

TEXT SEARCHABLE DOCUMENT

DATA EVALUATION RECORD STUDY 2

CHEM 129034

S-53482

\$162-1

Study ID 42684906

Fathulla, R. N. 1991. Aerobic Soil Metabolism of ¹⁴C-S-53482. Laboratory project ID HLA 6311-104. Unpublished study performed by Hazelton Laboratories America, Inc., WI, and submitted by Valent U.S.A. Corporation, CA

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7/21/93

Aerobic Soil Metabolism

CONCLUSIONS:

This study is scientifically sound and partially satisfies the data requirement by providing information about the aerobic soil metabolism of [Ph-¹⁴C]-V-53482. This study was conducted using only phenyl ring-labeled V-53482. To fully satisfy the aerobic soil metabolism data requirement, the registrant should submit an additional aerobic soil metabolism study using THP-ring labeled V-53482.

The structure of V-53482 consists of two major moieties; one of them was radiolabeled in this study (the phenyl ring). The other one is the 3,4,5,6-tetrahydrophthalimide (THPA) moiety. Results of the hydrolysis study demonstrated that at least two additional degradates could be present in metabolism processes (THPA and ¹⁴Δ-TPA). The presence of these products under aerobic soil metabolism processes cannot be confirmed if only phenyl-ring labeled V-53482 is used. To satisfy the data requirement, an additional study using [THP-¹⁴C]-V-53482 is required.

[¹⁴C]-V-53482 (uniformly phenyl ring labeled), at 0.26 µg/g, degraded with a registrant-calculated half-life of 11.9 days in a California sandy loam soil incubated in the dark at 22-26°C.



METHODOLOGY:

[¹⁴C]-V-53482 (uniformly phenyl ring labeled, specific activity 418 mCi/g, radiochemical purity 98.0%), was dissolved in 2 mL of acetone to make a stock solution at 159657 dpm/ μ L. A dilution of the stock solution (1165 μ L in 4 mL of acetone) was applied to a 20 g sample of soil (application rate 0.26 μ g/g or 241000 dpm/g soil). The fortification level is approximately three times the estimated maximum field application rate. The soil used was a California sandy loam (67% sand, 29% silt, 4% clay, 1.2% OM, pH 7.8). The soil was sieved through a 2-mm screen. The aerobic samples were adjusted to 75% of 1/3 bar and placed in sealed glass chambers which were connected to five volatile traps with charcoal, ethylene glycol, and two traps with 2-ethoxyethanol:ethanolamine (1:1), and water; there was a continuous flow of humidified air. The chambers were kept in the dark at 22-26°C (mean \pm standard deviation 25 \pm 0.710°C). At days 1, 3, 7, 14, 28, 59, 89, 120 and 181 duplicate samples were removed for analysis. Traps were sampled at days 1, 3, 7, 14, 28, 42, 59, 81, 89, 113, 120, 144, 175, and 181. At each test interval, the trapping media was removed and replaced with fresh media. The radioactivity in the traps was measured by LSC.

At each test interval, the soils were extracted three times with 40 mL of acetone: water (5:1, v/v) and three times with 40 mL of acetone: 0.01N HCl (9:1, v/v). All extraction mixtures were stirred for 15 minutes and centrifuged. The acetone:water extracts were combined and their radioactivity was measured by LSC. Similarly, the acetone:0.01N HCl extracts were combined and analyzed.

The combined extracts were tested by two dimensional TLC using the following solvent systems:

toluene:ethyl formate:formic acid (5:7:1)
dichloromethane:acetic acid (10:1)

The peaks in the plates were located by fluorescence quenching under UV light and an image scanner to measure radioactivity. Reference standard solutions were cochromatographed with the sample extracts for product identification.

Portions of the extracted soils were oxidized by combustion and the levels of ¹⁴CO₂ were determined by LSC. Duplicate samples from the traps were analyzed by LSC. The charcoal medium was oxidized by combustion and the ¹⁴CO₂ measured. The 2-ethoxyethanol:ethanolamine (1:1) was acidified with 4 N sulfuric acid and the ¹⁴CO₂ trapped in Carbo-Sorb:Perma-Fluor (1:1) was counted.

A number of selected samples of the acetone extracts were analyzed by HPLC in addition to two-dimensional TLC for product identity confirmation. The HPLC method used a gradient mobile phase of water and 1.5% tetrabutyl ammonium phosphate:acetonitrile (pH 6) in a C₁₈ column.

The combined acetone extracts of other selected samples were rotoevaporated and separated by TLC using the following solvent system:

dichloromethane:acetic acid (10:1).

The resulting individual bands were scrapped and eluted with methanol. They were further tested by two-dimensional TLC and HPLC using the same systems described above.

In addition, The combined acetone extracts of certain samples were tested by direct probe MS and HPLC to determine the identity of the unknown chemical in "region 3" of the two-dimensional TLC.

5-g samples from the extracted soils were refluxed with portions of acetonitrile:0.25 N HCl (4:1) (time and details not specified). The extracts were analyzed by two-dimensional TLC and autoradiography. The refluxed soils were additionally extracted with 0.5 N NaOH for 24 hours to determine the amounts of fulvic acid, humic acid, and humin components in the soil.

RESULTS:

[¹⁴C]-V-53482 (uniformly phenyl ring labeled, specific activity 418 mCi/g, radiochemical purity 98.0%), at 0.26 µg/g, degraded with a registrant-calculated half-life of 11.9 days in a California sandy loam soil incubated in the dark at 22-26°C.

V-53482 was 92.9% of the applied at day 0 and decreased to 18.0% by day 28 and was ≤3.7% of the applied from day 89 posttreatment. ¹⁴CO₂ comprised 2.3% of the applied at day 0 and 11.5% of the applied at day 181 posttreatment. Chromatographic analysis of the sample extracts showed the presence of at least five minor unidentified components, each one was ≤10.9% of the applied.

Soil-bound residues increased from 0.7% of the applied at day 0 to 52.7% by day 28 and 73.6% of the applied by day 181. Extensive reflux extraction of the soil-bound residues showed additional amounts of parent V-53482 at concentrations ≤9.2% of the applied. It also showed the presence of at least four other unidentified minor components, each one was ≤3.5% of the applied. The humic acid, fulvic acid, and humin fractions in the soil-bound residues ranged from 3.1-12.9%, 2.3-7.6%, and 7.4-24.9% of the applied, respectively.

Material balances ranged from 88.9% to 104.5% of the applied throughout the study.

COMMENTS:

- 1 The study was conducted using only phenyl ring-labeled V-53482. The structure of V-53482 consists of two major moieties; one of them was radiolabeled in this study (the

phenyl ring). The other one is the 3,4,5,6-tetrahydro-phthalimide (THPA) moiety. Results of the hydrolysis study demonstrated that at least two additional degradates could also be present in metabolism processes (THPA and Δ -TPA). The presence of these products could not be confirmed if only phenyl-ring labeled V-53482 is used.

2. The application rate 0.26 $\mu\text{g/g}$ is approximately 3 times the maximum proposed field application rate (0.094 lb ai/A). It was used to allow measurement of parent and metabolites at the levels required by Subdivision N Guidelines.
3. According to the study, all samples were extracted and tested immediately after collection, except the second extraction of Day 1, which was tested one week later. Samples were stored in a freezer (temperature not specified) for possible additional analysis.
4. Some experimental procedures were not discussed in detail in the study. Instead, some of the procedures were presented in a schematic format on pages 19-22 of the study.
5. Selected soils were tested to determine the availability of microorganisms. Results showed that the soils contained a viable microbial count when the study started.
6. The authors defined as "diffuse" any unresolved radioactivity detected by the scanner. The "diffuse" radioactivity represents a maximum of 15.3% of the applied at the sample interval 14 days in the acetone:water (5:1) extracts. It appears from the representative radioactivity scans that there are no additional spots in the chromatographic plates.
7. Only data from day 0 to 28 posttreatment was used to calculate the degradation half-life. The authors indicate that the data after day 28 "was not well described by first order kinetics."
8. In order to determine the recoveries of radioactivity of the two-dimensional TLC procedure, selected aliquots of sample extracts of acetone:water (5:1), acetone:0.1 N HCl (9:1), and reflux extraction of soil bound residues were analyzed by the method. The TLC plate was divided into various areas and scrapings analyzed by LSC. Recoveries ranged from 87.1% to 103.6%.
9. To determine the efficiency of the soil oxidation procedure, a number of samples of untreated soils were spiked with known amounts of ^{14}C -V-53482. The samples were oxidized by combustion and the $^{14}\text{CO}_2$ determined. The recoveries ranged from 97.3% to 103.1%.

10. The California sandy loam used in this study was somewhat alkaline (pH 7.8). The Hydrolysis study (MRID# 42684906) demonstrated that higher pH's favor degradation of parent V-53482.

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Table II
Individual Summary of Radioactivity Found among the Sample Matrices

Sample Interval (Day)	Sample Number	Individual Radioactivity Applied to Sample								Extracted Soil ^b	Traps for Volatile Components ^b			
		Acetone:Water (5:1) and Acetone:0.1N HCL (9:1) Extracts ^a									Ethylene			
		S-53482	Origin	Region 1	Region 2	Region 3	Region 4	Diffuse	Total		2-EE	Glycol	Charcoal	Total
0	AE-A	94.4	ND	ND	ND	ND	ND	5.3	99.7	0.7	NA	NA	NA	100.4
	AE-B	91.4	ND	ND	ND	ND	ND	7.7	99.1	0.6	NA	NA	NA	99.7
1	AE-14	86.7	0.2	ND	ND	ND	ND	3.3	90.2	7.7	0.1	ND	ND	98.0
	AE-22	86.0	0.1	ND	ND	ND	ND	3.8	89.9	7.8	0.1	ND	ND	97.8
3	AE-2	68.3	1.1	ND	ND	ND	ND	10.9	80.3	17.1	0.1	<0.1	ND	97.5
	AE-12	68.4	1.5	ND	ND	ND	ND	10.6	80.5	16.7	0.1	<0.1	ND	97.3
7	AE-24	62.0	2.4	0.5	0.4	ND	ND	6.7	72.0	25.6	0.2	<0.1	ND	97.8
	AE-17	58.0	2.3	0.3	0.2	ND	ND	10.9	71.7	25.9	0.2	<0.1	ND	97.8
14	AE-8	35.5	4.1	ND	ND	ND	ND	17.9	57.5	42.7	0.6	<0.1	ND	100.8
	AE-1	37.1	4.0	0.5	0.5	ND	ND	17.1	59.2	43.3	0.6	<0.1	ND	103.1
28	AE-13	18.4	10.9	ND	ND	1.4	ND	8.1	38.8	49.4	2.3	<0.1	<0.1	90.5
	AE-6	17.6	5.3	ND	ND	7.7	ND	10.3	40.9	56.0	2.3	<0.1	<0.1	99.2
59	AE-23	9.2	3.4	2.0	2.0	ND	ND	5.5	22.1	76.8	5.6	<0.1	<0.1	104.5
	AE-18	5.9	4.3	2.5	2.4	ND	ND	6.0	21.1	65.7	5.6	<0.1	<0.1	92.4
89	AE-26	3.7	2.5	ND	ND	5.6	0.6	4.6	17.0	69.9	7.7	<0.1	<0.1	94.6
	AE-7	2.7	2.5	1.0	0.2	4.5	0.3	4.5	15.7	70.1	7.7	<0.1	<0.1	93.5
120	AE-3	3.2	2.3	ND	ND	6.3	ND	0.3	12.1	71.3	9.2	<0.1	<0.1	92.6
	AE-25	3.8	2.4	ND	ND	4.7	0.3	1.9	13.1	76.5	9.2	<0.1	<0.1	98.8
181	AE-21	4.2	3.5	ND	ND	1.6	ND	0.6	9.9	80.0	11.5	0.3	<0.1	101.7
	AE-20	3.2	2.0	ND	ND	1.2	0.4	3.2	10.0	67.1	11.5	0.3	<0.1	88.9

NA Not applicable.

ND Not detected.

2-EE 2-Ethoxyethanol:ethanolamine (1:1).

^a Sum of values from Tables VIII and IX.

^b Values from Appendix C.

Table III

Mean Summary of Radioactivity Found among the Sample Matrices^a

Sample Interval (Day)	Mean Radioactivity Applied to Sample								Traps for Volatile Components				
	Acetone:Water (5:1) and Acetone:0.1N HCL (9:1) Extracts								Extracted Soil	Ethylene			
	S-53482	Origin	Region 1	Region 2	Region 3	Region 4	Diffuse	Total		2-EE	Glycol	Charcoal	Total
0	92.9	ND	ND	ND	ND	ND	6.5	99.4	0.7	NA	NA	NA	100.1
1	86.4	0.2	ND	ND	ND	ND	3.6	90.2	7.8	0.1	ND	ND	98.1
3	68.4	1.3	ND	ND	ND	ND	10.8	80.4	16.9	0.1	<0.1	ND	97.4
7	60.0	2.4	0.4	0.3	ND	ND	8.8	71.9	25.8	0.2	<0.1	ND	97.9
14	36.3	4.1	0.3	0.3	ND	ND	17.5	58.5	43.0	0.6	<0.1	ND	102.1
28	18.0	8.1	ND	ND	4.6	ND	9.2	39.9	52.7	2.3	<0.1	<0.1	94.9
59	7.6	3.9	2.3	2.2	ND	ND	5.8	21.8	71.3	5.6	<0.1	<0.1	98.7
89	3.2	2.5	0.5	0.1	5.1	0.5	4.6	16.5	70.0	7.7	<0.1	<0.1	94.2
120	3.5	2.4	ND	ND	5.5	0.2	1.1	12.7	73.9	9.2	<0.1	<0.1	95.8
181	3.7	2.8	ND	ND	1.4	0.2	1.9	10.0	73.6	11.5	0.3	<0.1	95.4

NA Not applicable.

ND Not detected.

2-EE 2-Ethoxyethanol:ethanolamine (1:1).

^a Mean of values in Table II.

Table IV

Individual Summary of Radioactivity Found among the Sample Matrices
Expressed Relative to the Field Application Rate of S-53482^a

Individual Radioactivity Expressed Relative to the Field Application Rate of S-53482 (µg/g)														
Sample Interval (Day)	Sample Number	Acetone:Water (5:1) and Acetone:0.1N HCL (9:1) Extracts								Extracted Soil	Traps for Volatile Components			
		S-53482	Origin	Region 1	Region 2	Region 3	Region 4	Diffuse	Total		Ethylene			Total
											2-EE	Glycol	Charcoal	
0	AE-A	0.082	ND	ND	ND	ND	ND	<0.01	0.086	<0.01	NA	NA	NA	0.087
	AE-B	0.079	ND	ND	ND	ND	ND	<0.01	0.086	<0.01	NA	NA	NA	0.086
1	AE-14	0.075	<0.01	ND	ND	ND	ND	<0.01	0.078	<0.01	<0.01	ND	ND	0.085
	AE-22	0.075	<0.01	ND	ND	ND	ND	<0.01	0.078	<0.01	<0.01	ND	ND	0.085
3	AE-2	0.059	<0.01	ND	ND	ND	ND	<0.01	0.070	0.015	<0.01	<0.01	ND	0.085
	AE-12	0.059	<0.01	ND	ND	ND	ND	<0.01	0.070	0.014	<0.01	<0.01	ND	0.084
7	AE-24	0.054	<0.01	<0.01	<0.01	ND	ND	<0.01	0.062	0.022	<0.01	<0.01	ND	0.085
	AE-17	0.050	<0.01	<0.01	<0.01	ND	ND	<0.01	0.062	0.022	<0.01	<0.01	ND	0.085
14	AE-8	0.031	<0.01	ND	ND	ND	ND	0.016	0.050	0.037	<0.01	<0.01	ND	0.087
	AE-1	0.032	<0.01	<0.01	<0.01	ND	ND	0.015	0.051	0.038	<0.01	<0.01	ND	0.089
28	AE-13	0.016	<0.01	ND	ND	<0.01	ND	<0.01	0.034	0.043	<0.01	<0.01	<0.01	0.078
	AE-6	0.015	<0.01	ND	ND	<0.01	ND	<0.01	0.035	0.049	<0.01	<0.01	<0.01	0.086
59	AE-23	<0.01	<0.01	<0.01	<0.01	ND	ND	<0.01	0.019	0.067	<0.01	<0.01	<0.01	0.091
	AE-18	<0.01	<0.01	<0.01	<0.01	ND	ND	<0.01	0.018	0.057	<0.01	<0.01	<0.01	0.080
89	AE-26	<0.01	<0.01	ND	ND	<0.01	<0.01	<0.01	0.015	0.061	<0.01	<0.01	<0.01	0.082
	AE-7	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.014	0.061	<0.01	<0.01	<0.01	0.081
120	AE-3	<0.01	<0.01	ND	ND	<0.01	ND	<0.01	0.010	0.062	<0.01	<0.01	<0.01	0.080
	AE-25	<0.01	<0.01	ND	ND	<0.01	<0.01	<0.01	0.011	0.066	<0.01	<0.01	<0.01	0.086
181	AE-21	<0.01	<0.01	ND	ND	<0.01	ND	<0.01	<0.01	0.069	<0.01	<0.01	<0.01	0.088
	AE-20	<0.01	<0.01	ND	ND	<0.01	<0.01	<0.01	<0.01	0.058	<0.01	<0.01	<0.01	0.077

NA Not applicable.

ND Not detected.

2-EE 2-Ethoxyethanol:ethanolamine (1:1).

^a All values in this table (including "Total" values) were calculated by multiplying the fractions of corresponding values in Table II by the study application rate (0.26 µg/g) and dividing these results by 3 (study application rate was three times the field application rate).

Table V

Mean Summary of Radioactivity Found among the Sample Matrices
Expressed Relative to the Field Application Rate of S-53482^a

Sample Interval (Day)	Mean Radioactivity Expressed Relative to the Field Application Rate of S-53482 ($\mu\text{g/g}$)									
	Acetone:Water (5:1) and Acetone:0.1N HCL (9:1) Extracts				Extracted		Traps for Volatile Components			
	S-53482	Origin	Region 1	Region 2	Region 3	Region 4	Diffuse	Total	Soil	Total
0	0.081	ND	ND	ND	ND	ND	<0.01	0.086	<0.01	NA
1	0.075	<0.01	ND	ND	ND	ND	<0.01	0.078	<0.01	ND
3	0.059	<0.01	ND	ND	ND	ND	<0.01	0.070	0.015	<0.01
7	0.052	<0.01	<0.01	<0.01	ND	ND	<0.01	0.062	0.022	<0.01
14	0.032	<0.01	<0.01	<0.01	ND	ND	0.016	0.051	0.038	<0.01
28	0.016	<0.01	ND	ND	<0.01	ND	<0.01	0.035	0.046	<0.01
59	<0.01	<0.01	<0.01	<0.01	ND	ND	<0.01	0.019	0.062	<0.01
89	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.015	0.061	<0.01
120	<0.01	<0.01	ND	ND	<0.01	<0.01	<0.01	0.011	0.064	<0.01
181	<0.01	<0.01	ND	ND	<0.01	<0.01	<0.01	<0.01	0.064	<0.01

NA Not applicable.

ND Not detected.

2-EE 2-Ethoxyethanol:ethanolamine (1:1).

a Means of all values (including "Total" values) in Table IV.

Table VI

Individual and Mean Relative Distribution of Radioactivity Detected on the
Two-Dimensional TLC Plate for the Acetone:Water (5:1) Sample Extract^a

Sample Interval (Day)	Sample Number	Percentage of Radioactivity Detected on TLC Plate													
		Individual							Mean						
		S-53482	Origin	Region 1	Region 2	Region 3	Region 4	Diffuse	S-53482	Origin	Region 1	Region 2	Region 3	Region 4	Diffuse
0	AE-A	94.7	ND	ND	ND	ND	ND	5.3	93.5	ND	ND	ND	ND	ND	6.5
	AE-B	92.3	ND	ND	ND	ND	ND	7.7							
1	AE-14	96.6	0.2	ND	ND	ND	ND	3.2	96.3	0.2	ND	ND	ND	ND	3.6
	AE-22	95.9	0.1	ND	ND	ND	ND	4.0							
3	AE-2	87.1	0.5	ND	ND	ND	ND	12.4	87.2	0.5	ND	ND	ND	ND	12.3
	AE-12	87.3	0.5	ND	ND	ND	ND	12.2							
7	AE-24	89.7	2.5	ND	ND	ND	ND	7.8	86.7	2.7	ND	ND	ND	ND	10.7
	AE-17	83.6	2.8	ND	ND	ND	ND	13.6							
14	AE-8	62.6	3.7	ND	ND	ND	ND	33.7	63.6	5.3	ND	ND	ND	ND	31.2
	AE-1	64.6	6.8	ND	ND	ND	ND	28.6							
28	AE-13	52.4	26.5	ND	ND	ND	ND	21.1	49.5	18.4	ND	ND	10.3	ND	21.9
	AE-6	46.6	10.3	ND	ND	20.5	ND	22.6							
59	AE-23	45.4	10.7	9.2	8.4	ND	ND	26.3	35.5	13.9	11.7	9.7	ND	ND	29.3
	AE-18	25.6	17.1	14.1	11.0	ND	ND	32.2							
89	AE-26	19.0	15.3	ND	ND	39.5	ND	26.2	16.9	15.0	4.5	0.9	36.9	ND	26.0
	AE-7	14.7	14.7	8.9	1.8	34.2	ND	25.7							
120	AE-3	24.7	19.2	ND	ND	53.5	ND	2.6	24.7	16.2	ND	ND	47.8	ND	11.4
	AE-25	24.7	13.1	ND	ND	42.0	ND	20.2							
181	AE-21	47.9	24.7	ND	ND	22.9	ND	4.5	39.6	22.9	ND	ND	17.9	3.1	16.7
	AE-20	31.2	21.0	ND	ND	12.8	6.2	28.8							

ND Not detected.

^a Solvent systems were: Dimension 1 [toluene:ethyl formate:formic acid (5:7:1)] and Dimension 2 [dichloromethane:acetic acid (10:1)].

Table VII

Individual and Mean Relative Distribution of Radioactivity Detected on the
Two-Dimensional TLC Plate for the Acetone:0.1N HCl (9:1) Sample Extract^a

Sample Interval (Day)	Sample Number	Percentage of Radioactivity Detected on TLC Plate													
		Individual							Mean						
		<u>S-53482</u>	<u>Origin</u>	<u>Region 1</u>	<u>Region 2</u>	<u>Region 3</u>	<u>Region 4</u>	<u>Diffuse</u>	<u>S-53482</u>	<u>Origin</u>	<u>Region 1</u>	<u>Region 2</u>	<u>Region 3</u>	<u>Region 4</u>	<u>Diffuse</u>
0	AE-A	91.2	ND	ND	ND	ND	ND	8.8	90.3	ND	ND	ND	ND	ND	9.8
	AE-B	89.3	ND	ND	ND	ND	ND	10.7							
1	AE-14	86.9	ND	ND	ND	ND	ND	13.1	88.9	ND	ND	ND	ND	ND	11.2
	AE-22	90.8	ND	ND	ND	ND	ND	9.2							
3	AE-2	69.3	7.4	ND	ND	ND	ND	23.3	67.8	10.0	ND	ND	ND	ND	22.3
	AE-12	66.2	12.6	ND	ND	ND	ND	21.2							
7	AE-24	64.6	8.5	5.0	4.1	ND	ND	17.8	64.8	7.2	3.9	3.1	ND	ND	21.2
	AE-17	64.9	5.8	2.7	2.1	ND	ND	24.5							
14	AE-8	58.5	19.8	ND	ND	ND	ND	21.7	56.7	13.1	2.1	2.3	ND	ND	25.9
	AE-1	54.9	6.4	4.2	4.5	ND	ND	30.0							
28	AE-13	33.9	32.2	ND	ND	13.5	ND	20.4	33.7	26.4	ND	ND	13.8	ND	26.2
	AE-6	33.4	20.5	ND	ND	14.1	ND	32.0							
59	AE-23	33.1	25.8	9.1	10.3	ND	ND	21.7	33.3	26.5	8.5	11.0	ND	ND	20.8
	AE-18	33.5	27.2	7.8	11.7	ND	ND	19.8							
89	AE-26	27.4	13.1	ND	ND	21.3	10.1	28.1	24.6	15.6	0.6	ND	19.6	7.9	31.9
	AE-7	21.7	18.0	1.2	ND	17.8	5.6	35.7							
120	AE-3	29.4	18.5	ND	ND	49.0	ND	3.1	32.5	23.4	ND	ND	37.4	3.6	3.2
	AE-25	35.5	28.3	ND	ND	25.8	7.1	3.3							
181	AE-21	33.7	52.6	ND	ND	6.6	ND	7.1	34.1	35.2	ND	ND	8.3	ND	22.5
	AE-20	34.4	17.7	ND	ND	10.0	ND	37.9							

ND Not detected.

^a Solvent systems were: Dimension 1 [toluene:ethyl formate:formic acid (5:7:1)] and Dimension 2 [dichloromethane:acetic acid (10:1)].

Table VIII

Individual and Mean Two-Dimensional TLC Distribution of Radioactivity in the Acetone:Water (5:1)
Extract Expressed as the Percentage of Radioactivity Applied to the Sample

Sample Interval (Day)	Sample Number	Percentage of Radioactivity Applied to Sample														
		Total Extract ^a	Individual Distribution ^b							Mean Distribution						
			S-53482	Origin	Region 1	Region 2	Region 3	Region 4	Diffuse	S-53482	Origin	Region 1	Region 2	Region 3	Region 4	Diffuse
0	AE-A	97.1	92.0	ND	ND	ND	ND	ND	5.1	90.7	ND	ND	ND	ND	ND	6.3
	AE-B	96.9	89.4	ND	ND	ND	ND	ND	7.5							
1	AE-14	86.1	83.2	0.2	ND	ND	ND	ND	2.8	82.9	0.2	ND	ND	ND	ND	3.1
	AE-22	86.0	82.5	0.1	ND	ND	ND	ND	3.4							
3	AE-2	71.1	61.9	0.4	ND	ND	ND	ND	8.8	62.2	0.4	ND	ND	ND	ND	8.8
	AE-12	71.5	62.4	0.4	ND	ND	ND	ND	8.7							
7	AE-24	61.3	55.0	1.5	ND	ND	ND	ND	4.8	53.1	1.6	ND	ND	ND	ND	6.6
	AE-17	61.1	51.1	1.7	ND	ND	ND	ND	8.3							
14	AE-8	45.5	28.5	1.7	ND	ND	ND	ND	15.3	29.5	2.5	ND	ND	ND	ND	14.4
	AE-1	47.2	30.5	3.2	ND	ND	ND	ND	13.5							
28	AE-13	28.3	14.8	7.5	ND	ND	ND	ND	6.0	14.4	5.3	ND	ND	3.1	ND	6.4
	AE-6	30.0	14.0	3.1	ND	ND	6.2	ND	6.8							
59	AE-23	15.3	6.9	1.6	1.4	1.3	ND	ND	4.0	5.3	2.0	1.7	1.5	ND	ND	4.3
	AE-18	14.2	3.6	2.4	2.0	1.6	ND	ND	4.6							
89	AE-26	11.0	2.1	1.7	ND	ND	4.3	ND	2.9	1.8	1.6	0.5	0.1	3.9	ND	2.8
	AE-7	10.1	1.5	1.5	0.9	0.2	3.5	ND	2.6							
120	AE-3	7.7	1.9	1.5	ND	ND	4.1	ND	0.2	2.0	1.3	ND	ND	3.8	ND	1.0
	AE-25	8.4	2.1	1.1	ND	ND	3.5	ND	1.7							
181	AE-21	6.3	3.0	1.6	ND	ND	1.4	ND	0.3	2.5	1.5	ND	ND	1.1	0.2	1.1
	AE-20	6.1	1.9	1.3	ND	ND	0.8	0.4	1.8							

ND Not detected.

a Total radioactivity recovered from the acetone:water (5:1) extract (Appendix C).

b Percentage of radioactivity detected on TLC plate (Table VI) multiplied by total radioactivity recovered from the acetone:water (5:1) extract divided by 100.

Table IX

Individual and Mean Two-Dimensional TLC Distribution of Radioactivity in the Acetone:0.1N HCl (9:1)
Extract Expressed as the Percentage of Radioactivity Applied to the Sample

Sample Interval (Day)	Sample Number	Percentage of Radioactivity Applied to Sample														
		Total Extract ^a	Individual Distribution ^b							Mean Distribution						
			S-53482	Origin	Region 1	Region 2	Region 3	Region 4	Diffuse	S-53482	Origin	Region 1	Region 2	Region 3	Region 4	Diffuse
0	AE-A	2.6	2.4	ND	ND	ND	ND	ND	0.2	2.2	ND	ND	ND	ND	ND	0.2
	AE-B	2.2	2.0	ND	ND	ND	ND	ND	0.2							
1	AE-14	4.0	3.5	ND	ND	ND	ND	ND	0.5	3.5	ND	ND	ND	ND	ND	0.5
	AE-22	3.9	3.5	ND	ND	ND	ND	ND	0.4							
3	AE-2	9.2	6.4	0.7	ND	ND	ND	ND	2.1	6.2	0.9	ND	ND	ND	ND	2.0
	AE-12	9.0	6.0	1.1	ND	ND	ND	ND	1.9							
7	AE-24	10.8	7.0	0.9	0.5	0.4	ND	ND	1.9	7.0	0.8	0.4	0.3	ND	ND	2.3
	AE-17	10.7	6.9	0.6	0.3	0.2	ND	ND	2.6							
14	AE-8	11.9	7.0	2.4	ND	ND	ND	ND	2.6	6.8	1.6	0.3	0.3	ND	ND	3.1
	AE-1	12.0	6.6	0.8	0.5	0.5	ND	ND	3.6							
28	AE-13	10.5	3.6	3.4	ND	ND	1.4	ND	2.1	3.6	2.8	ND	ND	1.5	ND	2.8
	AE-6	10.9	3.6	2.2	ND	ND	1.5	ND	3.5							
59	AE-23	7.0	2.3	1.8	0.6	0.7	ND	ND	1.5	2.3	1.9	0.6	0.8	ND	ND	1.5
	AE-18	7.0	2.3	1.9	0.5	0.8	ND	ND	1.4							
89	AE-26	5.9	1.6	0.8	ND	ND	1.3	0.6	1.7	1.4	0.9	0.1	ND	1.2	0.5	1.8
	AE-7	5.4	1.2	1.0	0.1	ND	1.0	0.3	1.9							
120	AE-3	4.5	1.3	0.8	ND	ND	2.2	ND	0.1	1.5	1.1	ND	ND	1.7	0.2	0.2
	AE-25	4.7	1.7	1.3	ND	ND	1.2	0.3	0.2							
181	AE-21	3.6	1.2	1.9	ND	ND	0.2	ND	0.3	1.3	1.3	ND	ND	0.3	ND	0.9
	AE-20	3.7	1.3	0.7	ND	ND	0.4	ND	1.4							

ND Not detected.

a Total radioactivity recovered from the acetone:0.1N HCl (9:1) extract (Appendix C).

b Percentage of radioactivity detected on TLC plate (Table VII) multiplied by total radioactivity recovered from the acetone:0.1N HCl (9:1) extract divided by 100.

Table XI

Individual Distribution of Radioactivity among the Fractions
of the Extracted Soil of Selected Samples

Sample Interval (Day)	Sample Number	Percent of Radioactivity Applied to Sample				
		Extracted Soil ^a	Extracted Soil Fractions			
			Reflux Extract	Humic Acid	Fulvic Acid	Humin ^b
14	AE-8	42.7	29.9	3.1	2.3	7.4
	AE-1	43.3	27.0	3.6	2.4	10.3
28	AE-13	49.4	29.8	4.6	4.5	10.5
	AE-6	56.0	31.6	4.4	4.1	15.9
59	AE-23	76.8	39.6	9.7	5.7	21.8
	AE-18	65.7	40.2	9.8	5.8	9.9
89	AE-26	69.9	38.4	11.0	6.2	14.3
	AE-7	70.1	35.0	10.2	6.5	18.4
120	AE-3	71.3	35.4	11.9	6.4	17.6
	AE-25	76.5	36.6	11.2	6.3	22.4
181	AE-21	80.0	35.2	12.9	7.0	24.9
	AE-20	67.1	34.4	11.8	7.6	13.3

a Values from Table II.

b The percent of applied radioactivity in the extracted soil minus the sum of the percent of applied radioactivity in the reflux extract, fulvic acid, and humic acid fractions.

Table XII

Individual and Mean Two-Dimensional TLC Distribution of Radioactivity in the Reflux Extract
Expressed as the Percentage of Radioactivity Applied to the Sample

Sample Interval (Day)	Sample Number	Total Extract ^a	Percentage of Radioactivity Applied to Sample						
			Individual Distribution ^b			Mean Distribution			
			S-53482	Origin	Region 1	Region 2	Region 3	Region 4	Diffuse
14	AE-8	29.9	9.2	14.3	0.7	1.2	2.3	0.9	1.3
	AE-1	27.0	8.0	13.3	ND	1.3	2.5	0.7	1.2
28	AE-13	29.8	NA	NA	NA	NA	NA	NA	NA
	AE-6	31.6	4.3	24.0	ND	ND	ND	ND	3.3
59	AE-23	39.6	3.7	26.4	0.9	0.6	ND	ND	8.0
	AE-18	40.2	3.4	25.4	0.9	1.0	3.5	0.8	5.2
89	AE-26	38.4	5.7	25.7	ND	ND	ND	ND	7.0
	AE-7	35.0	1.5	27.2	0.7	0.3	ND	ND	5.3
120	AE-3	35.4	2.0	29.8	ND	ND	1.6	ND	2.0
	AE-25	36.6	1.6	30.0	ND	ND	2.0	ND	3.0
181	AE-21	35.2	1.5	29.6	ND	ND	1.2	ND	2.9
	AE-20	34.4	1.8	28.7	ND	ND	1.7	ND	2.2

NA Not applicable.
ND Not detected.

^a Total radioactivity recovered from the reflux extract (Table XI).

^b Percentage of radioactivity detected on TLC plate multiplied by total radioactivity recovered from the reflux extract divided by 100.

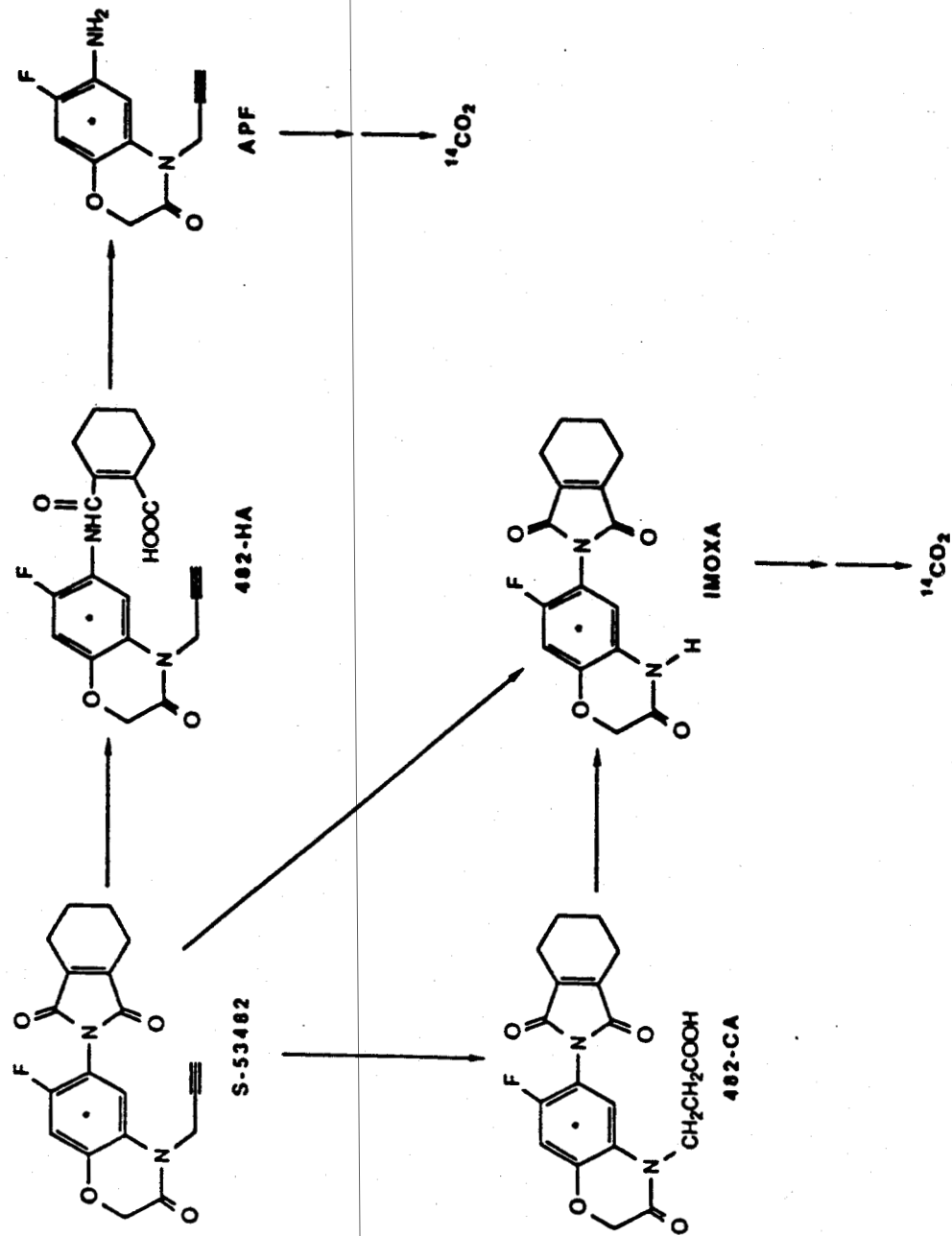


Figure 16. Proposed metabolic pathway for the aerobic degradation of ^{14}C -S-53482 on soil.

Study author(s)'s results and/or conclusions

RESULTS

Soil Microorganisms

The results of the analyses for the presence of microorganisms in the soil are below:

Microorganism Assay	Plate Count Per g of Soil	
	Sample Number	
	AE-10	AE-15
Aerobic	11,000,000	11,000,000
Anaerobic	46,000	44,000
Aerobic Spore	390,000	530,000
Anaerobic Spore	4,600	4,400
Yeast	<1,000	<1,000
Mold	3,000	50,000

The above data show that the study samples contained a viable microbial population at study initiation.

Definitive Study

Distribution of Applied Radioactivity Found among the Sample Matrices. The individual sample values for the applied radioactivity found among the sample matrices are in Table II; mean values are in Table III and presented graphically in Figure 3. Detailed data tables and calculation methods for the percentage of applied radioactivity found in the sample matrices are in Appendix C.

The mean material balance ranged from 94.2% to 102.1% throughout the 181-day study indicating that any test material lost through volatility or adsorption to the sample container was minimal. The amount of applied radioactivity in the sample extract decreased steadily from a mean of 99.4% (Day 0) to a low of 10.0% (Day 181).

Most of the recovered radioactivity was found in the extracted soil after Day 28. The decreases of radioactivity in the extract were paralleled by corresponding increases of radioactivity in the extracted soil and the 2-ethoxyethanol: ethanolamine (1:1) trap ($^{14}\text{CO}_2$). The mean radioactivity found in the extracted soil increased from 0.7% (Day 0) to 73.6% (Day 181) of that applied. By the end of the 181-day study, the cumulative radioactivity found in the 2-ethoxyethanol: ethanolamine (1:1) trap was 11.5% of that applied. The radioactivity detected in the ethylene glycol and charcoal traps did not exceed 0.3% of that applied.

The individual and mean summaries of recovered radioactivity expressed relative to the field application rate of S-53482 are in Tables IV and V, respectively. Except for the parent compound, the mean amount of radioactivity detected for any one TLC plate area of the sample extracts was less than 0.01 ppm. The mean amount of radioactivity detected in the traps for volatile components was less than 0.01 ppm throughout the study. The mean amount of radioactivity detected in the extracted soil ranged from less than 0.01 ppm (Day 0) to 0.064 ppm (Day 181).

Relative Distribution of Radioactivity Detected on the TLC Plate for the Sample Extracts. Radioactivity scans of the TLC plates indicated the presence of at least six components in the sample extracts: ^{14}C -S-53482, radioactivity at the origin designated as Origin, Regions 1 through 4. Any unresolved radioactivity detected by the scanner was designated as Diffuse. Representative radioactivity scans are in Appendix D [acetone:water (5:1)] and Appendix E [acetone: 0.1N HCl (9:1)]. The individual and mean sample values for the relative distribution of radioactivity detected on the TLC plate are in Table VI [acetone:water (5:1)] and Table VII [acetone:0.1N HCl (9:1)].

Two-Dimensional TLC Distribution of Radioactivity Expressed as the Percentage of Radioactivity Applied to the Sample. The individual and mean values for the 2-D TLC distribution of radioactivity expressed as the percentage of radioactivity applied to the sample are in Table VIII [acetone:water (5:1)] and Table IX [acetone:0.1N HCl (9:1)].

Radiolabeled S-53482 degraded under aerobic conditions. The mean extractable radioactivity corresponding to ^{14}C -S-53482 decreased from 92.9% (Day 0) to 3.7% (Day 181) of that applied. The mean applied radioactivity corresponding to any one of the remaining components (Origin and Regions 1 through 4) did not exceed 8.1%.

Degradation Half-Life of ^{14}C -S-53482 under Aerobic Conditions. The degradation of ^{14}C -S-53482 as measured in the sample extracts was assumed to follow first-order kinetics. The calculated half-life using linear regression analysis was 11.9 days. The data used in the linear regression analysis are in Table X; the linear regression line (y-intercept, 4.48; slope, -0.0581; correlation coefficient, -0.995) is in Figure 4 (because the test material degradation had exceeded two first-order half-lives by Day 28, and because the data after Day 28 was not well described by first-order kinetics, only data through Day 28 was used).

Identification and Characterization of Sample Components

Analysis of Day 14 Sample Extracts. The presence of ^{14}C -S-53482 in the Day 14 sample extracts was confirmed by 2-D TLC and HPLC using a reference standard of S-53482 (R_f values: x-axis, 0.67; y-axis, 0.78). The 482-HA (R_f values: x-axis, 0.28; y-axis, 0.56) and APF (R_f values: x-axis, 0.29; y-axis, 0.55) standards comigrated in the 2-D TLC system, however, the standards were separated by HPLC; the 482-HA and APF standards each coeluted with a component from Sample No. AE-1. No sample component comigrated or coeluted with the IMOXa (R_f values: x-axis, 0.48; y-axis, 0.59) and 482-CA standards. The HPLC cochromatography of the S-53482 (retention time, 42.8 minutes), 482-HA (retention time, 32.3 minutes), and APF (retention time, 23.7 minutes) reference standards and the combined extract of Sample No. AE-1 is shown in Figure 5.

Analysis of Day 59 Sample Extracts. The presence of ^{14}C -S-53482 in the Day 59 sample extracts was confirmed by 2-D TLC (Figure 6) and HPLC (Figure 7) using a reference standard of S-53482. The TLC plate scan and autoradiography revealed the presence of two components from isolated Band 2 (Region 1): ^{14}C -IMOXA and an unknown component (Figure 8). The presence of ^{14}C -IMOXA from isolated Band 2 was confirmed by HPLC (Figure 9). Two unidentified components from isolated Band 1 (Region 2) comigrated with the 482-CA standard using 2-D TLC (Figure 10). These two components had HPLC retention times similar to those of 482-HA and 482-CA when comparing a radiochromatogram (obtained from the isolated Band 1) with a U.V. chromatogram of a mixed reference standard solution of the two metabolites injected independently on the same day. (Figure 11). The TLC analysis of the isolated origin material revealed the presence of at least five components (Figure 12).

Analysis of Region 3 from Selected Sample Extracts. The HPLC and mass spectral analyses of isolated Region 3 were inconclusive. An HPLC chromatogram of this region is shown in Figure 13. Region 3 was assigned as unknown (Figure 16).

Analysis of Soil Bound Residues. Reflux of the extracted soil with acetonitrile: 0.25N HCl (4:1) released 40.0% to 70.0% of the radioactivity from the soil; the amount of applied radioactivity found in the extract ranged 27.0% to 40.2%. The amount of applied radioactivity found in the humic acid, fulvic acid, and humin fractions ranged from 3.1% to 12.9%, 2.3% to 7.6%, and 7.4% to 24.9%, respectively (Table XI).

Analysis of the reflux extract by 2-D TLC revealed the presence of at least six components: ^{14}C -S-53482, radioactivity at the origin designated as Origin, Regions 1 through 4. Any unresolved radioactivity detected by the scanner was designated as Diffuse (Table XII). The presence of ^{14}C -S-53482 in the reflux extract was confirmed by 2-D TLC using a reference standard of S-53482 (Figure 14). The mean applied radioactivity corresponding to any one of Regions 1 through 4 did not exceed 2.4% (less than 0.01 $\mu\text{g/g}$). The radioactivity from the reflux extract (mostly origin material) was separated by TLC; none of the components, however, were clearly resolved (Figure 15).

CONCLUSIONS

Radiolabeled S-53482 degraded on soil under the study conditions. The calculated degradation half-life of ^{14}C -S-53482 under aerobic conditions was 11.9 days. The radioactivity was distributed primarily among unchanged S-53482, CO_2 , and soil-bound residues. Several minor components (less than 0.01 $\mu\text{g/g}$) in the sample extracts of the soil were detected: 482-CA, 482-HA, APF, IMOXA, and unknown components of Regions 1 and 3. Radioactivity recovered as CO_2 accounted for 11.5% of the total amount of radioactivity applied to the sample by Day 181. At Day 181, a mean of 10% of the applied radioactivity was extractable; a mean of 3.7% of the extractable radioactivity corresponded to ^{14}C -S-53482. The applied radioactivity remaining in the soil at Day 181 after initial extraction was 73.6% of that applied; approximately 40% to 70% of the soil-bound radioactivity was released by reflux extraction. Based on the study results, a metabolic pathway of the aerobic degradation of ^{14}C -S-53482 was proposed (Figure 16).

The study results indicate that S-53482 will degrade on soil under aerobic conditions. Results indicate that when S-53482 is applied at a normal field application rate, degradates appear at rates less than 0.01 ppm, or as CO₂ or soil bound residues. Therefore, S-53482 may not be an environmental hazard or have a potential to leach in soil.