{14}CO_2$: The amount of carbon dioxide generated during the aerobic phase was 1.8% of the applied radioactivity.

Anaerobic Soil Phase (30 – 364 DAT): The majority of the applied radioactivity (AR) remained with the soil (extractable plus soil-bound radioactivity) during the 334 days of the anaerobic phase (75% of the AR at 364 DAT). The amount of mineralization to $^{14}CO_2$ and/or $^{14}CH_4$ was minor (3% of the AR at 364 DAT) and the balance of the AR remained in the water phase (23% of the AR at 364 DAT). After removal of the water, the 37 through 364 DAT soils were extracted with a neutral followed by an acidic extraction solvent and the residual soil-bound ^{14}C-residues (PES) were determined by oxidation. Detailed mass balance data, presented as a percent of the applied radioactivity or ppm, can be found in Table 7 and Table 8. Graphical presentations of the mass balance data can be found in Figure 6.

Water phase: The water phase contained 18% of the applied radioactivity at 37 DAT (7 days anaerobic) and reached 23% of the AR at 364 DAT (334 days anaerobic).

Soil Phase: The soil phase ranged from 83% of the applied radioactivity at 37 DAT (7 days anaerobic) and declined to 75% at 364 DAT (334 days anaerobic). This soil activity was characterized as:

Extractable radioactivity: Neutral and acidic extractions extracted 78% of the applied radioactivity at 37 DAT. Over time, as the activity in the water phase increased, the soil extractability decreased, with only 71% of the AR extractable at 364 DAT.

Soil-bound radioactivity: The soil-bound activity was 5% at 37 DAT and 4% of the AR by 364 DAT.

Evolved volatiles: This portion of radioactivity consisted of:

$^{14}CO_2$: The total amount of carbon dioxide generated during the anaerobic phase was 1% of the applied radioactivity.

$^{14}CH_4$: The maximum amount of evolved volatiles (presumed to be methane) was <0.1% of applied radioactivity at 364 DAT.

The following is a summary of the applied radioactivity distribution during the **[benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB)** aerobic and anaerobic soil phases. The average mass balance was 103.4% during the study. Individual sample mass balances ranged from 100.5% to 106.3% during the study.

Aerobic Phase (0 – 34 DAT): The majority of the applied radioactivity (AR) was extractable at the end of the aerobic phase (95% of the AR at 34 DAT) and only a small portion was bound (6.4% of AR) or converted to $^{14}CO_2$ (5.3% of AR). The

0, 15 and 34 DAT soils were extracted with neutral and acidic extract solutions and the residual soil-bound ^{14}C-residues (PES) were determined by oxidation. Detailed mass balance data, presented as a percent of the applied radioactivity or ppm, can be found in Table 9 and Table 10. Graphical presentations of the mass balance data can be found in Figure 7.

Soil Phase: The soil phase (extractable plus soil-bound radioactivity) was 103% of the applied radioactivity at day zero and 101% at 34 DAT. This activity was characterized as:

Extractable radioactivity: Neutral extraction extracted 101% of the applied radioactivity at time zero. Over time, the radioactivity became slightly more difficult to extract, with a neutral and acidic extract required to extract 95% of the AR at 34 DAT.

Soil-bound radioactivity: The soil-bound activity was minor at time zero, 2.6% of AR. As the extractability decreased the amount of soil-bound activity increased slightly, reaching 6% of the AR by 34 DAT.

Evolved $^{14}CO_2$: The amount of carbon dioxide generated during the aerobic phase was 5.3% of the applied radioactivity.

Anaerobic Soil Phase (34 – 363 DAT): The majority of the applied radioactivity (AR) remained with the soil (extractable plus soil-bound radioactivity) during the 329 days of the anaerobic phase (73% of the AR at 363 DAT). The amount of mineralization to $^{14}CO_2$ and/or $^{14}CH_4$ was 3X higher (7.3% of the AR at 363 DAT) than observed in the RB label. The balance of the AR remained in the water phase was 21% of the AR at 363 DAT. After removal of the water, the 41 thru 363 DAT soils were extracted with a neutral followed by an acidic extraction solvent and the residual soil-bound ^{14}C-residues (PES) were determined by oxidation. The 41 DAT PES (the highest PES value for this label) was subjected to more rigorous extraction conditions (acidic dismembrator released 1.4% of the AR) followed by base extractions to release additional activity into humin (2.2% of AR), humic acid (1.3% of AR) and fulvic acid (1.6% of AR) fractions. Detailed mass balance data, presented as a percent of the applied radioactivity or ppm, can be found in Table 9 and Table 10. Graphical presentations of the mass balance data can be found in Figure 7.

Water phase: The water phase contained 17% of the applied radioactivity at 41 DAT (7 days anaerobic) and reached 21% of the AR at 363 DAT (329 days anaerobic).

Soil Phase: The soil phase ranged from 83% of the applied radioactivity at 41 DAT (7 days anaerobic) and declined to 73% at 363 DAT (329 days anaerobic). This soil activity was characterized as:

Extractable radioactivity: Neutral and acidic extractions extracted 76% of the applied radioactivity at 41 DAT. Over time, as the activity in the water phase increased, the soil extractability decreased, with only 68% of the AR extractable at 363 DAT.

Soil-bound radioactivity: The soil-bound activity was 7% at 41 DAT and declined to 6% of the AR by 363 DAT.

Evolved volatiles: This portion of radioactivity consisted of:

$^{14}CO_2$: The total amount of carbon dioxide generated during the anaerobic phase was 2% of the applied radioactivity.

$^{14}CH_4$: The maximum amount of evolved volatiles (presumed to be methane) was <0.1% of applied radioactivity at 363 DAT.

The following is a summary of the applied radioactivity distribution during the **[phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP)** aerobic and anaerobic soil phases. The average mass balance was 103.1% during the study. Individual sample mass balances ranged from 100.1% to 106.4% during the study.

Aerobic Phase (0 – 34 DAT): The majority of the applied radioactivity (AR) was extractable at the end of the aerobic phase (87% of the AR at 34 DAT) and only a portion was bound (11.7% of AR) or converted to $^{14}CO_2$ (7.1% of AR). The 0, 15 and 34 DAT soils were extracted with neutral and acidic extract solutions and the residual soil-bound ^{14}C-residues (PES) were determined by oxidation. Detailed mass balance data, presented as a percent of the applied radioactivity or ppm, can be found in Table 11 and Table 12. Graphical presentations of the mass balance data can be found in Figure 8.

Soil Phase: The soil phase (extractable plus soil-bound radioactivity) was 105% of the applied radioactivity at day zero and 99% at 34 DAT. This activity was characterized as:

Extractable radioactivity: Neutral extraction extracted 102% of the applied radioactivity at time zero. The radioactivity became progressively more difficult to extract with the neutral extract and the acidic extract was required to extract 87% of the AR at 34 DAT.

Soil-bound radioactivity: The soil-bound activity was minor at time zero, 2.4% of AR. As the extractability decreased the amount of soil-bound activity increased significantly, reaching 11.7% of the AR by 34 DAT.

Evolved $^{14}CO_2$: The amount of carbon dioxide generated during the aerobic phase was 7.1% of the applied radioactivity.

Anaerobic Soil Phase (34 – 363 DAT): The majority of the applied radioactivity (AR) remained with the soil (extractable plus soil-bound radioactivity) during the

329 days of the anaerobic phase (75% of the AR at 363 DAT). The amount of mineralization to $^{14}CO_2$ and/or $^{14}CH_4$ was 3X higher (9.6% of the AR at 363 DAT) than observed in the RB label. The balance of the AR remained in the water phase was 17% of the AR at 363 DAT. After removal of the water, the 41 thru 363 DAT soils were extracted with a neutral followed by an acidic extraction solvent and the residual soil-bound ^{14}C-residues (PES) were determined by oxidation. The 41 DAT PES (the highest PES value) and the 363 DAT PES were subjected to more rigorous extraction conditions. In the 41 DAT PES, acidic dismembrator released 1.2% of the AR followed by base extractions to release additional activity into humin (3.9% of AR), humic acid (2.8% of AR) and fulvic acid (3.2% of AR) fractions. Detailed mass balance data, presented as a percent of the applied radioactivity or ppm, can be found in Table 11 and Table 12. Graphical presentations of the mass balance data can be found in Figure 8.

Water phase: The water phase contained 13% of the applied radioactivity at 41 DAT (7 days anaerobic) and reached 17% of the AR at 363 DAT (329 days anaerobic).

Soil Phase: The soil phase ranged from 82% of the applied radioactivity at 41 DAT (7 days anaerobic) and declined to 75% at 363 DAT (329 days anaerobic). This soil activity was characterized as:

Extractable radioactivity: Neutral and acidic extractions extracted 71% of the applied radioactivity at 41 DAT. Over time, as the activity in the water phase increased, the soil extractability decreased, with only 65% of the AR extractable at 363 DAT.

Soil-bound radioactivity: The soil-bound activity was 12% at 41 DAT and declined to 10% of the AR by 363 DAT.

Evolved volatiles: This portion of radioactivity consisted of:

$^{14}CO_2$: The total amount of carbon dioxide generated during the anaerobic phase was 2.5% of the applied radioactivity.

$^{14}CH_4$: The maximum amount of evolved volatiles (presumed to be methane) was <0.1% of applied radioactivity at 363 DAT.

Mass balance Summary: All three labels had acceptable mass balances. Trends were noted in neutral solvent extractability, bound residue and $^{14}CO_2$ formation. The neutral solvent extractability was RB>SB>RP. The bound residue formation was RP>SB>RB. The $^{14}CO_2$ formation was RP>SB>RB. These trends indicate that the RB label (*R*-isomer) was slightly less susceptible to degradation (less mineralized, less bound residue formation) than the SB (*S*-isomer). These trends suggest that the benzyl ring (RB and SB labels) was less susceptible to degradation (less mineralized, less bound residue formation) than the phenoxy ring (RP label).

6.3 Distribution of S-2200 in the Soil Extracts and Water

Aerobic Phase: The soil was extracted several times as described in Section 4.2.2 to yield a neutral and an acidic extract. The extractions were designed to exhaustively remove readily extractable S-2200 residues and avoid any hydrolytic breakdown, since S-2200 is resistant to hydrolysis under neutral (pH 7.0) and acidic (pH 4.0) conditions (References 8 & 9). The soil extracts were analyzed by HPLC-RAM as the primary method of quantitation. TLC-autoradiography analysis was the second analytical method and served to confirm the identity of degradates. Representative TLC chromatograms confirming S-2200 are presented in Appendix H.

S-2200 declined in all three aerobic soil systems (Figures 9 - 11). By 30 or 34 DAT, S-2200 had declined to 76, 77 and 73% of the AR in the RB, SB and RP soil extracts, respectively.

The study goal was to convert the test system to anaerobic conditions after S-2200 had degraded aerobically through one half-life or approximately 30 days (whichever came first). In order to meet this goal, the conversion to anaerobic conditions was done at 30 and 34 DAT.

Anaerobic Phase: The water was separated from the soil and analyzed after partition and concentration (37 or 41 DAT) or directly after concentration (44 or 48 DAT thru 363 or 364 DAT). The remaining soil was extracted several times as described in Section 4.2.8 to yield neutral and acidic extracts. The water and soil extracts were analyzed by HPLC-RAM as the primary method of quantitation. TLC-autoradiography analysis was the second analytical method and served to confirm the identity of degradates. Representative TLC chromatograms confirming S-2200 are presented in Appendix H.

S-2200 began the anaerobic phase (at 30 or 34 DAT) with 73 to 77% of the AR remaining as S-2200 and declined at a slower rate than observed in the aerobic phase (Figures 9 - 11). **RB study:** At 30 DAT, S-2200 represented 76% of the AR and had decreased slightly to 71% of the AR by 364 DAT. The amount of S-2200 in the water phase increased slightly during the study, indicating that S-2200 slowly desorbed off the soil during the anaerobic phase. S-2200 degraded only slightly once anaerobic conditions were established. The data suggests that S-2200 degradation under anaerobic conditions occurred in the soil phase. **SB study:** At 34 DAT, S-2200 represented 77% of the AR and had decreased slightly to 66% of the AR by 363 DAT S-2200. The amount of S-2200 in the water phase increased slightly during the study, indicating that S-2200 slowly desorbed off the soil during the anaerobic phase. S-2200 degraded only slightly once anaerobic conditions were established. The data suggests that S-2200 degradation under

anaerobic conditions occurred in the soil phase. **RP study:** At 34 DAT, S-2200 represented 73% of the AR and had decreased slightly to 68% of the AR by 363 DAT S-2200. The amount of S-2200 in the water phase increased slightly during the study, indicating that S-2200 slowly desorbed off the soil during the anaerobic phase. S-2200 degraded only slightly once anaerobic conditions were established. The data suggests that S-2200 degradation under anaerobic conditions occurred in the soil phase.

The data supports the conclusion that S-2200 declined significantly faster under aerobic conditions than anaerobic conditions. S-2200 declined with half-lives of 78 - 120 days in the aerobic soil (calculated using the 0, 14 or 15 and 30 or 34 day time points, first-order kinetics, r^2 values 0.99 - 1.00). S-2200 continued its decline at a much slower rate in the anaerobic water/soil system, but meaningful half-lives could not be calculated. Representative HPLC chromatograms from the 0, 30 or 34 and 363 or 364 DAT sampling intervals (beginning, middle and end of the study) are presented in Appendices E, F and G.

6.4 Distribution of S-2200 Degradates in the Soil Extracts and Water

Aerobic Phase: Soil extracts were analyzed by HPLC-RAM as the method of quantitation after extraction and concentration as described in section 4.2.2. **No major S-2200 degradates (>10% of the AR) were observed during the aerobic phase.** TLC-autoradiography analysis was used to confirm the identity of degradates. Representative TLC chromatograms confirming S-2200, Dx-CA-S-2200, 2-COOH-S-2200, 5-COOH-S-2200 and MCBX are presented in Appendix H. Summarized data for the RB study can be found in Tables 13 – 17 and are graphically represented in Figures 12 – 14. Summarized data for the SB study can be found in Tables 18 – 22 and are graphically represented in Figures 15 – 17. Summarized data for the RP study can be found in Tables 23 - 27 and are graphically represented in Figures 18 – 20.

([benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) degraded under aerobic conditions, to five identified degradation products, 2-COOH-S-2200 (6% of the AR) and 5-COOH-S-2200 (8% of the AR) and De-Xy-S-2200, DX-CA-S-2200 and MCBX at trace amounts (≤2% of the AR). Additionally, up to 5 unknowns were detected during the course of the aerobic phase, none > 2.1% of the AR (Table 17). The DX-CA-S-2200, MCBX, 2-COOH-S-2200 and 5-COOH-S-2200 degradates were tentatively identified in the soil extracts by HPLC co-chromatography with authentic standards and were confirmed by 2D-TLC co-chromatography (Appendix H).

[benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) also degraded under aerobic conditions, to five identified degradation products, DX-CA-S-2200 (4% of the AR), 2-COOH-S-2200 (4% of the AR) and 5-COOH-S-2200 (5% of the AR) and De-Xy-S-2200 and MCBX at trace amounts (≤2% of the AR). Additionally, up to 6 unknowns were detected during the course of the aerobic phase, none > 1.9% of the AR (Table 22). The DX-CA-S-2200, MCBX, 2-COOH-S-2200 and 5-COOH-S-2200 degradates were tentatively identified in the soil extracts by HPLC co-chromatography with authentic standards and were confirmed by 2D-TLC co-chromatography (Appendix H).

[phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) degraded under aerobic conditions, to three identified degradation products, 2-COOH-S-2200 (6% of the AR) and 5-COOH-S-2200 (6% of the AR) and MCBX at trace amounts (~1% of the AR). Additionally, up to 3 unknowns were detected during the course of the aerobic phase, none > 1.0% of the AR (Table 27). The MCBX, 2-COOH-S-2200 and 5-COOH-S-2200 degradates were tentatively identified in the soil extracts by HPLC co-chromatography with authentic standards and were confirmed by 2D-TLC co-chromatography (Appendix H).

Anaerobic Phase: The water was separated from the soil and analyzed after partition and concentration (37 or 41 DAT) or directly after concentration (44 or 48 DAT thru 363 or 364 DAT). The remaining soil was analyzed after extraction and concentration as described in section 4.2.8. **No major S-2200 degradates (>10% of the AR) were observed during the anaerobic phase.** S-2200 and degradates formed under aerobic conditions degraded much slower under anaerobic conditions. The soil extracts and water were analyzed by HPLC-RAM as the primary method of quantitation. TLC-autoradiography analysis was used for confirmation of degradates. Representative TLC chromatograms confirming S-2200, Dx-CA-S-2200, 2-COOH-S-2200, 5-COOH-S-2200 and MCBX are presented in Appendix H. Summarized data for the RB study can be found in Tables 13 – 17 and are graphically represented in Figures 12 – 14 Summarized data for the SB study can be found in Tables 18 – 22 and are graphically represented in Figures 15 – 17. Summarized data for the RP study can be found in Tables 23 - 27 and are graphically represented in Figures 18 – 20.

([benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB): The major S-2200 degradates formed under aerobic conditions, 2-COOH-S-2200 and 5-COOH-S-2200 did not degrade under anaerobic conditions. At 364 DAT, 2-COOH-S-2200 was 6% of the AR and 5-COOH-S-2200 8% of the AR. DX-CA-S-2200 increased during the anaerobic phase from 2% of the AR at 30 DAT to 5% of the AR at 364 DAT. De-Xy-S-2200 peaked at 3% of the AR at ca. 63 days and degraded to <0.1% by the

end of the study. MCBX was found at trace amounts throughout the study. Up to 9 unknowns were detected during the course of the anaerobic phase, none > 2.2% of the AR (Table 17). The De-Xy-S-2200, DX-CA-S-2200, MCBX, 2-COOH-S-2200 and 5-COOH-S-2200 degradates were tentatively identified in the soil extracts by HPLC co-chromatography with authentic standards and were confirmed by 2D-TLC co-chromatography (Appendix H).

[benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB): The major S-2200 degradates formed under aerobic conditions, DX-CA-S-2200, 2-COOH-S-2200 and 5-COOH-S-2200 did not degrade under anaerobic conditions. At 363 DAT, DX-CA-S-2200 had increased to 5% of the AR, 2-COOH-S-2200 was 5% of the AR and 5-COOH-S-2200 7% of the AR. DX-CA-S-2200 increased during the anaerobic phase from 4% of the AR at 34 DAT to 6% of the AR at 266 DAT. De-Xy-S-2200 peaked at 3% of the AR at ca. 48 days and degraded to <0.1% by the end of the study. MCBX increased as the soil developed towards strict anaerobic conditions and reached 3% of the AR at 363 DAT. Up to 8 unknowns were detected during the course of the anaerobic phase, none > 3.6% of the AR (Table 22). The De-Xy-S-2200, DX-CA-S-2200, MCBX, 2-COOH-S-2200 and 5-COOH-S-2200 degradates were tentatively identified in the soil extracts by HPLC co-chromatography with authentic standards and were confirmed by 2D-TLC co-chromatography (Appendix H).

[phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP): The major S-2200 degradates formed under aerobic conditions, 2-COOH-S-2200 and 5-COOH-S-2200 did not degrade under anaerobic conditions. At 363 DAT, 2-COOH-S-2200 was 6% of the AR and 5-COOH-S-2200 8% of the AR. MCBX was found at trace amounts throughout the study. Up to 6 unknowns were detected during the course of the anaerobic phase, none > 0.6% of the AR (Table 27). The MCBX, 2-COOH-S-2200 and 5-COOH-S-2200 degradates were tentatively identified in the soil extracts by HPLC co-chromatography with authentic standards and were confirmed by 2D-TLC co-chromatography (Appendix H).

6.5 Mass Balance and Distribution of the Soil-Bound Radioactivity (PES)

The radioactivity remaining in soil following the neutral and acidic extractions was termed as post extraction soil (PES) or soil-bound radioactivity. These unextractable residues reached their peak levels (5% to 12% of the AR) towards the end of the aerobic phase or during the earlier intervals of the anaerobic phase (0 - 41 DAT). The PES declined in all three radiolabels during the anaerobic phase (37, 41 - 363, 364 DAT) to 4 to 10% of the AR (Tables 7, 9 and 11). A 30 DAT PES (RB label), 41 DAT PES (RP and SB labels) and a 363 DAT PES (RP label) were

subjected to dismembrator extraction (5/1 methanol/0.5M HCl) (Section 4.2.9). The dismembrator extraction released about 1 to 2% of the AR (Table 28). The low amounts of activity observed in the dismembrator analysis confirm that the neutral and acidic extractions were efficient. The samples were concentrated and analyzed by HPLC. The analyses were difficult due to the low activity that could be injected. S-2200 was observed in trace levels and only hints of other degradates were detected (Table 29). The samples were finally fractionated into humin, humic acid and fulvic acid (Section 4.2.9).

The radioactivity remaining in the PES after the dismembrator extraction was fractionated into humin, humic acid and fulvic acid associated radioactive residues. These fractions are defined "operationally" by their solubility in alkali or acidic solutions: Humin – Part of the organic soil compounds not soluble in alkali solutions; Humic acid - Part of the organic soil compounds soluble in alkali solutions, but precipitated by acid; Fulvic acid - Part of the organic soil compounds soluble in alkali and acidic solutions. Collectively, these fractions make up the "soil humic substances", which arise from the microbial and abiotic degradation of biomolecules (lipids, proteins, carbohydrates, lignin) and exogenous compounds (such as S-2200). Slightly greater amounts of S-2200 radioactivity from the RP label were incorporated into the humin, humic acid and fulvic acid fractions than the RB and SB labels (Table 28). Incorporation into the humin fraction reached 2% to 4% of the AR during the study. The humic acid incorporated activity reached 1% to 3% of the AR during the study. The fulvic acid fraction contained 1% to 3% of the AR during the study. Around half of the fulvic acid activity was organosoluble (1% of the AR). The fulvic acid organosoluble activity was analyzed by HPLC. The analyses were difficult due to the low activity that could be injected. Trace amounts of S-2200 were observed.

6.6 Anaerobic Soil Degradation of S-2200

S-2200 was degraded by several routes. Oxidation of the methyl groups on the phenoxy ring formed 2-COOH-S-2200 and 5-COOH-S-2200. Cleavage of the ether linkage formed De-Xy-S-2200, which was subsequently oxidized to DX-CA-S-2200. These degradates (2-COOH-S-2200, 5-COOH-S-2200, De-Xy-S-2200, and DX-CA-S-2200) were formed primarily during the aerobic phase and did not degrade significantly (except for De-Xy-S-2200) during the anaerobic phase. *o*-Demethylation of the 2-methoxy group to form MCBX was observed only under anaerobic conditions. MCBX formed under anaerobic conditions appeared to be stable to further degradation (abiotic or biotic). Mineralization (degradation to bound residues unrelated to parent and $^{14}CO_2$ formation) were primarily significant during the aerobic phase, reaching maximums of 5 - 12% of the AR.

The anaerobic soil metabolism degradation pathway of S-2200 is summarized in Figure 21.

7. CONCLUSIONS

During agricultural use, circumstances may occur where S-2200 is applied to a terrestrial soil that may become waterlogged and in which anaerobic conditions may develop. S-2200 declined to 73 - 77% of the AR by the end of the aerobic phase (30/34 DAT), with an aerobic half-life estimated at 78 - 120 days. The principal degradation routes were oxidation of the methyl groups on the phenoxy ring to form 2-COOH-S-2200 and 5-COOH-S-2200 and cleavage of the ether linkage to form De-Xy-S-2200 which oxidized to Dx-CA-S-2200 2D-TLC analyses of the neutral and acidic soil extracts for all three labels confirmed the degradates. No extractable degradate exceeded 10% of applied radioactivity during the aerobic phase. S-2200 continued to degrade during the anaerobic phase (albeit much slower than under aerobic conditions), declining to an average of 66 - 71% of the AR in the water phase plus soil extracts after one year. As with S-2200, the concentrations of the degradates, 2-COOH-S-2200, 5-COOH-S-2200 and DX-CA-S-2200 did not change significantly under anaerobic conditions. Chiral HPLC analysis confirmed that the S-2200 *R*-isomer did not isomerize during the incubation.

Demethylation of S-2200 to form MCBX was observed in the SB label at the end of the study (3% of the AR at 363 DAT), while MCBX formation in the *R*-isomer studies remained low (<1% of AR).

The pattern of S-2200 decline and formation of degradates in the aerobic and anaerobic phases of the anaerobic soil study was similar to S-2200 aerobic soil and anaerobic aquatic studies.

8 REFERENCES

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TABLES

Table 1. Representative HPLC retention times and TLC R_f values for S-2200 and reference standards

Reference Standard[1]	**HPLC RT (minutes) Method #1**	**TLC R_f Value (CMT)**	**TLC R_f Value (TEA)**
De-Xy-S-2200 (RB, SB)	15.5	0.52	0.24
DX-CA-S-2200 (RB, SB)	17.6	0.13	0.17
DMP (RP)	30.9	0.56	0.67
2-COOH-S-2200 (RB, SB and RP)	28.9	0.50	0.35
5-COOH-S-2200 (RB, SB and RP)	31.4	0.33	0.35
MCBX (RB, SB and RP)	33.1	0.62	0.44
S-2200 (RB, SB and RP)	39.4	0.70	0.49

1) Retention time and R_f values of the standards presented in this table should be viewed as a guide of their relative mobilities rather than representing absolute values. The use of individual standards for [Benzyl-^{14}C]S-2167 (S-2200 *R*-isomer) - RB, [Benzyl-^{14}C]S-2354 (S-2200 *S*-isomer) - SB, and [Phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer) - RP analysis is indicated. HPLC retention times from chromatograms 37166X1 and RPH48D83W. TLC Rf values from chromatograms SB-363D-60Wc and RP-363D-84Wc.

CMT = Chloroform:Methanol:Trifluoroacetic acid, 90:10:0.1 (v:v:v)
TEA = Toluene:Ethyl acetate:Acetic acid, 5:7:1 (v:v:v)

Table 2. Characteristics of the test system soil. Soil characterization was conducted by AGVISE Laboratories, Inc. (Northwood, ND)

Property	Description
Soil classification (USDA classification)	Loam
Soil classification (International classification)	Clay Loam
Soil classification (BBA classification)	Silty Loam
Soil texture (USDA)	Sand 26%, Silt 49%, Clay 25%
Organic matter	2.0%
Soil pH (H_2O)	6.9
Bulk density (disturbed)	1.08 g/cm^3
1/3 bar moisture	21.3%
Cation Exchange Capacity (CEC)	8.7 meq/100 g of dry soil
Olsen Phosphorus	8 ppm
Salinity (electrical conductivity)	0.20 mmhos/cm
Sodium	7 ppm
Calcium	907 ppm
Magnesium	180 ppm
Total nitrogen	0.12%

Table 3. Dissolved oxygen, redox potential, and pH measurements - RB label

R-[Benzyl-^{14}C]S-2167 Sample Parameters																		
DAT	0			14			30, 0			37, 7			44, 14			63, 33		
Sample No.	48	49	Avg.	26	27	Avg.	28	29	Avg.	30	31	Avg.	33	34	Avg.	40	41	Avg.
pH (water phase)	NA	NA	NA	NA	NA	NA	NA	NA	NA	6.49	6.70	6.60	6.47	6.75	6.61	7.51	7.32	7.42
pH (soil phase)	NA	NA	NA	NA	NA	NA	NA	NA	NA	6.20	6.49	6.35	6.95	6.85	6.90	7.10	7.10	7.10
Dissolved Oxygen (ppm)	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.16	0.09	0.13	0.16	0.14	0.15	0.16	0.12	0.14
Redox potential	NA	NA	NA	NA	NA	NA	NA	NA	NA	259	157	208	188	156	172	162	153	158

DAT	86, 56			154, 124			259, 229			364, 334		
Sample No.	45	46	Avg.	35	42	Avg.	38	44	Avg.	36	37	Avg.
pH (water phase)	8.15	8.14	8.15	8.49	8.40	8.45	8.98	8.71	8.85	8.97	9.02	9.00
pH (soil phase)	7.60	7.42	7.51	8.23	8.06	8.15	8.32	8.42	8.37	8.77	8.83	8.80
Dissolved Oxygen (ppm)	0.04	0.06	0.05	0.06	0.16	0.11	0.06	0.07	0.07	0.06	0.03	0.05
Redox potential	123	129	126	79	93	86	-225	-145	-185	-192	-182	-187

'NA = Not analyzed.

Summary

	Max	**Min**	**Avg.**
pH (water)	9.02	6.47	7.86
pH (soil)	8.83	6.20	7.60
Dissolved Oxygen (ppm)	0.16	0.03	0.10
Redox potential	259	-225	54

Table 4. Dissolved oxygen, redox potential, and pH measurements - SB label

S-[Benzyl-^{14}C]S-2354 Sample Parameters																		
DAT	0			15			34, 0			41, 7			48, 14			64,30		
Sample No.	50	51	Avg.	52	53	Avg.	54	55	Avg.	56	57	Avg.	61	62	Avg.	67	69	Avg.
pH (water phase)	NA	NA	NA	NA	NA	NA	NA	NA	NA	6.72	6.63	6.68	5.94	6.72	6.33	7.19	7.44	7.32
pH (soil phase)	NA	NA	NA	NA	NA	NA	NA	NA	NA	6.40	6.31	6.36	5.91	6.37	6.14	6.85	7.06	6.96
Dissolved Oxygen (ppm)	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.14	0.16	0.15	0.22	0.11	0.17	0.14	0.08	0.11
Redox potential	NA	NA	NA	NA	NA	NA	NA	NA	NA	250	168	209	242	184	213	157	122	140

DAT	90, 56			153, 119			266, 232			363, 329		
Sample No.	71	72	Avg.	58	65	Avg.	59	68	Avg.	60	73	Avg.
pH (water phase)	7.40	8.28	7.84	8.31	7.81	8.06	8.90	8.89	8.90	8.92	8.93	8.93
pH (soil phase)	7.01	7.72	7.37	8.18	7.58	7.88	8.24	8.29	8.27	8.52	8.52	8.52
Dissolved Oxygen (ppm)	0.09	0.09	0.09	0.09	0.18	0.14	0.09	0.06	0.08	0.01	0.35	0.18
Redox potential	118	88	103	113	121	117	-158	-184	-171	-180	-186	-183

'NA = Not analyzed.

Summary

	Max	**Min**	**Avg.**
pH (water)	8.93	5.94	7.72
pH (soil)	8.52	5.91	7.35
Dissolved Oxygen (ppm)	0.35	0.01	0.13
Redox potential	250	-186	61

Table 5. Dissolved oxygen, redox potential, and pH measurements - RP label

R-[Phenoxy-^{14}C]S-2167 Sample Parameters																		
DAT	0			15			34, 0			41, 7			48, 14			64,30		
Sample No.	74	75	Avg.	76	77	Avg.	78	79	Avg.	80	81	Avg.	82	83	Avg.	94	95	Avg.
pH (water phase)	NA	NA	NA	NA	NA	NA	NA	NA	NA	6.83	6.93	6.88	6.76	6.95	6.86	7.51	7.58	7.55
pH (soil phase)	NA	NA	NA	NA	NA	NA	NA	NA	NA	6.56	6.60	6.58	6.92	6.87	6.90	7.44	7.32	7.38
Dissolved Oxygen (ppm)	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.09	0.14	0.12	0.06	0.11	0.09	0.07	0.11	0.09
Redox potential	NA	NA	NA	NA	NA	NA	NA	NA	NA	207	217	212	172	167	170	66	106	86

DAT	90, 56			153, 119			266, 232			363, 329		
Sample No.	89	90	Avg.	93	97	Avg.	92	96	Avg.	84	88	Avg.
pH (water phase)	7.98	7.65	7.82	7.60	7.79	7.70	9.04	8.96	9.00	8.84	8.74	8.79
pH (soil phase)	7.50	7.28	7.39	7.64	7.86	7.75	8.37	8.22	8.30	8.63	8.22	8.43
Dissolved Oxygen (ppm)	0.19	0.07	0.13	0.18	0.08	0.13	0.06	0.10	0.08	0.00	0.27	0.14
Redox potential	131	164	148	117	113	115	-214	-200	-207	-183	-178	-181

'NA = Not analyzed.

Summary

	Max	Min	Avg.
pH (water)	9.04	6.76	7.80
pH (soil)	8.63	6.56	7.53
Dissolved Oxygen (ppm)	0.27	0.00	0.11
Redox potential	217	-214	49

Table 6. Dissipation of the control substance [UL-^{14}C]-*D*-Glucose under anaerobic soil conditions (dosed at 311 days into the RB incubation)

Sample interval (days)	Percent of applied UL-^{14}C-*D*-Glucose						
	Sample ID	**$^{14}CO_2$**	**$^{14}CH_4$**	**Water phase**	**Soil phase (PES)**	**Biometer rinse**	**Total**
21	GLU-21D-4	21.7	6.8	33.4	30.6	NA	92.5

1) The workup method for sample #4 is described below.

Procedure for sample #4: Two consecutive headspace samples were taken (15 mL, oxidized, $^{14}CH_4$ analysis). Next, the physical parameters (O_2 = 0.03, redox = -164, pH water = 7.37, pH soil = 8.10) were taken. The NaOH solution (contains $Na_2{}^{14}CO_3$) volume was measured and aliquots taken for LSC analysis. Normally, during the course of the workup of the soil and water, any $^{14}CO_2$ trapped in the water or soil would be lost. Therefore, 25 mL of 0.5 M NaOH was added directly to the soil/water mixture to "trap" dissolved $^{14}CO_2$ (as $Na_2{}^{14}CO_3$). The water was decanted into a pre-weighed centrifuge bottle. The sample was centrifuged for 20 minutes at 7,500 rpm (10,819RCF). The water was separated from the residual soil, volume measured and aliquots taken for LSC analysis. The remaining soil phase (PES) was oxidized to determine residual soil activity.

Table 7. Summary of the mass balance data for the RB label [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer) as percent of applied radioactivity

Percent of applied radioactivity in isolated fractions[1] Time after application (days), Time after switch to anaerobic conditions (days)																	
	0			14			30, 0[2]			37, 7			44, 14			63, 33		
Sample No.	48	49	Avg.	26	27	Avg.	28	29	Avg.	30	31	Avg.	33	34	Avg.	40	41	Avg.
Water Phase	NA	NA	NA	NA	NA	NA	NA	NA	NA	16.9	18.6	17.7	20.2	19.5	19.9	22.3	22.7	22.5
Soil extracts																		
First soil ext.	100.9	100.7	100.8	87.8	86.7	87.3	81.6	80.8	81.2	63.6	60.7	62.1	58.5	57.3	57.9	57.2	55.7	56.4
Second soil ext.	NA	NA	NA	13.6	14.1	13.8	17.2	17.6	17.4	16.2	15.9	16.0	18.8	19.1	18.9	16.2	16.2	16.2
Total soil ext.	100.9	100.7	100.8	101.4	100.8	101.1	98.8	98.3	98.5	79.7	76.6	78.2	77.3	76.3	76.8	73.4	71.9	72.7
Soil-bound (PES)	1.4	1.5	1.5	2.2	2.2	2.2	5.0	5.0	5.0	4.6	4.6	4.6	4.3	4.3	4.3	3.6	3.8	3.7
Volatiles																		
$^{14}CO_2$	NA	NA	NA	0.8	0.8	0.8	1.8	1.8	1.8	1.8	1.8	1.8	1.9	2.4	2.2	2.1	2.1	2.1
$^{14}CH_4$	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Biometer/beaker rinse	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sample balance	102.3	102.2	102.3	104.5	103.9	104.2	105.5	105.1	105.3	103.1	101.6	102.3	103.7	102.6	103.2	101.4	100.5	100.9

	86, 56			154, 124			259, 229			364, 334		
Sample No.	45	46	Avg.	35	42	Avg.	38	44	Avg.	36	37	Avg.
Water Phase	24.5	23.7	24.1	24.6	24.7	24.6	23.1	23.9	23.5	22.5	23.2	22.9
Soil extracts												
First soil ext.	53.0	55.4	54.2	54.9	54.7	54.8	50.3	52.3	51.3	50.6	47.3	49.0
Second soil ext.	17.1	16.9	17.0	17.4	17.1	17.2	21.5	19.1	20.3	22.3	22.2	22.3
Total soil ext.	70.1	72.3	71.2	72.2	71.8	72.0	71.8	71.4	71.6	72.9	69.6	71.2
Soil-bound (PES)	3.9	4.0	4.0	3.5	3.8	3.6	3.8	4.0	3.9	3.9	4.5	4.2
Volatiles												
$^{14}CO_2$	2.2	2.3	2.2	2.6	2.6	2.6	2.8	2.8	2.8	2.7	2.9	2.8
$^{14}CH_4$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Biometer/beaker rinse	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sample balance	100.8	102.3	101.6	102.9	102.9	102.9	101.5	102.1	101.8	102.1	100.1	101.1

1) Duplicate samples were analyzed at each timepoint. Sample balance was determined after the second soil extract and consisted of activity from the following fractions: Total soil extract + Soil-bound (PES) + Water phase + $^{14}CO_2$ + $^{14}CH_4$ + Biometer rinse

2) $BaCl_2$ precipitation of NaOH trapped activity performed to confirm $^{14}CO_2$ as $Ba^{14}CO_3$.
NA = Not analyzed

Table 8. Summary of the mass balance data for the RB study ([benzyl-^{14}C]S-2167 (S-2200 *R*-isomer) as S-2200 equivalents (ppm, µg/g)

Applied radioactivity in isolated fractions (ppm)[1] Time after application (days), Time after switch to anaerobic conditions (days)										
Phase	0	14	30, 0	37, 7	44, 14	63, 33	86, 56	154, 124	259, 229	364, 334
Soil-bound	0.13	0.20	0.45	0.41	0.39	0.33	0.36	0.32	0.35	0.38
Soil Extractable	9.02	9.05	8.82	6.99	6.87	6.50	6.37	6.45	6.41	6.37
Water phase	NA	NA	NA	1.59	1.78	2.02	2.16	2.20	2.10	2.05
$^{14}CO_2$	NA	0.08	0.16	0.16	0.19	0.18	0.20	0.24	0.25	0.25
$^{14}CH_4$	NA	NA	NA	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Volatiles	NA	0.08	0.16	0.16	0.19	0.18	0.20	0.24	0.25	0.25
Biometer/beaker rinse	NA	NA	NA	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sample balance	9.15	9.32	9.42	9.16	9.23	9.03	9.09	9.21	9.11	9.05

1) Average of the duplicate samples analyzed at each timepoint.
NA = Not analyzed

Table 9. Summary of the mass balance data for the SB study ([benzyl-^{14}C]S-2354 (S-2200, *S*-isomer) as percent of applied radioactivity

Percent of applied radioactivity in isolated fractions[1] Time after application (days), Time after switch to anaerobic conditions (days)																		
	0			15			34, 0[2]			41, 7			48, 14			64, 30		
Sample No.	50	51	Avg.	52	53	Avg.	54	55	Avg.	56	57	Avg.	61	62	Avg.	67	69	Avg.
Water Phase	NA	NA	NA	NA	NA	NA	NA	NA	NA	15.9	17.8	16.9	17.2	18.1	17.7	19.6	20.0	19.8
Soil extracts																		
First soil ext.	100.9	100.8	100.9	82.5	83.1	82.8	76.2	76.2	76.2	60.8	59.5	60.2	56.3	56.7	56.5	53.2	53.8	53.5
Second soil ext.	NA	NA	NA	13.1	14.3	13.7	18.6	18.1	18.4	15.2	15.5	15.4	18.6	17.3	17.9	17.0	17.3	17.2
Total soil ext.	100.9	100.8	100.9	95.6	97.3	96.5	94.8	94.3	94.6	76.1	75.0	75.5	74.9	74.0	74.5	70.2	71.1	70.7
Soil-bound (PES)	2.5	2.6	2.6	4.9	4.5	4.7	6.2	6.6	6.4	7.1	6.9	7.0	6.9	6.6	6.8	5.7	5.1	5.4
Volatiles																		
$^{14}CO_2$	NA	NA	NA	3.7	3.7	3.7	5.3	5.3	5.3	5.4	5.4	5.4	5.5	5.5	5.5	5.7	5.7	5.7
$^{14}CH_4$	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Biometer/beaker rinse	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sample balance	103.4	103.5	103.4	104.2	105.5	104.8	106.3	106.1	106.2	104.4	105.2	104.8	104.6	104.2	104.4	101.3	101.9	101.6

	90, 56			153, 119			266, 232			363, 329		
Sample No.	71	72	Avg.	58	65	Avg.	59	68	Avg.	60	73	Avg.
Water Phase	22.2	22.0	22.1	22.7	21.0	21.9	22.9	23.3	23.1	22.3	19.7	21.0
Soil extracts												
First soil ext.	51.7	53.6	52.7	51.6	50.0	50.8	47.5	47.0	47.3	43.8	45.6	44.7
Second soil ext.	17.7	17.5	17.6	16.6	17.4	17.0	18.7	19.3	19.0	21.5	24.2	22.9
Total soil ext.	69.4	71.1	70.3	68.1	67.4	67.8	66.2	66.3	66.3	65.3	69.8	67.5
Soil-bound (PES)	5.3	5.2	5.2	5.2	5.6	5.4	5.1	5.2	5.2	5.6	5.5	5.5
Volatiles												
$^{14}CO_2$	6.3	6.1	6.2	6.7	6.7	6.7	7.2	7.2	7.2	7.4	7.2	7.3
$^{14}CH_4$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Biometer/beaker rinse	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sample balance	103.3	104.3	103.8	102.7	100.7	101.7	101.4	102.0	101.7	100.5	102.2	101.4

1) Duplicate samples were analyzed at each timepoint. Sample balance was determined after the second soil extract and consisted of activity from the following fractions: Total soil extract + Soil-bound (PES) + Water phase + $^{14}CO_2$ + $^{14}CH_4$ + Biometer rinse

2) $BaCl_2$ precipitation of NaOH trapped activity performed to confirm $^{14}CO_2$ as $Ba^{14}CO_3$.

NA = Not analyzed

Table 10. Summary of the mass balance data for the SB study ([benzyl-^{14}C]S-2354 (S-2200, *S*-isomer) as S-2200 equivalents (ppm, μg/g)

Applied radioactivity in isolated fractions (ppm)[1] Time after application (days), Time after switch to anaerobic conditions (days)										
Phase	0	15	34, 0	41, 7	48, 14	64, 30	90, 56	153, 119	266, 232	363, 329
Soil-bound	0.23	0.42	0.57	0.63	0.61	0.48	0.47	0.48	0.46	0.49
Soil Extractable	9.01	8.62	8.45	6.75	6.66	6.32	6.28	6.06	5.92	6.03
Water phase	NA	NA	NA	1.51	1.58	1.77	1.97	1.95	2.07	1.88
$^{14}CO_2$	NA	0.33	0.47	0.48	0.49	0.51	0.55	0.60	0.64	0.65
$^{14}CH_4$	NA	NA	NA	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Volatiles	NA	0.33	0.47	0.48	0.49	0.51	0.55	0.60	0.64	0.65
Biometer/beaker rinse	NA	NA	NA	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sample balance	9.24	9.37	9.49	9.37	9.33	9.08	9.28	9.09	9.09	9.06

1) Average of the duplicate samples analyzed at each timepoint.
NA = Not analyzed

Table 11. Summary of the mass balance data for the RP study ([phenoxy-^{14}C]S-2167 (S-2200, *R*-isomer) as percent of applied radioactivity

Percent of applied radioactivity in isolated fractions[1] Time after application (days), Time after switch to anaerobic conditions (days)																		
	0			15			34, 0[2]			41, 7			48, 14			64, 30		
Sample No.	74	75	Avg.	76	77	Avg.	78	79	Avg.	80	81	Avg.	82	83	Avg.	94	95	Avg.
Water Phase	NA	NA	NA	NA	NA	NA	NA	NA	NA	12.9	13.1	13.0	13.8	14.9	14.4	15.9	16.2	16.0
Soil extracts																		
First soil ext.	102.1	102.4	102.2	82.8	81.1	82.0	71.2	70.9	71.1	57.9	57.1	57.5	56.1	55.9	56.0	53.9	51.9	52.9
Second soil ext.	NA	NA	NA	10.8	11.2	11.0	15.1	16.5	15.8	14.1	12.4	13.2	15.2	14.3	14.7	14.7	15.0	14.9
Total soil ext.	102.1	102.4	102.2	93.6	92.3	93.0	86.4	87.4	86.9	72.0	69.4	70.7	71.3	70.3	70.8	68.7	66.9	67.8
Soil-bound (PES)	2.3	2.4	2.4	7.9	7.7	7.8	11.5	11.8	11.7	11.8	11.7	11.8	11.6	11.3	11.4	9.2	10.3	9.7
Volatiles																		
$^{14}CO_2$	NA	NA	NA	3.9	3.9	3.9	7.1	7.1	7.1	7.3	7.3	7.3	7.4	7.4	7.4	7.6	7.6	7.6
$^{14}CH_4$	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Biometer/beaker rinse	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sample balance	104.4	104.8	104.6	105.5	104.0	104.7	104.9	106.4	105.7	104.0	101.6	102.8	104.1	103.8	104.0	101.4	101.0	101.2

	90, 56			153, 119			266, 232			363, 329		
Sample No.	89	90	Avg.	93	97	Avg.	92	96	Avg.	84	88	Avg.
Water Phase	17.8	17.9	17.8	17.4	17.5	17.4	17.8	17.5	17.6	17.4	15.7	16.6
Soil extracts												
First soil ext.	51.4	51.3	51.4	50.1	50.9	50.5	43.7	45.3	44.5	43.7	42.6	43.1
Second soil ext.	15.7	15.3	15.5	15.4	15.7	15.6	20.5	20.6	20.5	22.1	21.8	22.0
Total soil ext.	67.1	66.7	66.9	65.5	66.7	66.1	64.2	65.9	65.0	65.8	64.4	65.1
Soil-bound (PES)	10.8	11.1	11.0	9.9	9.9	9.9	10.0	9.7	9.8	9.5	9.9	9.7
Volatiles												
$^{14}CO_2$	8.0	8.2	8.1	8.6	8.5	8.5	9.0	9.0	9.0	9.5	9.7	9.6
$^{14}CH_4$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Biometer/beaker rinse	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.2
Sample balance	103.7	103.9	103.8	101.4	102.6	102.0	100.9	102.1	101.5	102.2	100.1	101.2

1) Duplicate samples were analyzed at each timepoint. Sample balance was determined after the second soil extract and consisted of activity from the following fractions: Total soil extract + Soil-bound (PES) + Water phase + $^{14}CO_2$ + $^{14}CH_4$ + Biometer rinse

2) $BaCl_2$ precipitation of NaOH trapped activity performed to confirm $^{14}CO_2$ as $Ba^{14}CO_3$.

NA = Not analyzed

Table 12. Summary of the mass balance data for the RP study ([phenoxy-^{14}C]S-2167 (S-2200, *R*-isomer) as S-2200 equivalents (ppm, µg/g)

Applied radioactivity in isolated fractions (ppm)[1] Time after application (days), Time after switch to anaerobic conditions (days)										
Phase	0	15	34, 0	41, 7	48, 14	64, 30	90, 56	153, 119	266, 232	363, 329
Soil-bound	0.21	0.70	1.04	1.05	1.02	0.87	0.98	0.89	0.88	0.87
Soil Extractable	9.15	8.32	7.78	6.33	6.34	6.07	5.99	5.91	5.82	5.83
Water phase	NA	NA	NA	1.17	1.28	1.44	1.60	1.56	1.58	1.48
$^{14}CO_2$	NA	0.35	0.64	0.65	0.66	0.68	0.73	0.76	0.80	0.86
$^{14}CH_4$	NA	NA	NA	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total Volatiles	NA	0.35	0.64	0.65	0.66	0.68	0.73	0.76	0.80	0.86
Biometer/beaker rinse	NA	NA	NA	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Sample balance	9.37	9.37	9.46	9.20	9.31	9.06	9.29	9.13	9.09	9.06

1) Average of the duplicate samples analyzed at each timepoint.
NA = Not analyzed

Table 13. Radioactivity distribution in neutral soil extracts for the RB anaerobic soil study as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																	
	0			14			30, 0			37, 7			44, 14			63, 33		
Sample No.	48	49	Avg.	26	27	Avg.	28	29	Avg.	30	31	Avg.	33	34	Avg.	40	41	Avg.
De-Xy-S-2200	0.0	0.0	0.0	0.4	0.0	0.2	1.5	1.1	1.3	0.4	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	0.0	0.0	0.0	0.7	0.0	0.4	1.7	1.5	1.6	0.5	0.4	0.4	0.9	1.0	0.9	0.0	0.0	0.0
2-COOH-S-2200	0.0	0.0	0.0	2.3	2.3	2.3	4.6	4.4	4.5	2.3	1.9	2.1	1.5	1.9	1.7	1.3	1.3	1.3
5-COOH-S-2200	0.0	0.0	0.0	4.4	4.0	4.2	5.8	5.6	5.7	3.8	3.1	3.4	2.7	2.2	2.4	2.4	2.5	2.4
MCBX	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	99.6	99.2	99.4	79.2	80.0	79.6	65.6	65.1	65.4	56.2	55.3	55.7	53.5	52.2	52.8	53.4	52.0	52.7
Other unknowns	1.3	1.5	1.4	0.8	0.5	0.6	2.3	3.1	2.7	0.4	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Total	100.9	100.7	100.8	87.8	86.8	87.3	81.6	80.8	81.2	63.6	60.7	62.1	58.5	57.3	57.9	57.2	55.7	56.4

	86, 56			154, 124			259, 229			364, 334		
Sample No.	45	46	Avg.	35	42	Avg.	38	44	Avg.	36	37	Avg.
De-Xy-S-2200	0.0	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	0.7	0.5	0.6	0.5	0.7	0.6	1.2	1.2	1.2	1.4	1.9	1.7
2-COOH-S-2200	1.3	1.4	1.3	1.1	1.1	1.1	1.4	1.5	1.5	1.9	1.9	1.9
5-COOH-S-2200	2.1	2.3	2.2	1.9	1.9	1.9	2.0	2.2	2.1	2.3	2.5	2.4
MCBX	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.0	0.0	0.0
S-2200	48.0	49.9	49.0	50.3	50.1	50.2	45.0	46.7	45.8	44.9	41.1	43.0
Other unknowns	0.7	0.6	0.7	0.8	0.6	0.7	0.5	0.3	0.4	0.0	0.0	0.0
Total	53.0	55.4	54.22	54.9	54.7	54.8	50.3	52.3	51.3	50.6	47.3	49.0

1) Duplicate samples were analyzed at each timepoint.

Table 14. Radioactivity distribution in acidic soil extracts for the RB anaerobic soil study as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																	
	0			14			30, 0			37, 7			44, 14			63, 33		
Sample No.	48	49	Avg.	26	27	Avg.	28	29	Avg.	30	31	Avg.	33	34	Avg.	40	41	Avg.
De-Xy-S-2200	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	NA	NA	NA	1.2	1.1	1.1	0.9	0.5	0.7	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
2-COOH-S-2200	NA	NA	NA	0.3	0.2	0.2	1.3	1.4	1.3	0.9	0.7	0.8	1.0	1.3	1.2	0.0	0.6	0.3
5-COOH-S-2200	NA	NA	NA	1.7	1.5	1.6	2.7	2.7	2.7	2.4	2.2	2.3	2.6	2.8	2.7	2.3	2.1	2.2
MCBX	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	NA	NA	NA	9.2	9.8	9.5	10.6	11.1	10.8	11.5	12.3	11.9	13.8	14.3	14.0	13.2	12.6	12.9
Other unknowns	NA	NA	NA	0.7	0.7	0.7	1.5	1.2	1.4	1.1	0.6	0.8	0.7	0.0	0.4	0.0	0.0	0.0
Total	NA	NA	NA	13.1	13.4	13.2	17.0	17.0	17.0	16.1	15.8	15.9	18.1	18.5	18.3	15.5	15.3	15.4

	86, 56			154, 124			259, 229			364, 334		
Sample No.	45	46	Avg.	35	42	Avg.	38	44	Avg.	36	37	Avg.
De-Xy-S-2200	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
2-COOH-S-2200	0.4	0.4	0.4	0.3	0.4	0.3	0.4	0.5	0.4	0.5	0.5	0.5
5-COOH-S-2200	1.9	1.5	1.7	1.0	1.2	1.1	1.2	1.3	1.3	1.5	1.4	1.4
MCBX	0.3	0.3	0.3	0.2	0.2	0.2	0.4	0.3	0.3	0.0	0.4	0.2
S-2200	12.6	12.9	12.8	14.2	13.9	14.1	17.3	15.6	16.5	18.2	17.9	18.0
Other unknowns	1.4	1.3	1.3	0.8	0.9	0.9	1.3	1.3	1.3	1.6	1.1	1.3
Total	16.6	16.6	16.61	16.8	16.8	16.8	20.7	18.9	19.8	21.8	21.3	21.5

1) Duplicate samples were analyzed at each timepoint.

Table 15. Radioactivity distribution in total soil extracts for the RB anaerobic soil study as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																	
	0			14			30, 0			37, 7			44, 14			63, 33		
Sample No.	48	49	Avg.	26	27	Avg.	28	29	Avg.	30	31	Avg.	33	34	Avg.	40	41	Avg.
De-Xy-S-2200	0.0	0.0	0.0	0.4	0.0	0.2	1.5	1.1	1.3	0.4	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	0.0	0.0	0.0	1.9	1.1	1.5	2.7	2.1	2.4	0.7	0.4	0.6	0.9	1.0	0.9	0.0	0.0	0.0
2-COOH-S-2200	0.0	0.0	0.0	2.5	2.5	2.5	5.9	5.8	5.8	3.2	2.6	2.9	2.6	3.2	2.9	1.3	1.9	1.6
5-COOH-S-2200	0.0	0.0	0.0	6.1	5.5	5.8	8.4	8.2	8.3	6.2	5.3	5.8	5.2	5.0	5.1	4.7	4.6	4.6
MCBX	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	99.6	99.2	99.4	88.4	89.8	89.1	76.3	76.2	76.2	67.6	67.6	67.6	67.2	66.5	66.9	66.7	64.6	65.6
Other unknowns	1.3	1.5	1.4	1.5	1.2	1.4	3.8	4.3	4.1	1.5	0.6	1.0	0.7	0.0	0.4	0.0	0.0	0.0
Total	100.9	100.7	100.8	100.9	100.1	100.5	98.6	97.7	98.2	79.6	76.5	78.1	76.6	75.7	76.2	72.7	71.0	71.8

	86, 56			154, 124			259, 229			364, 334		
Sample No.	45	46	Avg.	35	42	Avg.	38	44	Avg.	36	37	Avg.
De-Xy-S-2200	0.1	0.6	0.3	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	0.7	0.5	0.6	0.6	0.7	0.6	1.2	1.2	1.2	1.4	1.9	1.7
2-COOH-S-2200	1.6	1.8	1.7	1.4	1.5	1.4	1.8	2.0	1.9	2.5	2.4	2.4
5-COOH-S-2200	4.0	3.8	3.9	3.0	3.1	3.1	3.2	3.5	3.4	3.8	3.9	3.8
MCBX	0.6	0.6	0.6	0.5	0.5	0.5	0.7	0.6	0.6	0.0	0.4	0.2
S-2200	60.6	62.9	61.7	64.6	64.1	64.3	62.3	62.2	62.3	63.1	59.0	61.0
Other unknowns	2.1	1.9	2.0	1.6	1.5	1.5	1.8	1.6	1.7	1.6	1.1	1.3
Total	69.7	72.0	70.83	71.6	71.5	71.6	71.0	71.2	71.1	72.4	68.7	70.5

1) Duplicate samples were analyzed at each timepoint.

Table 16. Radioactivity distribution in water extracts for the RB anaerobic soil study as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																	
	0			14			30, 0			37, 7			44, 14			63, 33		
Sample No.	48	49	Avg.	26	27	Avg.	28	29	Avg.	30	31	Avg.	33	34	Avg.	40	41	Avg.
De-Xy-S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	2.6	2.4	2.5	2.7	2.2	2.5
Dx-CA-S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.4	1.6	1.5	2.2	1.6	1.9	2.1	1.7	1.9
2-COOH-S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.4	2.9	2.6	3.3	3.3	3.3	3.6	4.1	3.9
5-COOH-S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.5	2.6	2.6	3.3	3.6	3.4	4.0	4.5	4.2
MCBX	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	7.0	7.4	7.2	8.2	8.6	8.4	10.0	10.2	10.1
Other unknowns	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.8	2.0	1.9	0.6	0.0	0.3	0.0	0.0	0.0
Total	NA	NA	NA	NA	NA	NA	NA	NA	NA	15.1	16.5	15.8	20.2	19.5	19.9	22.3	22.7	22.5

	86, 56			154, 124			259, 229			364, 334		
Sample No.	45	46	Avg.	35	42	Avg.	38	44	Avg.	36	37	Avg.
De-Xy-S-2200	1.4	1.4	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	2.8	2.8	2.8	3.8	4.1	4.0	3.6	3.9	3.8	3.6	3.9	3.8
2-COOH-S-2200	4.0	3.9	4.0	3.8	3.8	3.8	3.6	3.7	3.7	3.0	3.5	3.3
5-COOH-S-2200	4.8	4.6	4.7	4.9	5.0	5.0	4.8	5.4	5.1	4.0	5.1	4.5
MCBX	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	10.6	10.0	10.3	10.8	10.3	10.5	9.2	9.3	9.3	10.6	8.6	9.6
Other unknowns	0.8	1.0	0.9	1.3	1.4	1.4	1.9	1.5	1.7	1.4	2.1	1.8
Total	24.5	23.7	24.1	24.6	24.7	24.6	23.1	23.9	23.5	22.5	23.2	22.9

1) Duplicate samples were analyzed at each timepoint.

Table 17. Radioactivity distribution of the RB anaerobic soil study (total soil extracts and water extracts combined) as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																	
	0			14			30, 0			37, 7			44, 14			63, 33		
Sample No.	48	49	Avg.	26	27	Avg.	28	29	Avg.	30	31	Avg.	33	34	Avg.	40	41	Avg.
De-Xy-S-2200	0.0	0.0	0.0	0.4	0.0	0.2	1.5	1.1	1.3	0.4	0.0	0.2	2.6	2.4	2.5	2.7	2.2	2.5
Dx-CA-S-2200	0.0	0.0	0.0	1.9	1.1	1.5	2.7	2.1	2.4	2.1	2.0	2.1	3.1	2.6	2.8	2.1	1.7	1.9
2-COOH-S-2200	0.0	0.0	0.0	2.5	2.5	2.5	5.9	5.8	5.8	5.5	5.5	5.5	5.9	6.6	6.2	5.0	6.0	5.5
5-COOH-S-2200	0.0	0.0	0.0	6.1	5.5	5.8	8.4	8.2	8.3	8.7	7.9	8.3	8.5	8.5	8.5	8.6	9.0	8.8
MCBX	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	99.6	99.2	99.4	88.4	89.8	89.1	76.3	76.2	76.2	74.7	75.0	74.8	75.4	75.1	75.3	76.7	74.8	75.7
Other unknowns[2]	1.3	1.5	1.4	1.5	1.2	1.4	3.8	4.3	4.1	3.3	2.6	2.9	1.4	0.0	0.7	0.0	0.0	0.0
Total	100.9	100.7	100.8	100.9	100.1	100.5	98.6	97.7	98.2	94.8	93.0	93.9	96.9	95.3	96.1	95.0	93.7	94.4

	86, 56			154, 124			259, 229			364, 334		
Sample No.	45	46	Avg.	35	42	Avg.	38	44	Avg.	36	37	Avg.
De-Xy-S-2200	1.5	2.0	1.7	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	3.5	3.3	3.4	4.4	4.9	4.6	4.8	5.1	5.0	5.0	5.8	5.4
2-COOH-S-2200	5.7	5.7	5.7	5.1	5.3	5.2	5.4	5.7	5.6	5.4	5.9	5.7
5-COOH-S-2200	8.8	8.4	8.6	7.9	8.2	8.0	8.0	8.9	8.4	7.8	8.9	8.4
MCBX	0.6	0.6	0.6	0.5	0.5	0.5	0.7	0.6	0.6	0.0	0.4	0.2
S-2200	71.2	72.8	72.0	75.3	74.3	74.8	71.5	71.6	71.6	73.7	67.6	70.6
Other unknowns[2]	2.9	2.9	2.9	2.9	2.9	2.9	3.7	3.2	3.4	3.0	3.2	3.1
Total	94.2	95.7	95.0	96.2	96.1	96.2	94.1	95.1	94.6	94.9	91.9	93.4

1) Duplicate samples were analyzed at each timepoint.
2) There were a total of 5 unknowns observed during the aerobic phase (0-30 DAT), the highest individual unknown was 2.1% of the AR. There were a total of 9 unknowns observed during the anaerobic phase (37-364 DAT), the highest individual unknown was 2.2% of the AR.

Table 18. Radioactivity distribution in neutral soil extracts for the SB anaerobic soil study as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																	
	0			15			34, 0			41, 7			48, 14			64, 30		
Sample No.	50	51	Avg.	52	53	Avg.	54	55	Avg.	56	57	Avg.	61	62	Avg.	67	69	Avg.
De-Xy-S-2200	1.5	0.0	0.7	0.6	0.6	0.6	1.6	0.0	0.8	0.0	0.0	0.0	0.0	0.9	0.5	0.0	0.0	0.0
Dx-CA-S-2200	0.6	0.0	0.3	1.5	1.4	1.5	1.4	3.9	2.7	0.6	0.5	0.6	0.5	0.7	0.6	0.0	0.0	0.0
2-COOH-S-2200	0.0	0.0	0.0	1.6	1.3	1.4	2.5	3.0	2.8	2.0	1.7	1.8	1.5	1.7	1.6	1.9	1.5	1.7
5-COOH-S-2200	1.1	1.7	1.4	2.9	2.4	2.7	3.0	3.4	3.2	2.8	2.3	2.6	1.7	1.9	1.8	2.1	2.3	2.2
MCBX	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	93.9	94.3	94.1	75.3	77.4	76.3	67.2	64.4	65.8	55.2	54.3	54.7	52.6	51.5	52.1	49.3	50.1	49.7
Other unknowns	3.8	4.9	4.3	0.5	0.0	0.3	0.5	1.5	1.0	0.3	0.6	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Total	100.9	100.8	100.9	82.5	83.1	82.8	76.2	76.2	76.2	60.9	59.5	60.2	56.3	56.7	56.5	53.2	53.8	53.5

	90, 56			153, 119			266, 232			363, 329		
Sample No.	71	72	Avg.	58	65	Avg.	59	68	Avg.	60	73	Avg.
De-Xy-S-2200	0.0	0.5	0.3	0.3	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	0.7	0.7	0.7	0.5	0.7	0.6	1.5	1.4	1.5	1.6	1.3	1.4
2-COOH-S-2200	1.5	1.3	1.4	1.1	1.3	1.2	1.5	1.2	1.3	1.5	1.3	1.4
5-COOH-S-2200	2.1	1.7	1.9	1.4	1.9	1.6	1.9	1.5	1.7	1.8	1.8	1.8
MCBX	0.0	0.0	0.0	0.2	0.3	0.2	0.5	0.5	0.5	0.9	1.3	1.1
S-2200	47.5	49.1	48.3	47.6	45.0	46.3	42.1	41.9	42.0	37.9	39.9	38.9
Other unknowns	0.0	0.3	0.2	0.5	0.7	0.6	0.0	0.4	0.2	0.0	0.0	0.0
Total	51.7	53.6	52.7	51.6	50.0	50.8	47.5	47.0	47.3	43.8	45.6	44.7

1) Duplicate samples were analyzed at each timepoint.

Table 19. Radioactivity distribution in acidic soil extracts for the SB anaerobic soil study as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																	
	0			15			34, 0			41, 7			48, 14			64, 30		
Sample No.	50	51	Avg.	52	53	Avg.	54	55	Avg.	56	57	Avg.	61	62	Avg.	67	69	Avg.
De-Xy-S-2200	NA	NA	NA	0.0	0.8	0.4	1.5	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	NA	NA	NA	1.5	0.6	1.1	0.9	2.2	1.5	0.0	0.0	0.0	0.4	0.0	0.2	0.0	0.0	0.0
2-COOH-S-2200	NA	NA	NA	0.4	0.4	0.4	1.0	1.1	1.0	0.9	0.9	0.9	0.9	1.1	1.0	0.0	0.0	0.0
5-COOH-S-2200	NA	NA	NA	1.0	0.9	1.0	1.7	1.7	1.7	1.8	2.0	1.9	2.2	2.4	2.3	1.9	2.0	1.9
MCBX	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.2	0.0	0.0	0.0
S-2200	NA	NA	NA	9.1	10.2	9.7	12.0	10.9	11.5	11.8	11.2	11.5	11.3	12.0	11.6	14.1	14.4	14.2
Other unknowns	NA	NA	NA	1.0	0.8	0.9	0.7	1.4	1.0	1.2	1.0	1.1	2.0	0.5	1.3	0.0	0.0	0.0
Total	NA	NA	NA	13.0	13.8	13.4	17.8	17.2	17.5	15.6	15.2	15.4	17.2	16.1	16.7	15.9	16.4	16.2

	90, 56			153, 119			266, 232			363, 329		
Sample No.	71	72	Avg.	58	65	Avg.	59	68	Avg.	60	73	Avg
De-Xy-S-2200	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2-COOH-S-2200	0.7	0.4	0.6	0.3	0.5	0.4	0.5	0.4	0.4	0.5	0.0	0.3
5-COOH-S-2200	2.0	1.2	1.6	0.8	1.4	1.1	1.1	1.1	1.1	1.6	1.7	1.6
MCBX	0.0	0.0	0.0	0.2	0.2	0.2	0.0	0.5	0.3	0.9	1.2	1.1
S-2200	13.5	14.1	13.8	14.1	13.6	13.8	15.3	15.6	15.5	18.5	19.4	19.0
Other unknowns	1.6	1.3	1.4	0.8	1.1	0.9	1.5	1.5	1.5	1.5	2.1	1.8
Total	17.8	16.9	17.35	16.1	16.8	16.48	18.3	19.1	18.7	23.1	24.4	23.73

1) Duplicate samples were analyzed at each timepoint.

Table 20. Radioactivity distribution in total soil extracts for the SB anaerobic soil study as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																	
	0			15			34, 0			41, 7			48, 14			64, 30		
Sample No.	50	51	Avg.	52	53	Avg.	54	55	Avg.	56	57	Avg.	61	62	Avg.	67	69	Avg.
De-Xy-S-2200	1.5	0.0	0.7	0.6	1.5	1.0	3.1	0.0	1.5	0.0	0.0	0.0	0.0	0.9	0.5	0.0	0.0	0.0
Dx-CA-S-2200	0.6	0.0	0.3	3.1	2.0	2.5	2.3	6.1	4.2	0.6	0.5	0.6	1.0	0.7	0.8	0.0	0.0	0.0
2-COOH-S-2200	0.0	0.0	0.0	2.0	1.7	1.8	3.5	4.1	3.8	2.9	2.6	2.7	2.4	2.9	2.6	1.9	1.5	1.7
5-COOH-S-2200	1.1	1.7	1.4	3.9	3.3	3.6	4.8	5.0	4.9	4.6	4.4	4.5	3.8	4.3	4.1	4.0	4.3	4.1
MCBX	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.2	0.0	0.0	0.0
S-2200	93.9	94.3	94.1	84.4	87.6	86.0	79.2	75.3	77.3	67.0	65.5	66.2	63.9	63.5	63.7	63.3	64.4	63.9
Other unknowns	3.8	4.9	4.3	1.5	0.8	1.2	1.2	2.9	2.0	1.5	1.6	1.5	2.0	0.5	1.3	0.0	0.0	0.0
Total	100.9	100.8	100.9	95.5	96.9	96.2	94.0	93.4	93.7	76.5	74.7	75.6	73.5	72.9	73.2	69.2	70.2	69.7

	90, 56			153, 119			266, 232			363, 329		
Sample No.	71	72	Avg.	58	65	Avg.	59	68	Avg.	60	73	Avg.
De-Xy-S-2200	0.0	0.5	0.3	0.3	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	0.7	0.7	0.7	0.5	0.7	0.6	1.5	1.4	1.5	1.6	1.3	1.4
2-COOH-S-2200	2.2	1.7	1.9	1.4	1.9	1.6	2.0	1.6	1.8	2.1	1.3	1.7
5-COOH-S-2200	4.1	2.8	3.4	2.2	3.3	2.7	3.0	2.7	2.8	3.4	3.5	3.4
MCBX	0.0	0.0	0.0	0.5	0.4	0.4	0.5	1.0	0.8	1.8	2.5	2.2
S-2200	61.0	63.2	62.1	61.6	58.7	60.2	57.5	57.5	57.5	56.4	59.3	57.9
Other unknowns	1.6	1.6	1.6	1.3	1.8	1.5	1.5	1.9	1.7	1.5	2.1	1.8
Total	69.5	70.5	70.01	67.7	66.8	67.28	65.9	66.1	65.96	66.8	70.0	68.40

1) Duplicate samples were analyzed at each timepoint.

Table 21. Radioactivity distribution in water extracts for the SB anaerobic soil study as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																		
	0			15			34, 0			41, 7			48, 14			64, 30		
Sample No.	50	51	Avg.	52	53	Avg.	54	55	Avg.	56	57	Avg.	61	62	Avg.	67	69	Avg.
De-Xy-S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.1	1.2	1.1	2.9	2.7	2.8	1.9	2.6	2.2
Dx-CA-S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.4	1.8	1.6	2.5	2.4	2.5	2.8	2.2	2.5
2-COOH-S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.2	2.3	2.2	2.5	2.5	2.5	3.5	3.0	3.2
5-COOH-S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.8	1.9	1.8	1.9	2.3	2.1	3.0	2.9	3.0
MCBX	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	7.3	8.0	7.6	6.6	8.1	7.4	8.4	9.3	8.9
Other unknowns	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.3	0.9	1.1	0.6	0.0	0.3	0.0	0.0	0.0
Total	NA	NA	NA	NA	NA	NA	NA	NA	NA	15.0	16.1	15.6	17.2	18.1	17.7	19.6	20.0	19.8

	90, 56			153, 119			266, 232			363, 329		
Sample No.	71	72	Avg.	58	65	Avg.	59	68	Avg.	60	73	Avg.
De-Xy-S-2200	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	1.8	1.2	1.5	4.3	4.6	4.4	4.6	4.6	4.6	4.4	3.7	4.0
2-COOH-S-2200	3.3	3.1	3.2	3.0	3.1	3.1	3.5	3.2	3.3	3.4	2.5	2.9
5-COOH-S-2200	3.3	3.1	3.2	3.4	3.5	3.4	3.9	3.9	3.9	3.7	2.9	3.3
MCBX	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.4
S-2200	9.1	10.9	10.0	10.3	8.7	9.5	9.1	9.4	9.2	8.5	8.3	8.4
Other unknowns	4.6	3.7	4.2	1.7	1.1	1.4	1.8	2.3	2.1	1.9	2.0	2.0
Total	22.2	22.0	22.1	22.7	21.0	21.9	22.9	23.3	23.1	22.3	19.7	21.0

1) Duplicate samples were analyzed at each timepoint.

Table 22. Radioactivity distribution of the SB anaerobic soil study (total soil extracts and water extracts combined) as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																		
	0			15			34, 0			41, 7			48, 14			64, 30		
Sample No.	50	51	Avg.	52	53	Avg.	54	55	Avg.	56	57	Avg.	61	62	Avg.	67	69	Avg.
De-Xy-S-2200	1.5	0.0	0.7	0.6	1.5	1.0	3.1	0.0	1.5	1.1	1.2	1.1	2.9	3.6	3.3	1.9	2.6	2.2
Dx-CA-S-2200	0.6	0.0	0.3	3.1	2.0	2.5	2.3	6.1	4.2	2.0	2.3	2.1	3.5	3.1	3.3	2.8	2.2	2.5
2-COOH-S-2200	0.0	0.0	0.0	2.0	1.7	1.8	3.5	4.1	3.8	5.0	5.0	5.0	4.9	5.4	5.1	5.4	4.5	4.9
5-COOH-S-2200	1.1	1.7	1.4	3.9	3.3	3.6	4.8	5.0	4.9	6.4	6.3	6.3	5.8	6.6	6.2	7.0	7.2	7.1
MCBX	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.2	0.0	0.0	0.0
S-2200	93.9	94.3	94.1	84.4	87.6	86.0	79.2	75.3	77.3	74.3	73.5	73.9	70.5	71.7	71.1	71.7	73.7	72.7
Other unknowns[2]	3.8	4.9	4.3	1.5	0.8	1.2	1.2	2.9	2.0	2.8	2.6	2.7	2.6	0.5	1.6	0.0	0.0	0.0
Total	100.9	100.8	100.9	95.5	96.9	96.2	94.0	93.4	93.7	91.5	90.8	91.1	90.7	90.9	90.8	88.8	90.2	89.5

	90, 56			153, 119			266, 232			363, 329		
Sample No.	71	72	Avg.	58	65	Avg.	59	68	Avg.	60	73	Avg.
De-Xy-S-2200	0.0	0.5	0.3	0.3	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Dx-CA-S-2200	2.4	1.9	2.1	4.8	5.3	5.0	6.2	6.0	6.1	6.0	4.9	5.5
2-COOH-S-2200	5.5	4.8	5.2	4.4	5.0	4.7	5.4	4.8	5.1	5.5	3.8	4.6
5-COOH-S-2200	7.4	5.9	6.6	5.6	6.7	6.2	6.9	6.5	6.7	7.1	6.3	6.7
MCBX	0.0	0.0	0.0	0.5	0.4	0.4	0.5	1.0	0.8	2.2	2.9	2.5
S-2200	70.1	74.1	72.1	72.0	67.4	69.7	66.5	66.9	66.7	64.9	67.6	66.3
Other unknowns[2]	6.2	5.3	5.8	3.0	2.9	2.9	3.3	4.2	3.7	3.5	4.1	3.8
Total	91.7	92.5	92.1	90.4	87.8	89.1	88.8	89.4	89.1	89.1	89.7	89.4

1) Duplicate samples were analyzed at each timepoint.
2) There were a total of 6 unknowns observed during the aerobic phase (0-34 DAT), the highest individual unknown was 1.9% of the AR. There were a total of 8 unknowns observed during the anaerobic phase (41-363 DAT), the highest individual unknown was 3.6% of the AR.

Table 23. Radioactivity distribution in neutral soil extracts for the RP anaerobic soil study as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																		
	0			15			34, 0			41, 7			48, 14			64, 30		
Sample No.	74	75	Avg.	76	77	Avg.	78	79	Avg.	80	81	Avg.	82	83	Avg.	94	95	Avg.
DMP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0
2-COOH-S-2200	0.0	1.0	0.5	2.6	2.9	2.7	4.1	4.0	4.0	2.2	1.9	2.0	1.7	1.4	1.5	2.1	1.5	1.8
5-COOH-S-2200	1.2	1.1	1.2	3.7	4.1	3.9	4.4	4.1	4.2	2.8	2.7	2.8	2.2	1.8	2.0	2.5	2.1	2.3
MCBX	0.6	0.5	0.5	0.0	0.0	0.0	0.0	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	98.3	98.4	98.3	75.8	73.7	74.8	61.9	61.6	61.7	52.9	52.0	52.5	51.9	52.8	52.3	49.3	48.3	48.8
Other unknowns	2.0	1.4	1.7	0.7	0.4	0.5	1.0	0.8	0.9	0.0	0.0	0.0	0.3	0.0	0.2	0.0	0.0	0.0
Total	102.1	102.4	102.2	82.8	81.1	82.0	71.2	70.9	71.1	57.9	56.6	57.3	56.1	55.9	56.0	53.9	51.9	52.9

	90, 56			153, 119			266, 232			363, 329		
Sample No.	89	90	Avg.	93	97	Avg.	92	96	Avg.	84	88	Avg.
DMP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2-COOH-S-2200	1.6	1.7	1.6	1.5	1.6	1.5	1.4	1.6	1.5	1.7	1.4	1.6
5-COOH-S-2200	2.2	1.9	2.1	2.1	2.1	2.1	2.0	1.8	1.9	2.2	2.0	2.1
MCBX	0.3	0.5	0.4	0.3	0.3	0.3	0.4	0.4	0.4	0.0	0.7	0.3
S-2200	46.9	47.3	47.1	45.6	46.5	46.0	39.4	40.5	39.9	39.8	38.4	39.1
Other unknowns	0.4	0.0	0.2	0.6	0.5	0.6	0.5	1.0	0.8	0.0	0.0	0.0
Total	51.4	51.3	51.4	50.1	50.9	50.5	43.7	45.3	44.5	43.7	42.6	43.1

1) Duplicate samples were analyzed at each timepoint.
NA = Not analyzed

Table 24. Radioactivity distribution in acidic soil extracts for the RP anaerobic soil study as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																	
	0			15			34, 0			41, 7			48, 14			64, 30		
Sample No.	74	75	Avg.	76	77	Avg.	78	79	Avg.	80	81	Avg.	82	83	Avg.	94	95	Avg.
DMP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0
2-COOH-S-2200	NA	NA	NA	0.6	0.5	0.5	1.6	1.8	1.7	1.5	1.1	1.3	0.0	1.3	0.6	0.0	0.0	0.0
5-COOH-S-2200	NA	NA	NA	1.2	1.4	1.3	2.1	2.2	2.2	2.0	1.7	1.8	1.6	2.3	2.0	1.9	1.8	1.9
MCBX	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	NA	NA	NA	8.7	8.8	8.8	10.6	11.5	11.0	10.3	9.1	9.7	12.7	9.8	11.2	12.0	12.2	12.1
Other unknowns	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	NA	NA	NA	10.5	10.7	10.6	14.4	15.5	14.9	13.8	11.9	12.8	14.3	13.4	13.8	13.8	14.1	14.0

	90, 56			153, 119			266, 232			363, 329		
Sample No.	89	90	Avg.	93	97	Avg.	92	96	Avg.	84	88	Avg.
DMP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.0	0.0	0.0
2-COOH-S-2200	1.0	0.8	0.9	0.5	0.6	0.6	0.6	0.7	0.6	0.8	0.8	0.8
5-COOH-S-2200	1.6	1.7	1.7	1.3	1.3	1.3	1.2	1.3	1.3	1.4	1.6	1.5
MCBX	0.0	0.0	0.0	0.3	0.2	0.2	0.0	0.4	0.2	1.0	0.0	0.5
S-2200	13.3	12.1	12.7	12.8	13.0	12.9	17.5	17.7	17.6	20.6	20.5	20.6
Other unknowns	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.3	0.5	0.0	0.3
Total	15.9	14.6	15.2	14.9	15.0	14.9	19.9	20.5	20.2	24.3	22.9	23.6

1) Duplicate samples were analyzed at each timepoint.

NA = Not analyzed

Table 25. Radioactivity distribution in total soil extracts for the RP anaerobic soil study as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																		
	0			15			34, 0			41, 7			48, 14			64, 30		
Sample No.	74	75	Avg.	76	77	Avg.	78	79	Avg.	80	81	Avg.	82	83	Avg.	94	95	Avg.
DMP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0
2-COOH-S-2200	0.0	1.0	0.5	3.2	3.3	3.3	5.7	5.8	5.8	3.7	3.0	3.3	1.7	2.6	2.2	2.1	1.5	1.8
5-COOH-S-2200	1.2	1.1	1.2	4.9	5.6	5.2	6.5	6.3	6.4	4.8	4.5	4.6	3.8	4.1	4.0	4.4	3.9	4.2
MCBX	0.6	0.5	0.5	0.0	0.0	0.0	0.0	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	98.3	98.4	98.3	84.6	82.5	83.5	72.4	73.1	72.8	63.3	61.1	62.2	64.5	62.5	63.5	61.2	60.6	60.9
Other unknowns	2.0	1.4	1.7	0.7	0.4	0.5	1.0	0.8	0.9	0.0	0.0	0.0	0.3	0.0	0.2	0.0	0.0	0.0
Total	102.1	102.4	102.2	93.3	91.8	92.6	85.6	86.4	86.0	71.7	68.5	70.1	70.4	69.3	69.9	67.8	66.0	66.9

	90, 56			153, 119			266, 232			363, 329		
Sample No.	89	90	Avg.	93	97	Avg.	92	96	Avg.	84	88	Avg.
DMP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.0	0.0	0.0
2-COOH-S-2200	2.5	2.5	2.5	2.0	2.1	2.1	2.0	2.2	2.1	2.6	2.3	2.4
5-COOH-S-2200	3.8	3.7	3.7	3.4	3.3	3.4	3.3	3.1	3.2	3.5	3.6	3.6
MCBX	0.3	0.5	0.4	0.5	0.5	0.5	0.4	0.8	0.6	1.0	0.7	0.8
S-2200	60.2	59.3	59.8	58.4	59.4	58.9	56.9	58.2	57.5	60.4	59.0	59.7
Other unknowns	0.4	0.0	0.2	0.6	0.5	0.6	1.1	1.0	1.0	0.5	0.0	0.3
Total	67.2	65.9	66.6	65.0	65.9	65.4	63.7	65.8	64.7	68.0	65.5	66.8

1) Duplicate samples were analyzed at each timepoint.
NA = Not analyzed

Table 26. Radioactivity distribution in water extracts for the RP anaerobic soil study as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																		
	0			15			34, 0			41, 7			48, 14			64, 30		
Sample No.	74	75	Avg.	76	77	Avg.	78	79	Avg.	80	81	Avg.	82	83	Avg.	94	95	Avg.
DMP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0
2-COOH-S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.6	3.1	2.9	3.1	3.6	3.4	3.3	3.3	3.3
5-COOH-S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.3	2.3	2.3	2.9	3.2	3.0	4.0	3.9	3.9
MCBX	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	NA	NA	NA	NA	NA	NA	NA	NA	NA	6.9	7.1	7.0	7.8	8.1	8.0	8.6	9.0	8.8
Other unknowns	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.4	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Total	NA	NA	NA	NA	NA	NA	NA	NA	NA	12.3	12.6	12.4	13.8	14.9	14.4	15.9	16.2	16.0

	90, 56			153, 119			266, 232			363, 329		
Sample No.	89	90	Avg.	93	97	Avg.	92	96	Avg.	84	88	Avg.
DMP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2-COOH-S-2200	3.9	3.8	3.8	3.6	3.8	3.7	4.0	3.6	3.8	3.6	3.3	3.4
5-COOH-S-2200	4.2	4.0	4.1	4.0	4.0	4.0	4.6	4.5	4.5	4.3	3.9	4.1
MCBX	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	9.7	10.0	9.9	9.8	9.7	9.7	8.9	9.0	8.9	9.2	8.0	8.6
Other unknowns	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.4	0.4	0.4	0.5	0.5
Total	17.8	17.9	17.8	17.4	17.5	17.4	17.8	17.5	17.6	17.4	15.7	16.6

1) Duplicate samples were analyzed at each timepoint.
NA = Not analyzed

Table 27. Radioactivity distribution of the RP anaerobic soil study (total soil extracts and water extracts combined) as percent of applied radioactivity

Percent of applied radioactivity[1] Time after application (days), Time after switch to anaerobic conditions (days)																		
	0			15			34, 0			41, 7			48, 14			64, 30		
Sample No.	74	75	Avg.	76	77	Avg.	78	79	Avg.	80	81	Avg.	82	83	Avg.	94	95	Avg.
DMP	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.0	0.0	0.0	0.0	0.0	0.0
2-COOH-S-2200	0.0	1.0	0.5	3.2	3.3	3.3	5.7	5.8	5.8	6.2	6.1	6.2	4.8	6.2	5.5	5.5	4.8	5.1
5-COOH-S-2200	1.2	1.1	1.2	4.9	5.6	5.2	6.5	6.3	6.4	7.1	6.8	6.9	6.7	7.3	7.0	8.4	7.8	8.1
MCBX	0.6	0.5	0.5	0.0	0.0	0.0	0.0	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S-2200	98.3	98.4	98.3	84.6	82.5	83.5	72.4	73.1	72.8	70.2	68.2	69.2	72.3	70.7	71.5	69.8	69.5	69.7
Other unknowns[2]	2.0	1.4	1.7	0.7	0.4	0.5	1.0	0.8	0.9	0.4	0.0	0.2	0.3	0.0	0.2	0.0	0.0	0.0
Total	102.1	102.4	102.2	93.3	91.8	92.6	85.6	86.4	86.0	84.0	81.1	82.5	84.2	84.2	84.2	83.7	82.1	82.9

	90, 56			153, 119			266, 232			363, 329		
Sample No.	89	90	Avg.	93	97	Avg.	92	96	Avg.	84	88	Avg.
DMP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.0	0.0	0.0
2-COOH-S-2200	6.4	6.3	6.4	5.7	5.9	5.8	6.0	5.9	5.9	6.1	5.6	5.8
5-COOH-S-2200	8.0	7.7	7.8	7.4	7.3	7.4	7.9	7.5	7.7	7.9	7.5	7.7
MCBX	0.3	0.5	0.4	0.5	0.5	0.5	0.4	0.8	0.6	1.0	0.7	0.8
S-2200	69.9	69.4	69.6	68.2	69.1	68.7	65.8	67.2	66.5	69.6	67.0	68.3
Other unknowns[2]	0.4	0.0	0.2	0.6	0.5	0.6	1.4	1.5	1.4	0.9	0.5	0.7
Total	85.0	83.8	84.4	82.4	83.3	82.9	81.4	83.3	82.4	85.4	81.2	83.3

1) Duplicate samples were analyzed at each timepoint.
2) There were a total of 3 unknowns observed during the aerobic phase (0-34 DAT), the highest individual unknown was 1.0% of the AR. There were a total of 6 unknowns observed during the anaerobic phase (41-363 DAT), the highest individual unknown was 0.6% of the AR.
NA = Not analyzed

Table 28. Summary of the mass balance data for the anaerobic soil study soil-bound activity as percent of applied radioactivity

Percent of applied radioactivity in the PES				
Sample ID	RB 30D-28	SB 41D-56	RP 41D-81	RP 363D-88
Starting Sediment-bound (PES)	5.0	7.1	11.7	9.9
Dismembrator extract[1]	1.5	1.4	1.2	0.9
Humin (PFD)	1.5	2.2	3.9	3.6
Humic acid (HA)	0.9	1.3	2.8	2.7
Fulvic acid (FA)	1.2	1.6	3.2	2.1
FA organosoluble	0.8	0.8	1.4	0.8
FA aqueous	0.4	0.7	1.7	1.2
Total recovery[2]	5.1	6.5	11.2	9.3
Mass balance (%)	100.8	91.4	95.4	94.6

1) Selected sediment-bound residues (PES) were sequentially subjected to a dismembrator extraction (5/1 methanol/0.5M HCl, 15 minute pulsing) prior to a final Humin, Humic acid, Fulvic acid fractionation.
2) Total recovery = Dismembrator extract + Humin + Humic acid + Fulvic acid

Table 29. Radioactivity released from the anaerobic soil PES by Dismembrator extraction as percent of applied radioactivity

Percent of applied radioactivity[1]				
Sample ID	RB 30D-28	SB 41D-56	RP 41D-81	RP 363D-88
DMP	NA	NA	0.0	0.0
De-Xy-S-2200	0.0	0.0	NA	NA
Dx-CA-S-2200	0.0	0.0	NA	NA
2-COOH-S-2200	0.0	0.0	0.0	0.2
5-COOH-S-2200	0.4	0.0	0.2	0.0
MCBX	0.4	0.1	0.0	0.0
S-2200	0.4	0.7	0.3	0.3
Other unknowns	0.4	0.5	0.8	0.4
Total	1.5	1.4	1.2	0.9

1) Duplicate samples were analyzed at each timepoint. A dismembrator extraction was conducted on PES prior to a final Humin, Humic acid, Fulvic acid fractionation.
NA = Not applicable

Table 30. Decline of S-2200 under anaerobic conditions in water and soil

S-2200 rate estimate - Pseudo first order assumed (w/o fractionation S-2200)
RB label (combined soil extracts or soil extract plus water residues)

Exposure time Days	Hours	S-2200 remaining (%)	ln(S-2200)	K (hr^{-1})	t1/2 (hrs)	t1/2 (days)	t1/2 (years)	R^2
Aerobic Phase				-0.0003692	1877.2	78.2	0.21	0.9961
0	0	99.4	4.5989					
14	336	89.1	4.4896					
30	720	76.2	4.3338					
Anaerobic Phase				-0.0000083	83373.0	3473.9	9.51	0.6953
0	0	76.2	4.3338					
7	168	74.8	4.3154					
14	336	75.3	4.3212					
33	792	75.7	4.3270					
56	1344	72.0	4.2768					
124	2976	74.8	4.3153					
229	5496	71.6	4.2706					
334	8016	70.6	4.2573					

S-2200 rate estimate - Pseudo first order assumed (w/o fractionation S-2200)
SB label (combined soil extracts or soil extract plus water residues)

Exposure time Days	Hours	S-2200 remaining (%)	ln(S-2200)	K (hr^{-1})	t1/2 (hrs)	t1/2 (days)	t1/2 (years)	R^2
Aerobic Phase				-0.0002413	2872.0	119.7	0.33	0.9997
0	0	94.1	4.5442					
15	360	86.0	4.4545					
34	816	77.3	4.3471					
Anaerobic Phase				-0.0000157	44069.3	1836.2	5.03	0.7956
0	0	77.3	4.3471					
7	168	73.9	4.3024					
14	336	71.1	4.2639					
30	720	72.7	4.2866					
56	1344	72.1	4.2782					
119	2856	69.7	4.2440					
232	5568	66.7	4.2003					
329	7896	66.3	4.1939					

Table 30 (continued). Decline of S-2200 under anaerobic conditions in water and soil

S-2200 rate estimate - Pseudo first order assumed (w/o fractionation S-2200)
RP label (combined soil extracts or soil extract plus water residues)

Exposure time Days	Hours	S-2200 remaining (%)	ln(S-2200)	K (hr^{-1})	t1/2 (hrs)	t1/2 (days)	t1/2 (years)	R^2
Aerobic Phase				-0.0003661	1892.7	78.9	0.22	0.9867
0	0	98.3	4.5883					
15	360	83.5	4.4253					
34	816	72.8	4.2872					
Anaerobic Phase				-0.0000069	100486.8	4187.0	11.46	0.5241
0	0	72.8	4.2872					
7	168	69.2	4.2370					
14	336	71.5	4.2697					
30	720	69.7	4.2439					
56	1344	69.6	4.2432					
119	2856	68.7	4.2290					
232	5568	66.5	4.1968					
329	7896	68.3	4.2236					

FIGURES

Figure 1. Schematic representation of the aerobic incubation apparatus showing locations of the soil sample and volatiles traps. The units were placed in an incubation chamber at 20 ± 2 °C and in the dark.

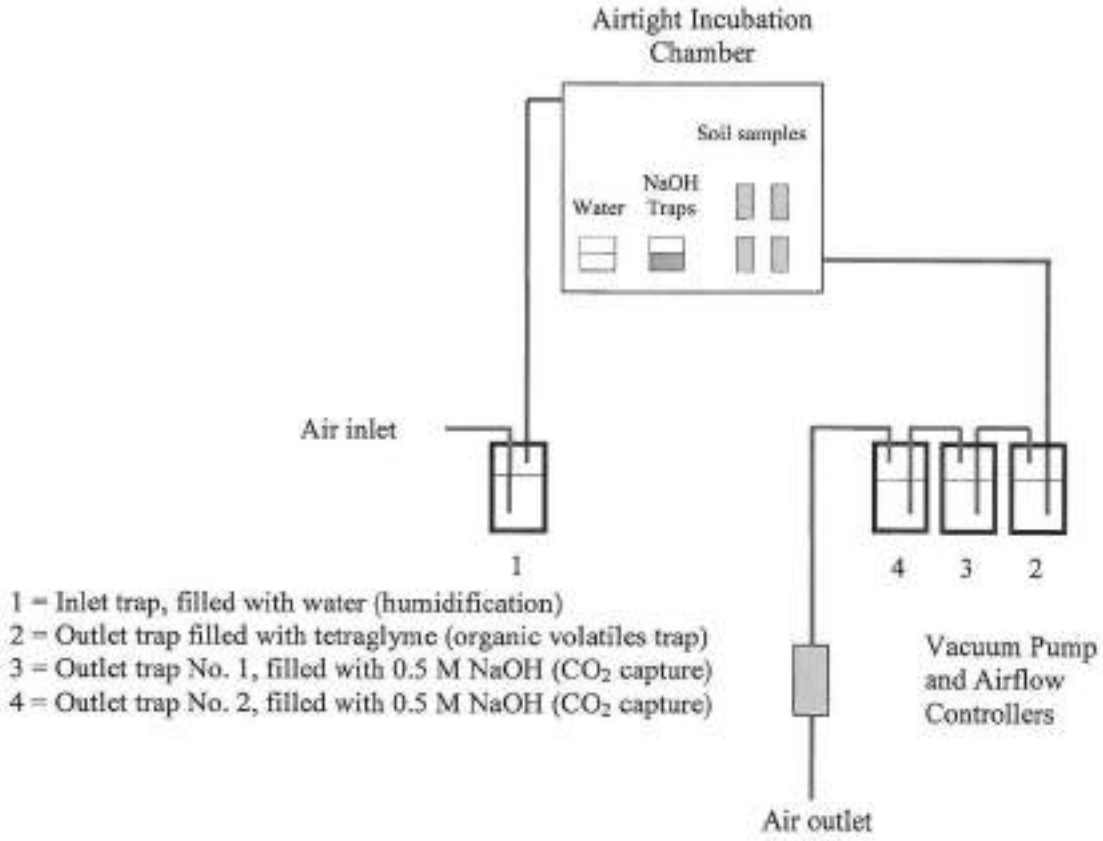


Figure 2. A schematic of the anaerobic incubation apparatus (biometer flask) showing the two chambers housing the test system and the NaOH solution used to absorb evolved CO_2. Also shown is the syringe valve used for the anaerobic treatment application and headspace gas sampling

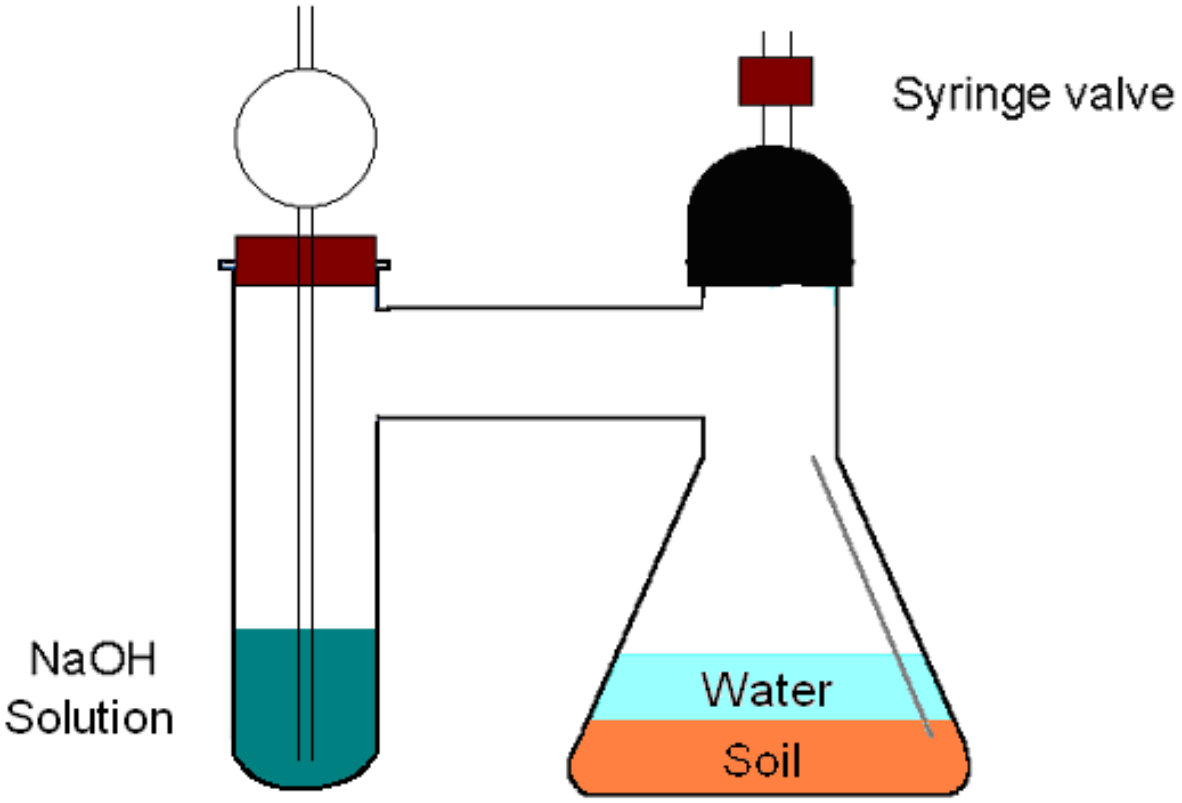


Figure 3. Summary of aerobic sample processing procedure

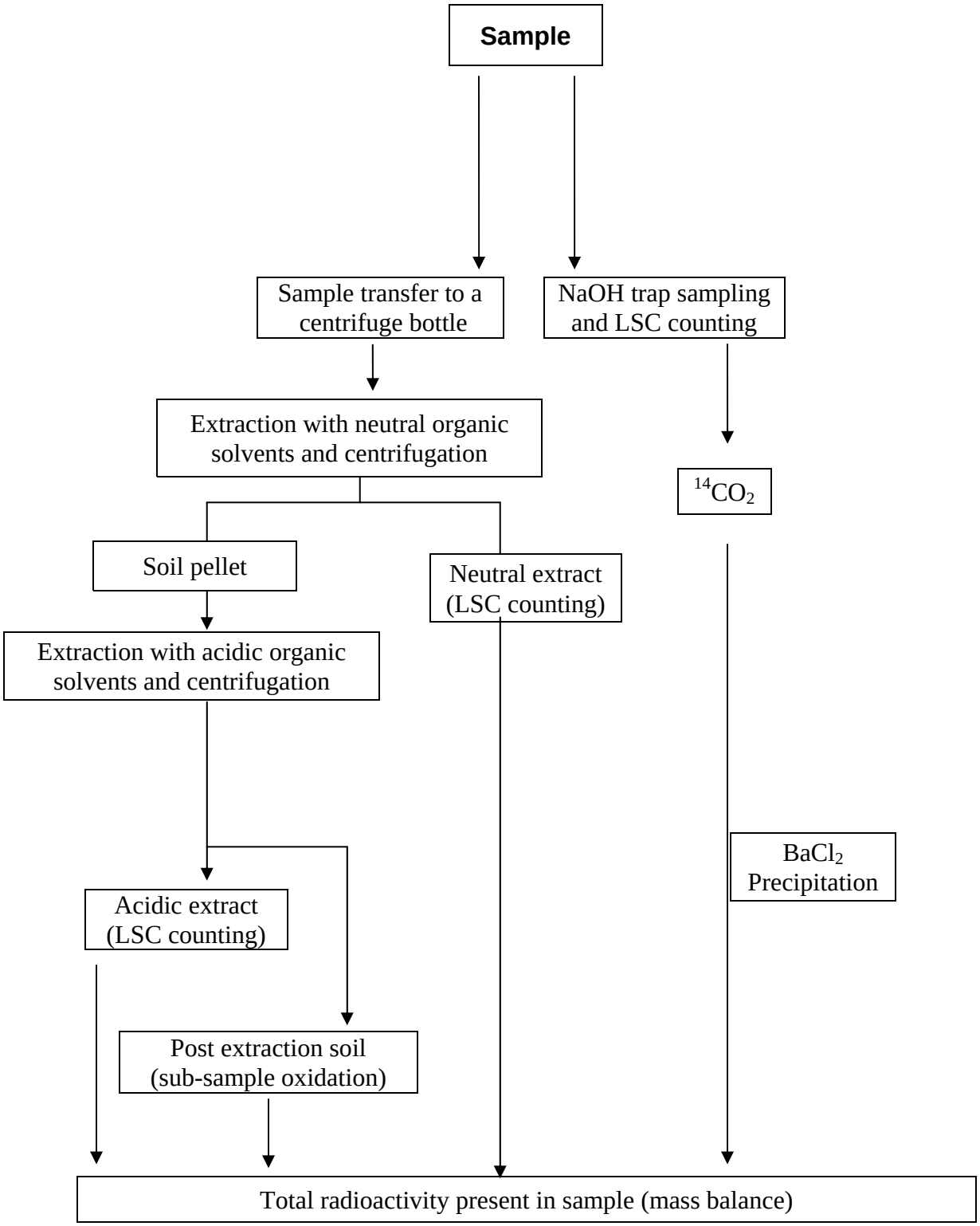


Figure 4. Summary of anaerobic sample processing procedure

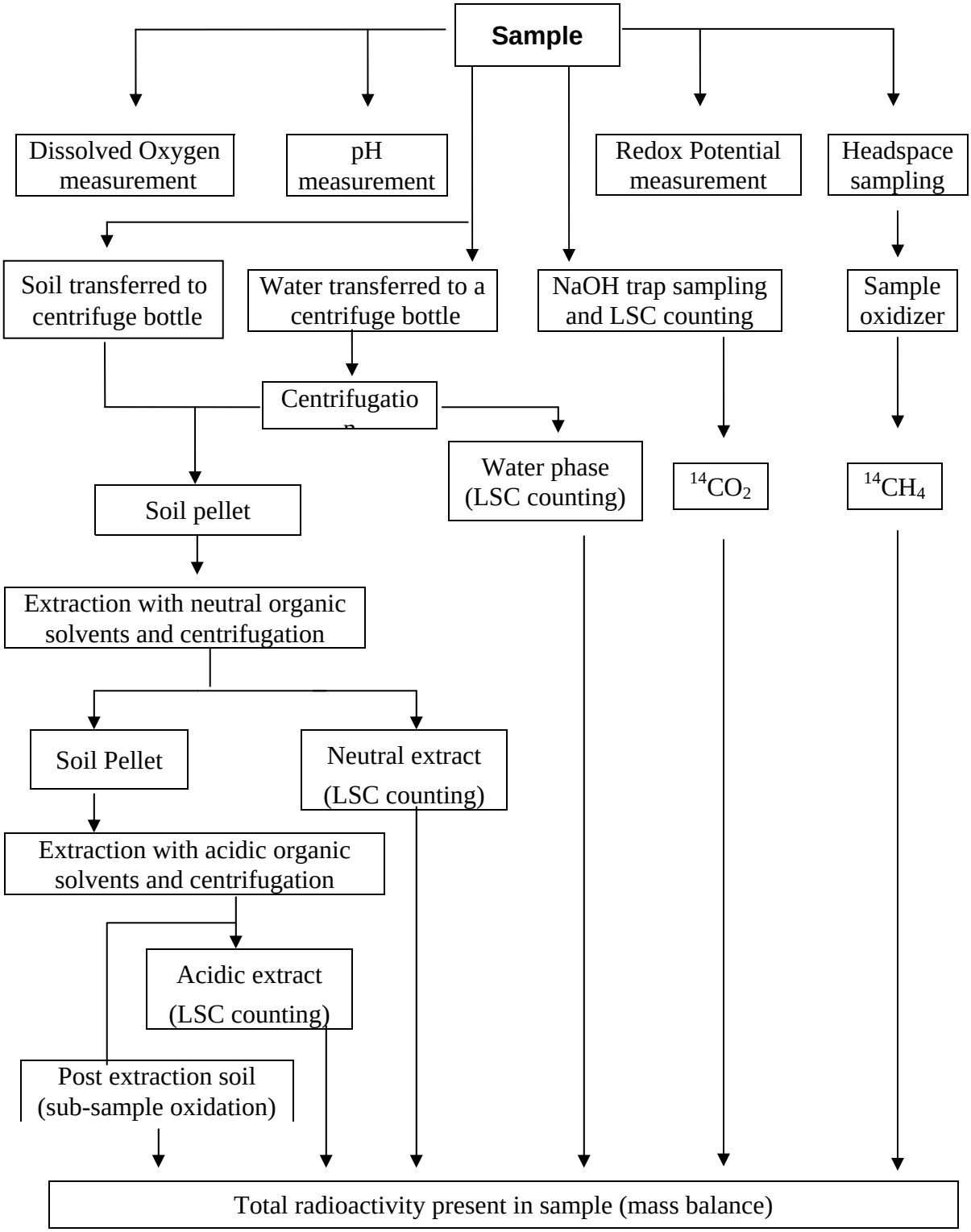


Figure 5. Flowchart summary of PES residues fractionation

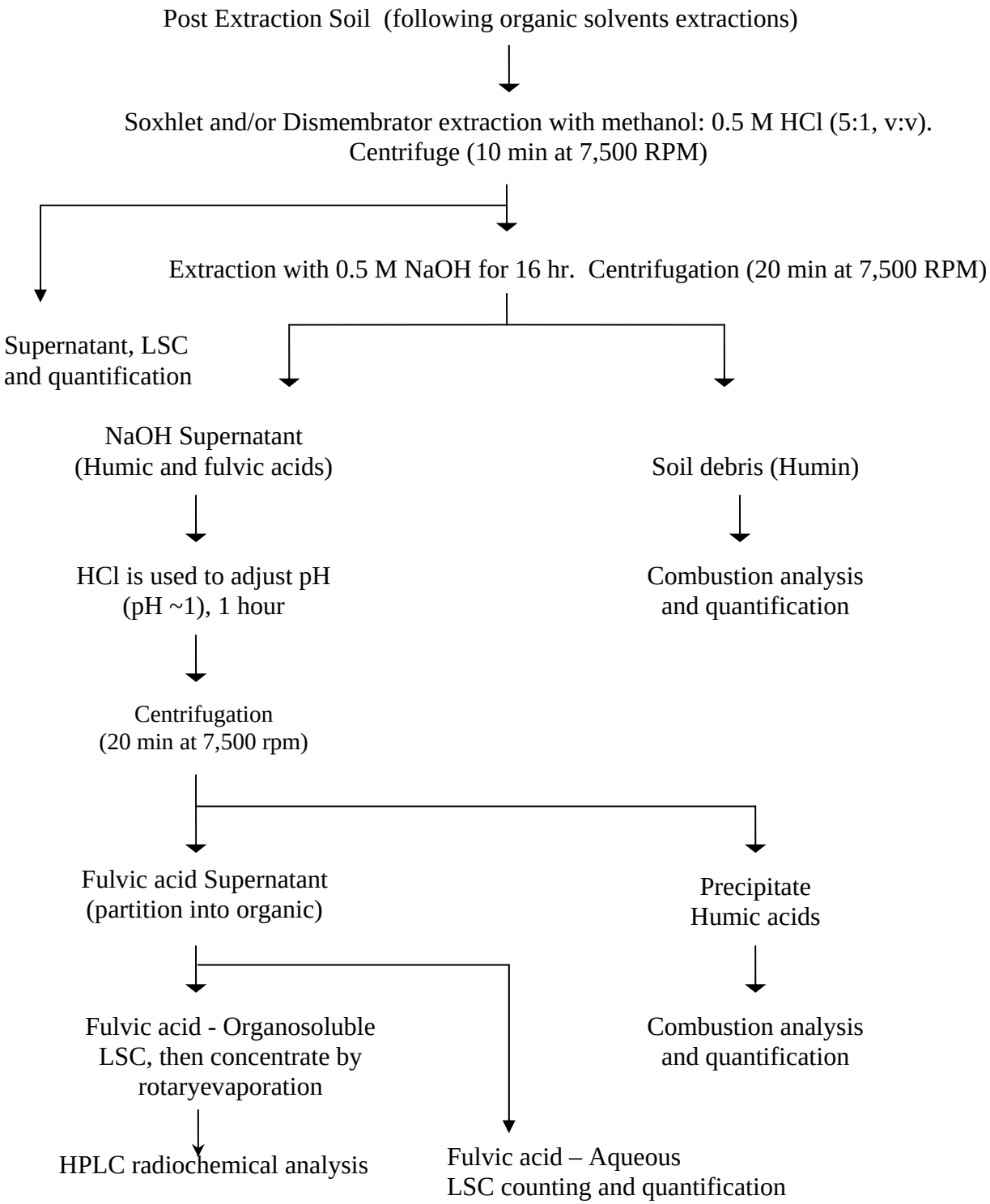


Figure 6. Summary of water, soil extracts, volatiles and soil-bound radioactivity for the RB anaerobic soil study (aerobic phase ended at 30 days)

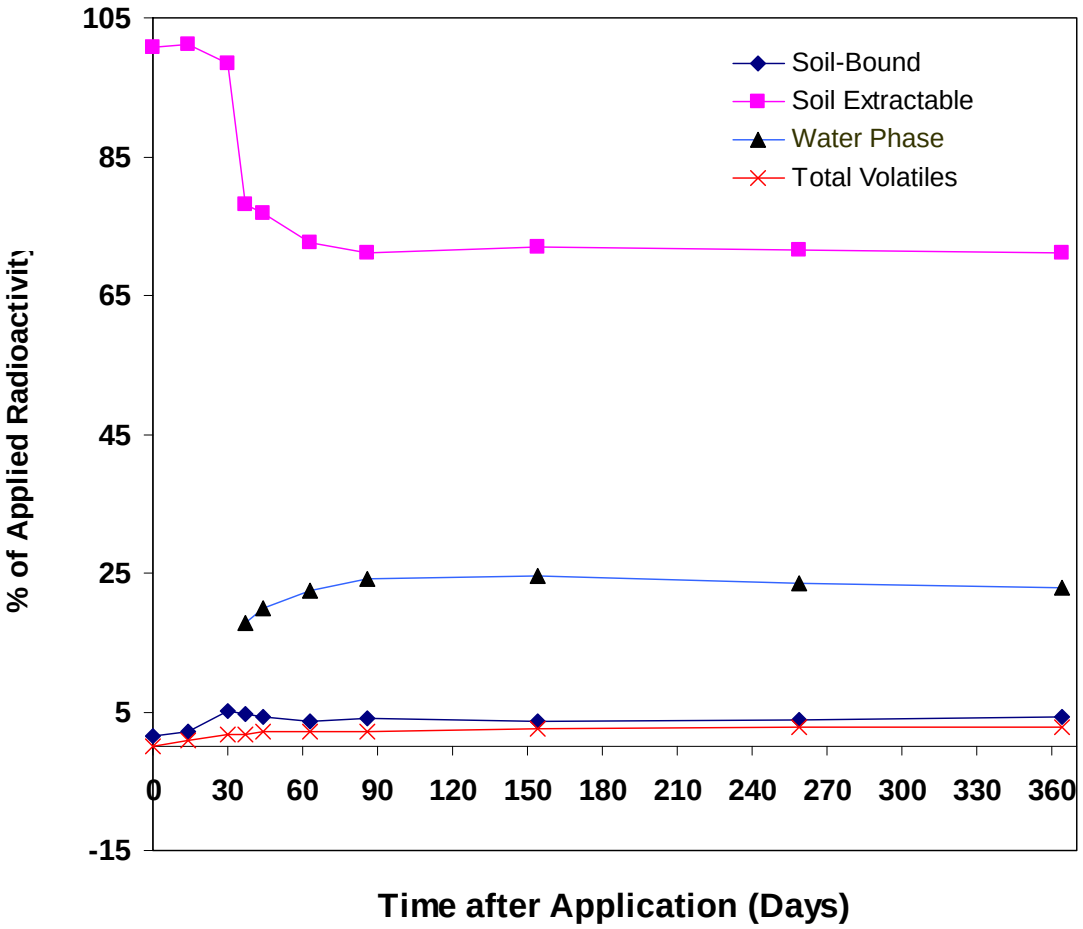


Figure 7. Summary of water, soil extracts, volatiles and soil-bound radioactivity for SB anaerobic soil study (aerobic phase ended at 34 days)

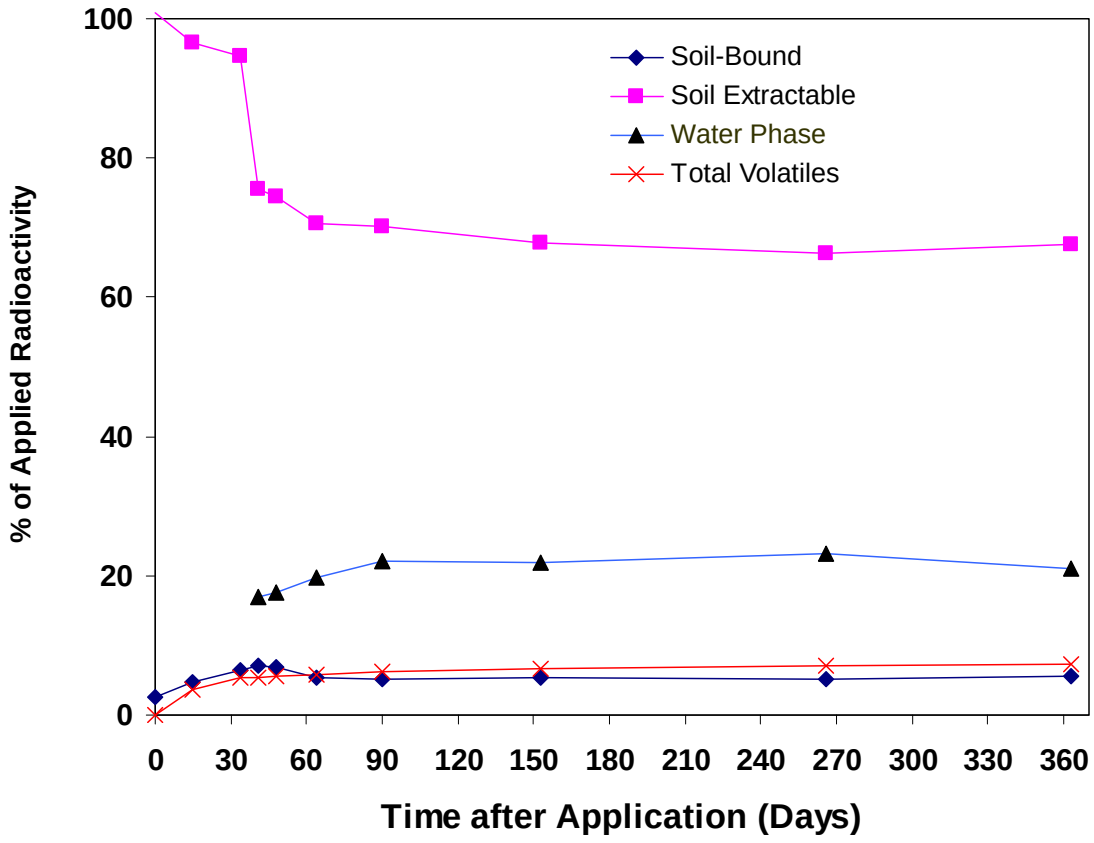


Figure 8. Summary of water, soil extracts, volatiles and soil-bound radioactivity for RP anaerobic soil study (aerobic phase ended at 34 days)

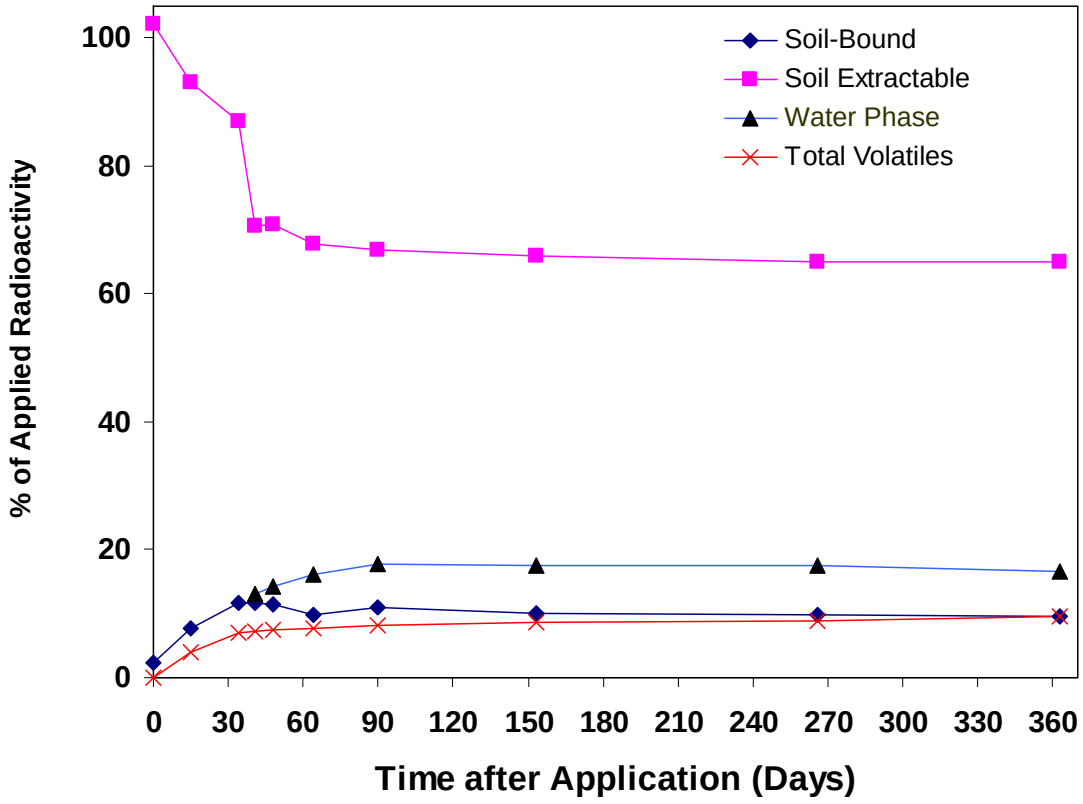


Figure 9. Dissipation of S-2200 from the soil phase of the three anaerobic soil studies - neutral and acidic Extracts (aerobic phase ended at 30 or 34 days)

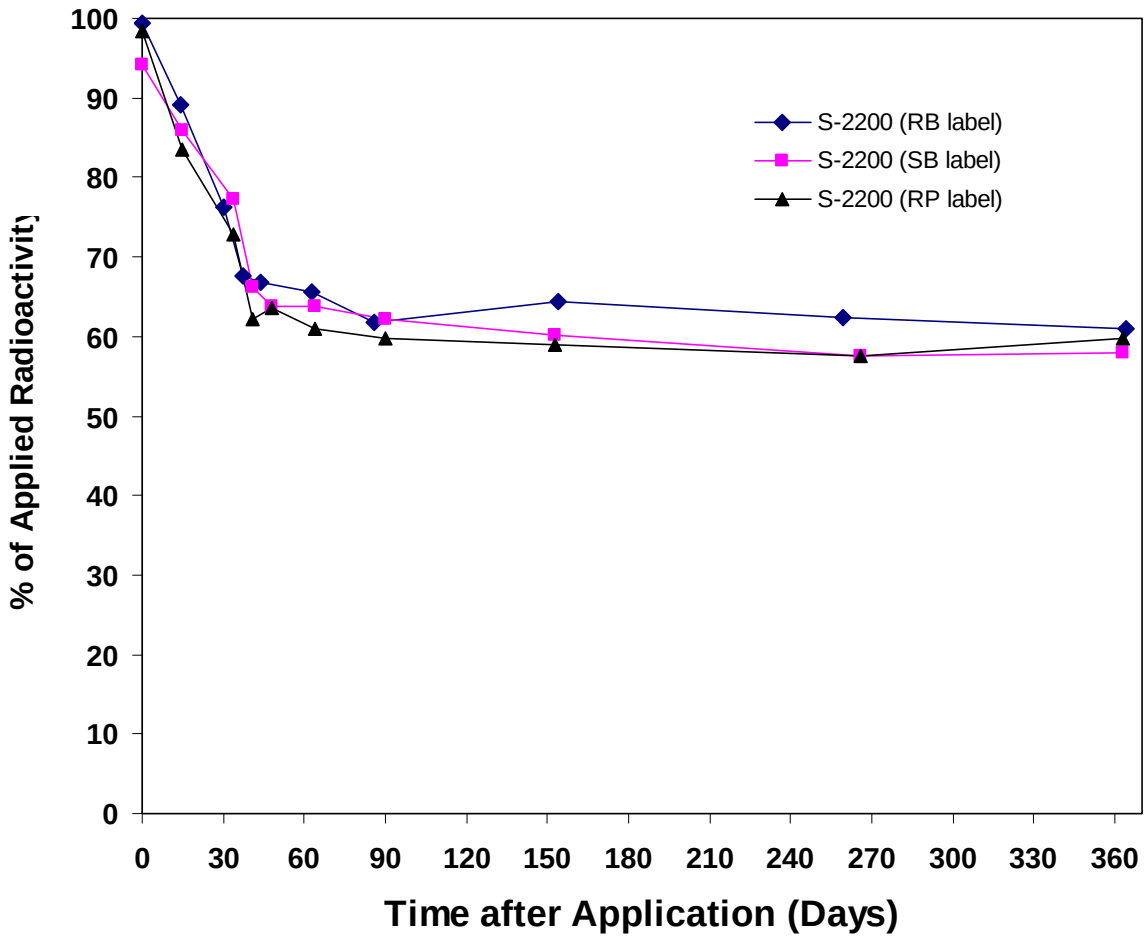


Figure 10. Dissipation of S-2200 from the water phase of the three anaerobic soil studies (aerobic phase ended at 30 or 34 days)

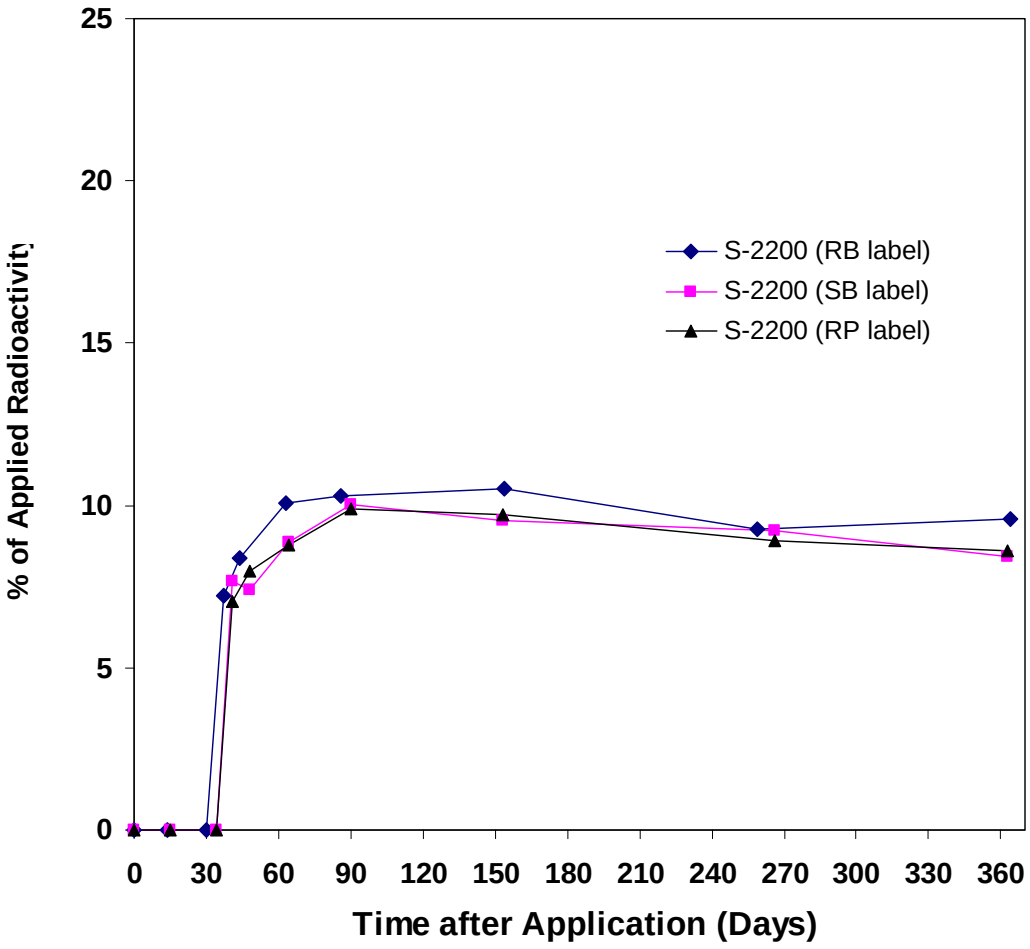


Figure 11. Dissipation of S-2200 from the three anaerobic soil studies (total soil extracts and water extracts combined (aerobic phase ended at 30 or 34 days)

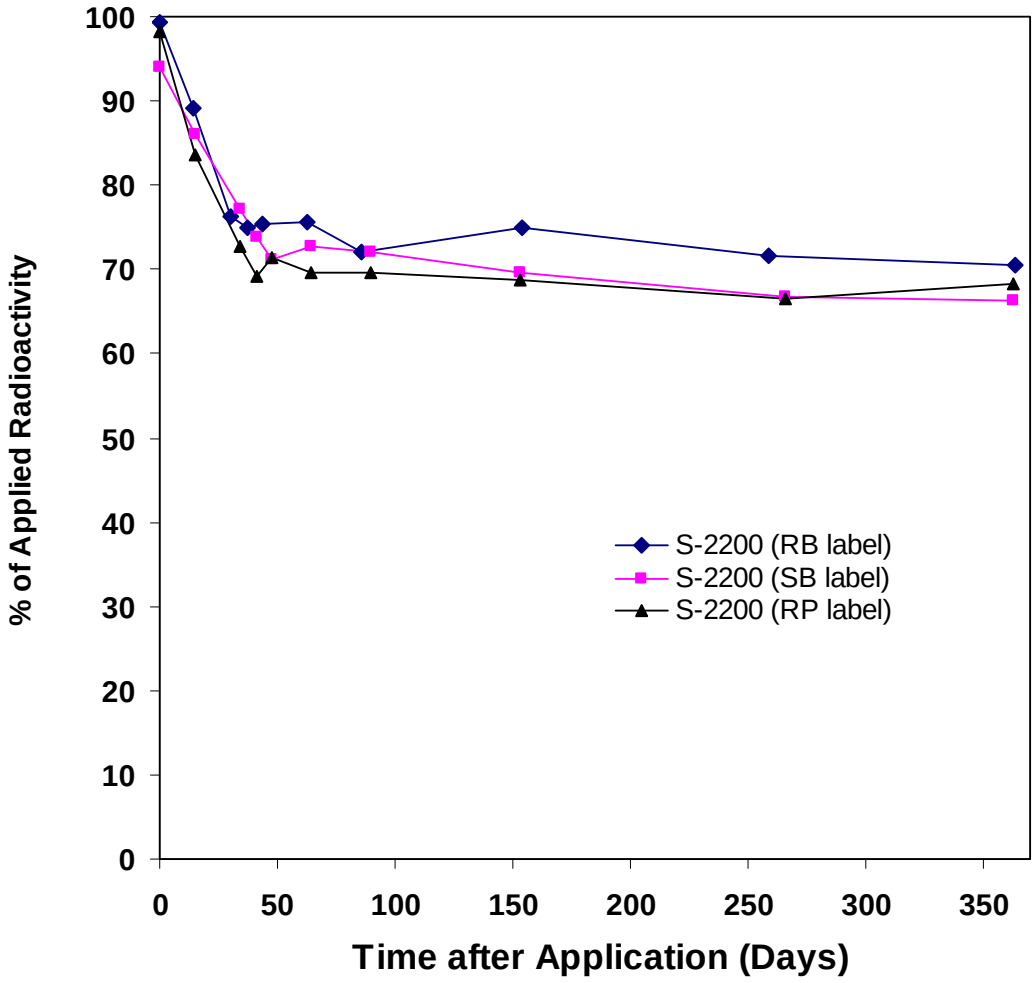


Figure 12. Formation and decline of S-2200 degradates in the soil of the RB anaerobic soil study - neutral and acidic extracts (aerobic phase ended at 30 days)

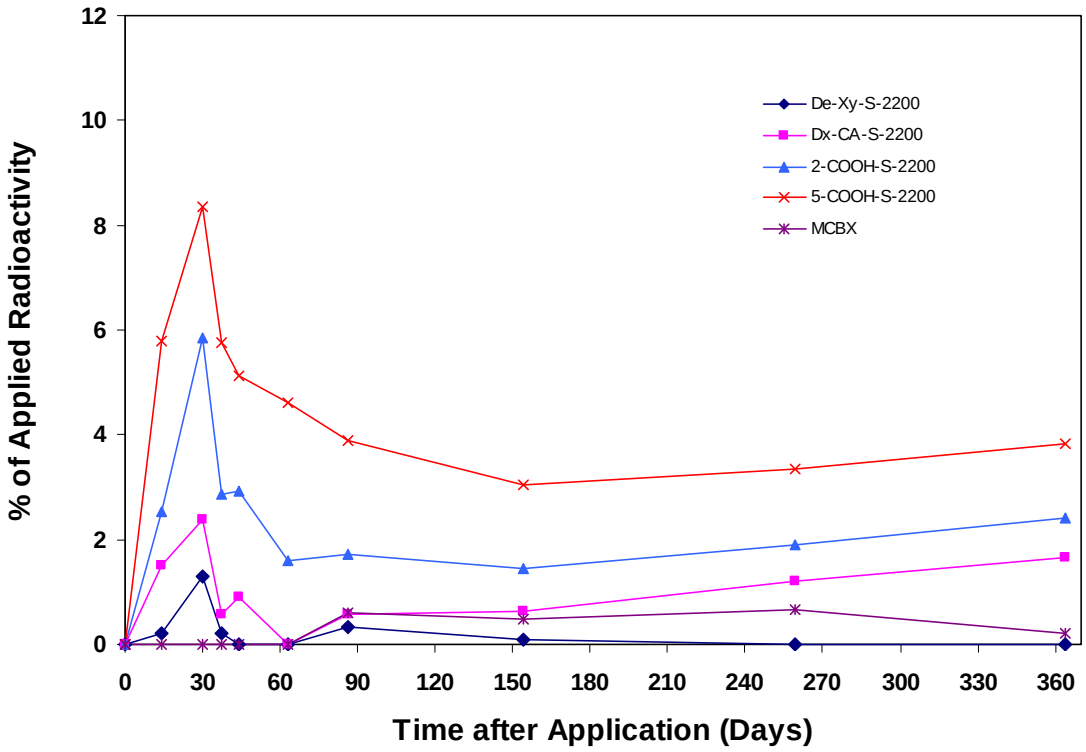


Figure 13. Formation and decline of S-2200 degradates in the water phase of the RB anaerobic soil study - (aerobic phase ended at 30 days)

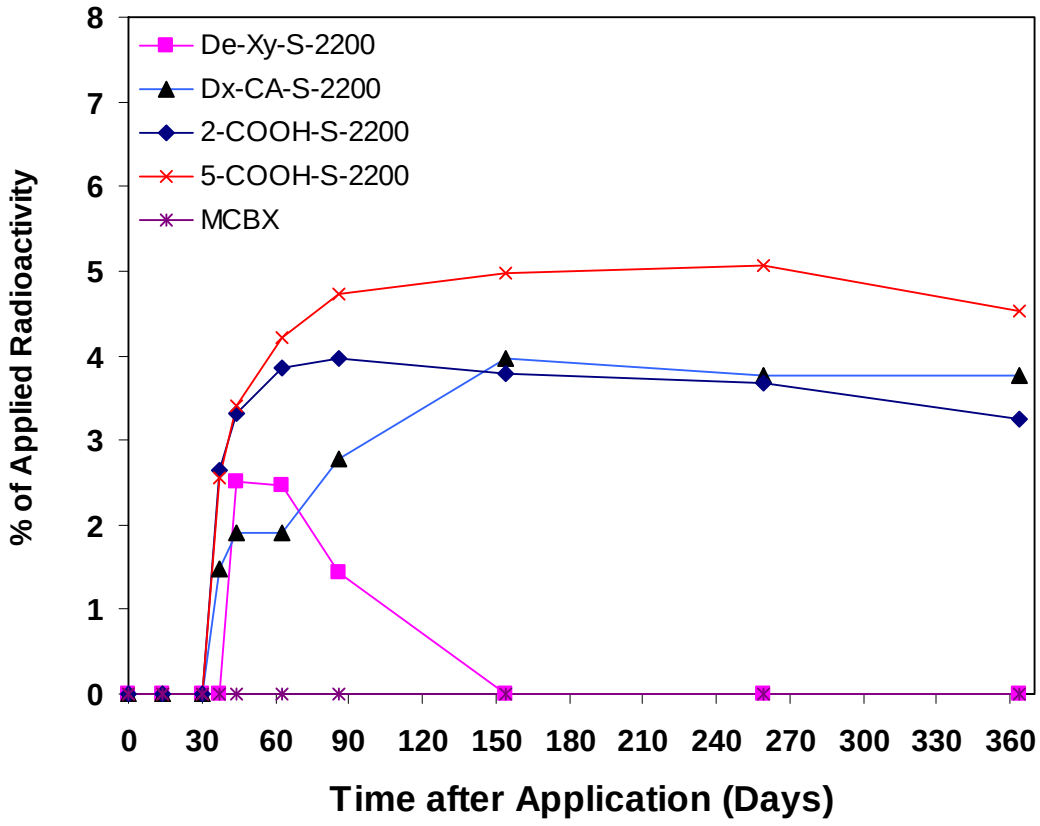


Figure 14. Formation and decline of S-2200 degradates in RB anaerobic soil study (total soil extracts and water extracts combined) - (aerobic phase ended at 30 days)

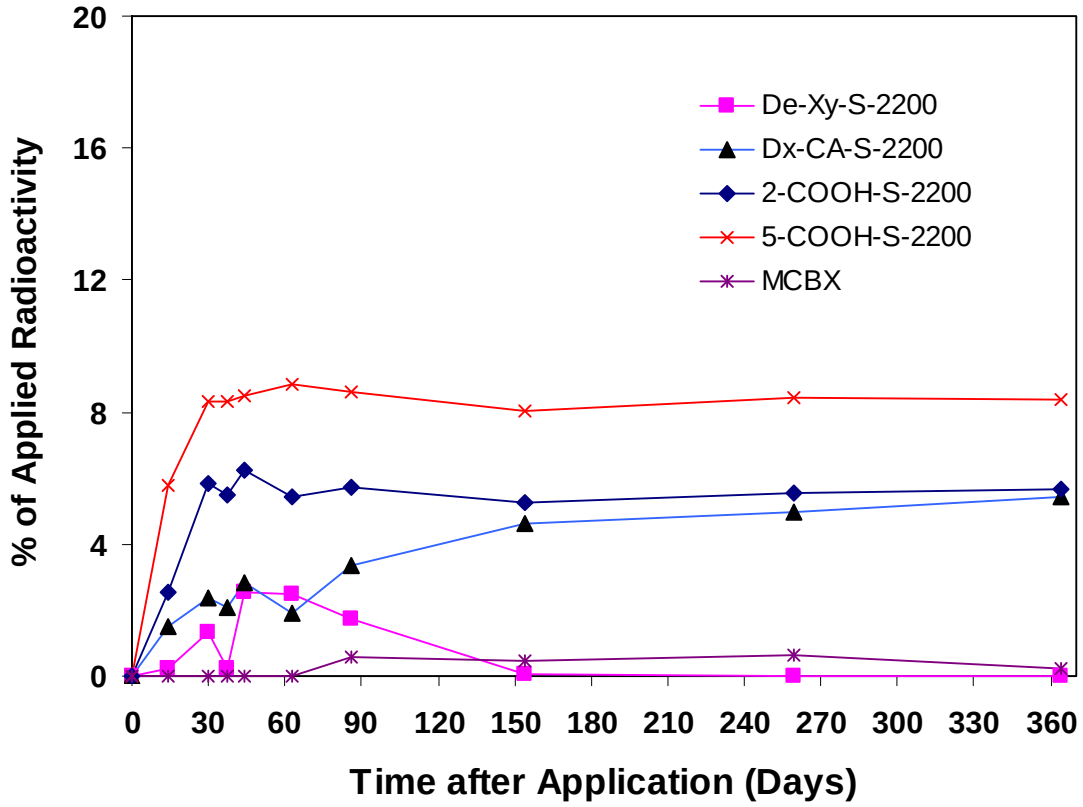


Figure 15. Formation and decline of S-2200 degradates in the soil of the SB anaerobic soil study - neutral and acidic extracts (aerobic phase ended at 34 days)

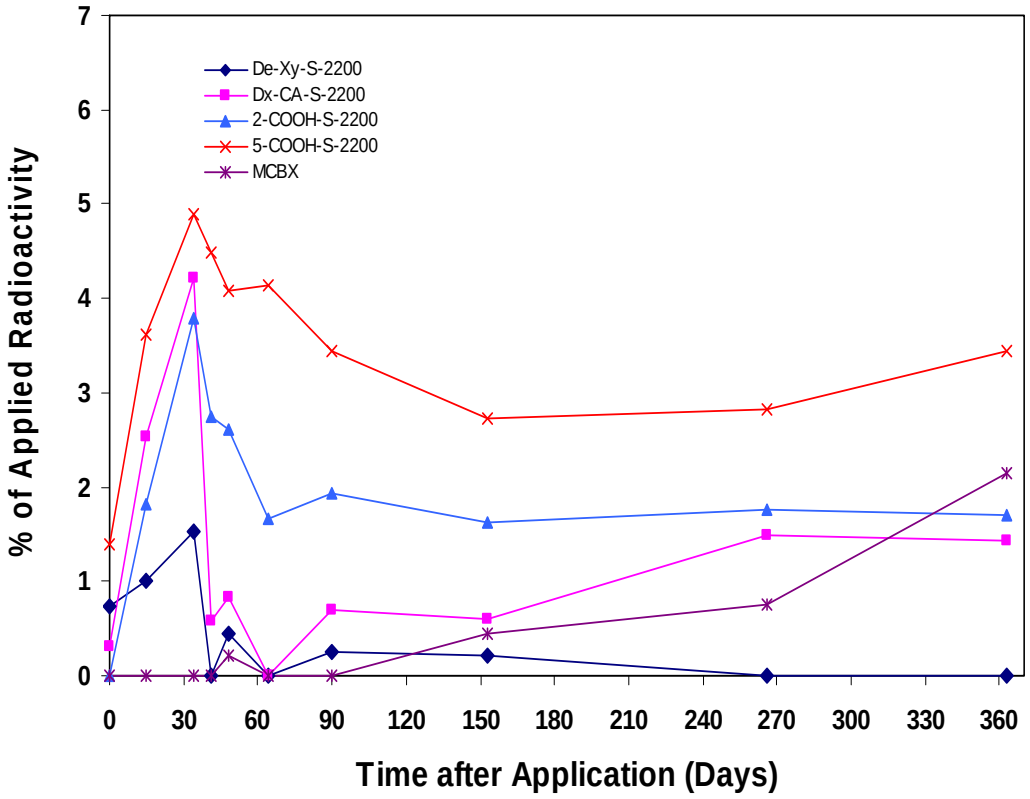


Figure 16. Formation and decline of S-2200 degradates in the water phase of the SB anaerobic soil study - (aerobic phase ended at 34 days)

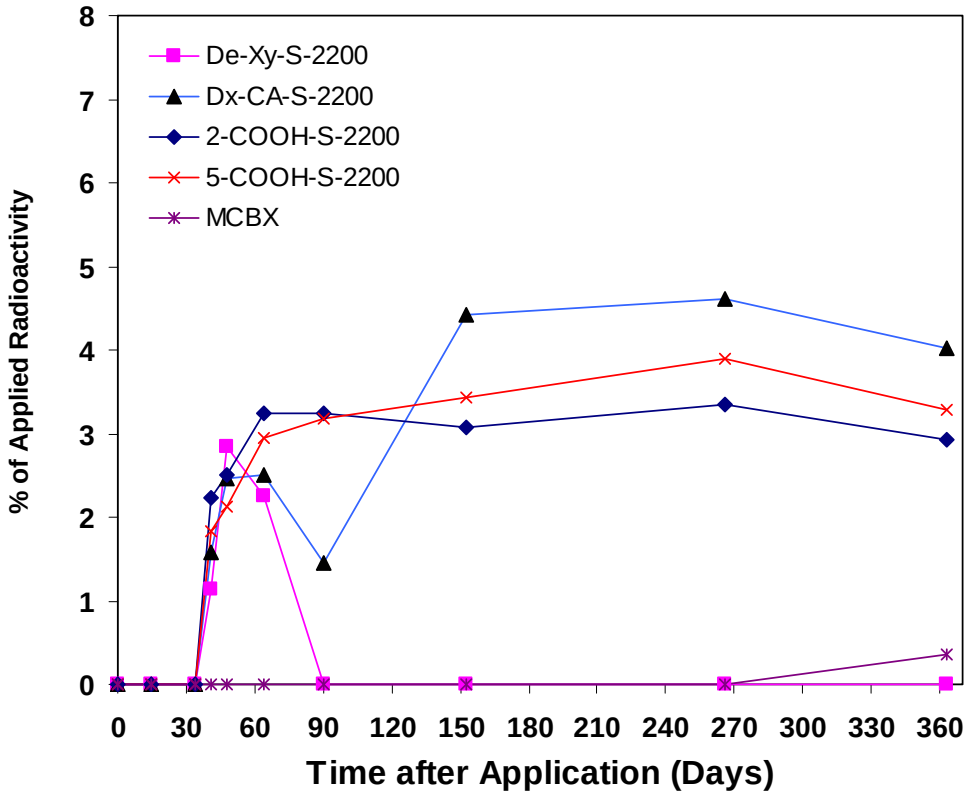


Figure 17. Formation and decline of S-2200 degradates in SB anaerobic soil study (total soil extracts and water extracts combined) - (aerobic phase ended at 34 days)

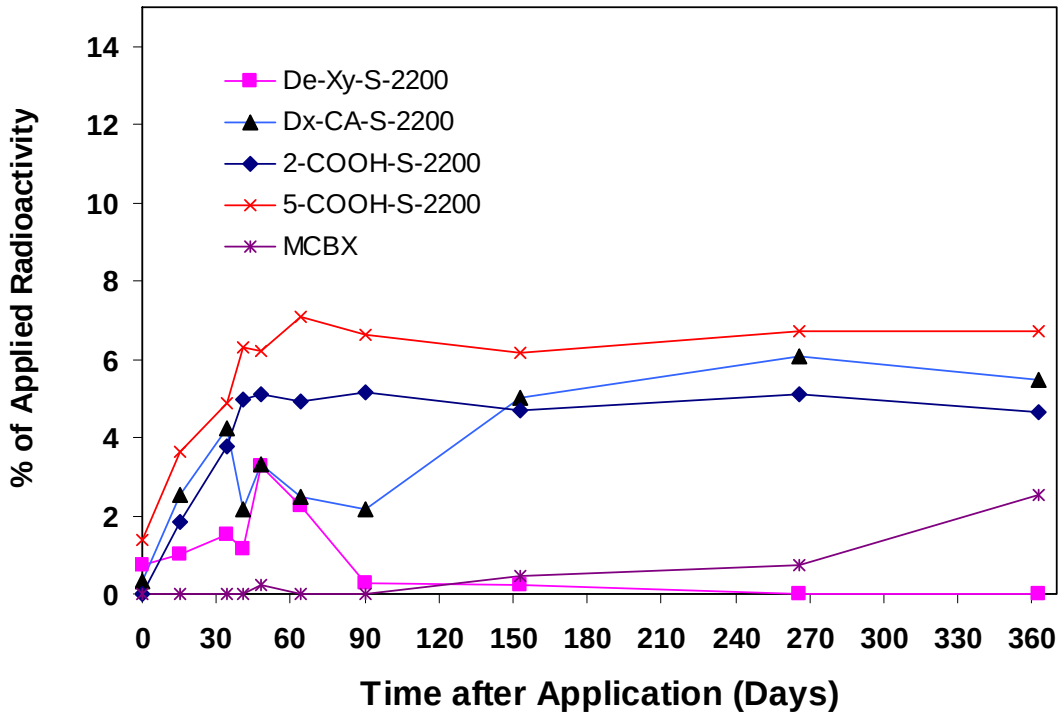


Figure 18. Formation and decline of S-2200 degradates in the soil of the RP anaerobic soil study - neutral and acidic extracts (aerobic phase ended at 34 days)

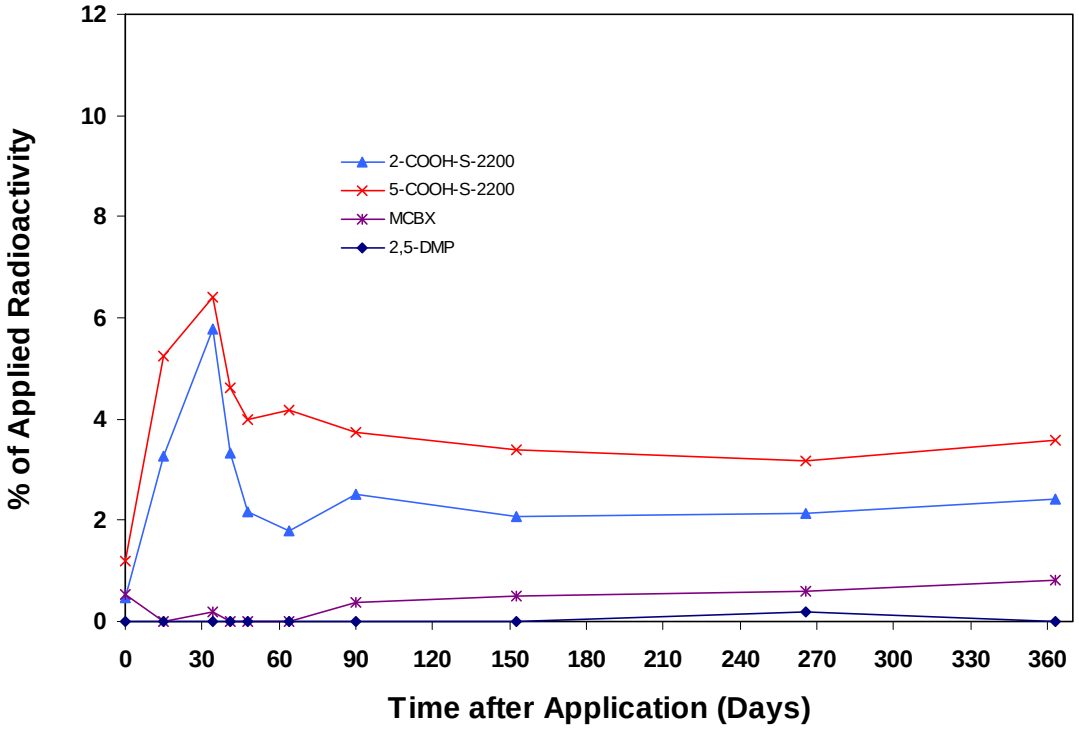


Figure 19. Formation and decline of S-2200 degradates in the water phase of the RP anaerobic soil study - (aerobic phase ended at 34 days)

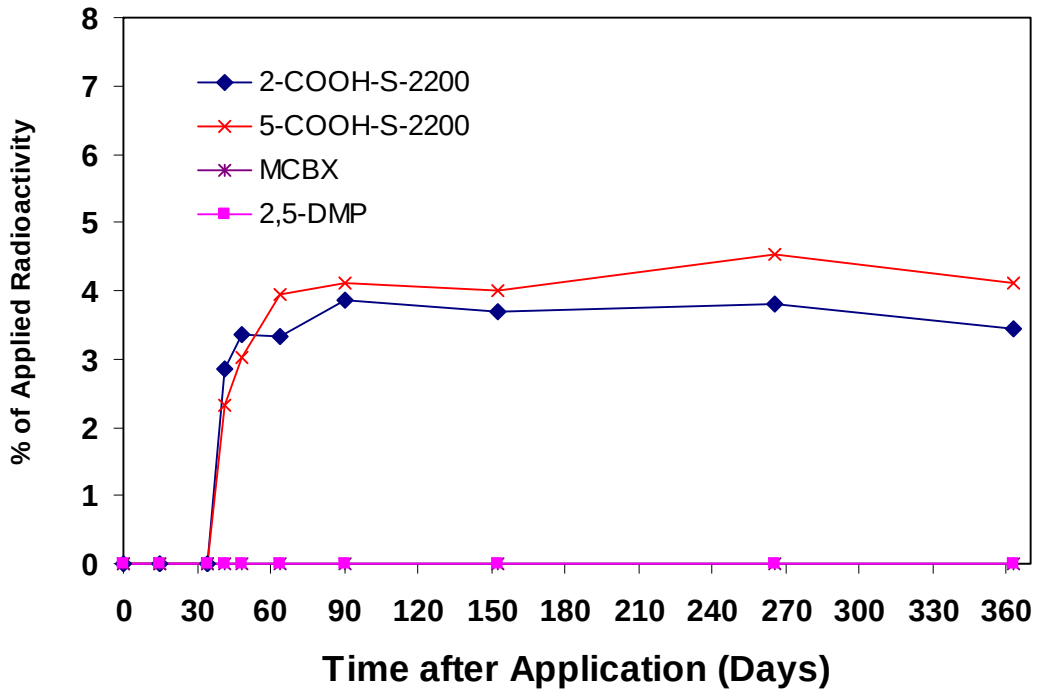


Figure 20. Formation and decline of S-2200 degradates in RP anaerobic soil study (total soil extracts and water extracts combined) - (aerobic phase ended at 34 days)

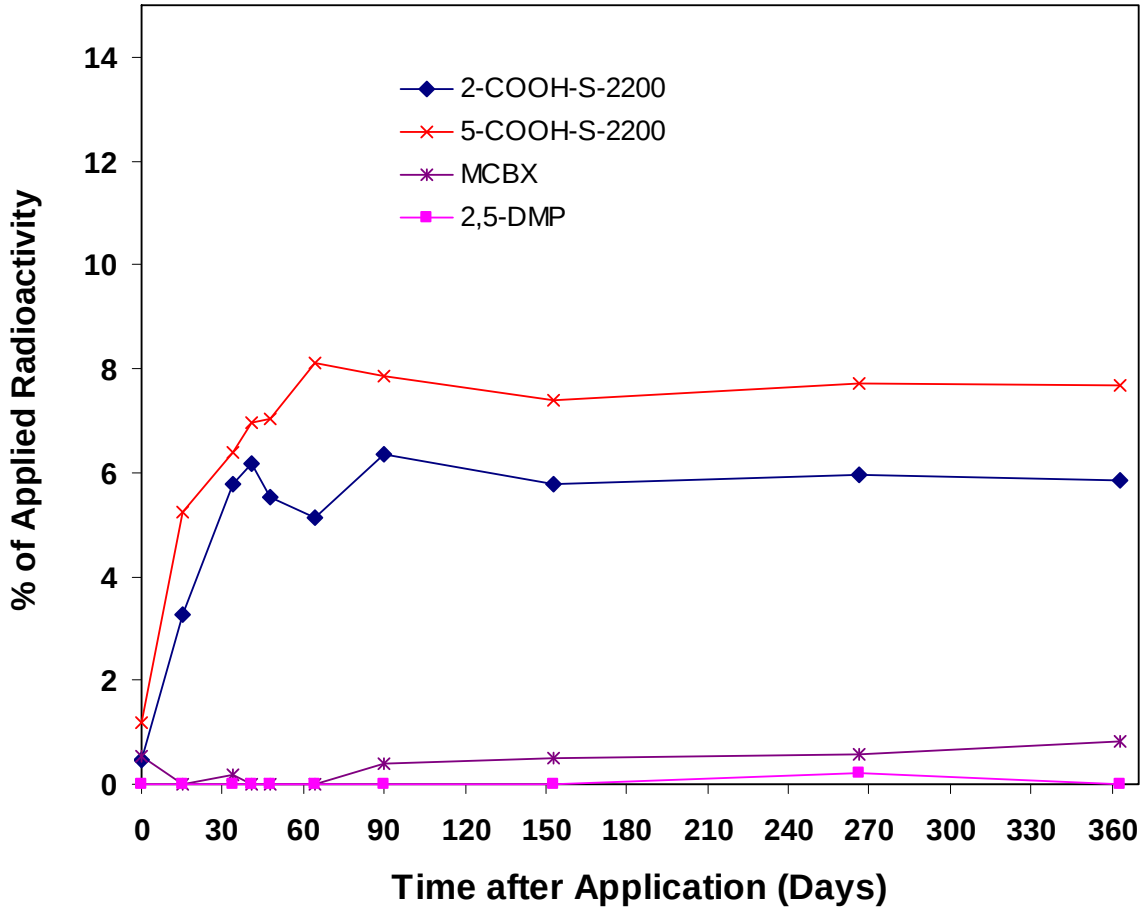
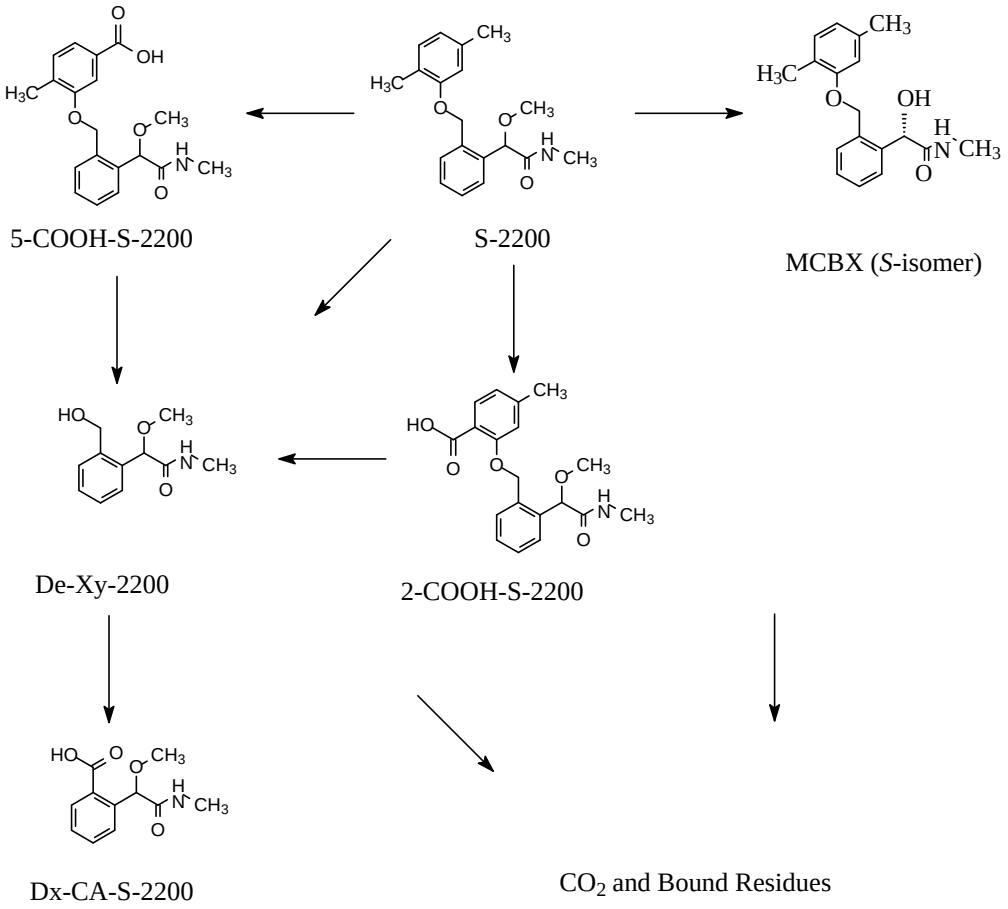


Figure 21. Proposed anaerobic soil degradation pathway of S-2200
(Compounds were primarily formed during the aerobic phase)



Appendix A. Study Protocol, Amendments and Deviations

VALENT U.S.A. CORPORATION

STUDY PROTOCOL

1 TITLE

S-2230 Anaerobic Soil Metabolism Study

2 STUDY NUMBER

Protocol No.: Valent 37166
Project No.: VP-37166

3. STUDY OBJECTIVE

The objective of this study is to determine the rate of degradation and route of dissipation (i.e., radiolabeled compounds of S-2230 - [Benzyl-^{14}C]S-2167 (R-isomer), [Benzyl-^{14}C]S-2384 (S-isomer), and [Phenoxy-^{14}C]S-2167 (R-isomer)) in a single U.S. soil under anaerobic conditions. The study was designed to meet the EPA data requirements for the registration of pesticides in the United States, as set forth in 40 CFR § 158.1320(e) at will be conducted in accordance with EPA Data Requirements and Transformation Guidelines, EPA/FIFRA §85-2300 Anaerobic Soil Metabolism.

4. JUSTIFICATION OF TEST SYSTEM

During agricultural use, circumstances may occur where the test substance is applied to a terrestrial soil that may become water-logged and in which anaerobic conditions may develop. The test system will consist of a sieved soil with a pH, organic carbon content, clay and texture representative of agricultural soils. The agricultural location from which the soil was collected will be presented in the Field Report. The soil (the moisture level of 50 ± 10% of the maximum water holding capacity (WHC) during the aerobic phase and will be waterlogged during the following anaerobic phase. The test system will be contained in an open beaker within an incubation chamber during the aerobic phase and quantitatively transferred to a closed biometer flask for the anaerobic phase (Figure 1). Figure 14 is the isomer of choice for environmental fate studies. The positions of labeling chosen are considered to be resistant to transformation and suitable for identification of degradates. Three separate studies will be conducted on a single soil with the *R* and *S* isomers of [Benzyl-^{14}C]S-2167 (R-isomer), [Benzyl-^{14}C]S-2384 (S-isomer), and [Phenoxy-^{14}C]S-2167 (R-isomer) to determine the effect of chirality on rate of degradation and metabolite determination. Studies are being conducted primarily with the *R* isomer since it is the biologically active isomer.

Appendix A. Study Protocol, Amendments and Deviations

5. STUDY CONDUCT

This study is intended to support the registration of the test substance and products containing the test substance. The intent is to submit this study to Regulatory Authorities. The study will be conducted in U.S. Environmental Protection Agency Good Laboratory Practice (GLP) Standards specified in 40 CFR Part 160.

5.1 Study Location

Valent Technical Center (VTC)
6560 Trinity Court
Dublin, CA 94568
USA

5.2 Study Director

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5.3 Study Sponsor

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Sponsor Representative

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Appendix A. Study Protocol, Amendments and Deviations

6. PROPOSED STUDY SCHEDULE

6.1 Experimental Start Date

The experimental start date will be the date that the Study Director signs the protocol. The date of the first application of the test substance to the test systems

6.2 Experimental Termination Date

The study may be terminated before 120 days after the anaerobic phase provided that "ultimate transformation pathway and ultimate mineralization" has been reached. Termination after 120 days and the anaerobic phase is possible if at least 90 % of parent has been transformed, but only if at least 5 % of [illegible] has been formed

7. MATERIALS

7.1 Test Substances

Sumitomo Chemical Company will supply the test substance. Records of the methods of synthesis, fabrication, and/or derivation of the test substance will be maintained by Sumitomo Chemical Company

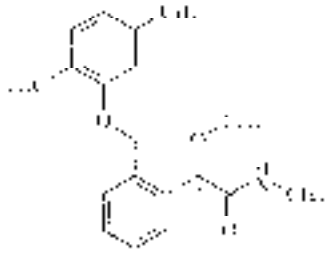
The S-2210 test substances include two chiral (*R* & *S*) and two differential label positions ([Benzyl-^{14}C]S-2367 (*R*-isomer), [Benzyl-^{14}C]S-2384 (*S*-isomer), and [Phenoxy-^{14}C]S-2367 (*R*-isomer)). Identical rate and route of degradation experiments will be conducted using the three radiolabeled test substances. Non-radiolabeled test substances will be characterized by

7.1.1 Non-radiolabelled S-2210

Future Code Name:	S-2210, V-101301
Isomer Code Name:	S-2367 (R-isomer of S-2207)
	S-2384 (S-isomer of S-2207)
Common Name:	Not available
Chemical Name:	(R)-2-(4-phenoxy-3-trifluoromethylphenyl)-2-[(2-pyridylthio)acetamido]propionic acid (IUPAC) (R)-2-(4-phenoxy-3-trifluoromethylphenyl)-2-[(2-pyridylthio)acetamido]propionic acid (CAS)
CAS Number:	[illegible]
Molecular Weight:	514.50
Molecular Formula:	$C_{24}H_{19}F_3N_2O_4S$ (non-radiolabeled)
Test Site & Charge Purity:	S-2210, [illegible]

Appendix A. Study Protocol, Amendments and Deviations

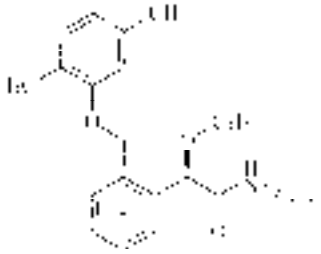
	[illegible]
	[illegible]
Water Solubility	[illegible]
Partition Coefficient	[illegible]
Chiral Isomers	[illegible]



2.1.2 [Benzyl-^{14}C] S-2167 (R isomer)

Chemical Name	[illegible]
	[illegible]
Test No.	[illegible]
Source	[illegible]
Valent Document No.	[illegible]
Specific Activity	[illegible]

Radiochemical Purity [illegible]



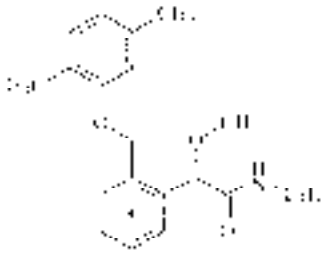
Purity: The chemical/enantiomeric purity was determined before preparation of the test substance. If the radiochemical purity was [illegible] by Valent Analytical

Appendix A. Study Protocol, Amendments and Deviations

7.1.3 Benzyl-^{14}C]S-2354 (S isomer)

Chemical Name:	(RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy-o-tolyl]acetamide (IUPAC)
Lot No.:	[RS20105-102] (radiochemical)
Source:	Synthesized in Chemical Dev., Ltd.
Valent Inventory No.:	406
Specific Activity:	4.32 GBq/mmol, 14 mCi/mmol, 801.308 dpm/µg

Radiolabeled Position of the Molecule: Uniformly radiolabeled in the benzyl ring

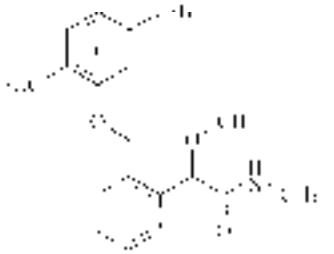


Purity: The radiochemical purity will be determined before preparation. Prior to test solutions, the radiochemical purity is 98% or greater. Valent Chemical purity at

7.1.4 [Phenoxy-^{14}C]S-2167 (R isomer)

Chemical Name:	(RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide (IUPAC)
Lot No.:	[RS20104-102] (radiochemical)
Source:	Synthesized in Chemical Dev., Ltd.
Valent Inventory No.:	404
Specific Activity:	4.44 GBq/mmol, 12 mCi/mmol, 850,980 dpm/µg

Radiolabeled Position of the Molecule: Uniformly radiolabeled in the phenoxy ring.



Appendix A. Study Protocol, Amendments and Deviations

Purity: The test chemical purity will be determined before preparation of the test solutions. If the radiochemical purity is <95%, then Valent will purify it.

7.2 Reference Substances (non-radiolabelled)

Advised all reference chemicals used in the study, their chemical names, structure, Valent inventory numbers and purity will be added to the protocol by amendment.

7.3 Storage Conditions

Where appropriate the test substances or solutions thereof will be stored in freezers (nominal -15 °C). Reference compounds, or solutions thereof, may be stored in a refrigerator (nominal 5 °C). Aqueous test solutions or solutions may be stored in freezers (nominal -15 °C) or in a refrigerator (nominal 5 °C).

7.4 Soil and Water

Soil from Replicate A (Pierce series) will be collected prior to test initiation and used to determine the rate of degradation and route of dissipation. This is the same soil used in the aerobic soil studies. The soil will be thoroughly mixed and passed through a 2 mm mesh sieve with a maximum of air drying. After sieving the moisture content at 1/3 bar for the entirety of the soil will be determined. If necessary, the soils will be stored in the dark in accordance with EPA OPPTS 850.1600 (1998), Soil Quality – Sampling – Part A: Soil storage conditions and length of storage. Bulk samples and subsamples of the soil stored under UHP N_2 gas (oxygen purged with nitrogen) will be used to maintain moisture or to water by the soil.

Soil characterization of material according to EPA will be performed by A&L Laboratories Inc. (Northwood, ND) and will include texture (ASU and USDA classification), pH, soil cation exchange capacity, organic carbon, saturated bulk density and water retention characteristics. The characterization results will be recorded in the final report. Soil microbial analyses conducted will be determined using the previously mentioned procedures (OCSPP [8] Compliance, Inc. (Northwood, ND)). Soil microbial analyses conducted will be determined by Stoneybrook Laboratories (Wareham, MA) during and at the end of the incubation. Soil moisture also will be determined at other times during the study.

Appendix A. Study Protocol, Amendments and Deviations

8. EXPERIMENTAL PROCEDURES

OVERVIEW The study involves the exposure of S-2200 to aerobic soil degradation followed by exposure to anaerobic aquatic degradation. All samples (Treatments A – C, each in 3 x) will be exposed to an aerobic and anaerobic phase.

Aerobic phase: Soil samples will be placed into glass beakers and the beakers placed into an incubation chamber. The incubation chamber will be placed in a dark, constant temperature walk-in incubator (25 ± 2 °C). After a suitable pre-incubation period (7-28 days), the samples will be dosed with the test substances ([Benzyl-^{14}C] S-2200 (R-isomer), [Benzyl-^{14}C] S-2200 (S-isomer), [Benzyl-^{14}C] S-2200 (racemate) and non-radiolabeled S-2200). The beakers will be returned to the incubation chamber in the dark at constant temperature walk-in incubator. The incubation chambers will be equipped for the collection of ^{14}CO$_2$ volatiles (using ethanol and/or ethylenediamine traps). The samples moisture level will be adjusted to 50% – 100% of the maximum water holding capacity (WHC) as needed throughout the study. At pre-determined intervals (pre-application, 0 hr, 24 hr, 48 hr, 72 hr, 96 hr, 120 hr, 144 hr, and day 30) duplicate samples will be removed from the incubation chamber for radiochemical analyses to determine mass balance, recoverability of radioactivity and estimate the aerobic degradation half-life. The day 14 analysis may be eliminated if data from the S-2200 aerobic soil study (MRID 45937101) indicates a half-life for parent in soil of less than 30 days. The traps will be analyzed for radiocarbon content at each sampling interval and, if necessary, between sampling intervals. If greater than 10% of the applied radioactivity is found in any sample, the residue(s) will be characterized and, if possible, identified.

Anaerobic phase: The establishment of anaerobic conditions will begin after S-2200 has degraded aerobically through one half-life or approximately 30 days (whichever comes first). Each soil sample will be quantitatively transferred to an anaerobic test system flask and the exposure conditions changed to promote the establishment of an anaerobic environment. Dry ground filter aided biosolids (sewage sludge) at a rate of 0.1% (w/w) of the soil dry weight is spiked into the first layer of aerobic soil (~ 1 cm) to ensure the survival of the microbial inoculum. Sterile DI H$_2$O (to the water holding capacity) will be added to overlay the soil (~ 3 cm depth) to establish anaerobic conditions. The actual procedures to be used in the study will be described in the final report. The soil and water will be sparged with 2 – 3 % N$_2$/98 – 97% H$_2$ nitrogen purged gas for the collection of ^{14}CO$_2$. To facilitate the formation of anaerobic conditions, the incubator headspace will be purged with a stream of nitrogen gas for 0.25 to 0.5 hr. The biometers will be periodically vented during the nitrogen purge to the atmosphere to facilitate the release of air trapped in the soil. The sealed biometers will be returned to the constant temperature walk-in incubator (20 – 25 °C). At pre-determined intervals during the anaerobic phase, duplicate flasks (a replicate of each treatment) will be removed from the incubator to confirm the establishment of anaerobic soil conditions, conduct radiochemical analyses, [illegible] mass balance

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will be calculated. If greater than 10% of the applied radioactivity is found in any sample, the results will be characterized and if possible identified.

8.1 Incubation Unit Preparation

Separate, identical water reports will be prepared for the treatments described below (Treatments A – C, see Table 3). For each experiment, soil samples (50 g dry weight) will be placed into glass beakers (quantity of beakers necessary, adjusted to 80 – 85% of the water holding capacity with sterile, HPLC grade water. The glass beakers will be placed into an incubation chamber. The incubation chamber will be placed into a dark, constant temperature walk-in incubator (20 ± 2 °C). For each radiolabeled study, 18 units will be needed for the specified sampling intervals, but up to a total of 21 units (including extra units) may be prepared. Additional units intended for use as untreated controls (Treatment A), solvent controls (Treatment B), [UL-^{14}C]-D-glucose controls (Treatment C), and non-radiolabeled S-2200 (Treatment D) will be prepared and placed into an incubation unit.

8.2 Preincubation Time

Prior to treatment with the test substance, soil units will be incubated under the experimental conditions for 2-28 days. The actual pre-incubation time will be given in the Report. The aerobic and anaerobic characteristics of the test soil are a prerequisite for this study. Therefore, the test system may be prepared and incubated for an established period of time before the Study Director signs the protocol. The study initiation date will be the date that the Study Director signs the protocol, that date will be before the test substance is added to the test system.

8.3 Test System Treatment and Maintenance

After an suitable pre-incubation period, some of the soil units will be dosed with the test substance (radiolabeled and/or non-radiolabeled S-2200). The test substance will be applied in water or a water miscible organic solvent (≤ 5% of soil dry weight basis). The rate, volume and method of application will be described in the study data and in the Final report. After dosing, all units will be immediately made aerobic and then anaerobic conditions as described below. The control substance [UL-^{14}C]-D-glucose will be applied in water only to Treatment C units after the establishment of anaerobic conditions. Untreated control substances will be added to the soil units or to the flasks as follows:

Treatment A: Untreated Controls. Samples will be used to measure the microbial biomass. Control samples will be analyzed to ensure that the level of test substance application is adequate at least one time zero. Other samples will be analyzed after the

Appendix A. Study Protocol, Amendments and Deviations

establishment of anaerobic conditions and at the end of sampling (i.e., at the last anaerobic phase sampling interval).

Treatment B: Organic solvent controls. Samples will be used to measure the effect of the organic solvent (acetonitrile) on the microbial biomass at the treatment concentration listed in Table 1. In preliminary work, it was determined that this assay can be done in water; therefore, Treatment B will not be performed.

Treatment C: [U-^{14}C]-D-glucose control. This treatment will be used to verify that anaerobic microbial activity in the anaerobic phase. Several harvest times will be selected with [U-^{14}C]-D-glucose after the establishment of anaerobic conditions has been indicated by low O_2 and negative redox potentials. Volatile production of $^{14}CH_4$ and $^{14}CO_2$ will be evaluated.

Treatment D: Non-radiolabeled S-2200 samples treated with non-radiolabeled S-[illegible] (AS-[illegible]). Samples will be used to measure the effect of non-radiolabeled S-2200 and S-[illegible] on microbial biomass at the test concentrations listed in Table 1.

Treatment E: [Benzyl-^{14}C]-S-2200 (Reference). Glass beakers will be dosed at the beginning of the aerobic phase. This treatment will be used to measure the test substance dissipation rate during the aerobic and anaerobic phases, and to [illegible] metabolite formation. Volatile production of $^{14}CO_2$ will be evaluated during the aerobic phase. Volatile production of $^{14}CH_4$ and $^{14}CO_2$ will be evaluated during the anaerobic phase.

Treatment F: Benzyl-^{14}C-S-2200 (Isomer). Glass beakers will be dosed at the beginning of the aerobic phase. This treatment will be used to measure the test substance dissipation rate during the aerobic and anaerobic phases, and its [illegible] metabolite formation. Volatile production of $^{14}CO_2$ will be evaluated during the aerobic phase. Volatile production of $^{14}CH_4$ and $^{14}CO_2$ will be evaluated during the anaerobic phase.

Treatment G: Phenoxy-^{14}C-S-2200 (Isomer). Glass beakers will be dosed at the beginning of the aerobic phase. This treatment will be used to measure the test substance dissipation rate during the aerobic and anaerobic phases, and its [illegible] metabolite formation. Volatile production of $^{14}CO_2$ will be evaluated during the aerobic phase. Volatile production of $^{14}CH_4$ and $^{14}CO_2$ will be evaluated during the anaerobic phase.

Aerobic Phase Maintenance: Four separate incubation chambers will be set up as described above containing Treatments A, B, C and D, untreated controls, organic solvent controls, [illegible] [U-^{14}C]-glucose controls, and non-radiolabeled S-2200, in separate

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chamber for Treatment 1. Purified air will be passed through each chamber and over the surface of the soil, using a negative pressure system. The chamber will contain a central sampling port for the collection of $^{14}CO_2$ and series of peripheral traps for the collection of $^{14}CO_2$ evolved, including $^{14}CO_2$. Petri dishes containing 50 mL of 1 M NaOH solution will be placed inside the chamber to capture evolved $^{14}CO_2$. A series of inlet lines are connected to the chamber outlet tubes. The chamber lid will not be sealed off CO_2, however it will be bubbled through water traps to help prevent ingress of atmospheric CO_2. The chamber will be flushed for a minimum of at least two consecutive NaOH traps to capture any $^{14}CO_2$ not associated with the NaOH traps inside the chamber. The continuous airflow will maintain aerobic conditions and evacuate any microbial volatiles into the outlet traps.

One of two open Petri dishes containing water will be placed inside the chamber to maintain high air humidity and prevent loss of soil moisture. The soil traps will be weighed at appropriate intervals and any water loss will be replaced with sterile HPLC grade water. The samples will be incubated in the dark at 20 ± 2 °C. Airflow rate will be increased for at least 0.5 h prior to opening the chamber to purge any ^{14}C volatiles.

Anaerobic Phase Maintenance: The sealed incubation vessels will be incubated in the dark at constant temperature with a target of 20 ± 2 °C.

When aerobic conditions are established, the control substance ^{14}C-glucose will be added to the Treatment 2 flasks at the concentration specified in Section 8.4.2. The control substance will be delivered in a target weight of 1 mL of sterile HPLC grade water. The ^{14}C-glucose will be taken up microbially through the aerobic respiration of the biomass flask. The control substance will be distributed into the water phase by gentle swirling the flask. At least one control duplicate flasks is replaced with a sealed flask to maintain anaerobic conditions and conduct volatiles analysis.

8.4 Test and Control Substances Application Rate

8.4.1 Test Substance

The target application rate of the test substance is 1.0 lb/A (1.12 kg/ha). Based on a soil mass equivalent to the total soil application rate of approximately 2.2 lb/A (2.5 kg/ha) equivalent to the total of all applications at 0.5 lb ai/A per application (1.0 lb ai/A/year) the target solution application rate is 2.0 mL/kg. A 1 mL aliquot equivalent to 8.96 µg applied to the surface of each 2.5 cm diameter soil core (20 g soil) will result in a target soil concentration of 0.896 ppm

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[illegible] µg S 2200 [illegible] dry soil). The actual soil concentration will be determined in the raw data and in the final report. Based on the specific activity of the test substances, the amount of radioactivity per treatment dose (non-isotopically enriched) would be about 360,000 dpm for [Benzoyl-^{14}C]S-2367 (Reference), 360,000 dpm for [Benzoyl-^{13}C]S-2367 (Reference), and 360,000 dpm for [Pyrimidine-^{15}N]S-2367 (Reference). The actual dose per replicate will be selected as isotopic dilution.

For treatments 2 & 3, the test material will be dissolved in a suitable delivery solvent. The appropriate volume (0.2% of sample dry weight basis) will be added uniformly to each treatment soil sample. For Treatment 3, the same amount of delivery solvent will be added to the solvent control soils. Dosing solution preparation will be described in the raw data and in the final report.

8.4.2 Control Substance

Since the purpose of the control substance is to verify anaerobic conditions and methanogenesis under conditions similar to those of the test substance, the control substance will be applied at the same molar rate as the test substance. Therefore, a molar equivalent of [U-^{14}C]-D-glucose will be applied to the specified treatments. The amount of [U-^{14}C]-D-glucose used per sample is calculated as follows: 418 µg S 2200 = 316.98 µg product x 180.2 µg glucose/product = 256 µg glucose.

8.5 Dosing Solution Verification and Dose Homogeneity

Aliquots of the dosing solution will be analyzed by LSC to verify the test substance concentration and homogeneity of the solution. The actual amount of radioactivity applied to the treated samples will be determined by LSC of all aliquots taken from the dosing solution before and after the dosing process. The radiochemical purity of the test substances will be determined by a solvent chromatographic method (HPLC) with radioactivity detection.

After dosing, the soil will be mixed to homogenize the sample. The uniform distribution of the test substance in the soil will be determined in a pilot homogeneity study. This mode of application is justified by its quantifiable delivery of test substance to the test system.

8.6 Soil Incubation

After addition of the test substances, the cans will be placed into an incubator

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[illegible] as described in Section 8.3. After quantitative transfer of the aerobic test samples to anaerobic biometers, the biometers will be placed into an incubation chamber in a walk-in incubator for anaerobic incubation, as described in Section 8.3.

8.7 Sampling Intervals and Test Duration

A sample in this study refers to soil in a glass beaker and/or the soil and water from an individual biometer flask. Sampling involves taking a glass beaker and/or biometer flask for one time use only and analyzing the total contents of the unit flask. Table 1 contains a proposed sampling schedule for Treatments A, B, C, D, E, F, and G. Treatments A and B samples will be used to measure the microbial biomass during the aerobic and anaerobic phases. Treatment C samples will be used to verify methanogenic enzymatic activity during the anaerobic phase. Treatment D samples will be used to measure the microbial biomass at the end of the aerobic phase. Duplicate samples will be taken from Treatments B, C, and G at each sampling interval [illegible] as will be taken from proposed sampling intervals for these treatments during the study with at least 6 during the anaerobic phase. Sampling intervals may be modified during the study depending on the projected half-life of the test compound. The study will be terminated when the formation and decline of the degradates have been established or the study may be terminated before 180 days (30 days of aerobic exposure plus 120 days of anaerobic exposure) provided that ultimate transformation pathways and ultimate mineralization has been reached. Termination after 120 days of the anaerobic study [illegible] presumes that at least 90% of the applied has been transformed, but even if that is not 90% it may be argued that the study will be concluded and patterns of persistence established for parent and patterns of formation and decline of degradates established for no more than one year. Whichever comes first. The actual duration of intervals and the sampling scheme will be described in the final report.

8.8 Microbial Analysis of Soils

Treatments A, C, or F will be used to measure the microbial biomass during the aerobic and anaerobic incubations.

8.9 Radiochemical Analysis of Soils

At scheduled intervals, duplicate ^{14}C-treated soil samples will be removed from the test Treatments B, C, and analyzed radiochemically for residues of $^{14}CO_2$, volatiles, and degradates. The soil will be extracted, multiple times with a solvent mixture. The radioactivity content of the extract and the post-extracted soil will be determined

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and for aerobic analysis separately. Mass balance will be calculated. Radioresidues in extracts will be identified by at least two methods and quantified by HPLC as the primary method. For HPLC analysis, extracts may be co-injected with authentic standards of test substance and available reference substances. The extraction method(s) developed during the study will be presented in the final report. HPLC and LSC are the methods of choice but other analytical methods such as mass spectrometry may be used as needed. Authentic standards of the test substance and reference substances will be used in the identification and analysis of microbial products.

The extraction method will be validated by fortifying each test soil with a known amount of one radiolabelled test substance. Five 0.25 AT samples may be used for this validation. The recovery of the test substance will be determined. It must be between 90 to 110%, to be acceptable. The analytical method used for quantification of degradates will have a maximum limit of detection (LOD) of 0.01 mg/kg, for each type of test substance parent, of any soil or water sample. Where the applied dose solution is lower, the limit of quantitation (LOQ) will also be specified in the final report.

8.9.1 Aerobic Phase Soil Analysis

Analysis will involve extraction of the soil to remove the majority of the residues at 0 DAT and around 1, 3 and 30 DAT. The soil extracts will be analyzed chromatographically. The extracted soil will be weighed, mixed, homogenized, and subsamples removed for combustion analysis.

8.9.2 Anaerobic Phase Soil and Water Analysis

Analysis will involve extraction of the soil to remove the majority of the residues through 120 day of the anaerobic phase.

(a) **Headspace sampling.** Using a gastight syringe, a known volume of headspace gas will be withdrawn (except for at 0 day) from the flask(s). The syringe will be used to inject the gas sample(s) into a suitable device to measure $^{14}CO_2$.

(ii) **pH, dissolved O_2, and oxidation reduction potential measurements.** After the headspace is sampled, the dissolved O_2, oxidation-reduction potential, and the pH of the water and the oxidation-reduction potential of the soil will be measured and recorded (3 reps/trt).

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[illegible]

(iii) Evolved $^{14}CO_2$ determination. Aliquots of the NaOH solution from reactions A and B. C only will be added to a scintillation cocktail vial for counting in a liquid scintillation counter (LSC).

(iv) **Soil water separation.** The entire aqueous phase will be transferred into centrifuge tubes, and the soil and water phases separated by centrifugation.

(a) **The water phase.** The water will be decanted from the soil and the water volume recorded. Aliquots will be removed and counted by LSC to determine its content of radioactivity. The water will be analyzed chromatographically either after concentration or extraction.

(b) **The soil phase.** The pelleted soil samples will be extracted and the soil extracts will be analyzed chromatographically. The post-extracted treated PES will be air-dried and subsamples will be removed for analysis by a sample oxidizer to determine its radioactive content. If the PES contains [illegible] of the applied radioactivity, it may be extracted using harsher conditions (such as Soxhlet extraction) and/or the solubilized residues will be fractionated into humin, humic acid and fulvic acid.

(v) **Bioreactor rinse.** After removal of the water, soil and carbon solution, the inner walls of the bioreactor flask will be rinsed with a water/polar organic solvent. The rinse volume will be measured, and aliquots dried and dissolved in scintillation cocktail for LSC analysis.

8.9.3 Combustion Analysis

Total ^{14}C-residues in soil sample extracts will be determined by combustion of such samples using a biological oxidizer (OX-500 Harvey instrument) or equivalent followed by LSC. Soil samples will be weighed and dried to a constant point for oxidation. Headspace gas samples collected with a gastight syringe will be injected into a biological oxidizer to determine the presence and amount of $^{14}CO_2$.

8.9.4 Identification of ^{14}C Residues

If 10% or greater of the applied radioactivity is found in any single extract, the residue(s) will be characterized and if possible identified. If necessary, the sample will be extracted with organic and/or aqueous solvents. Extracts will

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radioactivity that will be present in the final organic extracts will be analyzed by two independent methods. HPLC and TLC are the methods of choice but other analytical methods may be used as needed. A radioactivity detector connected in series to the HPLC may be used in the analysis. Fractions from HPLC corresponding to chromatogram peaks of pesticide residues may be collected on a fraction collector for further analysis. A storage phosphor screen and a digital imaging device may be used to generate an autoradiogram for each TLC plate. The autoradiograms may be used for the identification and quantification of the radioactive components of samples.

All test chemicals and solvents that will be purchased for the study will be listed in the final report.

9. CALCULATION OF RESULTS

The dissipation (DT50 for S-2200 in the test soil will be calculated based on the assumption that degradation follows single first-order kinetics (SFO):

$$C = C_0 e^{-kt} \quad \text{Eq. 1}$$

or

$$\ln(C) = \ln(C_0) - kt \quad \text{Eq. 2}$$

where C is the concentration at time t, C_0 is the concentration at time 0, and k is the degradation rate constant. The DT50 is achieved when $C = 0.5$ and Eq. 3 converts to

$$DT50 = \ln(0.5)/(-k) \quad \text{Eq. 3}$$

The rate constant, k, is determined by plotting the logarithm of the concentration versus time or by the use of the natural linear regression function in the Microsoft Excel Data Analysis Plus Pack. If degradation is first-order, the regression analysis line (function) experimental data should have a correlation coefficient (r) value in the range of 0.7 to 1.0. Other kinetic model calculations and statistical methods (e.g., Parameter Estimation for Degradation Kinetics Spreadsheet) will be documented in the final report.

Mass balance considerations referred to as material balance will be calculated as the sum of concentrations in the soil extracts, post-extracted soil, and volatile traps and/or headspace and reported as a percentage of the amount of radioactivity applied to the sample. Mass balances should be between 90 and 110%.

Appendix A. Study Protocol, Amendments and Deviations

10. SAMPLE IDENTIFICATION

Necessarily, study number, sampling interval, replicate groups, and sample type will be noted on properly sampled.

11. STATISTICAL ANALYSIS AND BIAS CONTROL

Conventional statistical software will be used to calculate linear regression equations and calculate best-fit parameter statistics. Statistical assistance may be required for calculation of application rates and reference standard deviations (coefficient of variation). If necessary, Hedges and Davis's Q-test will be used to reject outlying data points (Anal. Chem. 23, 617, 1951). Bias will be controlled by concurrent method blanks and matrix blanks for analysis. Handling suspensions will be controlled to the study standard.

12. FINAL REPORT

A final report will be prepared after the completion of the study which will reflect the findings. The report will include items described in current FIFRA Data Reporting Guidelines for Anaerobic Aquatic Metabolism Studies and GLP. The report will be reviewed by Valent Management and Valent Quality Assurance Unit and their comments will be addressed prior to the finalization of the report.

13 DATA AND RECORDS

All original raw data, the protocol, correspondence, and a copy or original of the final report or certified copies thereof, will be archived by Valent U.S.A. Corporation currently the archive location is in the VLC.

14. QUALITY ASSURANCE

Valent's QA unit will audit at least three times during the course of the study and the final study report. All findings of QA audits and reviews will be reported to the Study Director and Test Facility Management.

At the conclusion of the study, Valent's QA unit will prepare and sign a statement to be included with the final study report which specifies the dates inspections were made and findings reported to Management and to the Study Director.

15. PROTOCOL AMENDMENTS AND DEVIATIONS

Any changes or changes between an amendment to this protocol will be approved by the Study Director. The Study Director will assure that signed protocol amendments

Appendix A. Study Protocol, Amendments and Deviations

Out [illegible] description of the amendment, the reason for the [illegible], and the impact, if any, on the study. The Sponsor must approve protocol changes. The Sponsor must be notified of protocol amendments within [illegible] working days.

Changes from procedures outlined in this protocol, or its amendments, are deviations. All deviations must be recorded in writing as soon as possible after the occurrence and the Study Director must be notified. The written deviation must include a description of the deviations, the reason for the deviation, and the impact, if any, on the study. The written deviation will be placed in the study file.

16. STANDARD OPERATING PROCEDURES

If there is a conflict between existing Facility Standard Operating Procedures (SOPs) and this protocol, the protocol shall take precedence.

17 PROTOCOL APPROVAL

[illegible] [illegible]

[illegible]
Study Director
Valent U.S.A. Corporation

Date

[illegible] [illegible]

[illegible]
Study Director
Valent U.S.A. Corporation

Date

Protocol reviewed by:

[illegible] [illegible]

[illegible]
Quality Assurance Specialist
Valent U.S.A. Corporation

Date

Appendix A. Study Protocol, Amendments and Deviations

Table 1 Summary of proposed [illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]				
[illegible]		[illegible]		[illegible]	
[illegible]				[illegible]	
[illegible]	[illegible]			[illegible]	
	[illegible]			[illegible]	
	[illegible]			[illegible]	
	[illegible]	[illegible]		[illegible]	
	[illegible]			[illegible]	
	[illegible]			[illegible]	
	[illegible]	[illegible]		[illegible]	[illegible]

[illegible]

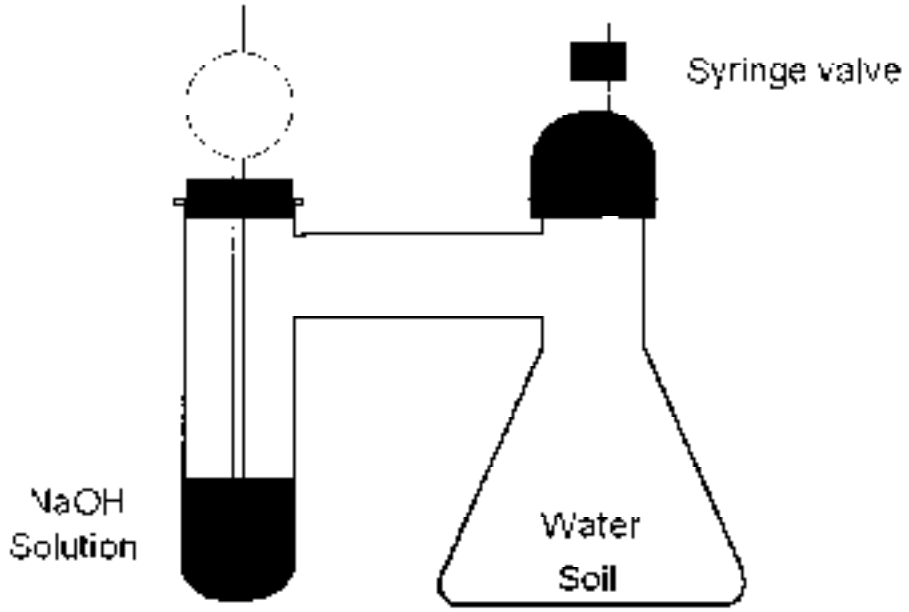
Appendix A. Study Protocol, Amendments and Deviations

Table 2 Summary of measurements and tests to be performed in treatment groups A-C during the anaerobic phase of the study.

Measurement/Analyses	Treatments A, B, & D	Treatment C	Treatments A, B, & C
Soil/Headspace sampling (H_2O_2 evaluation)	NA	yes	yes
Headspace sampling ($^{18}O_2$ Flux evaluation)	NA	yes	yes
Dissolved Oxygen	yes	yes	yes
Oxidation-reduction potential	yes	yes	yes
pH	yes	yes	yes
Volatiles analysis	NA	yes	yes
Soil pulse extraction	NA	no	yes
Water phase extraction	NA	no	yes

NA = not applicable.

Appendix A. Study Protocol, Amendments and Deviations



Appendix A. Study Protocol, Amendments and Deviations

VALENT U.S.A. CORPORATION

Protocol Amendment No. 1

1. **Protocol Number:** VP-37166

2. **Study Title:** S-2200: Anaerobic Soil Metabolism Study

3. **Nature of Amendment:** Change Sponsor Representative.

4. **Reason for the Amendment:** The Sponsor Representative retired.

5. **Description of the Amendment:** Change Sponsor Representative Section 5.3.:

   Sponsor Representative/Test Facility Manager

   Richard Allen, Ph.D.
   Manager, Valent Technical Center
   6560 Trinity Court
   Dublin, CA 94568
   US
   Telephone: (925) 948-2934
   E-mail: ralle@valent.com

6. **IMPACT ON STUDY:** None.

7. **AMENDMENT LOCATION:** Section 5.3

8. **APPROVED BY:**

   9/4/13
   Dr. Kirk Göhre Date
   Study Director
   Valent U.S.A. Corporation

   9/4/13.
   Dr. Richard Allen Date
   Sponsor's Representative/Test Facility Management
   Valent U.S.A. Corporation

Appendix A. Study Protocol, Amendments and Deviations

Valent USA Corporation

Protocol Deviation No. 1

1. **Protocol Number:** VP-37166

2. **Study Title:** S-2200: Anaerobic Soil Metabolism Study

3. **Nature of Deviations:** Address several deviations from the protocol.
a) Table 1 of the protocol states that a microbial analysis will be done midway through the anaerobic phase. **b)** Section 7.4 of the protocol states that soil characterization would be done by FAO and USDA classifications. **c)** Section 8 of the protocol states that the soil would be waterlogged with "sterile HPLC grade water (nitrogen purged)". **d)** Section 8.9.2 stated that the oxidation-reduction potential of the water will be measured. It was not done. **e)** Section 14 states that there would be 3 in process audits. Only two were done.

4. **Reason for the Deviations:**
a) It was decided that a microbial analysis during the transition phase (aerobic to anoxic to anaerobic) would be of limited value.
b) Agvise does not perform a FAO classification, instead characterizations were done by International, German BBA and USDA classifications.
c) The soils were waterlogged with sterile HPLC grade water then the whole system was nitrogen purged.
d) The measurement was not necessary.
e) Similar in process audits were done on concurrent S-22000 Efate studies.

5. **Impact on Study:** None.

6. **Deviation Locations:** Table 1, Sections 7.4, 8 and 8.9.2.

7. **Acknowledged by:**

Kirk Göhre, Ph.D.
Study Director
Valent USA Corporation

9/4/13
Date

Appendix B. Analytical Reference Standards

Valent Technical Center
6560 Trinity Court
Dublin, CA 94568-2628
(925) 256-2700
FAX (925) 948-2901

CERTIFICATE OF QUALITY
ANALYTICAL REFERENCE STANDARDS

Product: De-Xy-S-2200

Lot #: AS 2264a

Purity: 99.9%

Date of Analysis: July 26, 2010

Expiration Date: July 26, 2011

Storage Conditions: Under nitrogen in freezer

Characterization of this Analytical Reference Standard was performed in compliance with EPA FIFRA Good Laboratory Practices Standards (40 CFR, 160, August 17, 1989). Characterization data and a sub-sample of this material are archived at the Valent Technical Center, 6560 Trinity Court, Dublin, CA 94568.

Sample Archive Number: V-Arc-2089

Records reflecting method of fabrication or source of supply are maintained at the Valent Technical Center, Dublin, CA.

Manufacturer's Sample Identity: Sumitomo Lot CTS08001

John C. Whittington, Analytical Chemist DATE: August 10, 2010

Appendix B. Analytical Reference Standards

Valent Technical Center
6560 Trinity Court
Dublin, CA 94568-2628
(925) 256-2700
FAX (925) 948-2901

CERTIFICATE OF QUALITY
ANALYTICAL REFERENCE STANDARDS

Product: DX-CA-S-2200

Lot #: AS 2269a

Purity: 100%

Date of Analysis: July 28, 2010

Expiration Date: July 28, 2011

Storage Conditions: Under nitrogen in freezer

Characterization of this Analytical Reference Standard was performed in compliance with EPA FIFRA Good Laboratory Practices Standards (40 CFR, 160, August 17, 1989). Characterization data and a sub-sample of this material are archived at the Valent Technical Center, 6560 Trinity Court, Dublin, CA 94568.

Sample Archive Number: V-Arc-2094

Records reflecting method of fabrication or source of supply are maintained at the Valent Technical Center, Dublin, CA.

Manufacturer's Sample Identity: Sumitomo Lot CTS08031

John C. Whittington, Analytical Chemist DATE: August 10, 2010

Appendix B. Analytical Reference Standards

Valent Technical Center
6560 Trinity Court
Dublin, CA 94568-2628
(925) 256-2700
FAX (925) 948-2901

CERTIFICATE OF QUALITY
ANALYTICAL REFERENCE STANDARDS

Product: 2-COOH-S-2200

Lot #: AS 2268s

Purity: 99.0%

Date of Analysis: July 28, 2010

Expiration Date: July 28, 2011

Storage Conditions: Under nitrogen in freezer

Characterization of this Analytical Reference Standard was performed in compliance with EPA FIFRA Good Laboratory Practices Standards (40 CFR, 160, August 17, 1989). Characterization data and a sub-sample of this material are archived at the Valent Technical Center, 6560 Trinity Court, Dublin, CA 94568.

Sample Archive Number: V-Arc-2093

Records reflecting method of fabrication or source of supply are maintained at the Valent Technical Center, Dublin, CA.

Manufacturer's Sample Identity: Sumitomo Lot 252-001-60-2

John C. Whittington, Analytical Chemist DATE: August 10, 2010

Appendix B. Analytical Reference Standards

Valent Technical Center
6560 Trinity Court
Dublin, CA 94568-2628
(925) 256-2700
FAX (925) 948-2901

CERTIFICATE OF QUALITY
ANALYTICAL REFERENCE STANDARDS

Product: 5-COOH-S-2200

Lot #: AS 2267a

Purity: 99.6%

Date of Analysis: July 28, 2010

Expiration Date: July 28, 2011

Storage Conditions: Under nitrogen in freezer

Characterization of this Analytical Reference Standard was performed in compliance with EPA FIFRA Good Laboratory Practices Standards (40 CFR, 160, August 17, 1989). Characterization data and a sub-sample of this material are archived at the Valent Technical Center, 6560 Trinity Court, Dublin, CA 94568.

Sample Archive Number: V-Arc-2092

Records reflecting method of fabrication or source of supply are maintained at the Valent Technical Center, Dublin, CA.

Manufacturer's Sample Identity: Sumitomo Lot 252-001-55-2

John C. Whittington, Analytical Chemist

DATE: August 10, 2010

Appendix B. Analytical Reference Standards



CERTIFICATE OF QUALITY
ANALYTICAL REFERENCE STANDARDS

Appendix B. Analytical Reference Standards

CERTIFICATE OF QUALITY
ANALYTICAL REFERENCE STANDARDS

Appendix B. Analytical Reference Standards

[illegible]
[illegible]
[illegible]
[illegible]
[illegible]

CERTIFICATE OF QUALITY
ANALYTICAL REFERENCE STANDARDS

[illegible]

[illegible]

Purity [illegible]

Date of Analysis: July 1, 2011

Expiration Date: July 1, 2013

Storage Conditions: [illegible]

This certification of this Analytical Reference Standard was performed in compliance with EPA FIFRA Good Laboratory Practice Standards 40 CFR 160, August 17, 1989. Characterization data and assay samples of this material are retained at the Valent Technical Center, 6560 Trinity Court, Dublin, CA 94568.

Sample Retrieval Number: [illegible]

Instrument Setup methods, calibration standards, supply and maintenance of the Valent Technical Center, Walnut Creek, CA

Manufacturer's Sample Identification Number: CTS-5015

[illegible] Quality Control Supervisor

[illegible] Analytical Chemist

DATE: July 01, 2011

Appendix B. Analytical Reference Standards

[illegible]

CERTIFICATE OF QUALITY
ANALYTICAL REFERENCE STANDARDS

[illegible]

Certification of the Analytical Reference Standard was performed in accordance with EPA 40 CFR Good Laboratory Practices Standards 40 CFR, Part 160, Aug. 17, 1989, Good laboratory practice standards; Final rule and Federal Register notice dated the Valent Analytical Services, 5560 Tulare Court, Tulare, CA 93274.

[illegible]

Recommended storage conditions: Refrigerate at 0 to 4°C. Supply as manufactured by the Valent Technical Center, Tulare, CA.

Manufacturer's Storage Container Number: [illegible]

Signature: [illegible] Date: [illegible]
Analytical Chemist

Appendix B. Analytical Reference Standards



CERTIFICATE OF QUALITY
ANALYTICAL REFERENCE STANDARDS

Appendix B. Analytical Reference Standards

Valent Technical Center
6560 Trinity Court
Dublin, CA 94568-2628
(925) 256-2700
FAX (925) 948-2901

CERTIFICATE OF QUALITY
ANALYTICAL REFERENCE STANDARDS

Product: S-2200

Lot #: AS 2261a
Purity: 99.6%

Date of Analysis: July 8, 2010

Expiration Date: July 8, 2011

Storage Conditions: Under nitrogen in freezer

Characterization of this Analytical Reference Standard was performed in compliance with EPA FIFRA Good Laboratory Practices Standards (40 CFR, 160, August 17, 1989). Characterization data and a sub-sample of this material are archived at the Valent Technical Center, 6560 Trinity Court, Dublin, CA 94568.

Sample Archive Number: V-Arc-2079

Records reflecting method of fabrication or source of supply are maintained at the Valent Technical Center, Dublin, CA.

Manufacturer's Sample Identity: Sumitomo Lot 081103G

John C. Whittington, Analytical Chemist DATE: July 13, 2010

Appendix B. Analytical Reference Standards

Certificate of Analysis

SIGMA-ALDRICH

Product Name	2,5-Dimethylphenol, ≥99%
Product Number	D174602
Product Brand	ALDRICH
CAS Number	95-87-4
Molecular Formula	$(CH_3)_2C_6H_3OH$
Molecular Weight	122.16

TEST	SPECIFICATION	LOT MKAA2399 RESULTS
Appearance (Color)	White to Off-White	White
Appearance (Form)	Conforms to Requirements	Crystals
	Needles, Fibers, Crystals, Flakes or Crystalline	
	Flakes	
Infrared spectrum	Conforms to Structure	Conforms
Purity (GC)	≥99.0 %	99.8 %
Specification Date:		JAN 2009

Barbara Rajzer

Barbara Rajzer, Supervisor
Quality Control
Milwaukee, Wisconsin USA

Appendix C. Soil Characterization Reports

AGVISE LABORATORIES
604 Highway 15 West
P.O. Box 510
Northwood, ND 58267
(701) 587-6010
FAX (701) 587-6013
email: agvise@polarcomm.com
Homepage: www.agvise.com

AGVISE Soil Characterization Report

Submitting firm	= VALENT USA	
Protocol or Study No	= V-09-37114	
Sample ID.	= VTC-7-09A 0-3"	
Trial ID.	= NA	
Date Received	= 10-7-09	
Date Reported	= 10-19-2009	
AGVISE Lab No		09-1237
Percent Sand		26
Percent Silt		49
Percent Clay		25
USDA Textural Class (hydrometer method)		Loam
Percent Sand		45
Percent Silt		30
Percent Clay		25
International Text. Class (hyd. method)		Clay Loam
Percent Sand		23
Percent Silt		52
Percent Clay		25
German BBA Texture (hydrometer method)		Silty Loam
Bulk Density (disturbed) gm/cc		1.08
Cation Exchange Capacity (meq/100 g)		8.7
% Moisture at 1/10 Bar		27.0
% Moisture at 1/3 Bar		21.3
% Moisture at 15 Bar		9.8
% Organic Matter--Walkley Black		2.0
pH in 1:1 soil:water ratio		6.9
Olsen Phosphorus (ppm)		8
Total Nitrogen (Analyzer) (%)		0.12
Soluble Salts (mmhos/cm)		0.20

Appendix C. Soil Characterization Reports

AGVISE LABORATORIES
604 Highway 15 West
P.O. Box 510
Northwood, ND 58267
(701) 587-6010
FAX (701) 587-6013
email: agvise@polarcomm.com
Homepage: www.agvise.com

AGVISE Soil Characterization Report (page 2)

Submitting firm = VALENT USA
Protocol or Study No = V-09-37114
Sample ID. = VTC-7-09A 0-3"
Trial ID. = NA
Date Received = 10-7-09
Date Reported = 10-19-2009

AGVISE Lab No 09-1237

Base Saturation Data

Cation	Percent	ppm
Calcium	52.4	907
Magnesium	17.3	180
Sodium	0.3	7
Potassium	1.3	45
Hydrogen	28.7	25

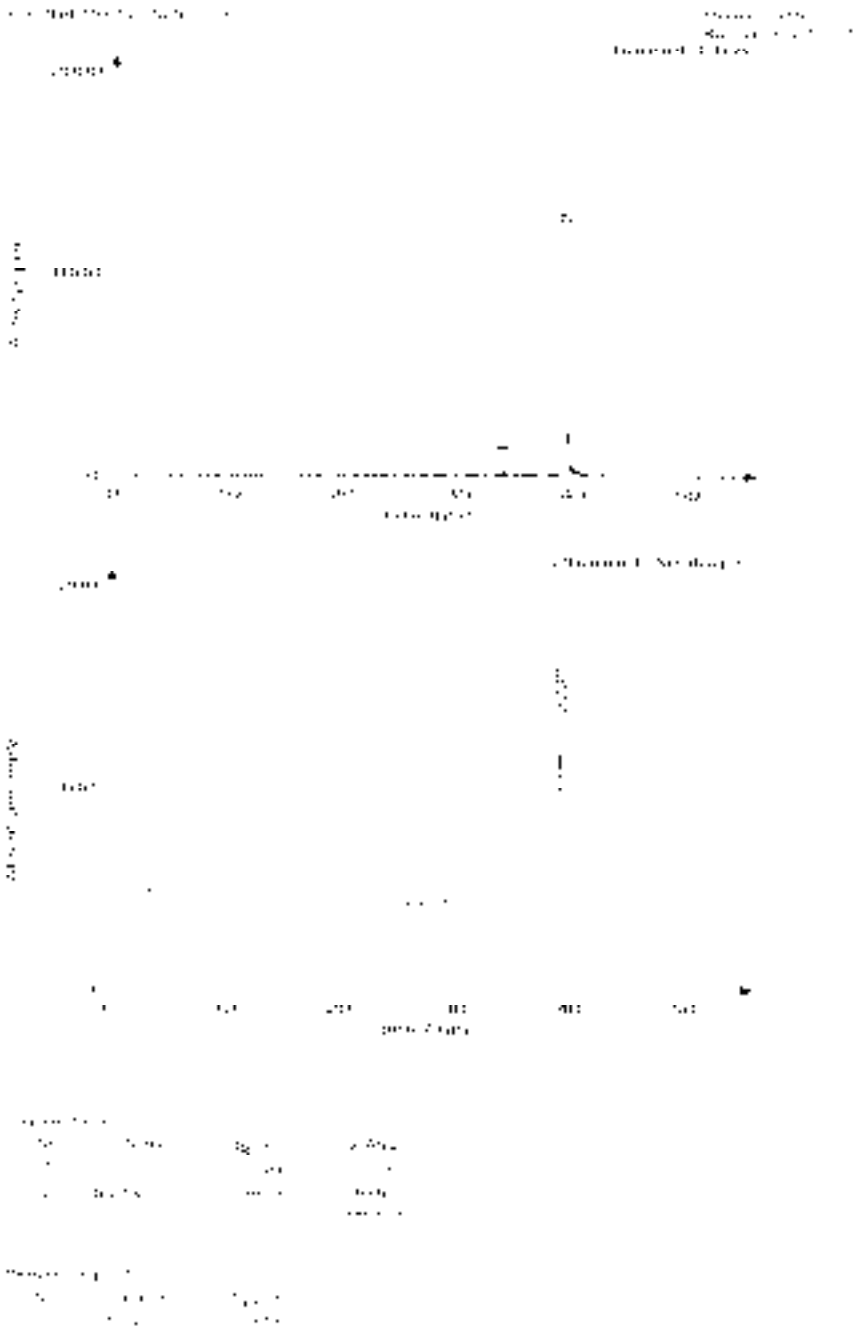
These tests were completed in compliance of 40 CFR Part 160.

Larry Wikoff
Analytical Investigator

10/20/09
Date

Appendix D. Test Substance Radiochemical Purity and Dosing Solution Data

HPLC analysis of [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) dosing solution (401G) - Radio and UV chromatograms



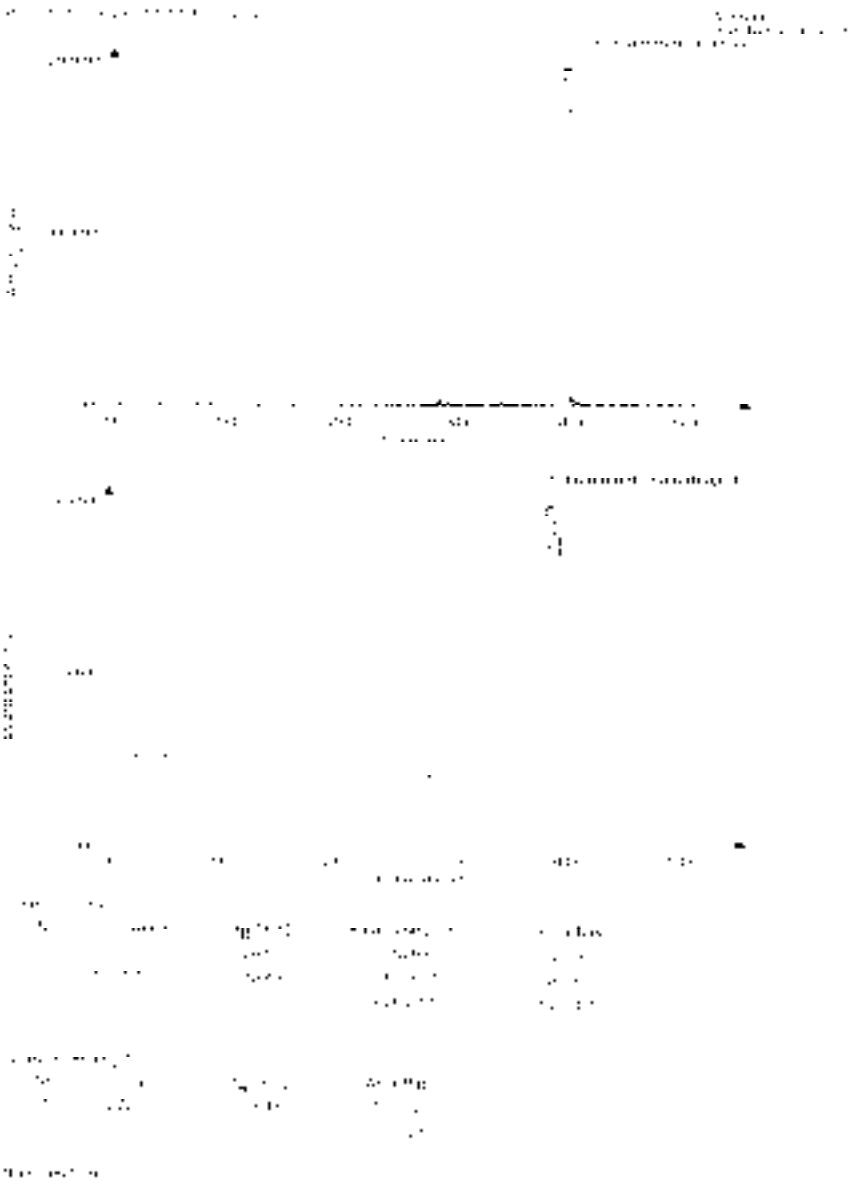
Appendix D. Test Substance Radiochemical Purity and Dosing Solution Data

HPLC analysis of [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) dosing solution (400D) – Radio and UV chromatograms



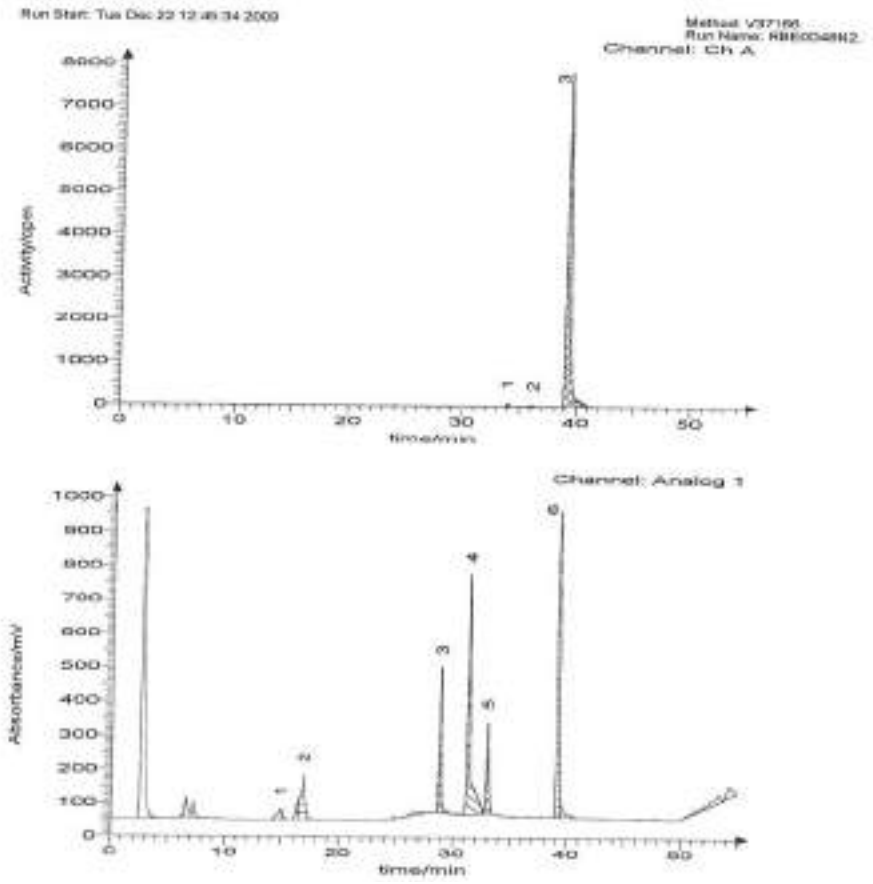
Appendix D. Test Substance Radiochemical Purity and Dosing Solution Data

HPLC analysis of [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) dosing solution (404D) – Radio and UV chromatograms



Appendix E [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) HPLC Chromatograms

Item #1. Radioactive and UV HPLC chromatograms for RB-0DAT-48N – 0 DAT neutral soil extract. Sample represents 100.9% of the applied dose. Cold standards co-injected.



Channel: Ch A

No	t_R [min]	%(Area)
1	34.10	0.83
2	36.24	0.47
3	39.39	98.70
-	-	100.00 *

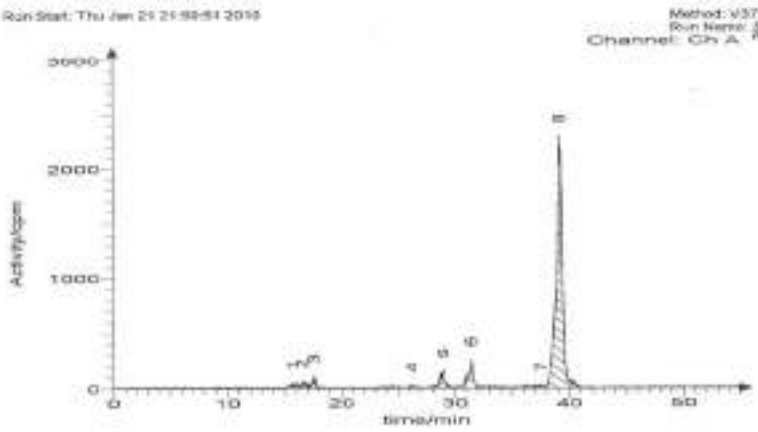
S-2200

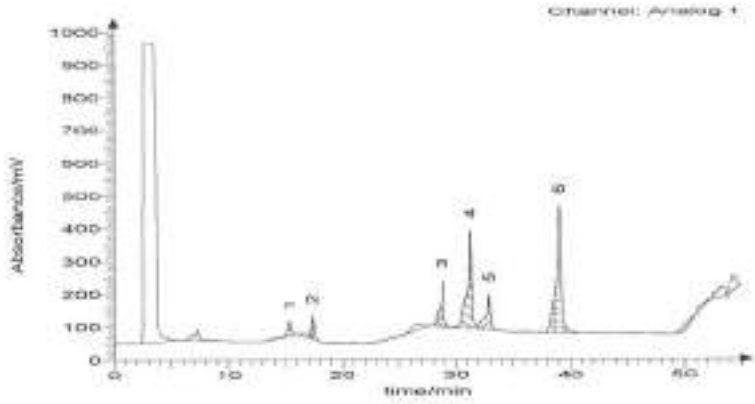
Channel: Analog 1

No	Name	t_R [min]
1	De-Xy-S-2200	14.86
2	De-CA-S-2200	18.78
3	2-COOH-S-2200	28.91
4	5-COOH-S-2200	31.52
5	MOBX	33.01
6	S-2200	39.22
-		-

Appendix E [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) HPLC Chromatograms

Item #2. Radioactive and UV HPLC chromatograms for RB-30DAT-28N – 30 DAT neutral soil extract. Sample represents 81.6% of the applied dose. Cold standards co-injected.





Run Start: Thu Jan 21 21:50:51 2010

Channel: Ch A

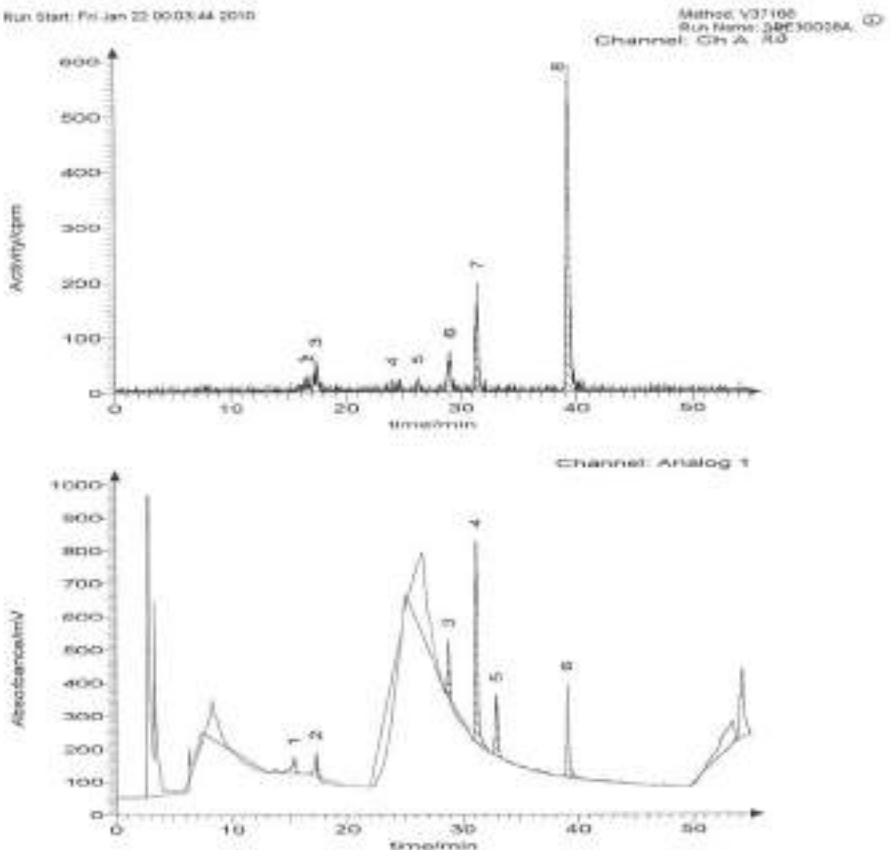
No	t_R [min]	%(Area)
1	15.88	1.88
2	16.73	1.19
3	17.47	2.13
4	26.20	0.61
5	28.89	5.67
6	31.32	7.07
7	37.33	1.14
8	39.17	80.44
-	-	100.00 *

Channel: Analog 1

No	Name	t_R [min]
1	Ox-Hy-S-2200	15.44
2	Ox-CA-S-2200	17.36
3	2-COOH-S-2200	26.73
4	5-COOH-S-2200	31.10
6	MCBX	32.75
8	S-2200	39.00
-		-

Appendix E [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) HPLC Chromatogram

Item #3 Radioactive and UV HPLC chromatograms for RB-30DAT-28A – 30 DAT acidic soil organic extract. Sample represents 17.0% of the applied dose. Cold standards co-injected.



Channel: Ch A

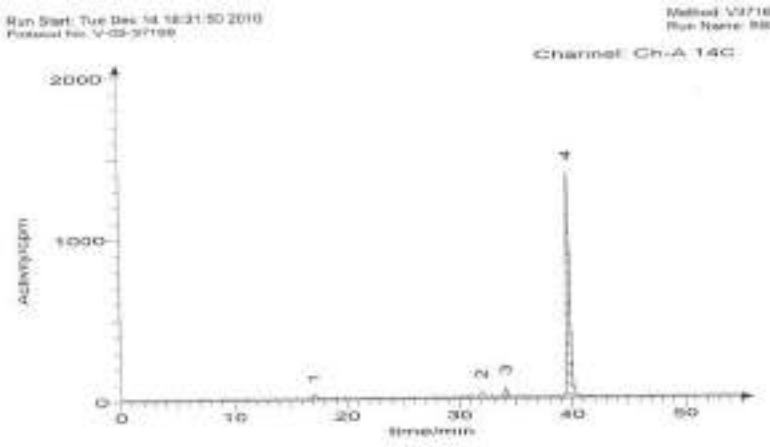
No	t_R [min]	S/(Area)	
1	16.28	1.61	
2	16.79	2.71	
3	17.48	5.54	θ_R-C A
4	24.48	2.62	
5	26.27	1.91	
6	29.04	7.38	1-COOH
7	31.47	15.69	5-4-COOH
8	39.38	62.54	S-23,00
-	--	100.00 *	

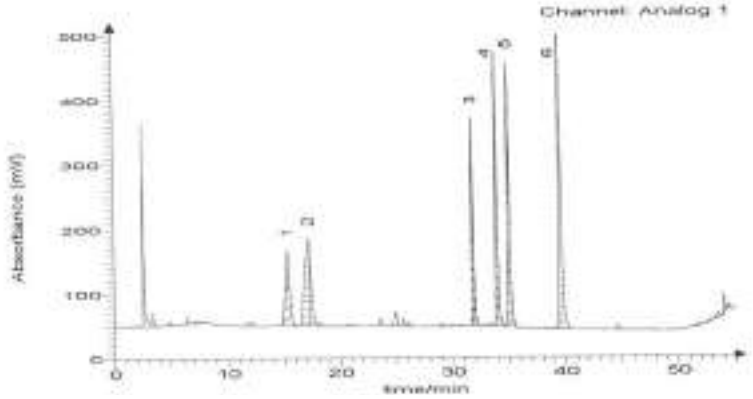
Channel: Analog 1

No	Name	t_R [min]
1	Di-Xy-S-2200	16.23
2	Di-CA-S-2200	17.36
3	2-COOH-S-2200	28.85
4	5-COOH-S-2200	31.32
5	MOBX	32.96
6	S-2200	39.17
-		--

Appendix E [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) HPLC Chromatogram

Item #4. Radioactive and UV HPLC chromatograms for RB-364DAT-36N – 364 DAT neutral soil extract. Sample represents 50.6% of the applied dose. Cold standards co-injected.





Channel: Ch-A 14C

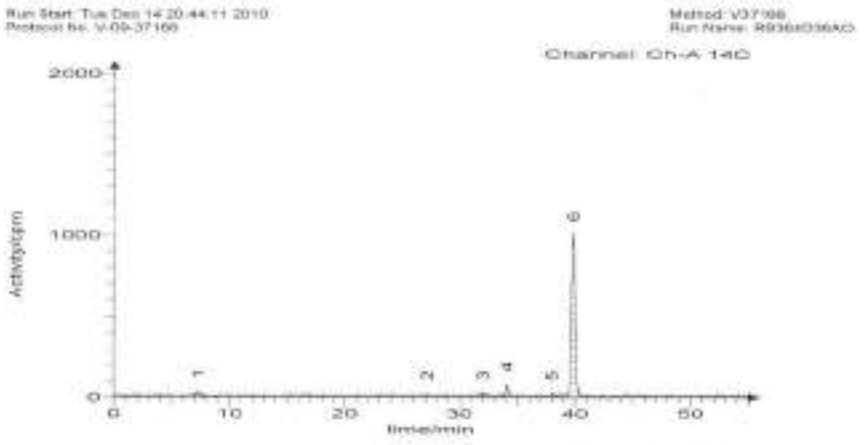
No	t_R [min]	%(Area)	
1	17.28	2.76	DX-CA
2	32.04	3.79	1-COOH
3	34.21	4.65	5-COOH
4	39.84	88.80	S-2200
*	--	100.00 *	

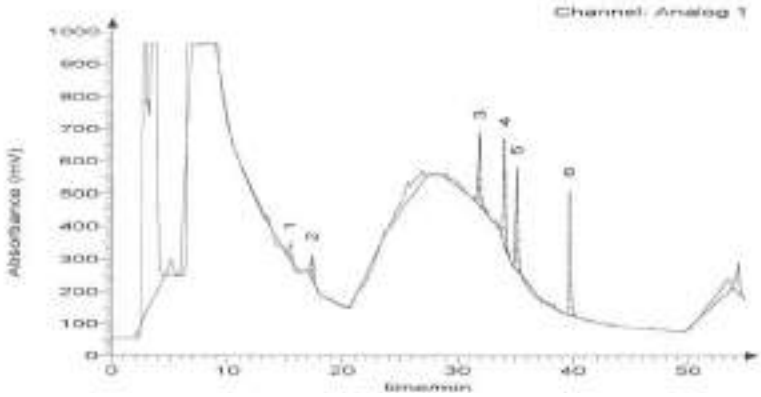
Channel: Analog 1

No	Name	t_R [min]
1	De-Xy-S-2200	19.34
2	De-CA-S-2200	17.18
3	2-COOH-S-2200	31.89
4	5-COOH-S-2200	34.05
5	MDBX	35.10
6	S-2200	39.68
*		--

Appendix E [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) HPLC Chromatogram

Item #5 Radioactive and UV HPLC chromatograms for RB-364DAT-36A – 364 DAT acidic soil organic extract. Sample represents 21.8% of the applied dose. Cold standards coinjected.





Channel: Ch-A 14C

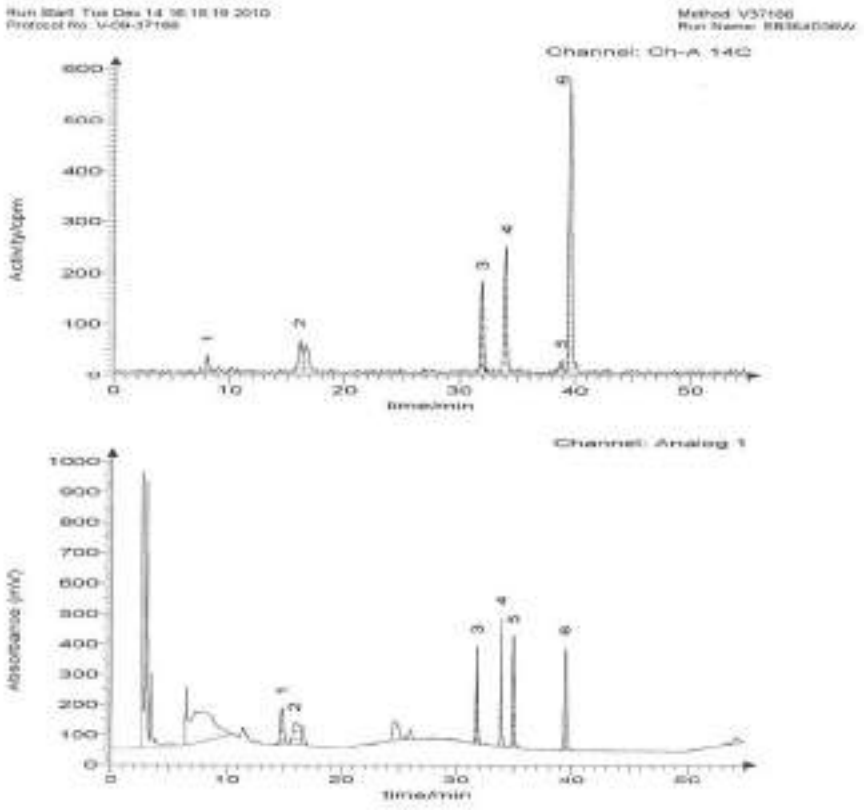
No	t_R [min]	%(Area)	
1	7.27	3.59	
2	27.79	1.37	
3	32.04	2.51	2-COOH
4	34.20	6.79	5-COOH
5	38.13	2.28	
6	39.90	83.47	S-2200
-	-	100.00 *	

Channel: Analog 1

No	Name	t_R [min]
1	De-Xy-5-2200	15.40
2	De-CA-5-2200	17.35
3	2-COOH-5-2200	31.90
4	5-COOH-5-2200	34.08
5	MCBX	35.13
6	S-2200	39.74
-		-

Appendix E [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) HPLC Chromatogram

Item #6. Radioactive and UV HPLC chromatograms for RB-364DAT-36W – 364 DAT water phase. Sample represents 22.5% of the applied dose. Cold standards coinjected.



Run Start: Tue Dec 14 16:19:15 2010
Protocol No. V-09-37166

Method: V37166
Run Name: RB364D36W

Channel: Ch-A 14C

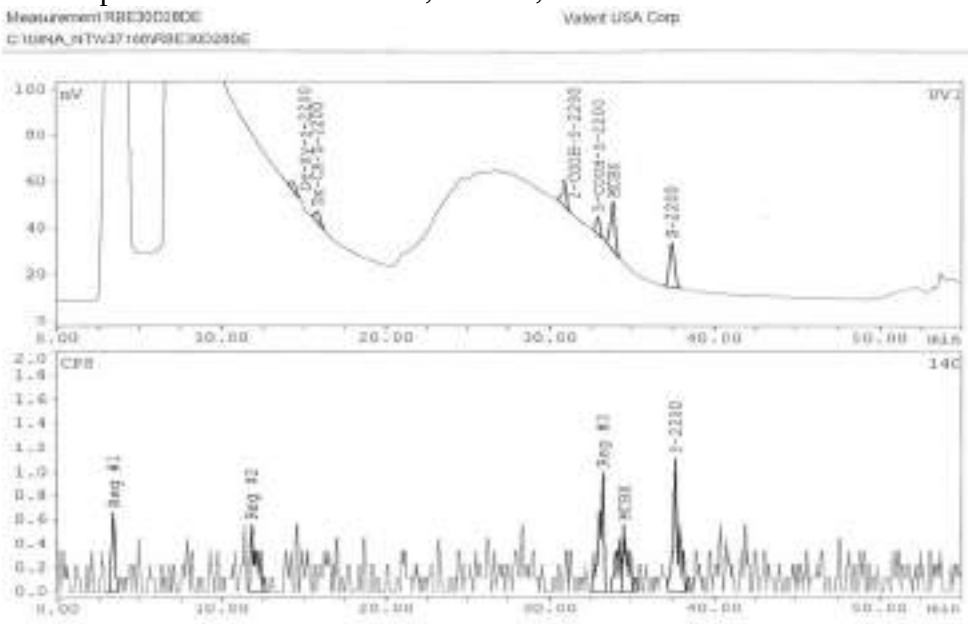
No	t_R [min]	%(Area)	
1	6.18	3.42	
2	16.40	15.67	Dx-CA
3	31.99	13.18	2-COOH
4	34.05	17.74	5-COOH
5	38.80	2.99	
6	39.01	46.94	S-2200
-	-	100.00*	

Channel: Analog 1

No	Name	t_R [min]
1	Dx-Xy-S-2200	14.67
2	Dx-CA-S-2200	16.27
3	2-COOH-S-2200	31.83
4	5-COOH-S-2200	33.90
5	MOBX	34.90
6	S-2200	38.44
-		-

Appendix E [benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) HPLC Chromatogram

Item #7 Radioactive and UV HPLC chromatograms for RB-30DAT-28D – 30 DAT dismembrator released extract. Sample represents 1.5% of the applied dose. Cold standards coinjected. Compounds observed: S-2200, MCBX, hint of 5-COOH.



Sample description

Measurement:	RBE30D28DE, injection:	3/1/2011 12:40 PM	
Method:	V37166.from:	3/1/2011 11:24 Add. Position:	100

Integration 14C

Substance	R/T min	Type	Area Counts	%Area %
Reg #1	3.43	BB	9.00000	10.23
Reg #2	11.80	BB	14.00000	15.91
Reg #3	33.36	BB	20.00000	22.73
MCBX	34.53	DB	21.00000	23.86
S-2200	37.67	BB	24.00000	27.27
Sum in ROI			88.00000	
Area			464.00000	

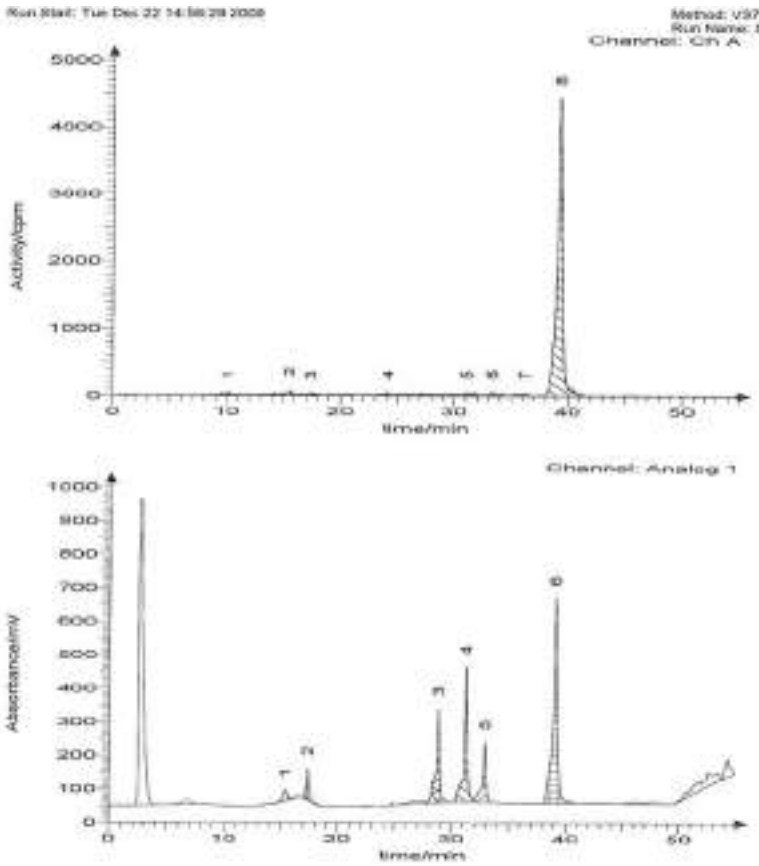
Integration UV1

Substance	R/T min	Type	Area mVmin	%Area %
Da-Xy-S-2200	14.30	BB	76.4483	5.84
Da-CA-S-2200	15.82	BB	97.4739	7.45
2-COOH-S-2200	30.93	BB	222.4313	16.99
5-COOH-S-2200	32.96	BB	120.2241	9.67
MCBX	33.67	BB	381.4364	29.14
S-2200	37.45	BB	401.9420	30.71
Sum in ROI			1308.9551	

KS 3/2/11

Appendix F [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) HPLC Chromatograms

Item #1. Radioactive and UV HPLC chromatograms for SB-0DAT-50N – 0 DAT neutral soil extract. Sample represents 100.9% of the applied dose. Cold standards co-injected.



Channel: Ch A

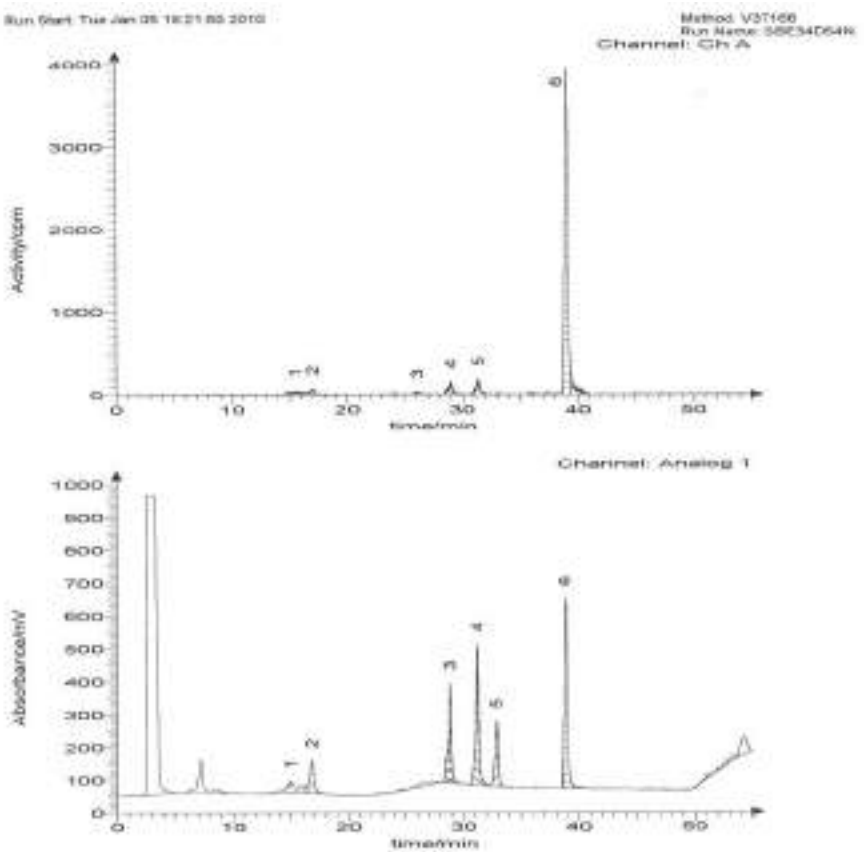
No	t_R [min]	%(Area)
1	10.02	0.87
2	15.47	1.47
3	17.82	0.69
4	26.17	0.72
5	31.52	1.08
6	33.81	1.31
7	35.97	0.88
8	39.29	93.04
–	–	100.00 *

Channel: Analog 1

No	Name	t_R [min]
1	De-Xy-S-2200	15.46
2	De-CA-S-2200	17.42
3	2-COOH-S-2200	28.77
4	5-COOH-S-2200	31.24
5	NCBA	32.87
6	S-2200	39.12
–		–

Appendix F [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) HPLC Chromatograms

Item #2 Radioactive and UV HPLC chromatograms for SB-34DAT-54N – 34 DAT neutral soil extract. Sample represents 76.2% of the applied dose. Cold standards co-injected.



Channel: Ch A

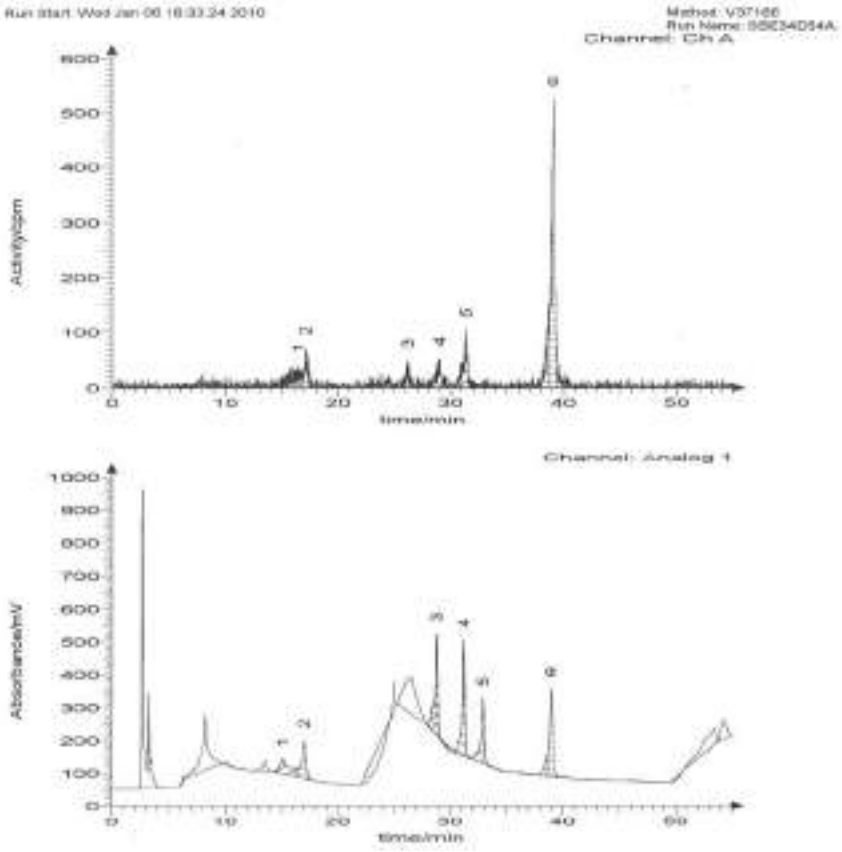
No	t_R [min]	%(Area)
1	15.52	2.07
2	16.92	1.89
3	28.13	0.65
4	29.90	3.23
5	31.41	3.99
6	39.09	88.16
-	-	100.00 *
-	-	110.84

Channel: Analog 1

No	Name	t_R [min]
1	De-Xy-S-2200	15.52
2	De-CA-S-2200	16.80
3	2-COOH-S-2200	28.62
4	5-COOH-S-2200	31.27
5	MCBX	32.89
6	S-2200	38.94
-		-
-		-

Appendix F [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) HPLC Chromatograms

Item #3. Radioactive and UV HPLC chromatograms for SB-34DAT-54A – 34 DAT acidic soil organic extract. Sample represents 17.8% of the applied dose. Cold standards co-injected.



Channel: Ch A

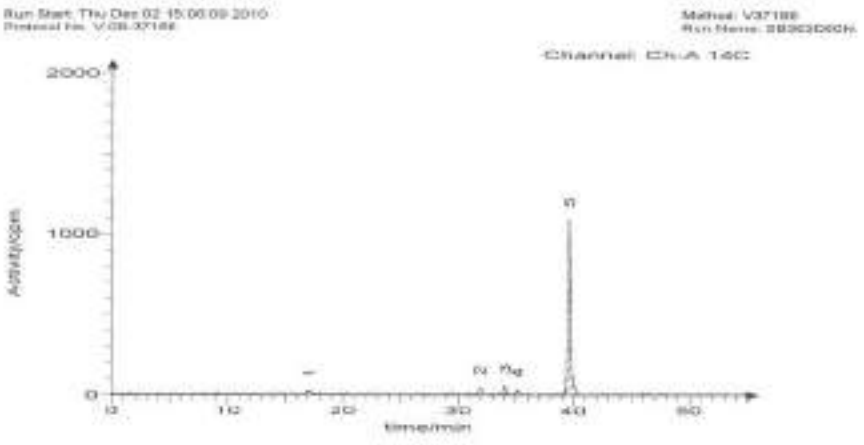
No	t_R [min]	%[Area]	
1	15.93	8.38	Dx-Xy
2	17.23	6.09	Dx-CA
3	28.19	3.77	
4	28.90	5.58	2-COOH
5	31.29	9.63	5-COOH
6	39.05	67.54	S-33
-	-	100.00 *	

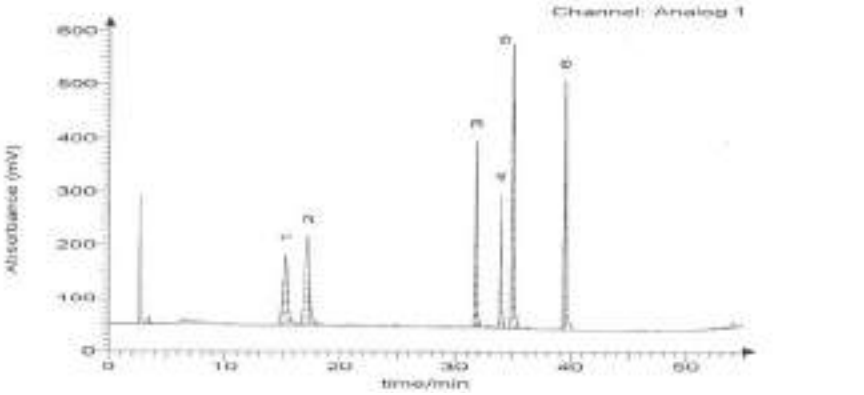
Channel: Analog 1

No	Name	t_R [min]
1	Dx-Xy-S-2200	15.47
2	Dx-CA-S-2200	17.00
3	2-COOH-S-2200	28.66
4	5-COOH-S-2200	31.23
5	MCBX	32.79
6	S-2200	38.89
-		-

Appendix F [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) HPLC Chromatograms

Item #4 Radioactive and UV HPLC chromatograms for SB-363DAT-60N – 363 DAT neutral soil extract. Sample represents 43.8% of the applied dose. Cold standards coinjected.





Channel: Ch-A 14C

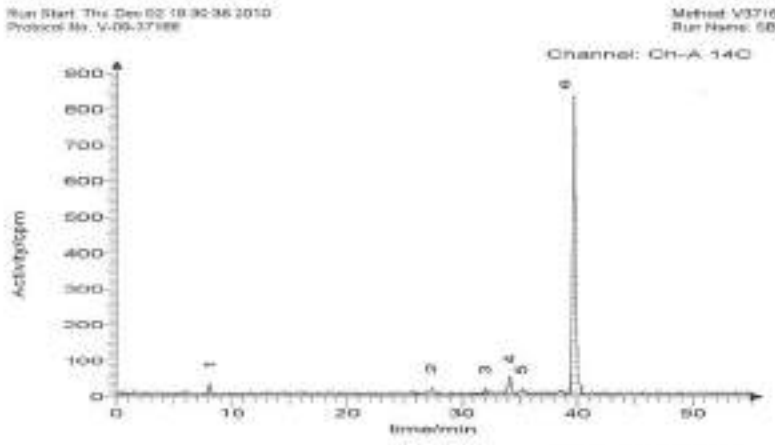
No	t_R [min]	%(Area)
1	17.21	3.67
2	32.01	3.47
3	34.14	4.14
4	35.18	2.06
5	39.67	86.65
-	--	100.00 *

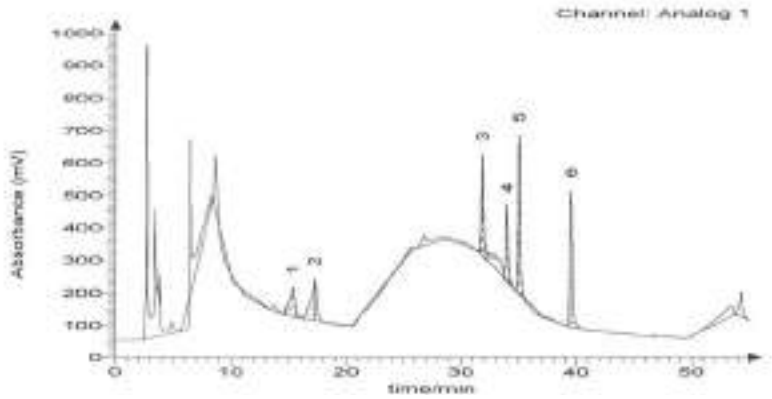
Channel: Analog 1

No	Name	t_R [min]
1	De-Ky-S-2200	15.30
2	De-CA-S-2200	17.17
3	3-COOH-S-2200	31.98
4	5-COOH-S-2200	34.00
5	MCBX	35.04
6	S-2200	39.51
-		--

Appendix F [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) HPLC Chromatograms

Item #5. Radioactive and UV HPLC chromatograms for SB-363DAT-60A – 363 DAT acidic soil organic extract. Sample represents 23.1% of the applied dose. Cold standards coinjected.





Channel: Ch-A 14C

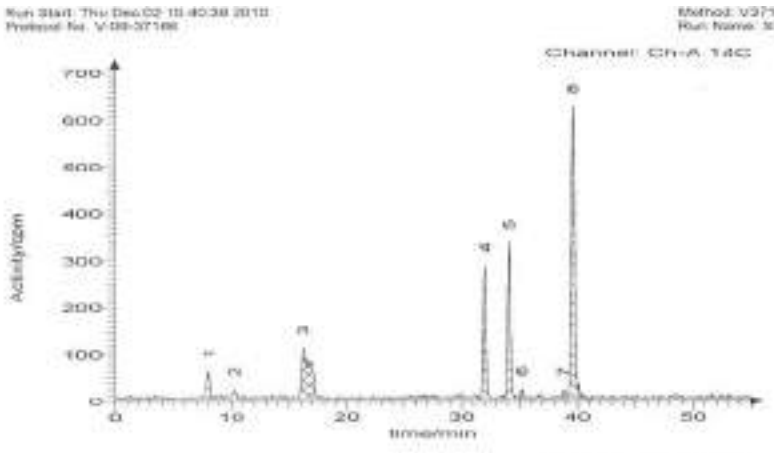
No	t_R [min]	%(Area)	
1	8.09	3.40	
2	27.25	2.66	
3	32.07	2.35	2-COOH
4	34.16	6.05	5-COOH
5	39.29	3.87	MCBX
6	39.78	80.18	S-2200
-	-	100.00 *	

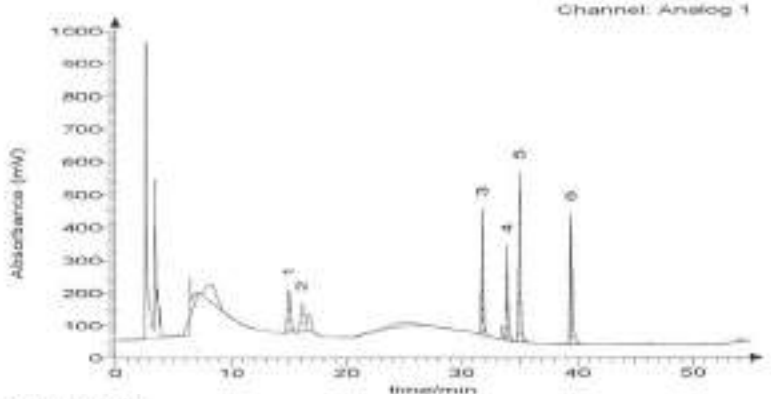
Channel: Analog 1

No	Name	t_R [min]
1	De-Xy-S-2200	15.26
2	De-CA-S-2200	17.18
3	2-COOH-S-2200	31.92
4	5-COOH-S-2200	34.06
5	MCBX	35.07
6	S-2200	39.62
-		-

Appendix F [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) HPLC Chromatograms

Item #6 Radioactive and UV HPLC chromatograms for SB-363DAT-60W – 363 DAT water phase. Sample represents 22.3% of the applied dose. Cold standards coinjected.





Channel: Ch-A 14C

No	t_R [min]	% Area
1	8.06	4.80
2	10.30	2.49
3	16.60	19.62
4	32.01	15.28
5	34.16	16.65
6	35.22	1.59
7	38.84	1.50
8	39.69	38.17
-	-	100.00 *

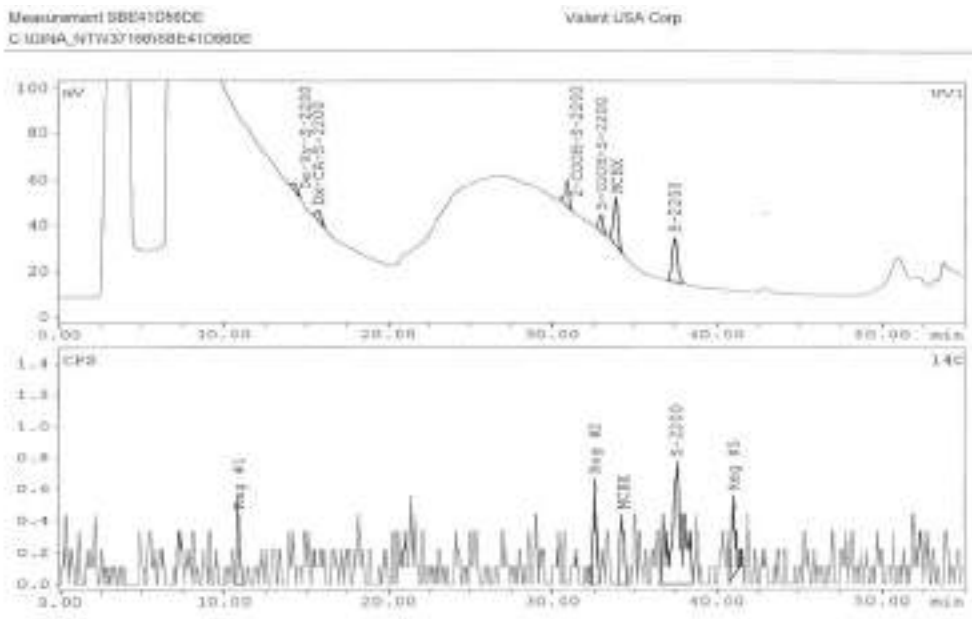
Dx-CA
2-COOH
5-COOH
MCBX
S-2200

Channel: Analog 1

No	Name	t_R [min]
1	Dx-Xy-S-2200	15.03
2	Dx-CA-S-2200	16.42
3	2-COOH-S-2200	31.85
4	5-COOH-S-2200	34.01
5	MCBX	35.09
6	S-2200	39.54
-		-

Appendix F [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) HPLC Chromatograms

Item #7. Radioactive and UV HPLC chromatograms for SB-41DAT-56D – 41 DAT dismembrator released extract. Sample represents 1.4% of the applied dose. Cold standards coinjected. Compounds observed: S-2200, hint of MCBX.



Sample description

Measurement:	SBE41D56DE, injection	3/1/2011 1:48 PM	
Method:	V37166 from 3/1/2011 11:24 AM Position:	103	

Integration 14C

Substance	R/T min	Type	Area Counts	%Area %
Reg #1	10.57	BB	7.00000	10.24
Reg #2	32.62	BB	10.00000	14.63
MCBX	34.30	BB	7.00000	10.24
S-2200	37.53	BB	37.00000	54.13
Reg #5	41.00	BB	7.35714	10.76
Sum in ROI			68.35714	
Area			421.00000	

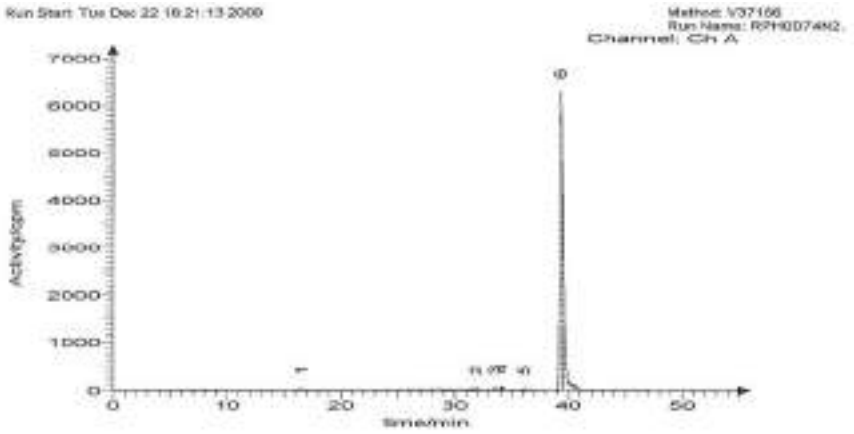
Integration UV1

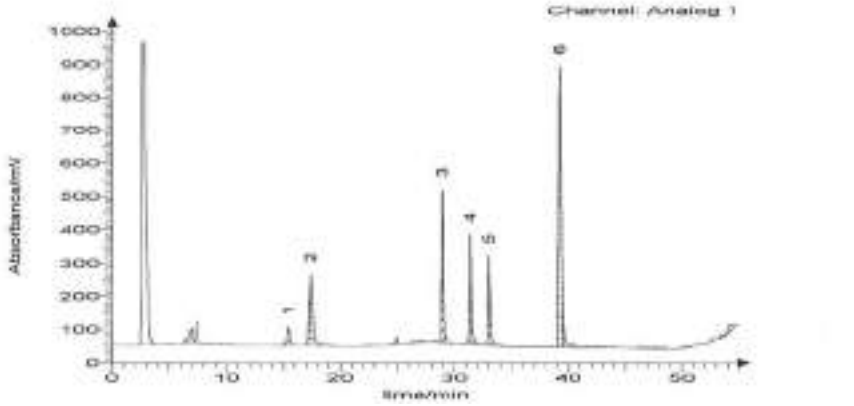
Substance	R/T min	Type	Area mVmin	%Area %
Dx-Xy-S-3300	14.25	BB	71.5975	5.50
Dx-CA-S-2200	15.75	BB	101.4355	7.79
2-COOH-S-2200	30.95	BB	200.2418	15.38
5-COOH-S-2200	33.02	BB	122.1251	9.38
MCBX	33.90	BB	373.3066	28.68

S-2200	37.48	BB	423.9621	32.57
Sum in ROI			1301.6748	

Appendix G [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) HPLC Chromatograms

Item #1. Radioactive and UV HPLC chromatograms for RP-0DAT-74N – 0 DAT neutral soil extract. Sample represents 102.1% of the applied dose. Cold standards co-injected.





Channel: Ch A

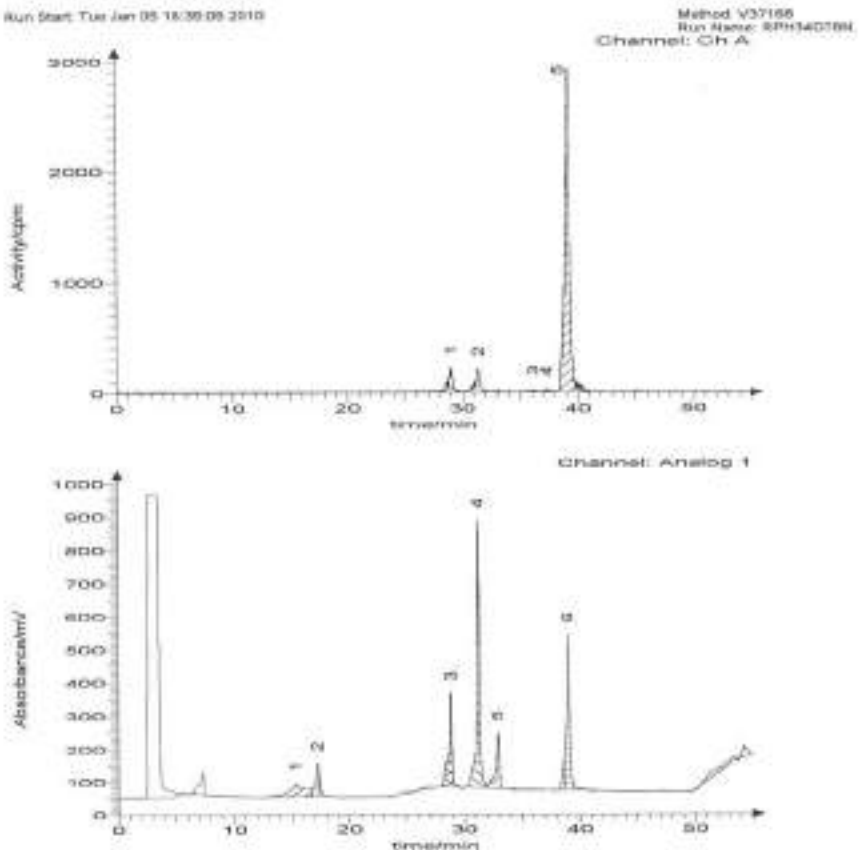
No	t_R [min]	%(Area)
1	16.51	0.43
2	31.67	1.22
3	33.51	0.56
4	34.18	1.01
5	36.26	0.46
6	39.51	96.39
-	-	100.00 *

Channel: Analog 1

No	Name	t_R [min]
1	De-Xy-S-2200	16.44
2	De-CA-S-2200	17.44
3	2-COOH-S-2200	28.97
4	5-COOH-S-2200	31.44
5	MOBX	33.14
6	S-2200	39.34
-		-

Appendix G [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) HPLC Chromatograms

Item #2 Radioactive and UV HPLC chromatograms for RP-34DAT-78N – 34 DAT neutral soil extract. Sample represents 71.2% of the applied dose. Cold standards co-injected.



Channel: Ch A

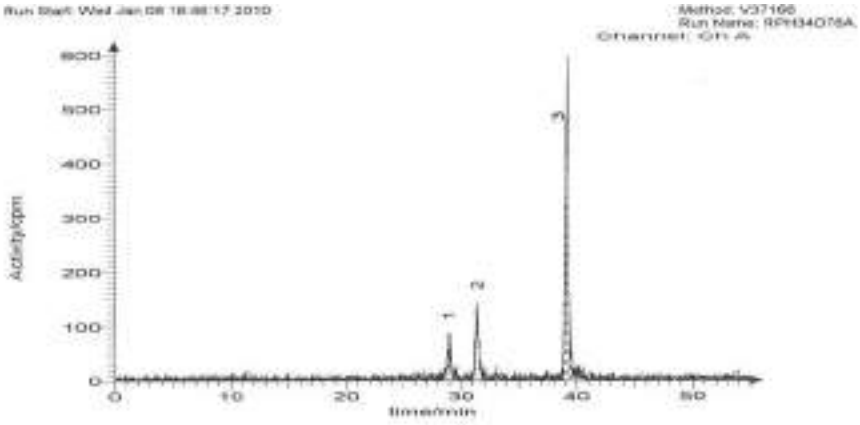
No	t_R [min]	%(Area)	
1	26.92	5.71	3-COOH
2	31.32	6.11	5-COOH
3	36.15	0.55	
4	37.39	0.79	
5	39.16	86.85	S-2167
-	-	100.00 *	

Channel: Analog 1

No	Name	t_R [min]
1	Ox-Xy-S-2200	16.48
2	Ox-CA-S-2200	17.23
3	3-COOH-S-2200	26.70
4	5-COOH-S-2200	31.23
5	MCBX	32.73
6	S-2200	38.97
-		-

Appendix G [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) HPLC Chromatograms

Item #3 Radioactive and UV HPLC chromatograms for RP-34DAT-78A – 34 DAT acidic soil organic extract. Sample represents 14.4% of the applied dose. Cold standards coinjected.



Channel: Ch A

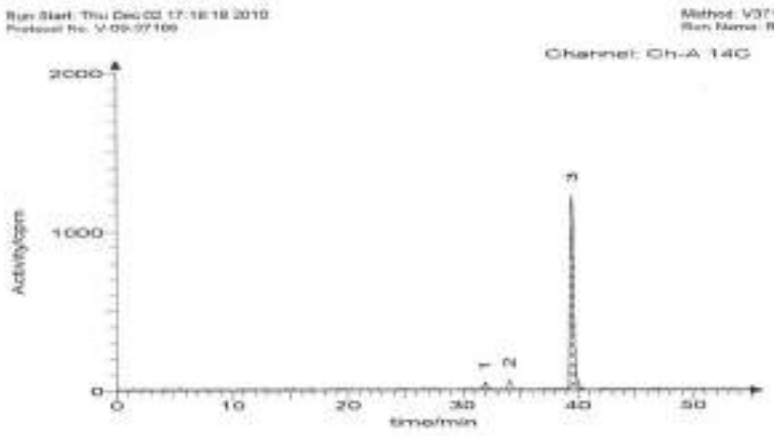
No	t_R [min]	%(Area)	
1	28.98	11.43	2-COOH
2	31.45	14.90	5-COOH
3	39.25	73.67	S-2200
-	--	100.00 *	
-	--	~~157.60~~	

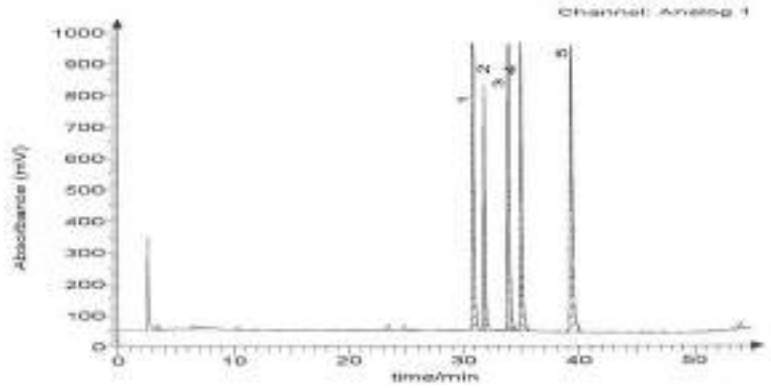
Channel: Analog 1

No	Name	t_R [min]
1	De-Xy-S-2200	15.41
2	De-CA-S-2200	17.15
3	2-COOH-S-2200	28.76
4	5-COOH-S-2200	31.31
5	MCBX	32.92
6	S-2200	39.08
-		--

Appendix G [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) HPLC Chromatograms

Item #4. Radioactive and UV HPLC chromatograms for RP-363DAT-84N – 363 DAT neutral soil extract. Sample represents 43.7% of the applied dose. Cold standards coinjected.





Channel: Ch-A 14C

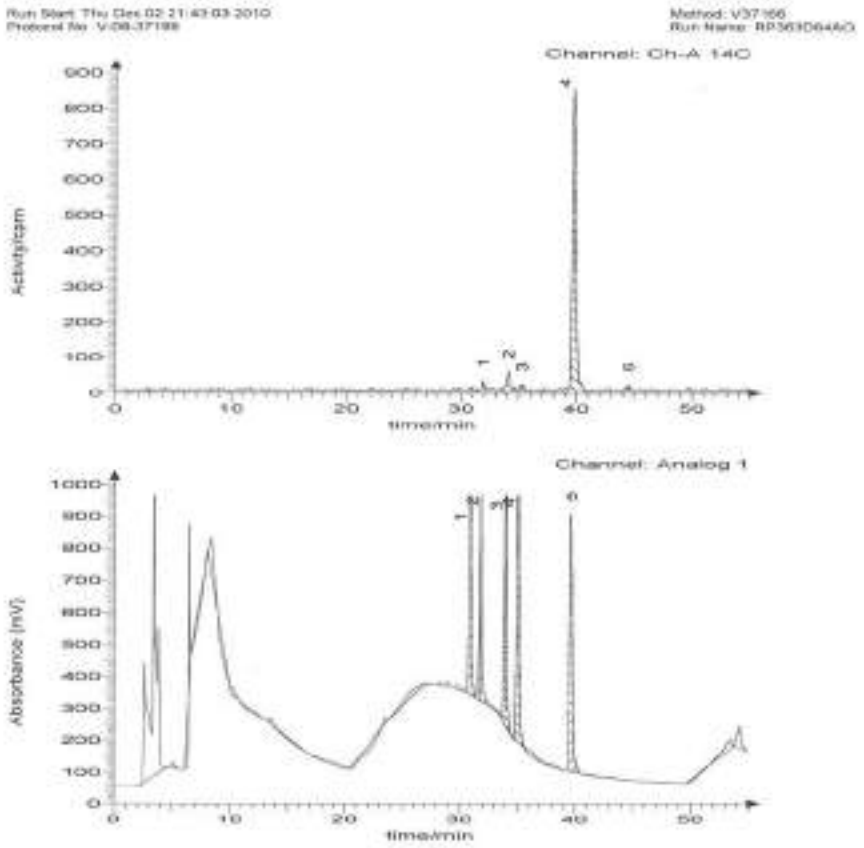
No	t_R [min]	%(Area)	
1	32.02	3.86	3-COOH
2	34.16	4.92	5-COOH
3	39.61	91.12	S-2200
-	--	100.00 *	

Channel: Analog 1

No	Name	t_R [min]
1	2,5-Dimethylphenol	30.92
2	2-COOH-S-2200	31.85
3	5-COOH-S-2200	34.01
4	MCBX	35.04
5	S-2200	39.45
-		--

Appendix G [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) HPLC Chromatograms

Item #5 Radioactive and UV HPLC chromatograms for RP-363DAT-84A – 363 DAT acidic soil organic extract. Sample represents 24.3% of the applied dose. Cold standards coinjected.



Channel: Ch-A 14C

No	t_R [min]	%(Area)
1	32.03	3.42
2	34.17	5.71
3	35.18	4.11
4	39.89	84.65
5	44.51	2.11
-	-	100.00 *

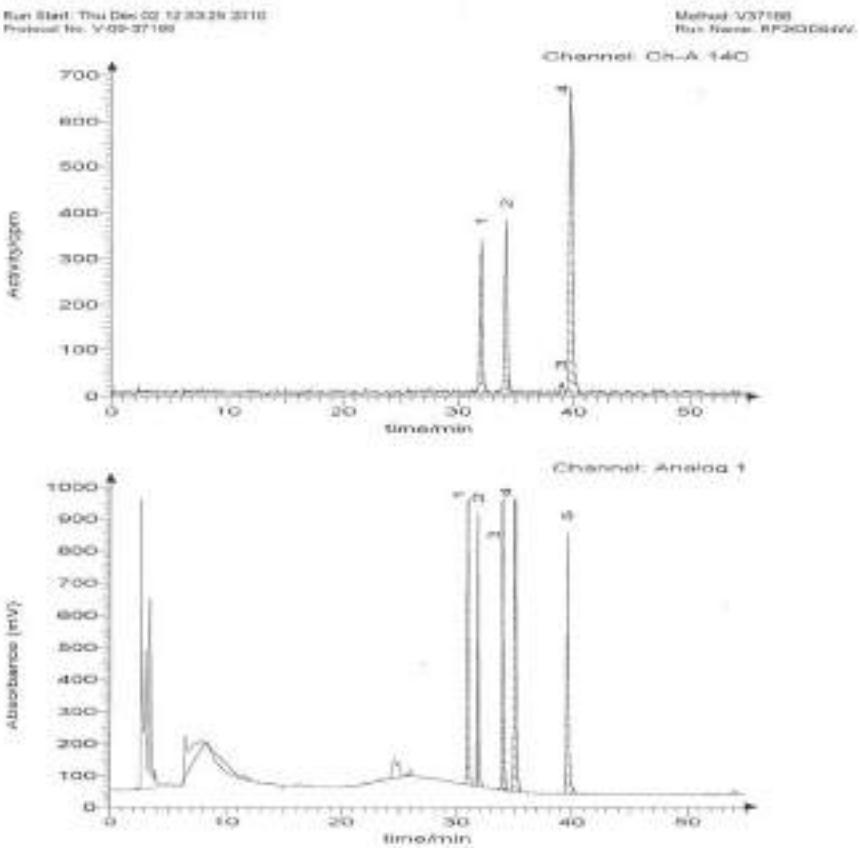
2-COOH
5-COOH
MCBX
S-2200

Channel: Analog 1

No	Name	t_R [min]
1	2,5-Dimethylphenol	30.93
2	2-COOH-S-2200	31.95
3	5-COOH-S-2200	34.03
4	MCBX	35.09
5	S-2200	39.73
-		-

Appendix G [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) HPLC Chromatograms

Item #6 Radioactive and UV HPLC chromatograms for RP-363DAT-84W – 363 DAT water phase. Sample represents 17.4% of the applied dose. Cold standards coinjected.



Channel: Ch-A 14C

No	t_R [min]	%(Area)	
1	32.04	20.40	3-COOH
2	34.19	24.79	5-COOH
3	36.86	2.30	
4	39.79	52.51	S-2200
-	--	100.00 *	

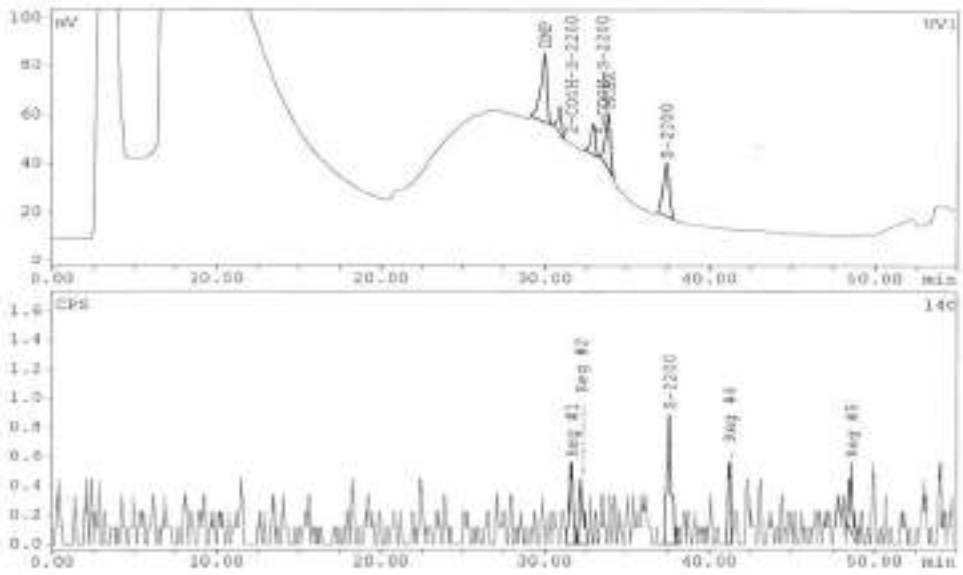
Channel: Analog 1

No	Name	t_R [min]
1	2,5-Dimethylphenol	30.96
2	2-COOH-S-2200	31.88
3	5-COOH-S-2200	34.04
4	MCBX	35.09
5	S-2200	39.63
-		--

Appendix G [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) HPLC Chromatograms

Item #7 Radioactive and UV HPLC chromatograms for RP-363DAT-88D – 363 DAT dismembrator released extract. Sample represents 0.9% of the applied dose. Cold standards coinjected. Compounds observed: S-2200, hint of 2-COOH.

Measurement RPH363D88DE Valent USA Corp Page 1
C:\GINA_NTW\37166\RPH363D88DE



Sample description

Measurement:	RPH363D88DE, injection :		3/1/2011 4:00 PM	
Method:	V37166 from:	3/1/2011 11:24 AM	Position:	100

Integration 14C

Substance	R/T min	Type	Area counts	%Area %
Reg#1	31.68	BD	9.00000	19.12
Reg#2	32.17	DS	7.00000	14.87
S-2200	37.60	BB	17.00000	36.12
Reg#4	41.27	BB	7.00000	14.87
Reg#5	48.62	BB	7.07143	15.02
Sum in ROI			47.07143	
Area			380.00000	

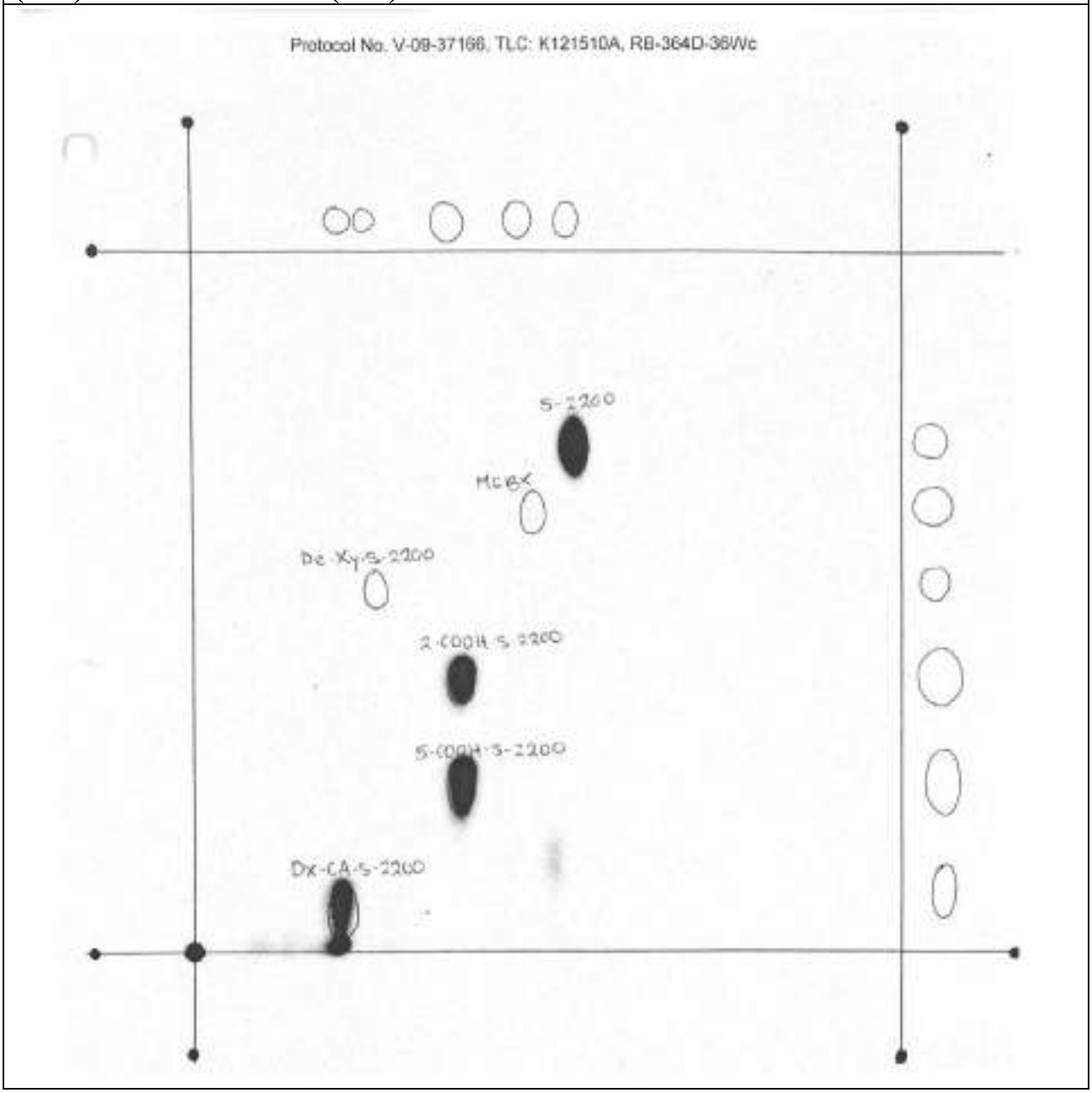
Integration UV1

Substance	R/T min	Type	Area mVmin	%Area %
DMP	30.00	BB	689.4434	33.04
2-COOH-S-2200	30.93	BB	192.9805	9.25
6-COOH-S-2200	33.00	BB	239.6541	11.49
MCBX	33.87	BB	439.0000	21.04
S-2200	37.43	BB	525.4782	25.18
Sum in ROI			2086.5661	

KS 3/2/11

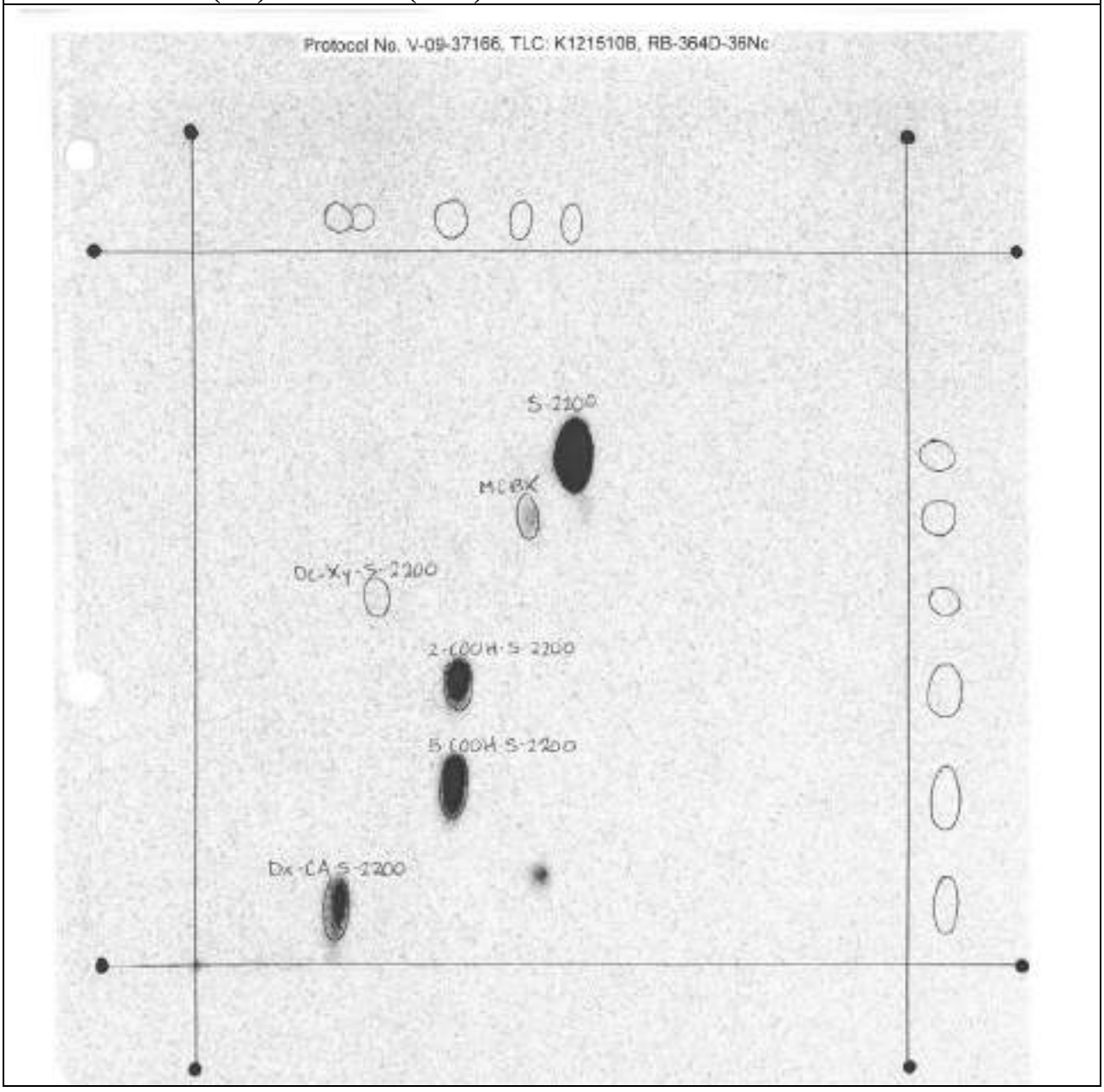
Appendix H Confirmation Data: 2D TLC, Chiral HPLC.

Figure 1 – 2D TLC Radiochromatogram for *R*-[Benzyl-^{14}C]S-2167 water fraction (364 DAT) fraction. Sample co-spotted with cold stds. First direction. Chloroform:Methanol:TFA (90:10:0.1); Second direction. Toluene:Ethyl acetate:Acetic acid (5:7:1). Sample represents 23% of the dose. Confirms S-2200 (48% of plate activity), Dx-CA (13%), 2-COOH-S-2200 (13%) and 5-COOH-S-2200 (17%).



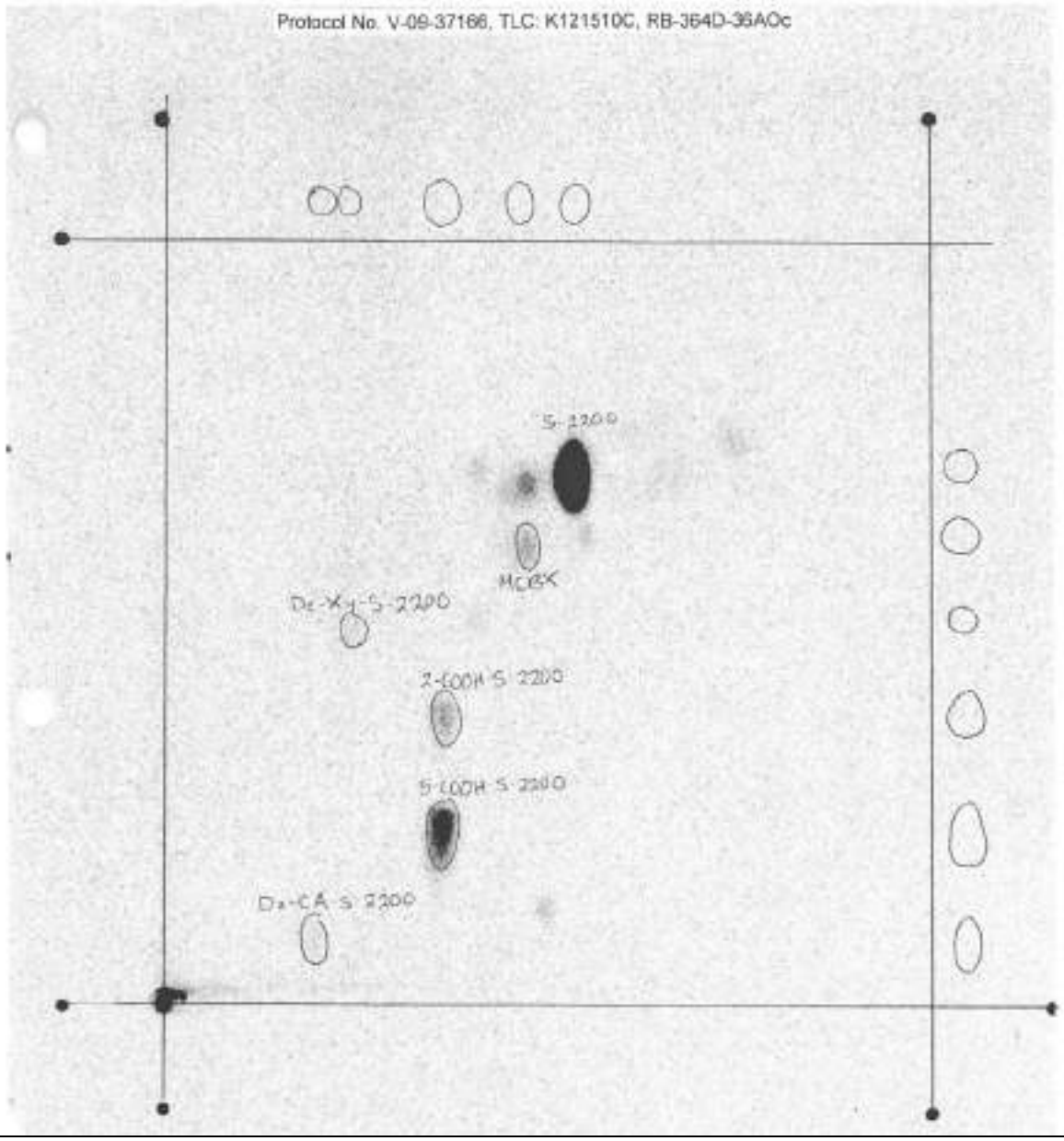
Appendix H Confirmation Data: 2D TLC, Chiral HPLC.

Figure 2 – 2D TLC Radiochromatogram for *R*-[Benzyl-^{14}C]S-2167 neutral fraction (364 DAT) fraction. Sample co-spotted with cold stds. First direction. Chloroform:Methanol:TFA (90:10:0.1); Second direction. Toluene:Ethyl acetate:Acetic acid (5:7:1). Sample represents 51% of the dose. Confirms S-2200 (92% of plate activity), Dx-CA (2%), 2-COOH-S-2200 (2%), 5-COOH-S-2200 (3%) and MCBX (0.3%).



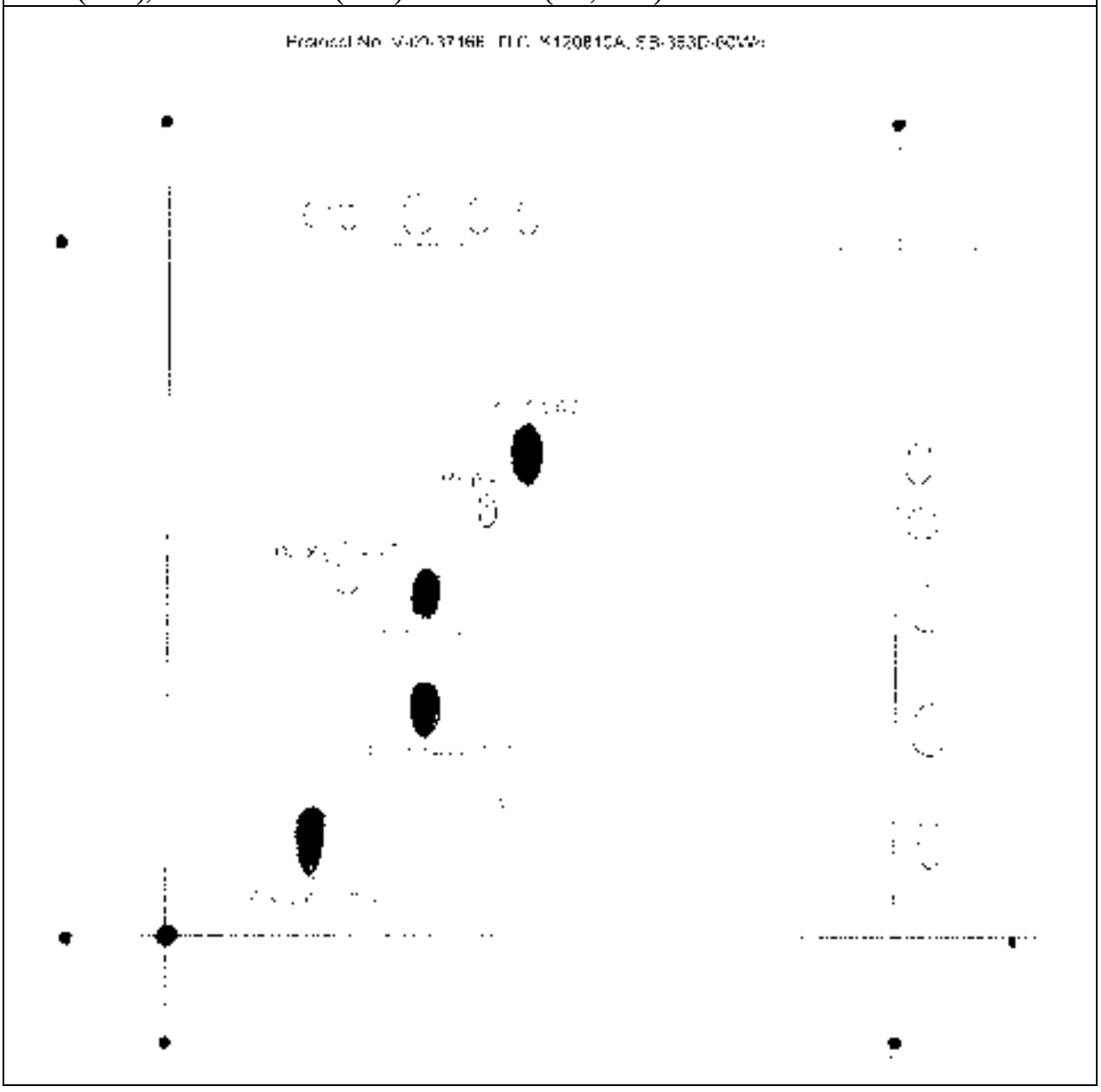
Appendix H Confirmation Data: 2D TLC, Chiral HPLC.

Figure 3 – 2D TLC Radiochromatogram for *R*-[Benzyl-^{14}C]S-2167 acidic fraction (364 DAT) fraction. Sample co-spotted with cold stds. First direction. Chloroform:Methanol:TFA (90:10:0.1); Second direction. Toluene:Ethyl acetate: Acetic acid (5:7:1). Sample represents 22% of the dose. Confirms S-2200 (83% of plate activity), Dx-CA (0.4%), 2-COOH-S-2200 (1%), 5-COOH-S-2200 (4%) and MCBX (0.9%).



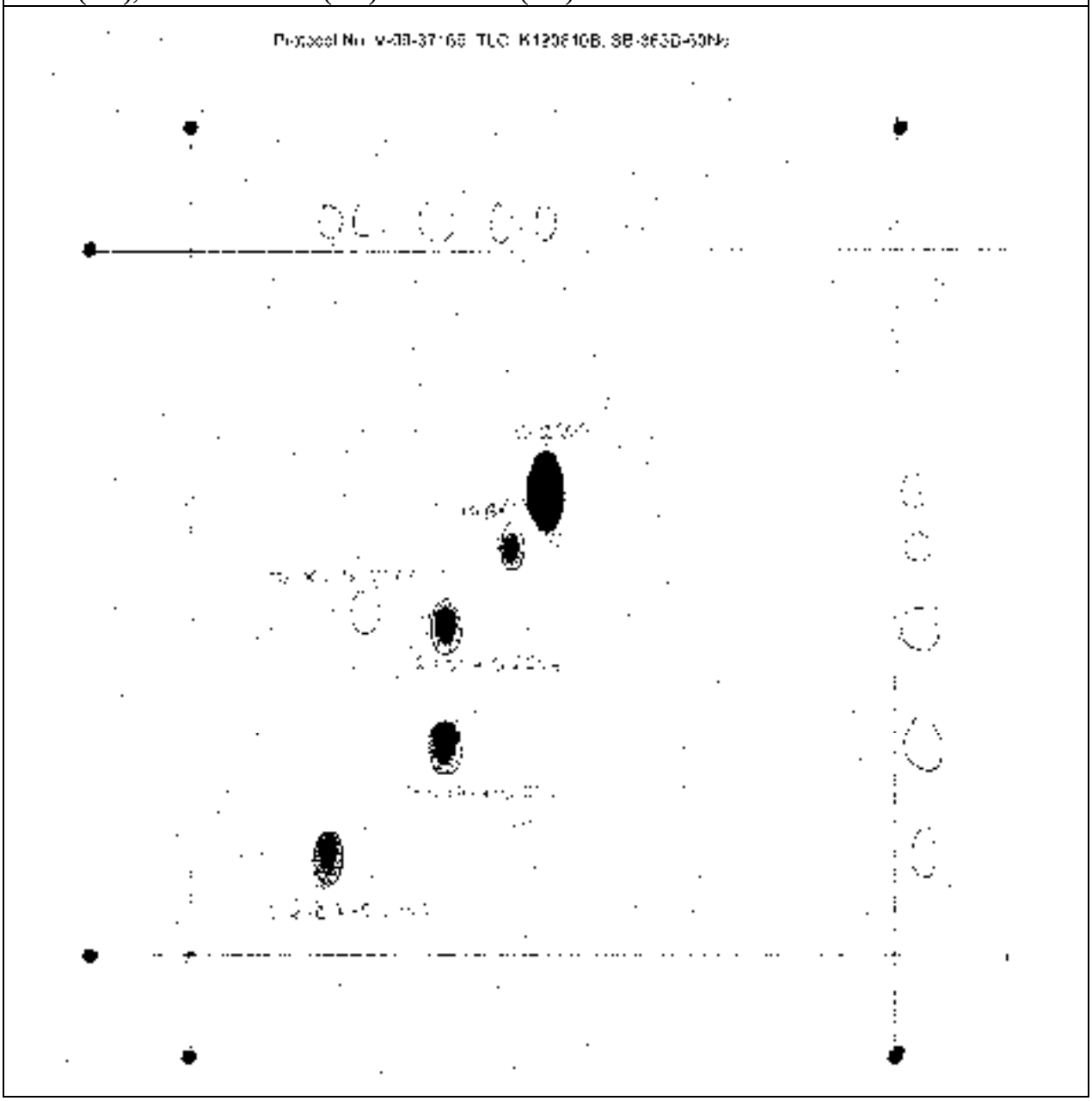
Appendix H Confirmation Data: 2D TLC, Chiral HPLC.

Figure 4 – 2D TLC Radiochromatogram for *S*-[Benzyl-^{14}C]S-2354 water fraction (363 DAT) fraction. Sample co-spotted with cold stds. First direction. Chloroform:Methanol:TFA (90:10:0.1); Second direction. Toluene:Ethyl acetate: Acetic acid (5:7:1). Sample represents 22% of the dose. Confirms S-2200 (38% of plate activity), 2-COOH-S-2200 (15%), 5-COOH-S-2200 (17%), Dx-CA-S-2200 (18%) and MCBX (1%, faint).



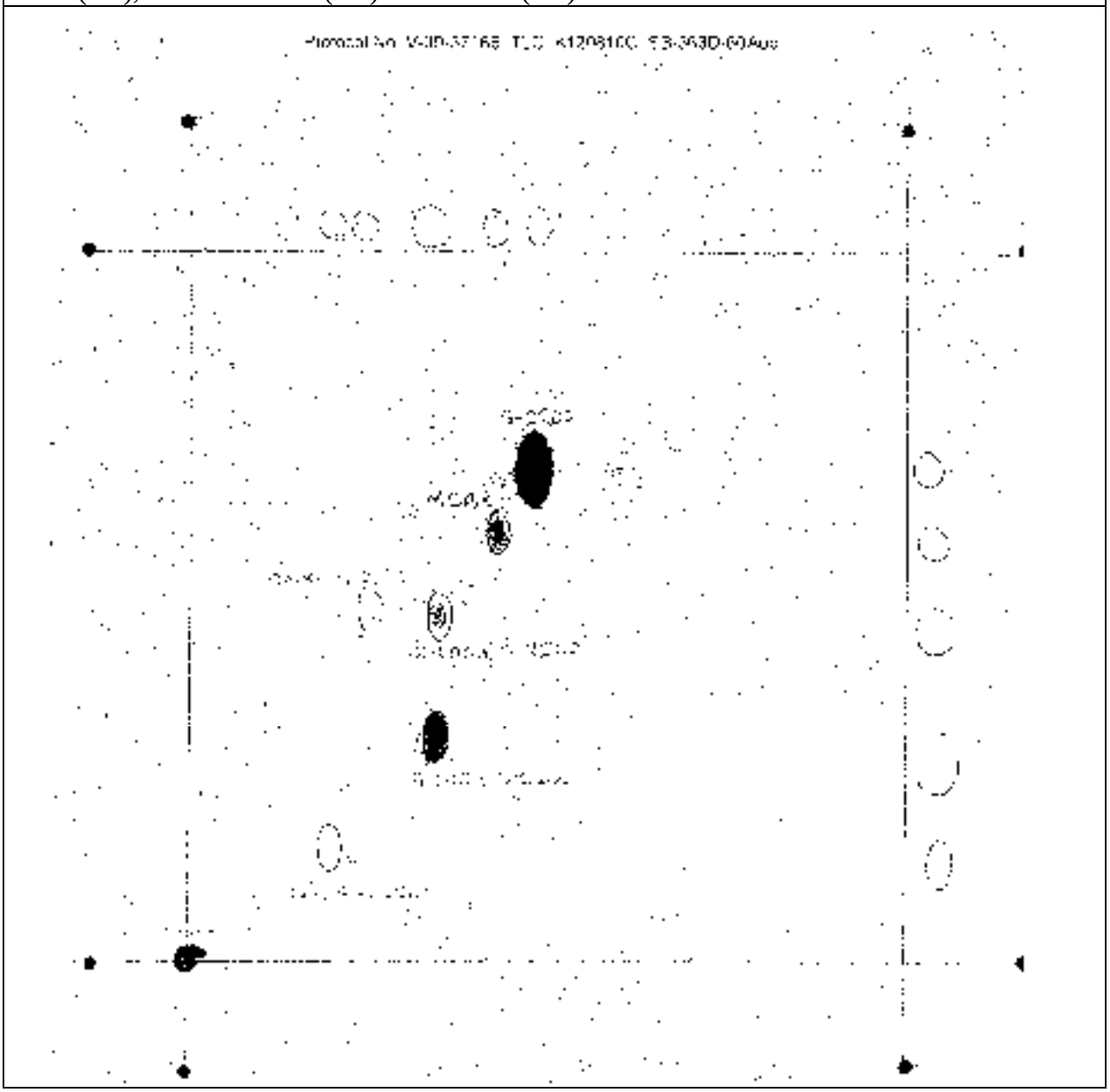
Appendix H Confirmation Data: 2D TLC, Chiral HPLC.

Figure 5 – 2D TLC Radiochromatogram for *S*-[Benzyl-^{14}C]S-2354 neutral fraction (363 DAT) fraction. Sample co-spotted with cold stds. First direction. Chloroform:Methanol:TFA (90:10:0.1); Second direction. Toluene:Ethyl acetate: Acetic acid (5:7:1). Sample represents 44% of the dose. Confirms S-2200 (91% of plate activity), 2-COOH-S-2200 (2%), 5-COOH-S-2200 (3%), Dx-CA-S-2200 (2%) and MCBX (1%).



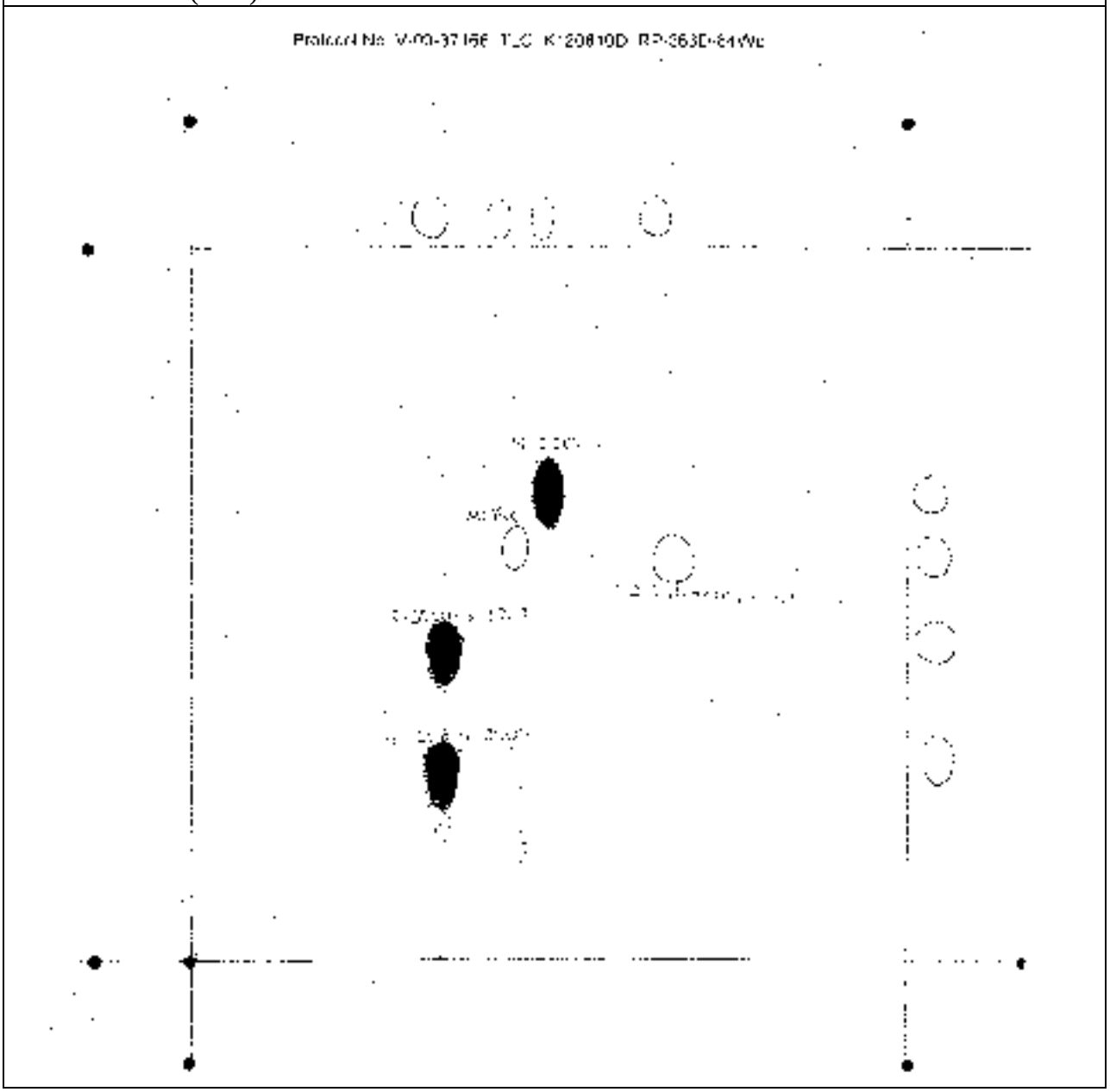
Appendix H Confirmation Data: 2D TLC, Chiral HPLC.

Figure 6 – 2D TLC Radiochromatogram for *S*-[Benzyl-^{14}C]S-2354 acidic fraction (363 DAT) fraction. Sample co-spotted with cold stds. First direction. Chloroform:Methanol:TFA (90:10:0.1); Second direction. Toluene:Ethyl acetate: Acetic acid (5:7:1). Sample represents 23% of the dose. Confirms S-2200 (86% of plate activity), 2-COOH-S-2200 (1%), 5-COOH-S-2200 (4%), Dx-CA-S-2200 (1%) and MCBX (2%).



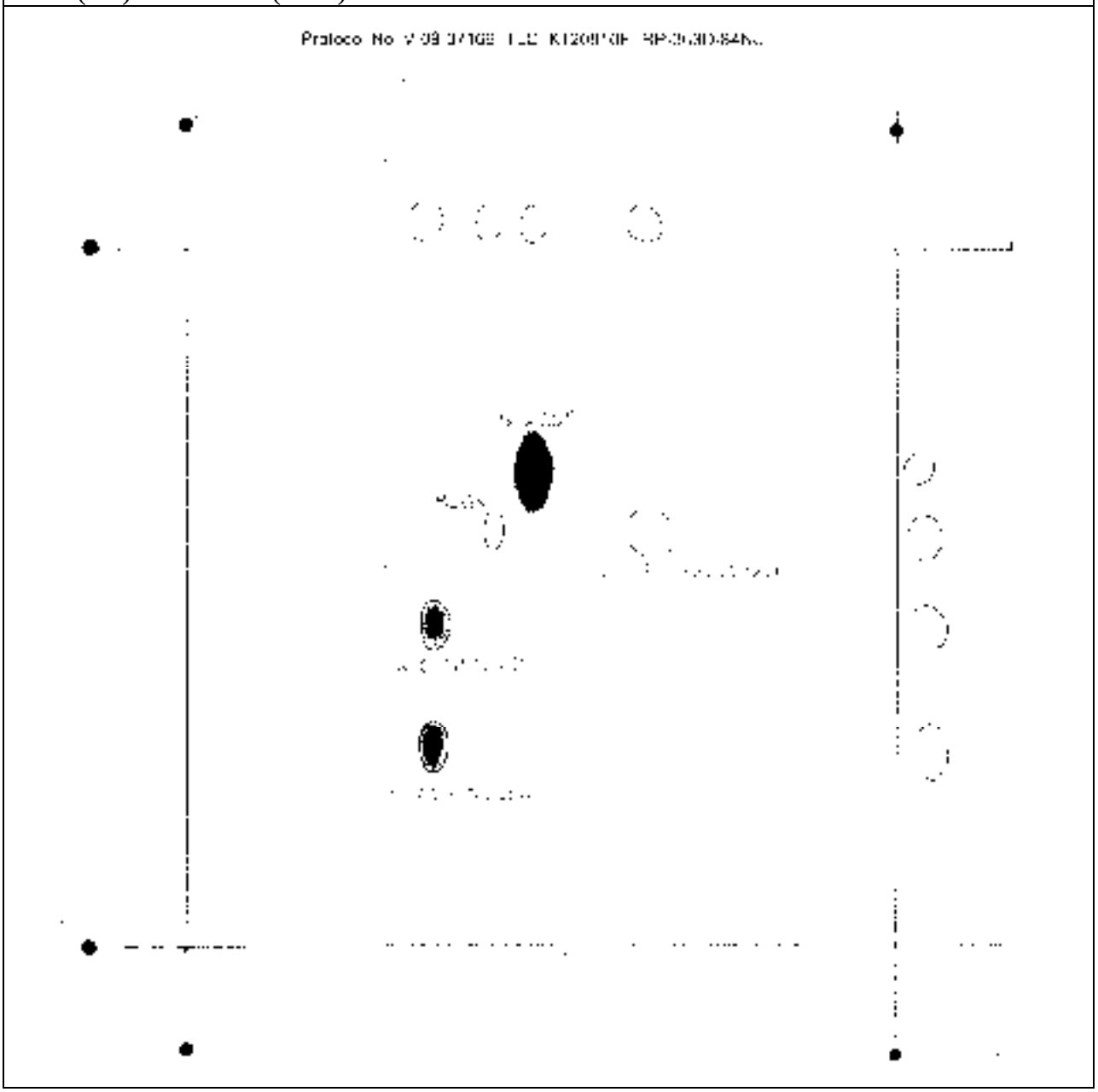
Appendix H Confirmation Data: 2D TLC, Chiral HPLC.

Figure 7 – 2D TLC Radiochromatogram for *R*-[Phenoxy-^{14}C]S-2167 water fraction (363 DAT) fraction. Sample co-spotted with cold stds. First direction. Chloroform:Methanol:TFA (90:10:0.1); Second direction. Toluene:Ethyl acetate: Acetic acid (5:7:1). Sample represents 17% of the dose. Confirms S-2200 (54% of plate activity), 2-COOH-S-2200 (19%) and 5-COOH-S-2200 (21%).



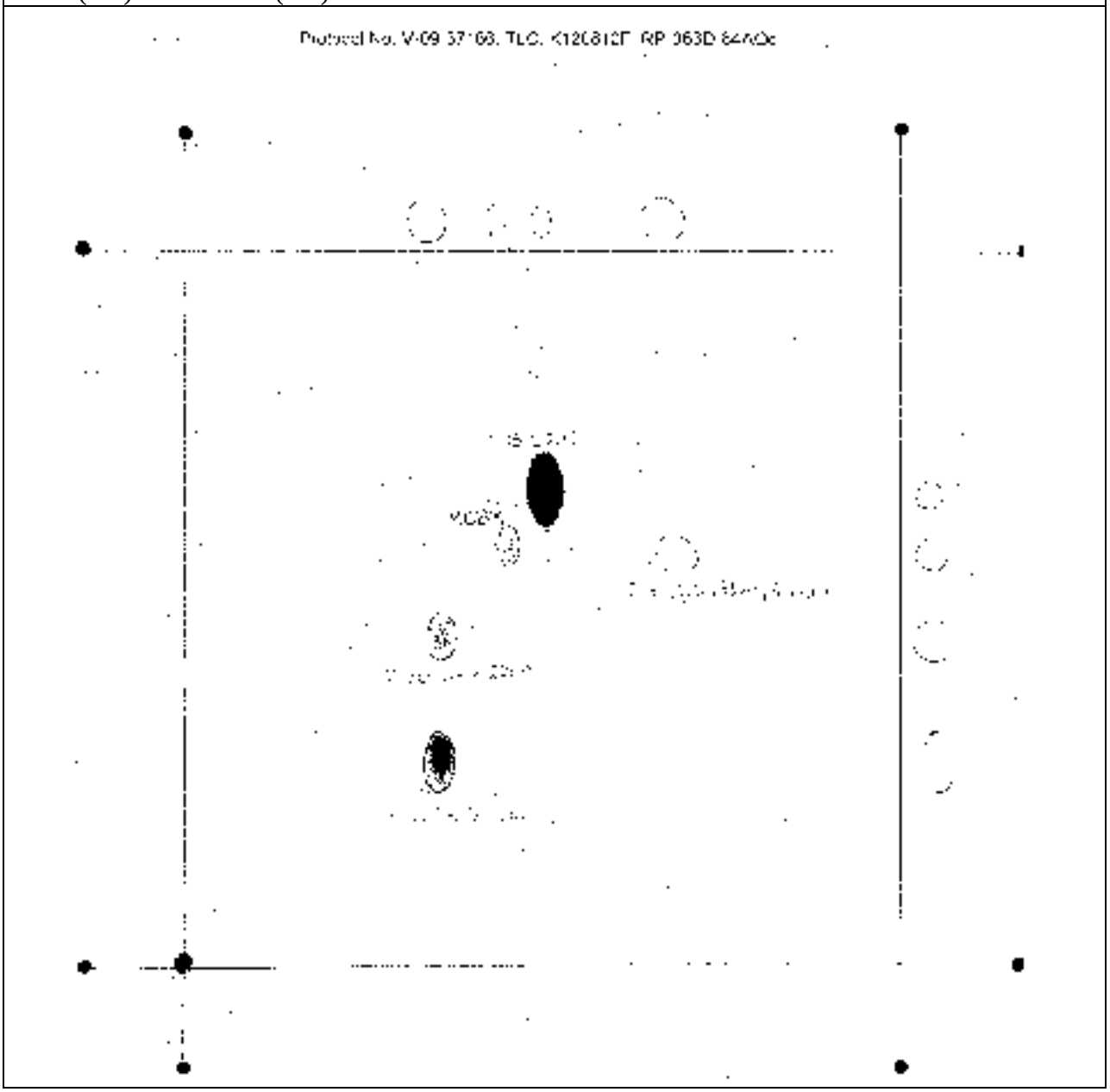
Appendix H Confirmation Data: 2D TLC, Chiral HPLC

Figure 8 – 2D TLC Radiochromatogram for *R*-[Phenoxy-^{14}C]S-2167 neutral fraction (363 DAT) fraction. Sample co-spotted with cold stds. First direction. Chloroform:Methanol:TFA (90:10:0.1); Second direction. Toluene:Ethyl acetate: Acetic acid (5:7:1). Sample represents 44% of the dose. Confirms S-2200 (93% of plate activity), 2-COOH-S-2200 (3%), 5-COOH-S-2200 (4%) and MCBX (0.4%).



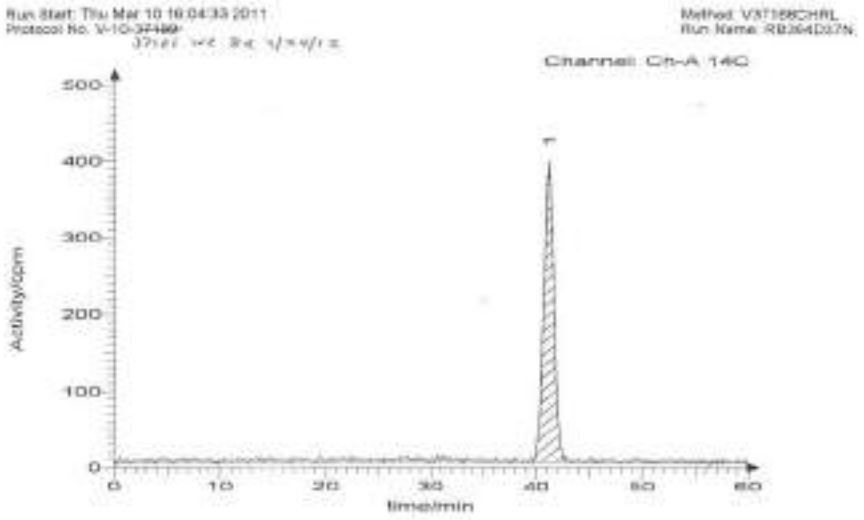
Appendix H Confirmation Data: 2D TLC, Chiral HPLC

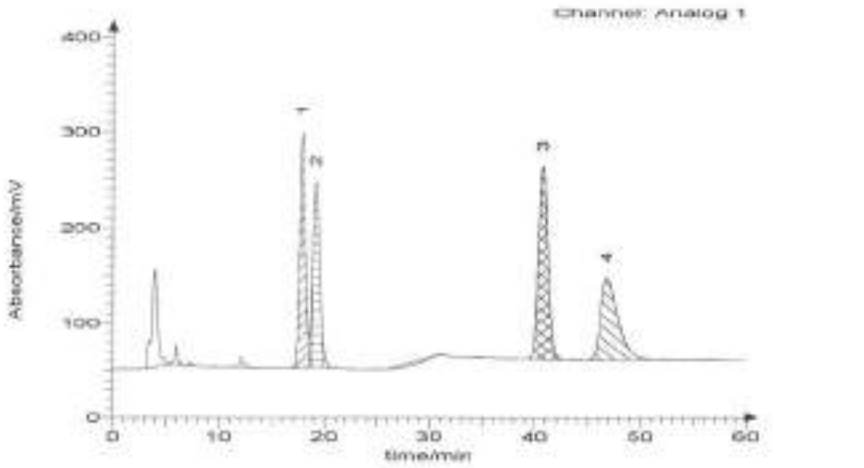
Figure 9 – 2D TLC Radiochromatogram for *R*-[Phenoxy-^{14}C]S-2167 acidic fraction (363 DAT) fraction. Sample co-spotted with cold stds. First direction. Chloroform:Methanol:TFA (90:10:0.1); Second direction. Toluene:Ethyl acetate: Acetic acid (5:7:1). Sample represents 24% of the dose. Confirms S-2200 (88% of plate activity), 2-COOH-S-2200 (1%), 5-COOH-S-2200 (6%) and MCBX (1%).



Appendix H Confirmation Data: 2D TLC, Chiral HPLC

Item #10. Chiral Radioactive and UV HPLC chromatograms for RB-364DAT-37N – 364 DAT neutral soil extract. Sample represents 47% of the applied dose. Cold standards coinjected. No isomerization of S-2200 detected.





Channel: Ch-A 14C

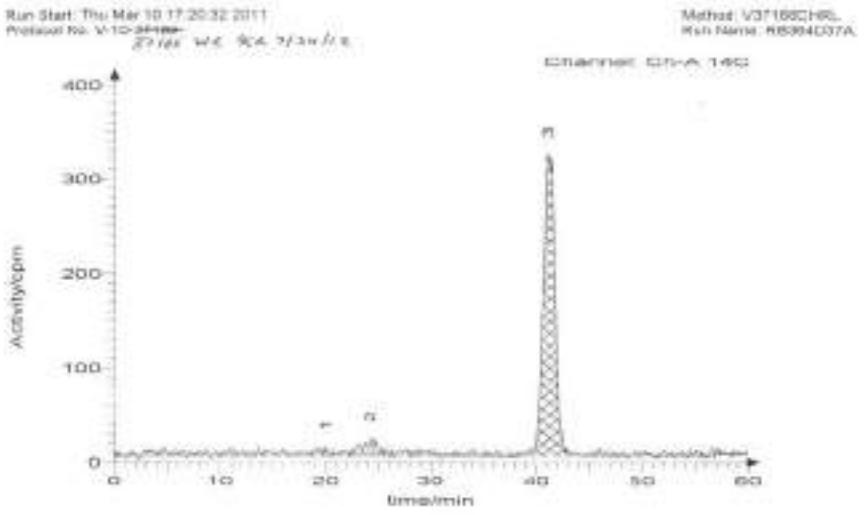
No	Name	Area [cpm]	Area-Base [cpm]	t_R [min]
1	S-2187	25888.60	24824.89	41.21

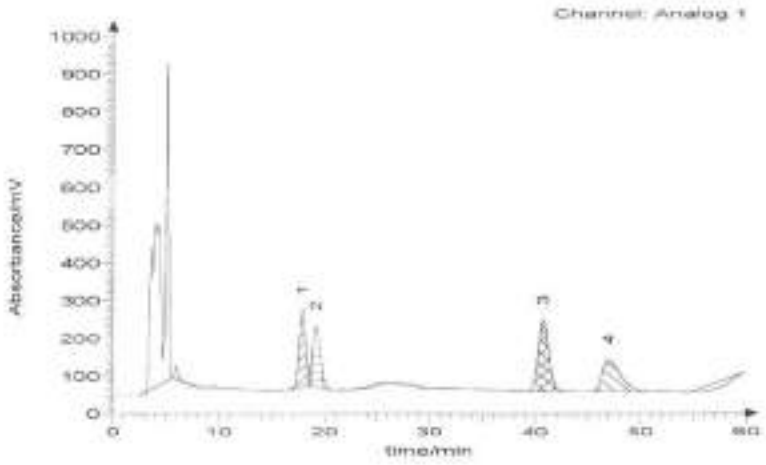
Channel: Analog 1

No	Name	Area [mV*min]	Area-Base [mV*min]	t_R [min]
1	R-MCBX	242.40	163.02	17.95
2	S-MCBX	247.89	149.73	19.28
3	S-2187	399.52	201.77	40.86
4	S-2354	447.08	180.89	47.25

Appendix H Confirmation Data: 2D TLC, Chiral HPLC

Item #11. Chiral Radioactive and UV HPLC chromatograms for RB-364DAT-37A – 364 DAT acidic soil organic extract. Sample represents 21% of the applied dose. Cold standards coinjected. No isomerization of S-2200 detected.





Channel: Ch-A 14C

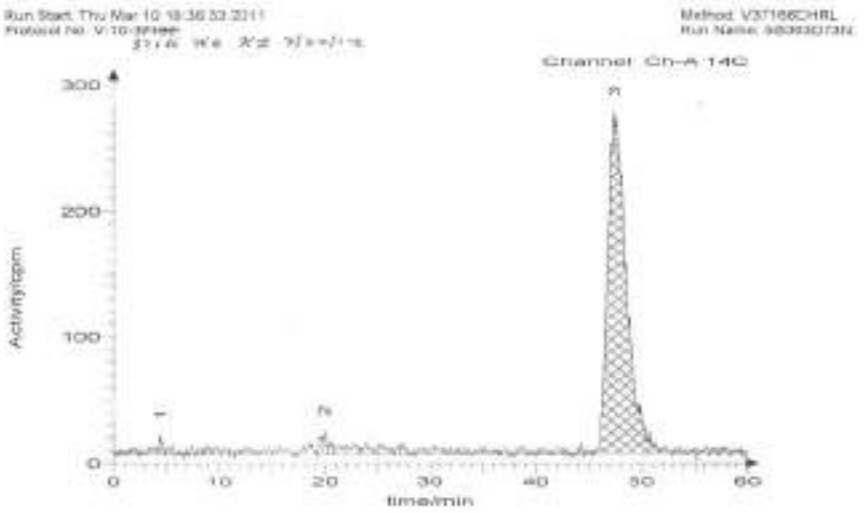
No	Name	t_R [min]	Area-Base [uAm]	%(Area-Base)
1		19.55	400.85	1.86
2		24.04	1605.58	7.13
3	S-2167	41.35	23034.06	91.01
-		-	25369.93	100.00 *

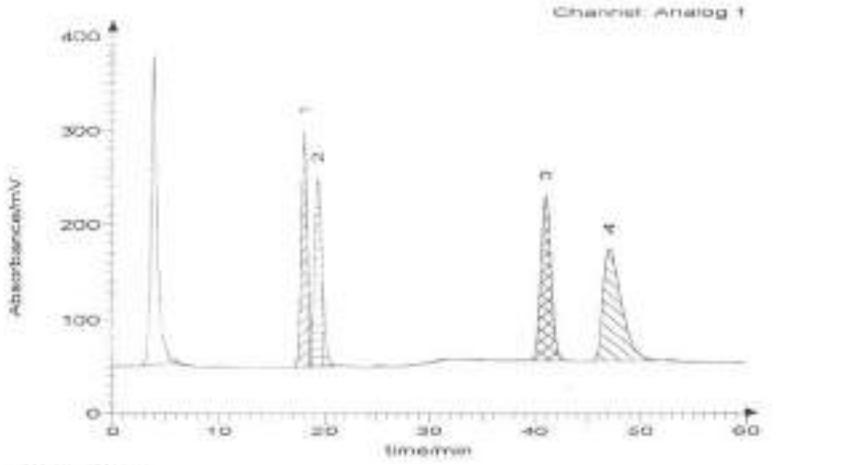
Channel: Analog 1

No	Name	t_R [min]
1	R-MCBX	17.99
2	S-MCBX	19.24
3	S-2167	40.91
4	S-2364	47.45
-		-
-		-

Appendix H Confirmation Data: 2D TLC, Chiral HPLC

Item #12. Chiral Radioactive and UV HPLC chromatograms for SB-363DAT-73N – 363 DAT neutral soil extract. Sample represents 46% of the applied dose. Cold standards coinjected. No isomerization of S-2200 detected. Hint of S-MCBX detected.





Channel: Ch-A 14C

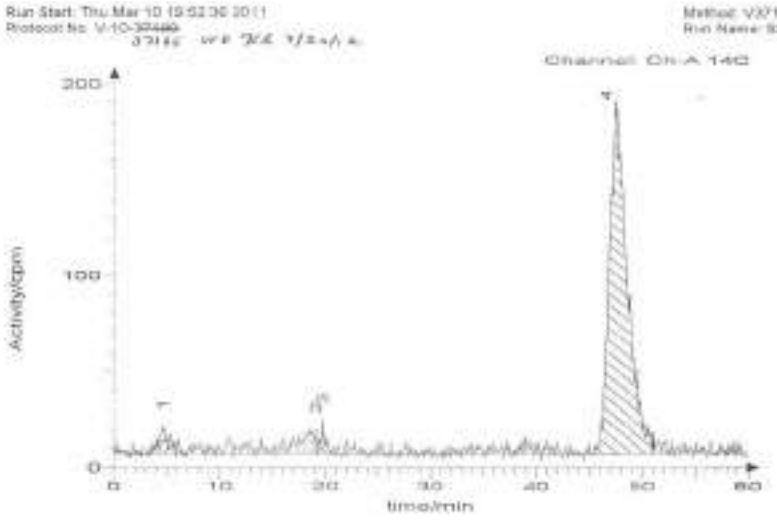
No	Name	t_R [min]	Area-Base [cpm]	%Area-Base
1		4.22	488.84	1.65
2		20.02	799.48	2.35
3	S-2364	47.06	32320.42	96.18
-		-	33609.75	100.00 *

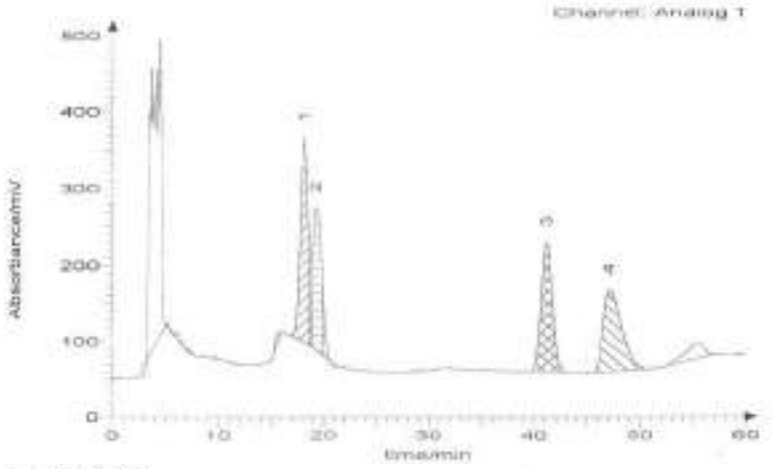
Channel: Analog 1

No	Name	t_R [min]
1	R-MCBX	16.13
2	S-MCBX	16.48
3	S-2167	41.13
4	S-2364	47.56
-		-

Appendix H Confirmation Data: 2D TLC, Chiral HPLC

Item #13. Chiral Radioactive and UV HPLC chromatograms for SB-363DAT-37A – 363 DAT acidic soil organic extract. Sample represents 24% of the applied dose. Cold standards coinjected. No isomerization of S-2200 detected.





Channel: Ch-A 14C

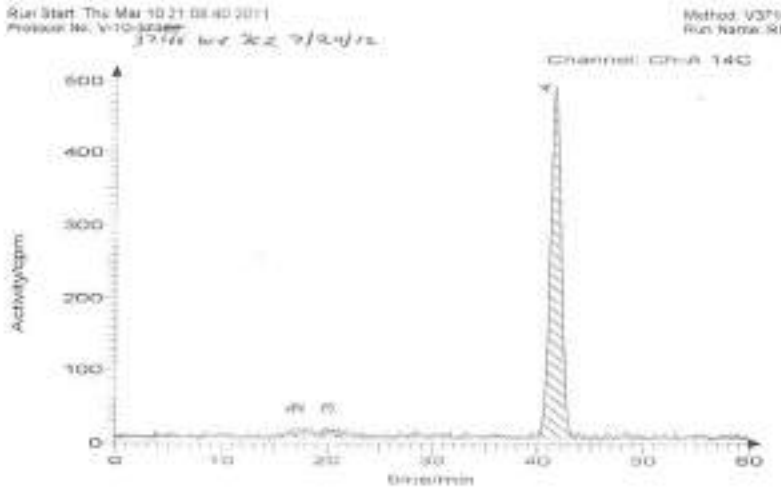
No	Name	t_R [min]	Area-Base [cpm]	%Area-Base
1		4.78	720.55	3.16
2		18.36	1010.59	4.45
3		19.62	488.05	2.15
4		47.99	20041.06	90.26
-		-	22988.34	100.00

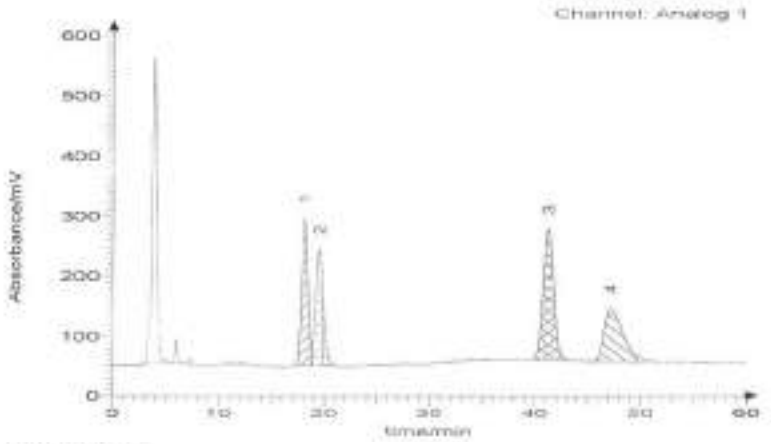
Channel: Analog 1

No	Name	t_R [min]
1	R-MCBX	16.13
2	S-MCBX	19.41
3	S-2197	41.22
4	S-2354	47.95

Appendix H Confirmation Data: 2D TLC, Chiral HPLC

Item #14. Chiral Radioactive and UV HPLC chromatograms for RP-363DAT-84N – 363 DAT neutral soil extract. Sample represents 44% of the applied dose. Cold standards coinjected. No isomerization of S-2200 detected.





Channel: Ch-A 14C

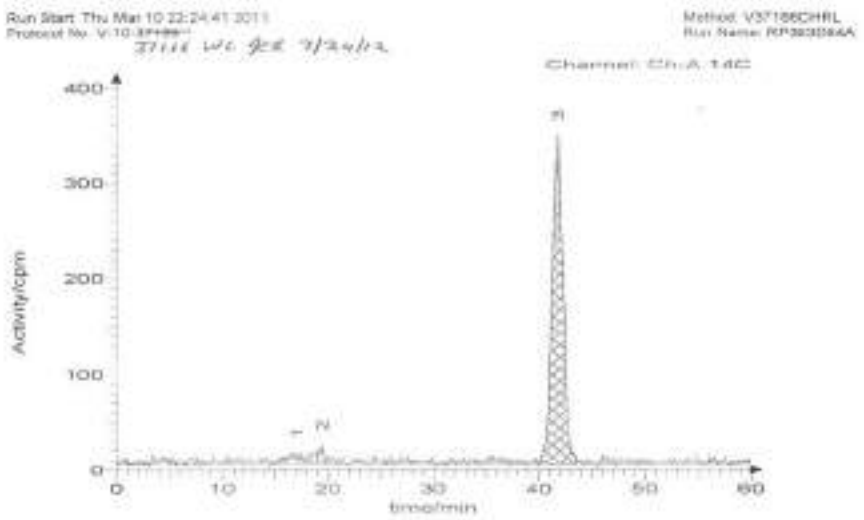
No	Name	t_R [min]	Area-Base [cpm]	%(Area-Base)
1		19.85	327.77	0.93
2		19.14	1017.08	2.88
3		20.40	971.87	2.73
4		41.70	33246.93	93.48
-		---	35563.66	100.00 *
-		---	39560.47	111.07

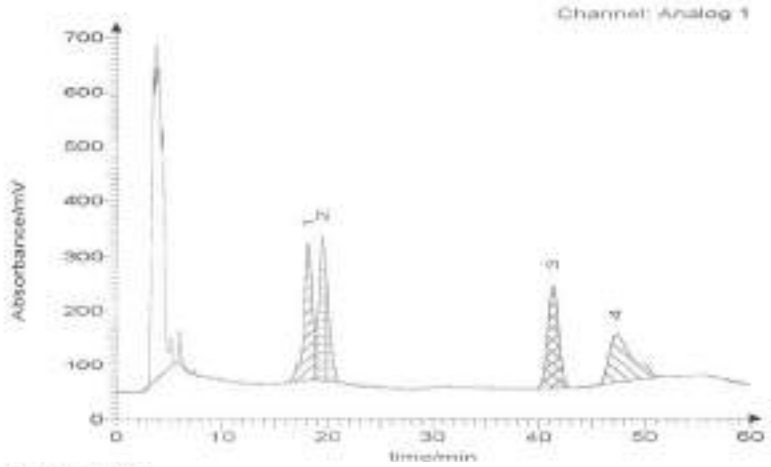
Channel: Analog 1

No	Name	t_R [min]
1	R-MCBX	19.19
2	S-MCBX	19.99
3	S-2197	41.34
4	S-2254	47.73
-		---

Appendix H Confirmation Data: 2D TLC, Chiral HPLC

Item #15. Chiral Radioactive and UV HPLC chromatograms for RP-363DAT-84A – 363 DAT acidic soil organic extract. Sample represents 24% of the applied dose. Cold standards coinjected. No isomerization of S-2200 detected.





Channel: Ch-A 14C

No	Name	t_R [min]	Area-Base [cpm]	%(Area-Base)
1		16.63	736.41	2.07
2		18.83	1051.08	4.10
3	S-2167	41.70	23892.65	93.83
-		-	25680.14	100.00 *

Channel: Analog 1

No	Name	t_R [min]
1	R-MCBX	18.11
2	S-MCBX	18.64
3	S-2167	41.42
4	S-2354	48.12
-		-

Appendix I Representative Data: ([benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) Exposure

Excel spreadsheet data for 0, 30 and 364 DAT samples

Sample ID: R-Be-0D-48 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(R)-benzyl-^{14}C]S-2167 401G **SA (dpm/ug):** 57151 **Sampling Date:** 12/14/2009
DAT: 0 **Soil:** **Baptistown, NJ (Penn Series - Loam)**

Sample initial conditions and weights (before incubation)				**Sample parameters (after incubation)**		
Applied ^{14}C (dpm)	Applied ^{14}C-S-2200 (ppm)	Soil dry wt. (g)	Init. water added (mL)	pH	Dissolved O_2 (ppm)	Redox potential (mV)
25568700	8.95	50	NA	NA	NA	NA

Sample Headspace data	Headspace volume (mL)	Headspace injected	Headspace LSC Reference No.	Headspace injection dpm	Total sample headspace (dpm)	% of applied
	NA	NA	NA	NA	NA	NA

Evolved $^{14}CO_2$ data	Trap NaOH Volume (mL)	Aliquot counted (mL)	LSC ref No.	Aliquot dpm	Trap dpm	% of applied
	NA	NA	NA	NA	NA	NA

Soil Phase data	Empty Tube wt.	Sample & tube wt. (g)	Sample Net wt.	LSC Reference No.	Total oxidized (g)	dpm/total oxidized	dpm/g	Sample dpm[1]	% of applied
PES after neutral extraction	50.30	118.53	68.23	Y199-Y201	1.14	6083	5341	366592	1.43

Extraction of the soil phase	Sample Vol. (mL)	Aliquot counted (mL)	LSC Reference No.	Aliquot dpm	Sample dpm	% of applied
1st extract (neutral)	290	1.0	Y95-Y97	88965	25799850	100.90
2nd extract (acidic)	NA	NA	NA	NA	NA	NA

Sample Balance Biometer/beaker rinse	$^{14}CO_2$	$^{14}CH_4$		Soil extract	Post extraction soil PES			Total % of appl.
NA	NA	NA		100.90	1.43			102.34

1) Total dpm corrected for the oxidation efficiency. NA = Not analyzed. 0.994

Appendix I Representative Data: ([benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) Exposure

Excel spreadsheet data for 0, 30 and 364 DAT samples

Sample ID: R-Be-0D-48 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**

Label: [(R)-benzyl-^{14}C]S-2167 401G **SA (dpm/ug):** 57151 **Sampling Date:** 12/14/2009

DAT: 0 **Soil:** **'Baptistown, NJ (Penn Series - Loam)**

HPLC analysis	**Soil phase**						**Total**
Compound (t_R)	1st extract (neutral)			2nd extract (acidic)			% of appl. In soil
(minutes)	% area	dpm	% appl.	% area	dpm	% appl.	
De-Xy							0.00
Dx-CA							0.00
2-COOH							0.00
5-COOH							0.00
MCBX							0.00
34.10	0.83	214139	0.84				0.84
36.24	0.47	121259	0.47				0.47
S-2200, 39.39	98.70	25464452	99.59				99.59
Total unknowns			1.31				1.31
2D TLC confirmation							

Unknown Summary

Soil extract Unknowns:		1.31	% of applied
Total Unknowns	2	1.31	% of applied
Highest single Unknown		0.84	% of applied

Extraction Method

Neutral extraction: Extract the soil 2X with 150 mL of 9:1 (v:v) acetone/water.

Appendix I Representative Data: ([benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) Exposure

Excel spreadsheet data for 0, 30 and 364 DAT samples

Sample ID: R-Be-30D-28 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(R)-benzyl-^{14}C]S-2167 401G **SA (dpm/ug):** 57151 **Sampling Date:** 1/13/2010
DAT: 30 **Soil:** **Baptistown, NJ (Penn Series - Loam)**

Sample initial conditions and weights (before incubation)				Sample parameters (after incubation)		
Applied ^{14}C (dpm)	Applied ^{14}C-S-2200 (ppm)	Soil dry wt. (g)	Init. water added (mL)	pH	Dissolved O_2 (ppm)	Redox potential (mV)
25568700	8.95	50	NA	NA	NA	NA

Sample Headspace data	Headspace volume (mL)	Headspace injected	Headspace LSC Reference No.	Headspace injection dpm	Total sample headspace (dpm)	% of applied
	NA	NA	NA	NA	NA	NA

Evolved $^{14}CO_2$ data	Trap NaOH Volume (mL)	Aliquot counted (mL)	LSC ref No.	Aliquot dpm	Trap dpm	% of applied
	NA	NA	NA	NA	NA	1.76

Soil Phase data	Empty Tube wt.	Sample & tube wt. (g)	Sample Net wt.	LSC Reference No.	Total oxidized (g)	dpm/total oxidized	dpm/g	Sample dpm[1]	% of applied
PES after acidic extraction	50.06	118.74	68.68	Y715-Y717	1.53	28534	18625	1281751	5.01

Extraction of the soil phase	Sample Vol. (mL)	Aliquot counted (mL)	LSC Reference No.	Aliquot dpm	Sample dpm	% of applied
1st extract (neutral)	280	1.0	Y459-Y461	74505	20861400	81.59
2nd extract (acidic)	390	1.0	Y465-Y467	11261	4391790	17.18
acid partition (aqueous)	36	1.0	Y618-Y620	969	34884	0.14
acid partition (organic)	340	1.0	Y612-Y614	12791	4348940	17.01

Sample Balance Biometer/beaker rinse	$^{14}CO_2$	$^{14}CH_4$		Soil extract	Post extraction soil PES			Total % of appl.
NA	1.76	NA		98.77	5.01			105.54

1) Total dpm corrected for the oxidation efficiency. NA = Not analyzed. 0.998

Appendix I Representative Data: ([benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) Exposure

Excel spreadsheet data for 0, 30 and 364 DAT samples

Sample ID: R-Be-30D-28 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(R)-benzyl-^{14}C]S-2167 401G **SA (dpm/ug :** 57151 **Sampling Date:** 1/13/2010
DAT: 30 **Soil: Baptistown, NJ (Penn Series - Loam)**

HPLC analysis	**Soil phase**						**Total**
Compound (t_R)	1st extract (neutral)			2nd extract (acidic)			% of appl. In soil
(minutes)	% area	dpm	% appl.	% area	dpm	% appl.	
De-Xy-S, 15.88	1.85	385936	1.51				1.51
16.26				1.61	70018	0.27	0.27
16.73, 16.75	1.10	229475	0.90	2.71	117856	0.46	1.36
Dx-CA, 17.47, 17.48	2.13	444348	1.74	5.54	240931	0.94	2.68
24.46				2.62	113942	0.45	0.45
26.20, 26.27	0.61	127255	0.50	1.91	83065	0.32	0.82
2-COOH, 28.89, 29.04	5.67	1182841	4.63	7.38	320952	1.26	5.88
5-COOH, 31.32, 31.47	7.07	1474901	5.77	15.69	682349	2.67	8.44
MCBX							0.00
37.33	1.14	237820	0.93				0.93
S-2200, 39.17, 39.36	80.44	16780910	65.63	62.54	2719827	10.64	76.27
Total unknowns			2.33			1.51	3.83

Unknown Summary

Soil extract Unknowns:		3.83	% of applied
Total Unknowns	5	3.83	% of applied
Highest single Unknown		1.36	% of applied

Extraction Method
Neutral extraction: Extract the soil 2X with 150 mL of 9:1 (v:v) acetone/water.
Acidic extraction: Extract the soil 2X with 150 mL of 5:1 (v:v) acetone/0.1 M HCl. Rinse with 100 mL acetone.

Appendix I Representative Data: ([benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) Exposure

Excel spreadsheet data for 0, 30 and 364 DAT samples

Sample ID: R-Be-30D-28 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(R)-benzyl-^{14}C]S-2167 401G **SA (dpm/ug):** 57151 **Sampling Date:** 1/13/2010
DAT: 30 **Soil:** **Baptistown, NJ (Penn Series - Loam)**

Fractionation of the PES										
Soil material	Empty Tube wt.	PES & tube wt.	Sample Net wt.	LSC Ref. No.	Total oxidized (g)	dpm/total oxidized	dpm/g	Sample dpm	% of applied	ppm
Post Extraction Soil	50.06	118.74	68.68	Y715-Y717	1.53	28,534	18,625	1,281,751	5.01	0.449

	Sample Vol. (mL)	Aliquot counted (mL)	LSC Ref. No.	Aliquot dpm	Sample dpm	% of applied[1]	ppm
Dismembrator extract	148	0.500	K162-K164	1,279	378,584	1.51	0.136

	Sample Net wt.	LSC Ref. No.	Mean dpm/g [2]	Sample dpm	% of applied[1]	ppm
Humin	68.26	K230-K232	5537	377,956	1.51	0.135
Humic acid	2.98	K218-K220	72,653	216,506	0.87	0.077

NaOH Extract	Sample Vol. (mL)	Aliquot counted (mL)	LSC Ref. No.	Aliquot dpm	Sample dpm	% of applied[1]	ppm
Fulvic acid (FA)	168	0.500	K174-K176	865	290,640	1.16	0.104
Fulvic acid -Organic	294	0.500	K186-K188	338	198,744	0.80	0.071
Fulvic acid -Aqueous	170	0.500	K198-K200	283	96,220	0.38	0.034

HPLC Analysis										**PES Sample Balance**	
Dismembrator Soil Extract					**Fulvic acid-Organic (Barely quantifiable)**					**Fraction**	**% of appl.**
Compound (t_R)	% area	dpm	% of appl.	ppm	Comp.	% area	dpm	% of appl.[2]	ppm	**Dismem.**	**1.51**
3.43	10.23	38,729	0.15	0.014	2.42	19.61	38,974	0.16	0.014	**Humin**	**1.51**
11.8	15.91	60,233	0.24	0.022	8.13	21.57	42,869	0.17	0.015	**Humic**	**0.87**
De-Xy-S-2200 15.25					15.25	27.45	54,555	0.22	0.020	**FA**	**1.16**
Dx-CA-S-2200											
2-COOH-S-2200											
5-COOH-S-2200, 33.25, 33.63	23.73	89,838	0.36	0.032		33.63	66,838	0.27	0.024		
MCBX, 34.53	23.86	90,330	0.36	0.032							
S-2200, 37.67	27.27	103,240	0.41	0.037							
Unks			0.40								
										Total	**5.06**

[1]This value is corrected to account for the sample aliquots taken for oxidation ((68.68/(68.68-1.53)) = 1.023 100.8 % Recovery

[2]This is the corrected total dpm from the oxidation summary spreadsheet using the oxidation efficiency.

Appendix I Representative Data: ([benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) Exposure

Excel spreadsheet data for 0, 30 and 364 DAT samples

Sample ID: R-Be-364D-36 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(R)-benzyl-^{14}C]S-2167 401G **SA (dpm/ug):** 57151 **Sampling Date:** 12/13/2010
DAT: 364 334 anaerobic **Soil:** **'Baptistown, NJ (Penn Series - Loam)**

Sample initial conditions and weights (before incubation)				**Sample parameters (after incubation)**			
Applied ^{14}C (dpm)	Applied ^{14}C (ppm)	Soil dry wt. (g)	Init. water (mL)	pH w	pH s	O_2 (ppm)	Redox pot. (mV)
25568700	8.95	50	95	8.97	8.77	0.06	-192

Sample Headspace data	Headspace Vol. (mL)	Headspace injected	Headspace LSC Reference No.	Headspace inject. dpm	Total sample headspace (dpm)	% of applied
	459	50	K659	229	2102	0.01

Evolved $^{14}CO_2$ data	Trap NaOH Volume (mL	Aliq. counted (mL	LSC ref No.	Aliq. dpm	Trap dpm	% of applied
	25	0.5	K661-K663	4718	235900	0.92
Aerobic Phase						1.76

Soil Phase data	Empty Tube wt.	Sample & tube wt. (g)	Sample Net wt.	LSC Reference No.	Total oxidized (g	dpm/total oxidized	dpm/g	Sample dpm[1]	% of applied
PES after acidic ext.	49.72	119.43	69.71	K687-K689	2.55	36130	14185	1009039	3.95

Extraction of the soil phase	Sample Vol. (mL)	Aliquot counted (mL)	LSC Reference No.	Aliquot dpm	Sample dpm	% of applied
1st extract (neutral)	295	0.5	K673-K675	21935	12941650	50.62
2nd extract (acidic)	397	0.5	K681-K683	7176	5697744	22.28
acid partition (aqueous)	26	0.5	K699-K701	470	24440	0.10
acid partition (organic)	316	0.5	K693-K695	8800	5561600	21.75
Water phase	Sample Vol. (mL)	Aliquot counted (mL)	LSC Reference No.	Aliquot dpm	Sample dpm	% of applied
Water phase	89	0.5	K667-K669	32384	5764352	22.54
Water phase conc.	2.50	0.020	K679	43479	5434875	21.26

Sample Balance Biometer/beaker rinse	$^{14}CO_2$	$^{14}CH_4$	Water Phase	Soil extract	Post extraction soil PES			Total % of appl.
0.01	2.68	0.01	22.54	72.90	3.95			102.09

1) Total dpm corrected for the oxidation efficiency. NA = Not analyzed. 0.980

Appendix I Representative Data: ([benzyl-^{14}C]S-2167 (S-2200 *R*-isomer, RB) Exposure

Excel spreadsheet data for 0, 30 and 364 DAT samples

Sample ID: R-Be-364D-36 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(R)-benzyl-^{14}C]S-2167 401G **SA (dpm/ug):** 57151 **Sampling Date:** 12/13/2010
DAT: 364 334 anaerobi **Soil:** **'Baptistown, NJ (Penn Series - Loam)**

HPLC analysis	Soil phase						Total	Water phase			Total
Compound (t_R)	1st extract (neutral)			2nd extract (acidic)			% of appl. In soil	organic extract			% of appl. water/soil
(minutes)	% area	dpm	% appl.	% area	dpm	% appl.		% area	dpm	% appl.	
7.27				3.58	199105	0.78	0.78				0.78
8.18								3.42	197141	0.77	0.77
De-Xy											0.00
Dx-CA 17.28, 16.46	2.78	359778	1.41				1.41	15.87	914803	3.58	4.98
27.29				1.37	76194	0.30	0.30				0.30
2-COOH 32.04, 32.04, 31.99	3.79	490489	1.92	2.51	139596	0.55	2.46	13.18	759742	2.97	5.44
5-COOH 34.21, 34.20, 34.06	4.63	599198	2.34	6.79	377633	1.48	3.82	17.74	1022596	4.00	7.82
MCBX											0.00
38.12, 38.80				2.28	126804	0.50	0.50	2.95	170048	0.67	1.16
S-2200 39.84, 39.90, 39.61	88.80	11492185	44.95	83.47	4642268	18.16	63.10	46.84	2700022	10.56	73.66
Total unknowns			0.00			1.57	1.57			1.44	3.01

Unknown Summary

Soil extract Unknowns:	3	1.57	% of applied
Highest single soil Unknown		0.78	% of applied
Water Unknowns	2	1.44	% of applied
Highest single water Unknown		0.77	% of applied
Total Unknowns	4	3.01	% of applied
Highest single Unknown		1.16	% of applied

Extraction Method

Neutral extraction: Extract the soil 2X with 150 mL of 9:1 (v:v) methanol/water.
Acidic extraction: Extract the soil 2X with 150 mL of 5:1 (v:v) methanol/0.5 M HCl. Rinse with 100 mL methanol.

Appendix J Representative Data: [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) Exposure

Excel spreadsheet data for 0, 34 and 363 DAT samples

Sample ID: S-Be-0D-50 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(S)-benzyl-^{14}C]S-2354 400D **SA (dpm/ug):** 51415 **Sampling Date:** 12/1/2009
DAT: 0 **Soil: 'Baptistown, NJ (Penn Series - Loam)**

Sample initial conditions and weights (before incubation)				Sample parameters (after incubation)		
Applied ^{14}C (dpm)	Applied ^{14}C (ppm)	Soil dry wt. (g)	Init. water added (mL)	pH	Dissolved O_2 (ppm)	Redox pot. (mV)
22976400	8.94	50	NA	NA	NA	NA

Sample Headspace data	Headspace Vol. (mL)	Headspace injected	Headspace LSC Reference No.	Headspace injection dpm	Total sample headspace (dpm)	% of applied
	NA	NA	NA	NA	NA	NA

Evolved $^{14}CO_2$ data	Trap NaOH Volume (mL	Aliq. counted (mL	LSC ref No.	Aliq. dpm	Trap dpm	% of applied
	NA	NA	NA	NA	NA	NA

Soil Phase data	Empty Tube wt.	Sample & tube wt. (g)	Sample Net wt.	LSC Reference No.	Total oxidized (g)	dpm/total oxidized	dpm/g	Sample dpm[1]	% of applied
PES after neutral ext.	49.90	117.91	68.01	Y70-Y72	1.58	13089	8289	575269	2.50

Extraction of the soil phase	Sample Vol. (mL)	Aliquot counted (mL)	LSC Reference No.	Aliquot dpm	Sample dpm	% of applied
1st extract (neutral)	290	1.0	Y64-Y66	79922	23177380	100.87
2nd extract (acidic)	NA	NA	NA	NA	NA	NA

Sample Balance	$^{14}CO_2$	$^{14}CH_4$		Soil extract	Post extraction soil			Total
Biometer/beaker rinse					PES			% of applied
NA	NA	NA		100.87	2.50			103.38

1) Total dpm corrected for the oxidation efficiency. NA = Not analyzed. 0.980

Appendix J Representative Data: [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) Exposure

Excel spreadsheet data for 0, 34 and 363 DAT samples

Sample ID: S-Be-0D-50 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(S)-benzyl-^{14}C]S-2354 400D **SA (dpm/ug):** 51415 **Sampling Date:** 12/1/2009
DAT: 0 **Soil: 'Baptistown, NJ (Penn Series - Loam)**

HPLC analysis	**Soil phase**						**Total**
Compound (t_R)	1st extract (neutral)			2nd extract (acidic)			% of appl. In soil
(minutes)	% area	dpm	% appl.	% area	dpm	% appl.	
10.02	0.87	201643	0.88				0.88
De-Xy, 15.47	1.47	340707	1.48				1.48
Dx-CA, 17.62	0.63	146017	0.64				0.64
24.17	0.72	166877	0.73				0.73
2-COOH							0.00
5-COOH, 31.52	1.08	250316	1.09				1.09
MCBX							0.00
33.61	1.31	303624	1.32				1.32
35.97	0.88	203961	0.89				0.89
S-2200, 39.29	93.04	21564234	93.85				93.85
Total unknowns			3.81				3.81
2D TLC confirmation							

Unknown Summary

Soil extract Unknowns:		3.81	% of applied
Total Unknowns	4	3.81	% of applied
Highest single Unknown		1.32	% of applied

Extraction Method
Neutral extraction: Extract the soil 2X with 150 mL of 9:1 (v:v) acetone/water.

Appendix J Representative Data: [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) Exposure

Excel spreadsheet data for 0, 34 and 363 DAT samples

Sample ID: S-Be-34D-54 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(S)-benzyl-^{14}C]S-2354 400D **SA (dpm/ug):** 51415 **Sampling Date:** 1/4/2010
DAT: 34 **Soil:** **'Baptistown, NJ (Penn Series - Loam)**

Sample initial conditions and weights (before incubation)					Sample parameters (after incubation)		
Applied ^{14}C (dpm)	Applied ^{14}C (ppm)		Soil dry wt. (g)	Init. water added (mL)	pH	Dissolved O_2 (ppm)	Redox pot.(mV)
22976400	8.94		50	NA	NA	NA	NA

Sample Headspace data	Headspace Vol. (mL)	Headspace injected	Headspace LSC Reference No.	Headspace injection dpm	Total sample headspace (dpm)	% of applied
	NA	NA	NA	NA	NA	NA

Evolved $^{14}CO_2$ data	Trap NaOH Volume (mL)	Aliq. counted (mL)	LSC ref No.	Aliq. dpm	Trap dpm	% of applied
	NA	NA	NA	NA	NA	5.26

Soil Phase data	Empty Tube wt.	Sample & tube wt. (g)	Sample Net wt.	LSC Reference No.	Total oxidized (g)	dpm/total oxidized	dpm/g	Sample dpm[1]	% of applied
PES after acidic ext.	50.32	118.16	67.84	Y362-Y364	1.33	27455	20658	1421364	6.19

Extraction of the soil phase	Sample Vol. (mL)	Aliquot counted (mL)	LSC Reference No.	Aliquot dpm	Sample dpm	% of applied
1st extract (neutral)	280	1.0	Y304-Y306	62554	17515120	76.23
2nd extract (acidic)	385	1.0	Y316-Y318	11108	4276580	18.61
acid partition (aqueous)	38	1.0	Y344-Y346	1448	55024	0.24
acid partition (organic)	305	1.0	Y350-Y352	13395	4085475	17.78

Sample Balance	$^{14}CO_2$	$^{14}CH_4$		Soil extract	Post extraction soil			Total
Biometer/beaker rinse					PES			% of appl.
NA	5.26	NA		94.84	6.19			106.29

1) Total dpm corrected for the oxidation efficiency. NA = Not analyzed. 0.986

Appendix J Representative Data: [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) Exposure

Excel spreadsheet data for 0, 34 and 363 DAT samples

Sample ID: S-Be-34D-54 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(S)-benzyl-^{14}C]S-2354 400D **SA (dpm/ug):** 51415 **Sampling Date:** 1/4/2010
DAT: 34 **Soil:** **'Baptistown, NJ (Penn Series - Loam)**

HPLC analysis	**Soil phase**						**Total**
Compound (t_R)	1st extract (neutral)			2nd extract (acidic)			% of appl. In soil
(minutes)	% area	dpm	% appl.	% area	dpm	% appl.	
De-Xy, 15.52, 15.93	2.07	362563	1.58	8.38	342363	1.49	3.07
Dx-CA, 16.92, 17.23	1.89	331036	1.44	5.09	207951	0.91	2.35
26.13, 26.19	0.65	113848	0.50	3.77	154022	0.67	1.17
2-COOH, 28.96, 28.90	3.23	565738	2.46	5.58	227970	0.99	3.45
5-COOH, 31.41, 31.29	3.99	698853	3.04	9.63	393431	1.71	4.75
MCBX							0.00
S-2200 39.09, 39.05	88.18	15444833	67.22	67.54	2759330	12.01	79.23
Total unknowns			0.50			0.67	1.17

Unknown Summary

Soil extract Unknowns:		1.17	% of applied
Total Unknowns	1	1.17	% of applied
Highest single Unknown		1.17	% of applied

Extraction Method

Neutral extraction: Extract the soil 2X with 150 mL of 9:1 (v:v) acetone/water.
Acidic extraction: Extract the soil 2X with 150 mL of 5:1 (v:v) acetone/0.1 M HCl. Rinse with 100 mL acetone.

Appendix J Representative Data: [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) Exposure

Excel spreadsheet data for 0, 34 and 363 DAT samples

Sample ID: S-Be-363D-60 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(S)-benzyl-^{14}C]S-2354 400D **SA (dpm/ug):** 51415 **Sampling Date:** 11/29/2010
DAT: 363 329 anaerobic **Soil:** **'Baptistown, NJ (Penn Series - Loam)**

Sample initial conditions and weights (before incubation)				Sample parameters (after incubation)			
Applied ^{14}C (dpm)	Applied ^{14}C (ppm)	Soil dry wt. (g)	Init. water added (mL)	pH w	pH s	O_2 (ppm)	Redox pot. (mV)
22976400	8.94	50	95	8.92	8.52	0.01	-180

Sample Headspace data	Headspace Vol. (mL)	Headspace injected	Headspace LSC Reference No.	Headspace injection dpm	Total sample headspace (dpm)	% of applied
	465	50	K544	0	0	0.00

Evolved $^{14}CO_2$ data	Trap NaOH Volume (mL)	Aliq.counted (mL)	LSC ref No.	Aliq. dpm	Trap dpm	% of applied
	25	0.5	K546-K548	9716	485800	2.11
Aerobic Phase						5.26

Soil Phase data	Empty Tube wt.	Sample & tube wt. (g)	Sample Net wt.	LSC Reference No.	Total oxidized (g)	dpm/total oxidized	dpm/g	Sample dpm[1]	% of applied
PES after acidic ext.	49.76	118.40	68.64	K630-K632	2.64	48737	18475	1278349	5.56

Extraction of the soil phase	Sample Vol. (mL)	Aliquot counted (mL)	LSC Reference No.	Aliquot dpm	Sample dpm	% of applied
1st extract (neutral)	293	0.5	K570-K572	17162	10056932	43.77
2nd extract (acidic)	396	0.5	K582-K584	6234	4937328	21.49
acid partition (aqueous)	32	0.5	K602-K604	1029	65856	0.29
acid partition (organic)	287	0.5	K614-K616	9234	5300316	23.07

Water phase	Sample Vol. (mL)	Aliquot counted (mL)	LSC Reference No.	Aliquot dpm	Sample dpm	% of applied
Water phase	91	0.5	K558-K560	28146	5122572	22.29

Sample Balance Biometer/beaker rinse	$^{14}CO_2$	$^{14}CH_4$	Water Phase	Soil extract	Post extraction soil PES			Total % of appl.
0.00	7.38	0.00	22.29	65.26	5.56			100.50

1) Total dpm corrected for the oxidation efficiency. NA = Not analyzed. 0.992

Appendix J Representative Data: [benzyl-^{14}C]S-2354 (S-2200 *S*-isomer, SB) Exposure

Excel spreadsheet data for 0, 34 and 363 DAT samples

Sample ID: S-Be-363D-60 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(S)-benzyl-^{14}C]S-2354 400D **SA (dpm/ug):** 51415 **Sampling Date:** 11/29/2010
DAT: 363 329 anaerobi **Soil:** **'Baptistown, NJ (Penn Series - Loam)**

HPLC analysis	Soil phase						Total	Water phase			Total
Compound (t_R)	1st extract (neutral)			2nd extract (acidic)			% of appl. In soil				% of appl. water/soil
(minutes)	% area	dpm	% appl.	% area	dpm	% appl.		% area	dpm	% appl.	
8.09, 8.06				3.60	190811	0.83	0.83	4.80	245883	1.07	1.90
10.30								2.40	122942	0.54	0.54
De-Xy											0.00
Dx-CA 17.21, 16.60	3.67	369089	1.61				1.61	19.62	1005049	4.37	5.98
27.25				2.96	156889	0.68	0.68				0.68
2-COOH 32.01, 32.07, 32.0	3.47	348976	1.52	2.35	124557	0.54	2.06	15.28	782729	3.41	5.47
5-COOH 34.14, 34.18, 34.16	4.14	416357	1.81	6.95	368372	1.60	3.42	16.65	852908	3.71	7.13
MCBX 35.18, 35.29, 35.22	2.08	209184	0.91	3.97	210423	0.92	1.83	1.58	80937	0.35	2.18
38.84								1.50	76839	0.33	0.33
S-2200 39.67, 39.78, 39.69	86.65	8714332	37.93	80.16	4248733	18.49	56.42	38.17	1955286	8.51	64.93
Total unknowns			0.00			1.51	1.51			1.94	3.45

Unknown Summary

Soil extract Unknowns:	2	1.51	% of applied
Highest single soil Unknown		0.83	% of applied
Water Unknowns	3	1.94	% of applied
Highest single water Unknown		1.07	% of applied
Total Unknowns	4	3.45	% of applied
Highest single Unknown		1.90	% of applied

Extraction Method

Neutral extraction: Extract the soil 2X with 150 mL of 9:1 (v:v) methanol/water.
Acidic extraction: Extract the soil 2X with 150 mL of 5:1 (v:v) methanol/0.5 M HCl. Rinse with 100 mL methanol.

Appendix K Representative Data: [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) Exposure

Excel spreadsheet data for 0, 34 and 363 DAT samples

Sample ID: R-Ph-0D-74 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**

Label: [(R)-phenoxy-^{14}C]S-2167 404D **SA (dpm/ug):** 51839 **Sampling Date:** 12/1/2009

DAT: 0 **Soil:** **'Baptistown, NJ (Penn Series - Loam)**

Sample initial conditions and weights (before incubation)					**Sample parameters (after incubation)**		
Applied ^{14}C (dpm)	Applied ^{14}C (ppm)		Soil dry wt. (g)	Init. water added (mL)	pH	Dissolved O_2 (ppm)	Redox pot. (mV)
23203500	8.95		50	NA	NA	NA	NA

Sample Headspace data	Headspace Vol. (mL)	Headspace injected	Headspace LSC Reference No.	Headspace injection dpm	Total sample headspace (dpm)	% of applied
	NA	NA	NA	NA	NA	NA

Evolved $^{14}CO_2$ data	Trap NaOH Volume (mL)	Aliq. counted (mL)	LSC ref No.	Aliq. dpm	Trap dpm	% of applied
	NA	NA	NA	NA	NA	NA

Soil Phase data	Empty Tube wt.	Sample & tube wt. (g)	Sample Net wt.	LSC Reference No.	Total oxidized (g)	dpm/total oxidized	dpm/g	Sample dpm[1]	% of applied
PES after neutral ext.	51.33	118.88	67.55	Y76-Y78	1.08	8558	7909	545186	2.35

Extraction of the soil phase	Sample Vol. (mL)	Aliquot counted (mL)	LSC Reference No.	Aliquot dpm	Sample dpm	% of applied
1st extract (neutral)	300	1.0	Y58-Y60	78945	23683500	102.07
2nd extract (acidic)	NA	NA	NA	NA	NA	NA

Sample Balance	$^{14}CO_2$	$^{14}CH_4$		Soil extract	Post extraction soil			Total
Biometer/beaker rinse					PES			% of appl.
NA	NA	NA		102.07	2.35			104.42

1) Total dpm corrected for the oxidation efficiency. NA = Not analyzed. 0.980

Appendix K Representative Data: I [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) Exposure

Excel spreadsheet data for 0, 34 and 363 DAT samples

Sample ID: R-Ph-0D-74 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**

Label: [(R)-phenoxy-^{14}C]S-2167 404D **SA (dpm/ug):** 51839 **Sampling Date:** 12/1/2009

DAT: 0 **Soil:** **'Baptistown, NJ (Penn Series - Loam)**

HPLC analysis	**Soil phase**						**Total**
Compound (t_R)	1st extract (neutral)			2nd extract (acidic)			% of appl. In soil
(minutes)	% area	dpm	% appl.	% area	dpm	% appl.	
16.51	0.43	101839	0.44				0.44
2-COOH							0.00
5-COOH, 31.67	1.22	288939	1.25				1.25
MCBX, 33.51	0.56	132628	0.57				0.57
34.18	1.01	239203	1.03				1.03
36.26	0.48	113681	0.49				0.49
S-2200, 39.51	96.29	22804842	98.28				98.28
Total unknowns			1.96				1.96

2D TLC confirmation

Unknown Summary

Soil extract Unknowns:		1.96	% of applied
Total Unknowns	3	1.96	% of applied
Highest single Unknown		1.03	% of applied

Extraction Method

Neutral extraction: Extract the soil 2X with 150 mL of 9:1 (v:v) acetone/water.

Appendix K Representative Data: I [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) Exposure

Excel spreadsheet data for 0, 34 and 363 DAT samples

Sample ID: R-Ph-34D-78 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(R)-phenoxy-^{14}C]S-2167 404D **SA (dpm/ug):** 51839 **Sampling Date:** 1/4/2010
DAT: 34 **Soil: 'Baptistown, NJ (Penn Series - Loam)**

Sample initial conditions and weights (before incubation)					Sample parameters (after incubation)		
Applied ^{14}C (dpm)	Applied ^{14}C (ppm)		Soil dry wt. (g)	nit. water added (mL	pH	Dissolved O_2 (ppm)	Redox pot. (mV)
23203500	8.95		50	NA	NA	NA	NA

Sample Headspace data	Headspace Vol. (mL)	Headspace injected	Headspace LSC Reference No.	Headspace injection dpm	Total sample headspace (dpm)	% of applied
	NA	NA	NA	NA	NA	NA

Evolved $^{14}CO_2$ data	Trap NaOH Volume (mL	Aliq. counted (mL	LSC ref No.	Aliq. dpm	Trap dpm	% of applied
	NA	NA	NA	NA	NA	7.11

Soil Phase data	Empty Tube wt.	Sample & tube wt. (g)	Sample Net wt.	LSC Reference No.	Total oxidized (g	dpm/total oxidized	dpm/g	Sample dpm[1]	% of applied
PES after acidic ext.	49.29	116.67	67.38	Y368-Y370	1.14	44552	38944	2661309	11.47

Extraction of the soil phase	Sample Vol. (mL)	Aliquot counted (mL)	LSC Reference No.	Aliquot dpm	Sample dpm	% of applied
1st extract (neutral)	285	1.0	Y310-Y312	57982	16524870	71.22
2nd extract (acidic)	400	1.0	Y322-Y324	8781	3512400	15.14
acid partition (aqueous)	38	1.0	Y332-Y334	2430	92340	0.40
acid partition (organic)	300	1.0	Y338-Y340	11122	3336600	14.38

Sample Balance	$^{14}CO_2$	$^{14}CH_4$		Soil extract	Post extraction soil			Total
Biometer/beaker rinse					PES			% of appl.
NA	7.11	NA		86.35	11.47			104.94

1) Total dpm corrected for the oxidation efficiency. NA = Not analyzed. 0.986

Appendix K Representative Data: I [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) Exposure

Excel spreadsheet data for 0, 34 and 363 DAT samples

Sample ID: R-Ph-34D-78 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**

Label: [(R)-phenoxy-^{14}C]S-2167 404D **SA (dpm/ug):** 51839 **Sampling Date:** 1/4/2010

DAT: 34 **Soil: 'Baptistown, NJ (Penn Series - Loam)**

HPLC analysis	**Soil phase**						**Total**
Compound (t_R)	1st extract (neutral)			2nd extract (acidic)			% of appl. In soil
(minutes)	% area	dpm	% appl.	% area	dpm	% appl.	
2-COOH, 28.92, 28.98	5.71	943570	4.07	11.43	381373	1.64	5.71
5-COOH, 31.32, 31.45	6.11	1009670	4.35	14.90	497153	2.14	6.49
MCBX							0.00
36.15	0.55	90887	0.39				0.39
37.39	0.79	130546	0.56				0.56
S-2200, 39.16, 39.26	86.85	14351850	61.85	73.67	2458073	10.59	72.45
Total unknowns			0.95			0.00	0.95

Unknown Summary

Soil extract Unknowns:		0.95	% of applied
Total Unknowns	2	0.95	% of applied
Highest single Unknown		0.56	% of applied

Extraction Method

Neutral extraction: Extract the soil 2X with 150 mL of 9:1 (v:v) acetone/water.
Acidic extraction: Extract the soil 2X with 150 mL of 5:1 (v:v) acetone/0.1 M HCl. Rinse with 100 mL acetone.

Appendix K Representative Data: I [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) Exposure

Excel spreadsheet data for 0, 34 and 363 DAT samples

Sample ID: R-Ph-363D-84 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**

Label: [(R)-phenoxy-^{14}C]S-2167 404D **SA (dpm/ug):** 51839 **Sampling Date:** 11/29/2010

DAT: 363 329 anaerobi **Soil:** **Baptistown, NJ (Penn Series - Loam)**

Sample initial conditions and weights (before incubation)				Sample parameters (after incubation)			
Applied ^{14}C (dpm)	Applied ^{14}C (ppm)	Soil dry wt. (g)	Init. water added (mL)	pH w	pH s	O_2 (ppm)	Redox pot. (mV)
23203500	8.95	50	95	8.84	8.63	0.00	-183

Sample Headspace data	Headspace Vol. (mL)	Headspace injected	Headspace LSC Reference No.	Headspace injection dpm	Total sample headspace (dpm)	% of applied
	457	50	K545	3	27	0.00

Evolved $^{14}CO_2$ data	Trap NaOH Vol. (mL)	Aliq. counted (mL)	LSC ref No.	Aliq. dpm	Trap dpm	% of applied
	25	0.5	K552-K554	10892	544600	2.35
Aerobic Phase						7.11

Soil Phase data	Empty Tube wt.	Sample & tube wt. (g)	Sample Net wt.	LSC Reference No.	Total oxidized (g	dpm/total oxidized	dpm/g	Sample dpm[1]	% of applied
PES after acidic ext.	49.37	117.97	68.60	K636-K638	2.52	80458	31940	2208785	9.52

Extraction of the soil phase	Sample Vol. (mL)	Aliquot counted (mL)	LSC Reference No.	Aliquot dpm	Sample dpm	% of applied
1st extract (neutral)	295	0.5	K576-K578	17193	10143870	43.72
2nd extract (acidic)	395	0.5	K588-K590	6487	5124730	22.09
acid partition (aqueous)	30	0.5	K608-K610	1057	63420	0.27
acid partition (organic)	310	0.5	K620-K622	9095	5638900	24.30

Water phase	Sample Vol. (mL)	Aliquot counted (mL)	LSC Reference No.	Aliquot dpm	Sample dpm	% of applied
Water phase	89	0.5	K564-K566	22719	4043982	17.43

Sample Balance	$^{14}CO_2$	$^{14}CH_4$	Water Phase	Soil extract	Post extraction soil			Total
Biometer/beaker rinse					PES			% of appl.
0.01	9.46	0.00	17.43	65.80	9.52			102.22

1) Total dpm corrected for the oxidation efficiency. NA = Not analyzed. 0.992

Appendix K Representative Data: I [phenoxy-^{14}C]S-2167 (S-2200 *R*-isomer, RP) Exposure

Excel spreadsheet data for 0, 34 and 363 DAT samples

Sample ID: R-Ph-363D-84 **S-2200 Anaerobic Soil Degradation Study (V-09-37166)**
Label: [(R)-phenoxy-^{14}C]S-2167 404D **SA (dpm/ug):** 51839 **Sampling Date:** 11/29/2010
DAT: 363 329 anaero **Soil:** **Baptistown, NJ (Penn Series - Loam)**

HPLC analysis	Soil phase						Total	Water phase			Total
Compound (t_R)	1st extract (neutral)			2nd extract (acidic)			% of appl. In soil				% of appl. water/soil
(minutes)	% area	dpm	% appl.	% area	dpm	% appl.		% area	dpm	% appl.	
2,5-Dimethylphenol											
2-COOH 32.02, 32.03, 32.0	3.96	401697	1.73	3.42	192850	0.83	2.56	20.40	824972	3.56	6.12
5-COOH 34.16, 34.17, 34.1	4.92	499078	2.15	5.71	321981	1.39	3.54	24.79	1002503	4.32	7.86
MCBX 35.18				4.11	231759	1.00	1.00				1.00
38.98								2.30	93012	0.40	0.40
S-2200 39.61, 39.89, 39.79	91.12	9243094	39.83	84.65	4773329	20.57	60.41	52.51	2123495	9.15	69.56
44.51				2.11	118981	0.51	0.51				0.51
Total unknowns			0.00			0.51	0.51			0.40	0.91

Unknown Summary

Soil extract Unknowns:	1	0.51	% of applied
Highest single soil Unknown		0.51	% of applied
Water Unknowns	1	0.40	% of applied
Highest single water Unknown		0.40	% of applied
Total Unknowns	2	0.91	% of applied
Highest single Unknown		0.51	% of applied

Extraction Method

Neutral extraction: Extract the soil 2X with 150 mL of 9:1 (v:v) methanol/water.
Acidic extraction: Extract the soil 2X with 150 mL of 5:1 (v:v) methanol/0.5 M HCl. Rinse with 100 mL methanol.

Appendix L Determination of Microbial Biomass by Standard Plate Counts

Item #1 - Viscient report 12709.6319

Study Title

Determination of Microbial Biomass by
Standard Anaerobic Plate Counts

Principal Investigator

Sean P. McLaughlin

Study Completed On

10 January 2011

Submitted To

Valent USA, Corporation
6560 Trinity Court
Dublin, California 94568

Performing Laboratory

Smithers Viscient
790 Main Street
Wareham, Massachusetts 02571 - 1037

Laboratory Project ID

Smithers Viscient Study No. 12709.6319
Sponsor Protocol/Project No. V-09-37166

Appendix L Determination of Microbial Biomass by Standard Plate Counts

Item #1 - Viscient report 12709.6319

GOOD LABORATORY PRACTICE STATEMENT

The study conduct, raw data and report presented for **"Determination of Microbial Biomass by Standard Anaerobic Plate Counts"** were produced and compiled in accordance with all pertinent U.S. EPA FIFRA (40 CFR Part 160) Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (OECD/GLP (92)32).

SMITHERS VISCIENT

Sean P. McLaughlin
Principal Investigator

Date

Appendix L Determination of Microbial Biomass by Standard Plate Counts

Item #1 - Viscient report 12709.6319

QUALITY ASSURANCE DOCUMENTATION

The study conduct, raw data and report for **"Determination of Microbial Biomass by Standard Anaerobic Plate Counts"** were inspected by the Quality Assurance Unit (QAU) at Springborn Smithers Laboratories to determine adherence with the laboratory standard operating procedures. This report is an accurate representation of the raw data. Dates of study inspections, inspection types, and dates reported to the Study Director and to Management are listed below.

Inspection Date	Inspection Type	Reported to Study Director/Management
12/7/10	In-life, Plating	12/14/10
1/4/11	Data	1/10/11
1/5/11	Final Report	1/10/11

SMITHERS VISCIENT

Kevin Jackson
Quality Assurance Auditor

10 Jan 11
Date

Appendix L Determination of Microbial Biomass by Standard Plate Counts

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KEY STUDY PERSONNEL

The following Smithers Viscient personnel were responsible for the conduct of the work and reporting of the study results.

Sean F. McLaughlin	Principal Investigator
Andrew Griffith	Chemist II
Jennifer M. Jackson	Technical Report Writer
Nancy R. Lentz, M.S.	Director, Environmental Fate and Metabolism

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COMPANY NAMES

On 8 November 2010, the legal name of Springborn Smithers Laboratories, LLC was changed to Smithers Viscient, LLC. The legal entity did not change.

Some of the activities described in this report were undertaken before the name change. Consequently, the name Springborn Smithers Laboratories, LLC may appear throughout this report. However, all inquiries regarding this report and the data contained herein can still be addressed to Smithers Viscient, 790 Main Street, Wareham, MA 02571.

Appendix L Determination of Microbial Biomass by Standard Plate Counts

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Appendix L Determination of Microbial Biomass by Standard Plate Counts

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1.0 INTRODUCTION

Enrichment techniques provide a means for isolating different microbial types from nature by taking advantage of their specific requirements. The inoculation of growth medium, for example, and incubation at a defined temperature promotes growth of different microorganisms depending on the growth conditions. Such cultures are often termed mixed cultures or mixed microbial populations. The premise for enumeration by plate counts is based on assuming that each colony counted arises from a single microbial propagule (bacterium, spore, hypha, etc.). Typically, culture enrichment enumeration techniques express microbial load as the number of colony forming units per volume/weight of initial sample (cfu/g or cfu/mL).

2.0 METHODS

This work was done in accordance with Smithers Viscient Standard Operating Procedure Number 7.26 entitled "Performing Standard Plate Counts". This SOP meets the requirements of the U.S. EPA FIFRA guidelines (40 CFR, Part 160).

3.0 SAMPLES

Information on the soil sample tested is summarized in the following table.

Valent Description	SV Sample ID	Date Received	Date(s) Tested
A-363D-7	120210-A	2 December 2010	7 to 10 December 2010
B-363D-14	120210-B	2 December 2010	7 to 10 December 2010
D-363D-23	120210-C	2 December 2010	7 to 10 December 2010

4.0 SUMMARY

The temperature during the standard plate count tests was approximately 25 ± 2°C. Approximately 1.0 grams of dry weight sediment from each of the samples was placed into 99 mL of sterile maximum recovery diluent, shaken at 200 rpm for 20 minutes, diluted and

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plated with AC agar, using the pour-plate method with agar overlay. The agar plates were placed in an anaerobic BBL gas pack system for approximately 72 hours. The results for the sediment tested are summarized in the following table.

Valent Description	SV Sample ID	Anaerobic[a] Plate Counts (cfu/mL)
A-363D-7	120210-A	9.6×10^5
B-363D-14	120210-B	7.7×10^5
D-363D-23	120210-C	1.1×10^6

[a] Anaerobic = Anaerobic colonies on AC agar.

The above values are typical for microbial sediment populations under anaerobic conditions.

5.0 ARCHIVAL STATEMENT

The raw data and a copy of the biomass report produced during this portion of the study are stored in the archives at Smithers Viscient (SV), located in Wareham, Massachusetts.

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6.0 PROCEDURE DEVIATIONS

No deviations occurred during this study.

Appendix L Determination of Microbial Biomass by Standard Plate Counts

Item #2 - Viscient report 12709.6320

Study Title

Determination of Microbial Biomass by
Standard Anaerobic Plate Counts

Principal Investigator

Sean P. McLaughlin

Study Completed On

23 February 2011

Submitted To

Valent USA, Corporation
6560 Trinity Court
Dublin, California 94568

Performing Laboratory

Smithers Viscient
790 Main Street
Wareham, Massachusetts 02571 - 1037

Laboratory Project ID

Smithers Viscient Study No. 12709.6320
Sponsor Protocol/Project No. V-09-37166

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GOOD LABORATORY PRACTICE STATEMENT

The study conduct, raw data and report presented for **"Determination of Microbial Biomass by Standard Anaerobic Plate Counts"** were produced and compiled in accordance with all pertinent U.S. EPA FIFRA (40 CFR Part 160) Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (OECD/GLP (92)32).

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Sean P. McLaughlin
Principal Investigator

27 Feb 2011
Date

Appendix L Determination of Microbial Biomass by Standard Plate Counts

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QUALITY ASSURANCE DOCUMENTATION

The study conduct, raw data and report for **"Determination of Microbial Biomass by Standard Anaerobic Plate Counts"** were inspected by the Quality Assurance Unit (QAU) at Smithers Viscient to determine adherence with the laboratory standard operating procedures. This report is an accurate representation of the raw data. Dates of study inspections, inspection types, and dates reported to the Study Director and to Management are listed below.

Inspection Date	Inspection Type	Reported to Study Director/Management
1/7/11	In life: Plate Counts	1/7/11
2/22/11	Data & Final report	2/23/11

SMITHERS VISCIENT

Janice E. Dean
Janice E. Dean
Quality Assurance Auditor

23 FEB 2011
Date

Appendix L Determination of Microbial Biomass by Standard Plate Counts

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KEY STUDY PERSONNEL

The following Smithers Viscient personnel were responsible for the conduct of the work and reporting of the study results.

Sean P. McLaughlin	Principal Investigator
Andrew Griffith	Microbiologist
Kathryn T. Janelle	Technical Report Writer
Kalumbu Malekani	Director, Environmental Fate and Metabolism

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Appendix L Determination of Microbial Biomass by Standard Plate Counts

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1.0 INTRODUCTION

Enrichment techniques provide a means for isolating different microbial types from nature by taking advantage of their specific requirements. The inoculation of growth medium, for example, and incubation at a defined temperature promotes growth of different microorganisms depending on the growth conditions. Such cultures are often termed mixed cultures or mixed microbial populations. The premise for enumeration by plate counts is based on assuming that each colony counted arises from a single microbial propagule (bacterium, spore, hypha, etc.). Typically, culture enrichment enumeration techniques express microbial load as the number of colony forming units per volume/weight of initial sample (cfu/g or cfu/mL).

2.0 METHODS

This work was done in accordance with Smithers Viscient Standard Operating Procedure Number 7.26 entitled "Performing Standard Plate Counts". This SOP meets the requirements of the U.S. EPA FIFRA guidelines (40 CFR, Part 160).

3.0 SAMPLES

Information on the soil sample tested is summarized in the following table.

Valent Description	SMV Sample ID	Date Received	Date(s) Tested
A-368D-101	122010-A	20 December 2010	3 to 7 January 2011

4.0 SUMMARY

The temperature during the standard plate count tests was approximately 25 ± 2°C. One gram of dry weight sediment from sample 122010-A was placed into 99 mL of sterile maximum recovery diluent, shaken at 200 rpm for 20 minutes, diluted and plated with AC agar, using the pour-plate method with agar overlay. The agar plates were placed in an anaerobic BBL gas pack system for

Appendix L Determination of Microbial Biomass by Standard Plate Counts

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approximately 72 hours. The results for the sediment tested are summarized in the following table.

Valent Description	SMV Sample ID	Anaerobic* Plate Counts (cfu/mL)
A-368D-101	122010-A	7.1×10^5

* Anaerobic = Anaerobic colonies on AC agar.

The above value is typical for a microbial sediment population under anaerobic conditions.

5.0 ARCHIVAL STATEMENT

The raw data and a copy of the biomass report produced during this portion of the study are stored in the archives at Smithers Viscient (SMV), located in Wareham, Massachusetts.

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6.0 PROCEDURE DEVIATIONS

No deviations occurred during this study.