DATA EVALUATION RECORD
STUDY 4

CHEM 129034 S-53482 §163-1

Study ID 42684909

Fathulla, R. N. 1992. Column Leaching Characteristics of [Phe-^{14}C]-V-53482 in Typical Agricultural Soils (Aged Soils). Unpublished study performed by Hazelton Laboratories America, Inc., WI, and submitted by Valent U.S.A. Corporation, CA

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CONCLUSIONS:

This study is scientifically sound and partially satisfies the data requirement by providing information about the mobility of aged [^{14}C-Ph]-V-53482. This study was conducted using only phenyl ring-labeled V-53482. If the Aerobic Soil Metabolism study using [^{14}C-THP]-V-53482 shows the presence of major degradates, the registrant should submit a mobility study using aged [^{14}C-THP]-V-53482 to satisfy the mobility on soils of aged V-53482 data requirement.

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 two additional degradates could be present in metabolism processes (THPA and Δ^1-TPA). The presence of these products under aerobic soil metabolism conditions could not be confirmed since only phenyl-ring labeled V-53482 was used.

In addition, the parent compound was aged in soils for 30 days under aerobic conditions. After this period only 31.3% of the radioactivity remained as parent compound. According to Subdivision N Guidelines, the test substance should be applied to the soil and incubated aerobically for 30 days or one half-life,

whichever is shorter. The registrant indicated that 30 days was the test interval when the largest amount of polar metabolites were produced in the aerobic soil metabolism study.

Since the results of the aerobic soil metabolism showed that there were no major metabolites formed during aerobic incubation, no additional data will be required on the mobility of aged [^{14}C-Ph]-V-53482. However, a new study is required on the mobility of aged [^{14}C-THP]-V53482.

[Ph-^{14}C]-V-53482 (uniformly ring labeled) residues remain mainly in the soil of leaching columns packed with a California College sandy loam and a Mississippi silt loam soils. The California College sandy loam soil was treated with 0.249-0.260 µg/g, then aged for 30 days in the dark at 25-26°C and 75% field moisture capacity.

In the soil columns packed with California College sandy loam soil 52.9-53.3% of the applied was recovered from the uppermost column section (section number 0), 6.2-6.4% of the applied from section number 1, 2.8-3.6% of the applied from section number 2, 2.3-2.6% of the applied from section number 3, 1.9-2.4% of the applied from section number 4, and 1.9-2.0% of the applied from section number 5. Residues in the leachate totaled 27.8-28.1% of the applied. Most of the leachate radioctivity was located in the second leachate fraction, with 16.7-17.4% of the applied.

In the soil columns packed with Mississippi silt loam soil 58.6-61.9% of the applied was recovered from the uppermost column section (section number 0), 18.1-22.0% of the applied from section number 1, 4.5-4.8% of the applied from section number 2, 1.9-2.1% of the applied from section number 3, 1.3-1.5% of the applied from section number 4, and 1.1% of the applied from section number 5. Residues in the leachate totaled 6.1-6.5% of the applied. Most of the leachate radioactivity was located in the second leachate fraction, with 2.8-3.1% of the applied.

METHODOLOGY:

Aging of V-53482:

[Ph-^{14}C]-V-53482 (uniformly phenyl-ring labeled, radiochemical purity 98.6%, specific activity 551 mCi/g) was dissolved in 1000 µL of acetonitrile, then diluted (200 µL to 21.5 mL of acetonitrile). The resulting fortification solution averaged 294222 dpm/10 µL.

A California College sandy loam soil (68.6% sand, 23% silt, 8.4% clay, 0.79% O.M., pH 8.1) was sieved (2 mm) and divided in 20-gram portions. The soils were fortified with 210-220 µL of fortification solutions. Also, 1.25 g of water were added to adjust soil moisture to 75% of field moisture capacity. The

applied concentration of test material (0.249-0.260 μg/g) was calculated to be about three times the maximum application rate.

The treated soils were then aged in the dark for 30 days in sealed glass chambers connected to three traps for volatiles as follows: charcoal, ethylene glycol, 2-ethoxyethanol:ethanolamine (1:1), and water. A continuous flow of humidified air was created by vacuum. The system was maintained at 25-26°C. Volatile traps were sampled at days 15 and 30.

After 30 days the test samples were removed and analyzed. The soils were extracted three times with portions of acetone: water (5:1, v/v) and three times with portions of acetone:0.1 N HCl (9:1, v/v). The amount of radioactivity in the two types of extracts was determined by LSC. The combined extracts were analyzed by two-dimensional TLC using the following solvent systems:

toluene:ethyl formate:formic acid (5:7:1)
hexane:ethyl acetate (4:3)

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 $^{14}CO_2$ were determined by LSC.

Soil Column Leaching:

Each glass column was cut into six 6-cm segments. The inner diameter was 2-inches (5.1-cm). Two columns per soil type were filled with untreated soil to a uniform density. Two soils were used: a California College sandy loam soil (described above), and a Mississippi silt loam (31.6% sand, 58% silt, 10.4% clay, 1.35% O.M., pH 6.5). Series of traps for volatiles were located at the top and the bottom of the column. Each series had traps of charcoal, ethylene glycol, 2-ethoxyethanol:ethanolamine (1:1), and water media in that order. The radioactivity in each of the traps was determined by LSC. Three 30-day aged soil samples were combined and added to each column (total of about 60 g of aged soil per column). The aged soil was covered with about 10 g of unfortified soils. Approximately 20 inches of 0.01 N to 0.02 N $CaCl_2$ solution were applied to the columns. The application rate averaged 0.4 cm/hour.

The amount of radioactivity was determined by LSC for each leachate fraction. Those leachates containing ≥1% of the applied radioactivity were extracted by filtration of a portion through C_{18} extraction columns. The organic extracts were eluted with methanol. The amount of radioactivity in the organic and aqueous phase was determined by LSC.

The leachate extracts were cochromatographed with standard solutions by two-dimensional TLC using the same solvent system described before. Selected extracts were analyzed by two-dimensional TLC using the following solvent systems:

ethyl ether
dichloromethane:acetic acid (10:1)

The presence of V-53482 in the leachate extracts was confirmed by analysis by HPLC using a gradient mobile phase of acetonitrile:water and both UV and radioactivity detection.

Duplicate portions of the soil sections were homogenized, oxidized by combustion, and the $^{14}CO_2$ determined by LSC. The soil sections containing ≥10% of the applied radioactivity plus four sections containing <10% of the applied radioactivity were extracted with three portions of acetone:water (5:1) (Extract 1), then with three portions of acetone:1 N HCl (9:1) (Extract 2). The extracts were counted by LSC; portions of the soils were combusted and the resulting $^{14}CO_2$ measured by LSC. Extracts 1 and 2 above were analyzed by two-dimensional TLC system using the same solvent systems used for leachate extracts.

The soil extracts were also applied on a silica gel TLC plate and developed with 100% ethyl ether. For those TLC scans in which the origin material was ≥10% of the applied, the region was scraped and extracted with methanol concentrated and applied to another plate. It was developed with chloroform:methanol:formic acid:water (60:70:4:2). An image scan showed a diffused region from the origin to the center of the plate. Selected soil extracts were concentrated and tested by HPLC (method described above) to confirm the presence of V-53482.

Those extracted soils containing ≥10% of the applied radioactivity were refluxed three times with acetonitrile:methanol: 0.1 N HCl (50:30:20) for 1 hour. The reflux extracts were combined, counted by LSC, concentrated by evaporation and filtered through Centricon 3 concentrators. The filtrates were analyzed by one-dimensional TLC using chloroform:methanol:formic acid:water (60:70:4:2). A diffused region was observed from the origin to the center of the plate. The amount of radioactivity in the refluxed soils was determined by combustion and measuring the resulting $^{14}CO_2$. Refluxed soils with ≥10% of the applied radioactivity were further extracted for humic and fulvic acids determination.

RESULTS:

Radioactivity in the aged soil:

After 30 days aging, 31.3% of the radioactivity remained as V-53482. About 5.9% of the applied radioactivity was found in the 2-ethoxyethanol:ethanolamine (1:1) trap. Several components (≤5.1% of the applied) were detected in the TLC plates of the organic extracts of the soil. About 38.1% of the applied radioactivity was

found in the extracted soils. The recoveries after 30 days were 98.3-98.8% of the applied.

Radioactivity in the soil columns and leachate:

The recoveries of radioactivity of the soil and leachates ranged from 95.8-98.2%. The radioactivity found in the traps was ≤0.1% of the applied. The radioactivity found in the walls of the columns was ≤0.3% of the applied.

The soils of the California College sandy loam soil columns contained 68.6-69.7% of the applied radioactivity. Most of this radioactivity (52.9-53.3% fo the applied) remained in the uppermost column section. The corresponding leachates contained 27.8-28.1% of the applied radioactivity.

In contrast, the soils from the Mississippi silt loam soil columns contained 89.2-89.7% of the applied radioactivity. Most of this radioactivity (58.6-61.9% of the applied) remained in the uppermost column section. Leachates contained a smaller fraction (6.1-6.5%) of the applied radioactivity.

TLC autoradiographs show the presence of several (at least 9) minor components in the leachate extracts at ≤2.0% of the applied (totals ≤3.2% of the applied). V-53482 was a maximum of 1.8% of the applied. The following degradates were identified by TLC cochromatography: 482-CA, IMOXA, 482-HA/APF. The presence of V-53482 and 482-CA was confirmed by HPLC.

TLC autoradiographs of the soil extracts show the presence of V-53482 and several (at least 7) other minor components at ≤2.2% of the applied (total ≤3.1% of the applied). Origin material was ≤9.0% of the applied (total 12.8% of the applied). V-53482 comprised a total of 13.8% of the applied in the Mississippi silt loam soil, and 9.2% of the applied for the California College sandy loam soil. Other minor components identified in the soil extracts were: 482-CA, IMOXA, and 482-HA/APF. The presence of V-53482 was confirmed by HPLC.

Further analysis of the extracted soils of the uppermost section of the columns show the presence of 3.7-4.5% of humic acid, 7.6-10.4% of fulvic acid, and 11.1-17.3% of humin.

COMMENTS:

1. This 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-tetrahydrophthalimide (THPA) moiety. Results of the hydrolysis study demonstrated that two additional degradates could be be present in metabolism processes (THPA and $^{1}\Delta$-TPA). The

presence of these products under aerobic soil metabolism conditions cannot be confirmed, and their mobility cannot be assessed if only phenyl-ring labeled V-53482 is used.

2. The parent compound was aged in soils for 30 days under aerobic conditions. After this period only 31.3% of the radioactivity remained as parent compound. According to Subdivision N Guidelines, the test substance should be applied to the soil and incubated aerobically for 30 days or one half-life, whichever is shorter. The registrant indicated that 30 days was the test interval when the largest amount of polar metabolites were produced in the aerobic soil metabolism study.

3. A continuous flow of humidified air in the aerobic aging system was created by vacuum. The flow was measured three times during the process (days 0, 15 and 30). A wide range of flow rates was observed from 32.1-289 mL/min.

4. The efficiency of the soil oxidation procedure was determined using the two soil types, fortified at three levels of radioactivity. The oxidation efficiency ranged from 98.1 to 103.8%.

5. In order to determine the recoveries of radioactivity of the two dimensional TLC procedure, one representative soil and one representative leachate extract were tested. The TLC plate was divided into various areas and scrapings analyzed by LSC. The recoveries were 97.1 to 100.5%.

6. According to the study, the quantity of soil adhered to the columns and containers was small. Rinses of acetone were collected and counted by LSC. The amount of radioactivity found was ≤0.3% of the applied.

7. The registrant used two-dimensional TLC to determine the identity of some of the metabolites present in the soil and leachate samples. HPLC was used to confirm the identity of parent V-53482, and 482-CA. EFGWB prefers that samples be identified by a chromatographic technique such as LSC or LC, and positive identification confirmation be performed with a diferent technique such as MS.

8. The California College sandy loam used in one of the columns and for the aerobic aging process had a pH of 8.1. The Hydrolysis study (MRID# 42684906) demonstrated that higher pH's favor degradation of parent V-53482. EFGWB prefers that the studies be conducted using soils with pH's between 4 and 8.

9. The applied concentration of test material (0.249-0.260 µg/g) was calculated to be about three times the maximum application rate.

10. Two soil types were used in this study: a California College sandy loam, and a Mississippi silt loam. The California College sandy loam is the soil that was used in the aerobic soil metabolism study. According to the registrant, the Mississippi silt loam is representative of the soil at the intended application sites. This herbicide is intended for application in soybeans.

11. The registrant did not attempt to calculate K_d values for V-53482 residues. In a protocol amendment it was stated that K_d values are specific to particular compounds and that in this study a number of compounds were added to the columns for analysis.

der60d
jlm

Table I

Mean Summary of Radioactivity Found in Soil Matrices and Volatile Traps During Aging Process

Sample Interval (Day)	Mean Radioactivity Applied to Sample (%)																
	Radioactivity Detected on the TLC plates for Combined Soil Extracts[a]												Extracted Soil[b]	Ethylene Glycol Trap[b]	2-E:E Trap[b]	Charcoal Trap[b]	Material Balance[c]
	V-53482	Origin	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Area 7	Area 8	Unresolved	Total					
0	93.5	ND	ND	ND	2.0	1.5	1.7	ND	ND	ND	0.1	98.8	2.0	NA	NA	NA	100.8
30	31.3	1.6	4.6	0.7	3.3	1.2	2.8	1.4	3.1	ND	4.7	54.7	38.1	<0.1	5.9	<0.1	98.7

ND Not detected.
NA Not applicable.
2-E:E 2-Ethoxyethanol:ethanolamine (1:1)

a TLC plates were developed in toluene:ethyl formate:formic acid (5:7:1) (Dimension 1) and hexane:ethyl acetate (4:3) (Dimension 2).
b Values from Appendix C.
c Sum of mean values.

Area 1 = 482-CA
Area 5 = IMIDA
Area 6 = 482-HA/APF
Areas 2, 3, 4 and 7 = Unknowns

Table II

Summary of the Total Radioactivity Found among the Soil Column Matrices

Column Number	Radioactivity Applied to Column (%)											
	Leachate[a]			Soil[b]			Ethylene Glycol Trap	2-E:E Trap	Charcoal Trap	Fortification Container	Column Walls	Column Total
	Leachate Number	Individual	Total	Section Number	Individual	Total						
California College Sandy Loam												
1	1	2.1	28.1	0	52.9	69.7	<0.1	ND	<0.1	0.1	0.3	98.2
	2	16.7		1	6.2							
	3	6.4		2	3.6							
	4	2.9		3	2.6							
				4	2.4							
				5	2.0							
2	1	1.4	27.8	0	53.3	68.6	<0.1	ND	<0.1	<0.1	0.1	96.5
	2	17.4		1	6.4							
	3	6.3		2	2.8							
	4	2.7		3	2.3							
				4	1.9							
				5	1.9							
Mississippi Silt Loam												
1	1	ND	6.1	0	58.6	89.7	ND	ND	ND	0.1	<0.1	95.9
	2	2.8		1	22.0							
	3	1.8		2	4.8							
	4	1.5		3	1.9							
				4	1.3							
				5	1.1							
2	1	ND	6.5	0	61.9	89.2	<0.1	ND	ND	<0.1	0.1	95.8
	2	3.1		1	18.1							
	3	1.9		2	4.5							
	4	1.5		3	2.1							
				4	1.5							
				5	1.1							

ND Not detected.

a Individual leachate values from Table D-1.
b Individual soil values from Table E-1.

Table III

Mean Summary of Radioactivity Found in the Leachate[a]

Leachate Number	Extracted Leachate	Mean Radioactivity Applied to Soil Column (%) Leachate Extract V-53482	Origin	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Area 7	Unresolved	Total[b]	Total Leachate[c]
California College Sandy Loam													
1	1.2	ND	0.4	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	ND	0.2	0.7	1.9
2	2.6	1.8	1.8	0.9	0.4	1.0	0.3	1.6	1.8	2.0	3.2	14.8	17.4
3	1.2	0.3	0.6	0.3	0.2	0.4	0.1	0.4	0.7	1.1	1.4	5.5	6.7
4	0.9	<0.1	0.2	0.1	<0.1	0.3	<0.1	0.3	0.3	0.1	0.7	2.0	2.9
Mississippi Silt Loam													
1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	ND
2	2.3	<0.1	0.3	0.2	<0.1	<0.1	<0.1	<0.1	<0.1	ND	0.2	0.6	2.9
3	1.1	0.1	0.3	0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	0.2	0.8	1.9
4	1.0	ND	0.2	0.1	<0.1	<0.1	<0.1	0.1	<0.1	ND	0.2	0.6	1.6

ND Not detected.
NA Not applicable.

a Mean of values in Table D-IV.
b Sum of values in this table ("Mean Radioactivity Applied to Soil Column (%), Leachate Extract")
c Sum of values in this table ["Mean Radioactivity Applied to Soil Column (%), Leachate Extract, Total" + "Mean Radioactivity Applied to Soil Column (%), Extracted Leachate"].
Area 3 = 482-CA
Area 6 = IMOXA
Area 7 = 482-HA/APF
Areas 1, 2, 4 and 5 = Unknowns

Table IV

(Leachate Extract[a])
Mean Distribution of Radioactivity Detected on the TLC Plate

Leachate Number	Radioactivity Detected on TLC Plate (%)									
	V-53482	Origin	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Area 7	Unresolved
					California College Sandy Loam					
1	ND	65.7	11.2	1.4	0.6	1.1	1.0	1.0	ND	18.2
2	12.4	12.2	6.0	2.5	6.3	1.7	11.2	12.2	14.0	21.6
3	5.1	11.3	4.7	3.2	6.7	1.9	7.0	12.6	20.6	27.0
4	1.7	10.4	5.3	2.2	16.1	1.8	12.5	10.9	4.5	34.8
					Mississippi Silt Loam					
1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	0.3	39.2	26.0	1.7	1.9	0.3	2.9	2.8	ND	25.1
3	7.8	35.6	14.4	1.7	3.3	0.6	4.0	8.0	2.3	22.5
4	ND	27.7	9.8	1.5	3.6	3.0	14.5	2.0	ND	38.1

ND Not detected.
NA Not applicable.

a Mean of values in Table D-V.

Area 3 = 482-CA
Area 6 = IMOXA
Area 7 = 482-HA/APF
Areas 1, 2, 4 and 5 = Unknowns

Table V

Mean Summary of Radioactivity Found in the Soil[a]

Section Number	Mean Radioactivity Applied to Soil Column (%)											
	Extracted Soil	Soil Extract										Total Soil[c]
		V-53482	Origin	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Unresolved	Total[b]	
						California College Sandy Loam						
0	34.7	8.7	4.1	2.2	0.4	0.7	0.5	0.8	ND	1.2	18.6	53.3
1	3.4	0.5	1.2	0.5	0.2	0.1	0.1	ND	<0.1	0.4	3.0	6.4
2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3.2[a]
3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.5[a]
4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.2[a]
5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.0[a]
						Mississippi Silt Loam						
0	37.2	9.3	9.0	1.8	0.7	0.7	0.3	0.4	ND	1.1	23.3	60.5
1	9.3	4.1	2.5	1.0	0.6	0.6	0.6	0.1	0.3	1.0	10.8	20.1
2	2.0	0.4	1.3	0.3	0.3	0.1	0.1	0.2	ND	0.3	3.0	5.0
3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.0[a]
4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.4[a]
5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.1[a]

ND Not detected.
NA Not applicable.

Area 2 = 482-CA
Area 3 = IMOXA
Area 4 = 482-HA/APF
Areas 1, 5 and 6 = Unknowns

a Mean of values in Table E-V.
b Sum of values in this table ("Mean Radioactivity Applied to Soil Column (%), Soil Extract).
c Sum of values in this table ("Mean Radioactivity Applied to Soil Column (%), Soil Extract, Total" + "Mean Radioactivity Applied to Soil Column (%), Extracted Soil".

Table VI

Mean Distribution of Radioactivity Detected on the TLC Plate for the Combined Soil Extracts[a]

Section Number	Radioactivity Detected on TLC Plate (%)								
	V-53482	Origin	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Unresolved
				California College Sandy Loam					
0	47.1	22.0	11.6	2.4	3.9	2.6	4.3	ND	6.3
1	16.5	40.4	16.6	5.4	2.7	3.6	ND	0.8	14.1
2	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	NA	NA	NA	NA	NA	NA	NA	NA	NA
				Mississippi Silt Loam					
0	40.4	38.8	7.7	3.1	2.8	1.4	1.4	ND	4.6
1	37.0	23.2	9.5	5.9	6.1	5.3	1.0	2.3	9.9
2	12.4	48.6	8.5	8.8	3.6	4.9	4.7	ND	8.7
3	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	NA	NA	NA	NA	NA	NA	NA	NA	NA

ND Not detected.
NA Not applicable.

a Mean of values in Table E-VI.

Area 2 = 482-CA
Area 3 = IMOXA
Area 4 = 482-HA/APF
Areas 1, 5 and 6 = Unknowns

Table VII

Distribution of Radioactivity among the Fractions of the Extracted Soil of Selected Soil Sections

Column Number	Section Number	Percent of Radioactivity Applied to Sample: Total Extracted Soil[a]	Extracted Soil Fractions: Reflux Extract[b]	Humic Acid[b]	Fulvic Acid[b]	Humin[c]
			California College Sandy Loam			
1	0	35.0	6.4	3.7	7.6	17.3
2	0	34.3	10.5	4.2	8.5	11.1
Mean		34.7	8.5	4.0	8.0	14.2
			Mississippi Silt Loam			
1	0	36.2	9.6	3.7	9.8	13.1
2	0	38.2	10.0	4.5	10.4	13.3
Mean		37.2	9.8	4.1	10.1	13.2

a Values from Table E-V.
b Values from Appendix F.
c 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.

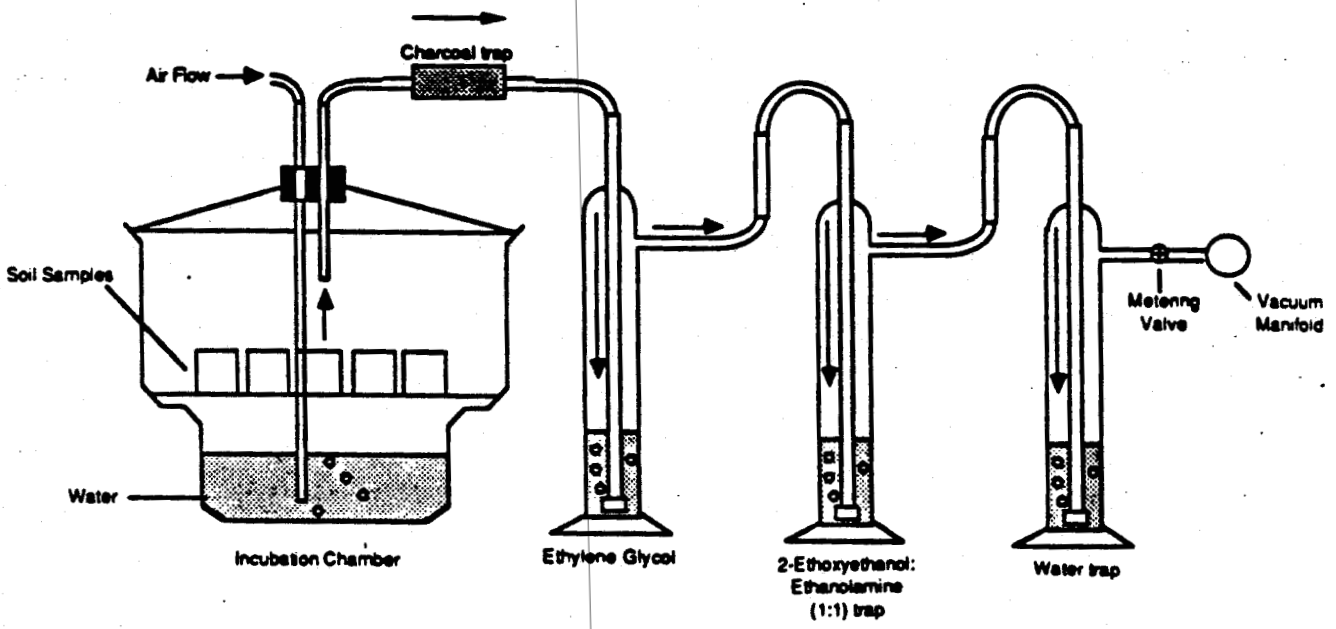

Figure 2. Schematic diagram of the aerobic incubation apparatus used for the test material aging phase.

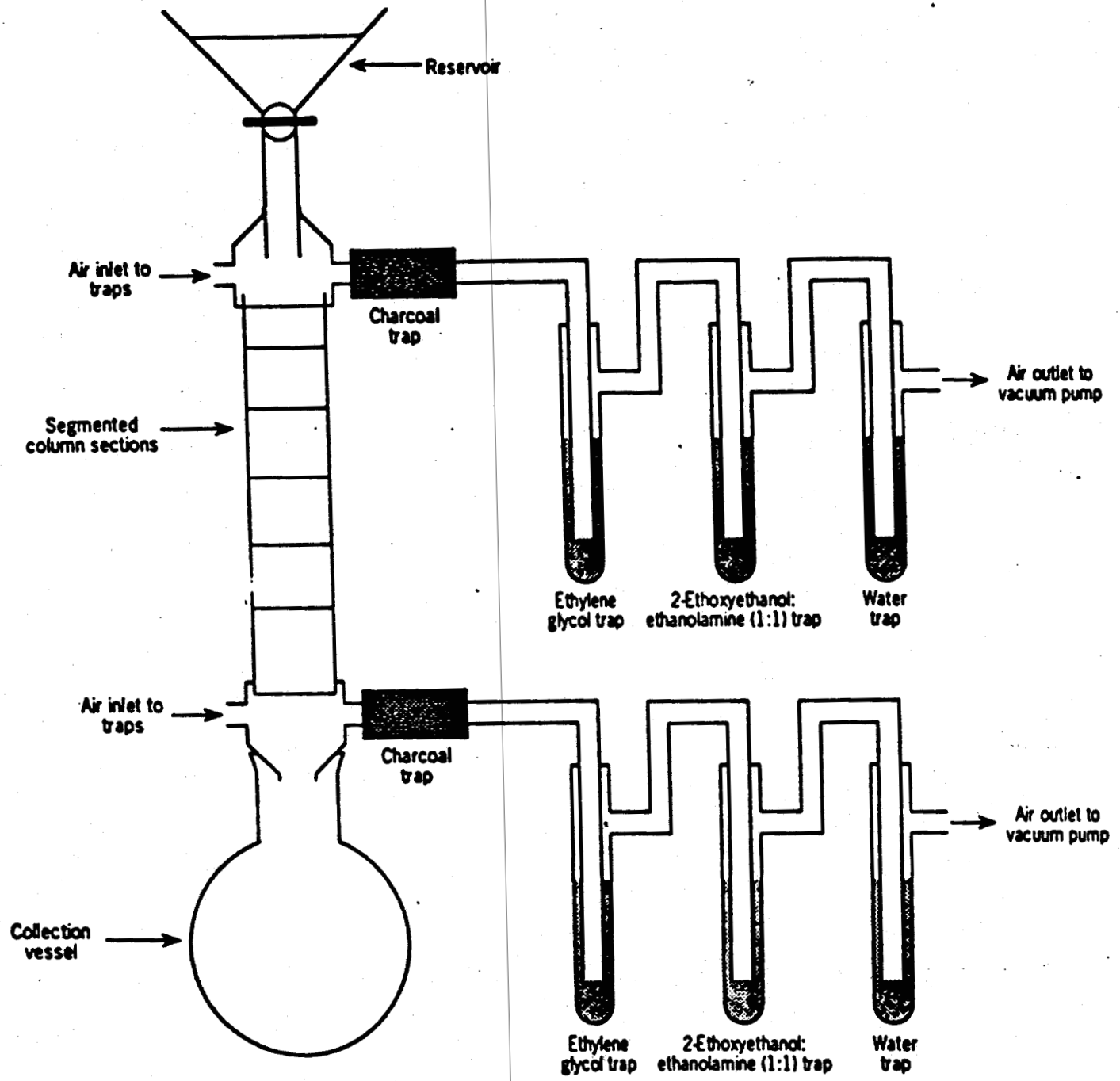

Figure 3. Schematic diagram of the soil column leaching apparatus.

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

RESULTS

Test Material Aging Phase

Distribution of Applied Radioactivity Found among the Sample Matrices. The mean values for the applied radioactivity found among the sample matrices are in Table I and the individual values are in Appendix C. 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 98.7% to 100.8% throughout the 30-day incubation period indicating that any amount of test material lost through volatility or adsorption to the sample container was small. Most of the recovered radioactivity was found in the organic extract of the soil. Over the course of the incubation period, the amount of applied radioactivity in the extract decreased from a mean of 98.8% (Day 0) to a low of 54.7% (Day 30).

The decreases of radioactivity in the extract were paralleled by a corresponding increase of radioactivity in the extracted soil. The mean radioactivity found in the extracted soil increased from 2.0% (Day 0) to a high of 38.1% (Day 30) of that applied. By the end of the 30-day incubation period, the cumulative radioactivity found in the 2-ethoxyethanol:ethanolamine (1:1) trap was only 5.9% of that applied. Less than 0.1% was detected in the ethylene glycol and charcoal traps.

Relative Distribution of Radioactivity Detected on the TLC Plate for the Organic Extract of the Soil. Radioanalytic imaging system scans of the TLC plates indicated the presence of at least ten components in the organic extract: [Phe-^{14}C]-V-53482, material at the origin (Origin), and Areas 1 through 8. A small amount (a mean of 4.7% or less) of radioactivity, not corresponding to any distinct peak or region, was also observed at each interval (Diffuse). Representative scans are in Figures 4 and 5.

TLC Distribution of Radioactivity Expressed as the Percentage of Radioactivity Applied to the Sample. The mean values for the TLC distribution of radioactivity expressed as the percentage of radioactivity applied to the sample are in Table I and the individual values are in Appendix C. Radiolabeled V-53482 degraded under aerobic conditions. The mean extractable radioactivity corresponding to [Phe-^{14}C]-V-53482 decreased from 93.5% (Day 0) to 31.3% (Day 30) of that applied. The mean applied radioactivity corresponding to the four metabolites (482-CA, 482-HA, APF and IMOXA) increased from 2.0% or less (Day 0) to 4.6% or less (Day 30). The mean radioactivity corresponding to Diffuse did not exceed 4.7%.

Material Balance

The values for the total applied radioactivity found among the column matrices are in Table II. The total applied radioactivity recovered for the four columns ranged from 95.8% to 98.2%, indicating that any amount of test material lost through volatility, adsorption to the column walls, or experimental error was small. The recovered radioactivity ranged from 6.1% to 28.1% of that applied for the leachate, and 68.6% to 89.7% for the soil. Less than 0.1% of the applied radioactivity was found in the traps for volatile components. A small amount of radioactivity (no more than 0.3% of that applied for any one soil column) was found on the column and soil container walls.

Leachate

Summary of Radioactivity Found in the Leachate. The mean summary values for the applied radioactivity found in the leachate are in Table III; individual values are found in Appendix D. Detailed data tables and calculation methods for the percentage of applied radioactivity found in the leachate are in Appendix D. Leachate from California College Sandy Loam and Mississippi Silt Loam soil contained 28.0% and 6.3%, respectively, of the applied radioactivity. Most of the leachate radioactivity could be adsorbed onto C_{18} extraction columns and subsequently eluted with methanol. No more than 2.6% of the applied radioactivity remained in the extracted leachate after the C_{18} extraction.

Relative Distribution of Radioactivity Detected on the TLC Plate for the Leachate Extract. Autoradiographs of the TLC plates indicated the presence of at least nine components in the leachate extract: [Phe-^{14}C]-V-53482, material at the origin (Origin), and Areas 1 through 7. A small amount of radioactivity (0.2% to 3.2%) not corresponding to any distinct area was also observed (Unresolved) in leachate extracts.

Because of the low amount of radioactivity, some areas of radioactivity detected by autoradiography were not visible on radioactivity imaging scans. However, based on the autoradiographs, the areas not visible on the scans were quantified by an imaging scanner. Representative imaging scans are in Figures 6 and 7. The mean values for the relative distribution of radioactivity detected on the TLC plate are in Table IV; individual values are in Appendix D.

TLC Distribution of Radioactivity Expressed as the Percentage of Radioactivity Applied to the Column. The mean values for the TLC distribution of radioactivity expressed as the percentage of radioactivity applied to the column are in Table III; indivdual values are in Appendix D. The mean total extractable radioactivity in the leachate corresponding to [Phe-^{14}C]-V-53482 was, 2.1% (California College Sandy Loam) and 0.1% (Mississippi Silt Loam); 482-CA (Area 3) was 1.7% (California College Sandy Loam) and <0.1% (Mississippi Silt Loam); IMOXA (Area 6) was 2.8% (California College Sandy Loam) and 0.1% (Mississippi Silt Loam);

482-HA/APF (Area 7) was 3.2% (California College Sandy Loam) and <0.1% (Mississippi Silt Loam); the unknowns (Areas 1, 2, 4, and 5) did not exceed 2.3% (California College Sandy Loam), 0.4% (Mississippi Silt Loam) and unresolved radioactivity was 0.2% and 3.2% of that applied. The mean applied radioactivity corresponding to Origin and Areas 1 through 7 less than 0.01 ppm of applied radioactivity.

Confirmation of [Phe-^{14}C]-V-53482 and Metabolites in the Leachate Extract. The presence of [Phe-^{14}C]-V-53482 in the leachate was confirmed by 2-D TLC and HPLC. Figure 15 shows the coelution of the V-53482 reference standard and radioactive leachate component. The presence of four metabolites: 482-HA, 482-CA, APF and IMOXA in the leachate was also confirmed by 2-D TLC (Figures 6, 7 and 10). Attempts were also made to confirm the metabolite 482-HA by HPLC (Figure 18).

Soil

Summary of Radioactivity Found in the Soil. Mean summary values for the applied radioactivity found in the soil are in Table V. Individual data tables and calculation methods for the percentage of applied radioactivity found in the soil are in Appendix E.

Most of the applied radioactivity (69.2%, California College Sandy Loam and 89.5%, Mississippi Silt Loam) remained in the soil matrix after the leaching phase. Most of the soil-bound radiocarbon, 53.3% (California College Sandy Loam) to 60.5% (Mississippi Silt Loam), was found in the uppermost column section (section 0). The soil-bound radiocarbon in soil sections 1 to 5 ranged from 6.4% to 2.0% (California College Sandy Loam), and 20.1% to 1.1% (Mississippi Silt Loam). The soil sections 2 to 5 for the sandy loam, and 3 to 5 for the silt loam each contained activity less than 0.01 ppm of that applied (parent equivalent). These soil sections were therefore not analyzed. An attempt was made to extract additional radioactivity from section 0 by refluxing and base extraction (Table VII). Refluxing (acetonitrile:methanol:0.1N HCl) removed an additional 8.5% of applied radioactivity for the California College Sandy Loam, and 9.8% for the Mississippi Silt Loam.

Additional radioactivity was removed by extraction with 0.5M NaOH. Based on solubility characteristics, this additional radioactivity was characterized as humic acid (4.0% applied for sandy loam, and 4.1% for silt loam), fulvic acid (8.0% applied for sandy loam, and 10.1% for the silt loam), or humin (14.2% applied for sandy loam, and 13.2% for silt loam).

Relative Distribution of Radioactivity Detected on the TLC Plate for the Soil Extract. Autoradiographs of the TLC plates indicated the presence of at least eight components in the soil extract: [Phe-^{14}C]-V-53482, material at the origin (Origin), and Areas 1 through 6. A small amount of radioactivity (a mean of 1.2% or less of that applied) not corresponding to any distinct area was also observed (Unresolved) in individual soil extracts.

Because of the low amount of radioactivity, some areas of radioactivity detected by autoradiography were not visible on radioactivity imaging scans. However, based on the autoradiographs, the areas not visible on the scans were quantified by an imaging scanner. Representative imaging scans are in Figures 8, 9 and 10. The mean values for the relative distribution of radioactivity detected on the TLC plate are in Table VI; individual values are in Appendix E.

TLC Distribution of Radioactivity Expressed as the Percentage of Radioactivity Applied to the Column. The mean values for the TLC distribution of radioactivity applied to the column are in Table V; individual values are in Appendix E. The mean total extractable radioactivity in the soil sections 0 and 1 (California College Sandy Loam) correspondinf to: [Phe-^{14}C]-V-53482 was 9.2%; 482-CA (Area 2) was 0.6%; IMOXA (Area 3) was 0.8%; 492-HA/APF (Area 4) was 0.6%; origin was 5.3%, and Areas 1, 5, and 6 (Unknowns) was <0.1% to 2.7%.

The mean total extractable radioactivity in the soil section 0, 1, and 2 (Mississippi Silt Loam) corresponding to: [Phe-^{14}C]-V-53482 was 13.8%; 482-CA (Area 2) was 1.6%; IMOXA (Area 3) was 1.4%; 482-HA/APF (Area 4) was 1.0%; origin was 12.8%, and Areas 1, 5, and 6 (Unknowns) was 0.3% to 3.1%.

Confirmation of [Phe-^{14}C]-V-53482 in the Soil Extract. The presence of [Phe-^{14}C]-V-53482 in the soil was confirmed by 2-D TLC and HPLC. Figures 16 and 17 show the coelution of the V-53482 reference standard and radioactive soil component.

CONCLUSIONS

Under aged leaching conditions most of radioactivity applied as V-53482 and its metabolites remained in the soil matrix after leaching (89.5% and 69.2% for the silt loam and sandy loam, respectively), and most of that stayed at the applied aged soil layer and the first section of soil column (average of 80.3% for Mississippi Silt Loam and 59.4% for California College Sandy Loam). It leached, however, more readily in the sandy loam soil than silt loam soil (28.0% and 6.3%, respectively)

Radiolabeled V-53482 degraded on soil, under aged leaching conditions. The majority of the radioactivity was incorporated to the soil organic matter. About 9.2% to 13.8% of the soil radioactivity was [Phe-^{14}C]-V-53482. Several minor components were observed; 482-CA, 482-HA, APF, IMOXA, and unknown components. All these components were less than 0.01% ppm when converted to the field application rate. The mean amount of radioactivity corresponding to [Phe-^{14}C]-V-53482 in the leachate at the end of the leaching phase ranged from less than 0.1% to 2.1%. Several minor components were observed; 482-CA, 482-HA, APF, IMOXA, and unknown components. All of these components were less than 0.01 ppm when converted to the field application rate. Volatile components were not detected above 0.1% of the applied radioactivity. Based on the results of this study, and the short degradation half-life of the test material in a soil metabolism study (11.9 days), the potential for the [Phe-^{14}C]-V-53482 to leach in the soil is low.