DATA EVALUATION RECORD

STUDY 3

CHEM 041403 Pebulate §162-1

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 40637101
Daly, D., and W. Cranor. 1988. Aerobic soil metabolism of ^{14}C-Tillam. ABC Final Report #33875. Unpublished study performed by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO, and submitted by Stauffer Chemical Co., Mountain View, CA.

STUDY ID 40637102
McBain, J. B. 1988. Metabolism of tillam in aerobic soil. Addendum I, ABC Project No. 33875. Unpublished study performed and submitted by ICI Americas Inc., Wilmington, DE.

STUDY ID 92138010
Calderbank, A. 1990. Phase 3 summary of MRID 40637101-02 consisting of "Aerobic soil metabolism of ^{14}C-Tillam." Report No. ABC. 33875 and Addendum: Document No. PMS-187. ICI Americas, Wilmington, DE.

DIRECT REVIEW TIME = 10

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CONCLUSIONS:

Metabolism - Aerobic Soil

1. This study is unacceptable at this time for the following reasons:

    1. The material balance was incomplete; up to 54.9% of the applied radioactivity was not recovered (material balance range = 45.1 to 111%, mean = 84.2%, N = 24) despite the fact that volatilization was monitored throughout the length of the study (See Table XXII, page 3.12).

       In order for this study to fulfill the aerobic soil metabolism data requirement, the registrant must provide data to conclusively demonstrate that the applied radioactivity that was lost from the system, at each time period, had been volatilized.

    2. No storage stability data was provided. The "Methods and Materials" section does not clearly indicate when soil extractions/analyses were performed in relation to when sampled. The registrant should provide information related to storage of samples before analysis.

3. Pebulate appears to dissipate from aerobic soil with a half-life of ≈1 month; the only significant nonvolatile degradate may be pebulate sulfoxide. However, because the material balance of samples taken after 1 month posttreatment is poor (up to ≈55% of the applied is not accounted for), the long-term persistence of pebulate and its degradates in soil cannot be accurately determined.

METHODOLOGY:

Samples of sieved (2 mm) sandy loam soil (58% sand, 30% silt, 12% clay, 2.6% organic matter, pH 8.1, CEC 11.6 meq/100 g) were weighed (250-g) into biometer flasks, adjusted to 2% moisture, and incubated at 25 ± 1°C for 6 days. Twenty-four flasks of soil were each treated with ≈5 ppm of [^{14}C]pebulate (Tillam; radiochemical purity 99.6%, specific activity 3.35 x 10^4 dpm/μg) that was dissolved in acetone. Two additional flasks of untreated soil served as controls. The soils were mixed by stirring, moistened to 75% of field capacity, and mixed again. The sidearm of each flask was filled with 1 N potassium hydroxide and fitted with a urethane foam plug for trapping volatiles. The flasks were sealed, placed in an environmental chamber, and attached to a "static" air-flow system (Figure 7). A humidified air-flow through the static system created a partial

vacuum, which theoretically drew CO_2 evolved from the soil through the urethane plug and into the trapping solution. Duplicate soil samples were collected at 0, 1, 3, 7, and 14 days, and 1, 2, 3, 4, 6, 9, and 12 months posttreatment; the urethane plug was changed at each sampling interval. The trapping solution was changed at each sampling interval and at 7, 8, and 10 months posttreatment.

Soil samples were extracted twice with acetone, then with methanol. Aliquots of the extracts were analyzed alone using LSC; additional aliquots were combined and analyzed for total extractable radioactivity using LSC. Unextractable [^{14}C]residues in extracted soils were quantified by LSC following combustion. The urethane foam plugs were extracted with ethyl acetate and total radioactivity was quantified using LSC. Soil and plug extracts were stored frozen in an amber bottle at -22°C when not in use. [^{14}C]Residues in the gas trapping solutions were quantified by LSC, and CO_2 content in the solutions was determined using a carbon analyzer equipped with an infrared gas analyzer/carbon dioxide detector.

Additional aliquots of the combined soil extracts were quantified and separated using two-dimensional TLC on silica gel plates developed in toluene:acetone (5:1) and methylene chloride. Those extracts found to contain high concentrations of polar [^{14}C]residues were also analyzed using one-dimensional TLC developed in the polar solvent system of butanol:acetic acid:water (15:8:1). Plug extracts were analyzed for specific compounds using one-dimensional TLC with either toluene:acetone (5:1) or methylene chloride as solvents. Nonradiolabeled standards were cochromatographed. Standards were identified by exposure to iodine vapors, and radioactive residues were detected using autoradiography. Radioactive compounds were scraped from the plates, desorbed from the silica gel, and quantitated by LSC.

Four additional flasks of soil were autoclaved for 1.5 hours prior to treatment and incubation as described above. The sterile soils were sampled at 1 and 3 months posttreatment and analyzed as previously described.

DATA SUMMARY:

Pebulate (radiochemical purity 99.6%), at ≈5 ppm, degraded with a calculated half-life of 35.5 days in sandy loam soil that was incubated in the dark at 25 ± 1°C and 75% of field moisture capacity for 12 months in a static system (Table II, Figure 2). Pebulate decreased from an average 4.8 ppm immediately posttreatment to 2.1 ppm at 1 month and 0.1 ppm

at 12 months (Table II). The major nonvolatile degradate was

pebulate sulfoxide,

which accumulated to a maximum of 0.79-0.94 ppm at 1 month. There were six unidentified nonpolar extractable degradates: Unknowns 1 and 2 each comprised a maximum 0.04 ppm at 1 to 4 months; Unknown 3 was isolated only at time 0; and Unknowns 4, 5, and 6 together comprised 0.08 ppm at time 0 and decreased to ≤0.01 ppm at 2 months (Table II). Unknowns 3-6 were characterized by the study author as impurities in the test substance. Polar degradates, which comprised up to 0.25 ppm, cochromatographed primarily (>90% of origin material) with

butylethylamine and butylamine;

however, the polar solvent system used by the study author did not resolve these two compounds separately (Table III). By 12 months posttreatment, $^{14}CO_2$ in the trapping solution and volatiles in the urethane foam plug were a maximum 1.66 and 0.01 ppm (35.2 and 0.27% of the applied), respectively (Table XXII). Unextractable residues increased from 0.09-0.13 ppm immediately posttreatment to a maximum 1.37-1.4 ppm at 4 months, then decreased to 1.02-1.14 ppm at 12 months (Table XXII). Material balances decreased from 104-111% of the applied at time 0 to 89.8-91.1% at 1 month and 45.1-61.0% at 12 months (Table XXII).

In sterilized soil, pebulate degraded with a calculated half-life of 82.5 days (Table IV). Pebulate sulfoxide was the major degradate.

REVIEWERS COMMENTS:

1. The material balance was incomplete; up to ≈55% of the applied radioactivity was not accounted for. The study authors stated that it was probable that the missing material was untrapped volatiles, since pebulate and its degradates are known to be volatile. Several experimental designs were employed by the study authors in an attempt to obtain an acceptable aerobic soil metabolism study. Experiments employing flow-through and static trapping systems were conducted. The static experiment is reported here; the experiment using a flow-through system was terminated because the material balance was "inadequate"; at 4 months.

   If the study authors are correct and the missing material was volatilized pebulate, then the trapping systems that

were employed in the experiments were inadequate. The trapping efficiencies for the foam plug were not reported.

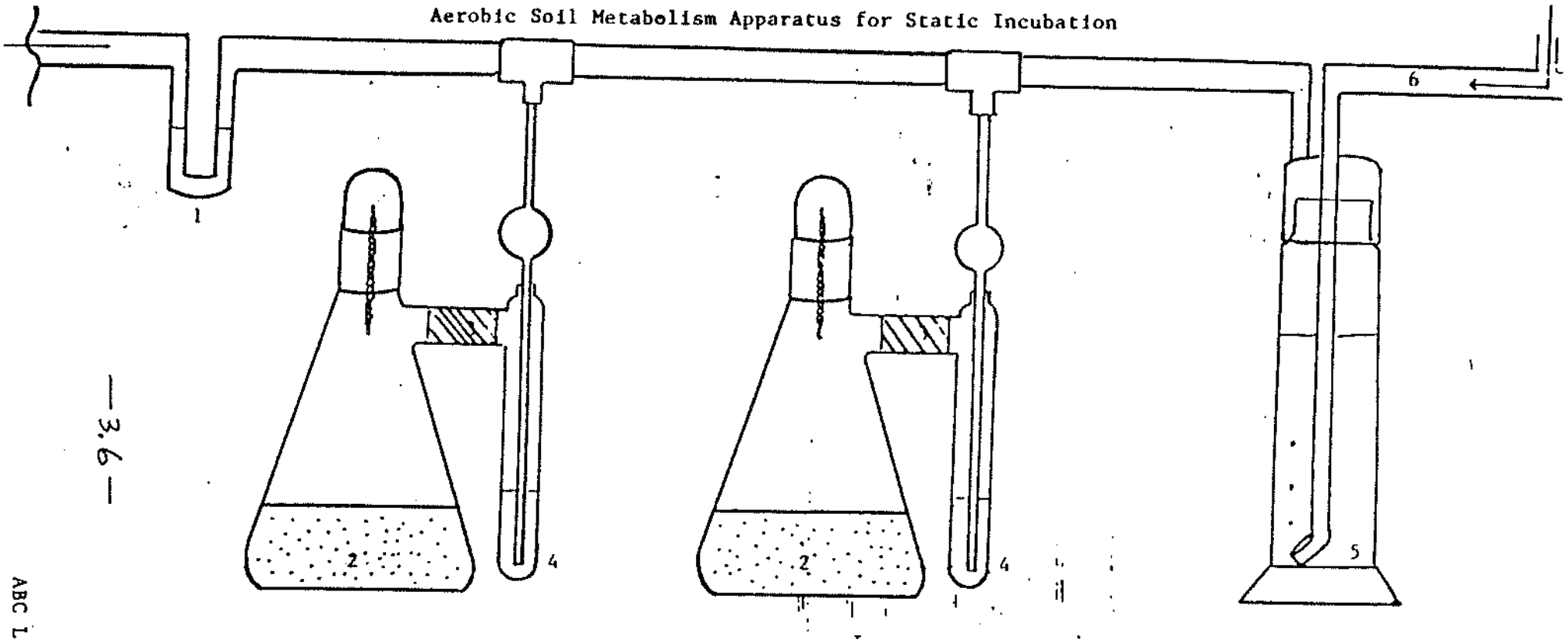
2. No storage stability data was provided. The "Methods and Materials" section does not clearly indicate when soil extractions/analyses were performed in relation to when they were sampled. It appears that after the soil samples were extracted, they were stored in amber bottles and stored at -22°C. However, the length of storage was not stipulated. If the samples were not extracted or analyzed immediately after sampling, then a storage stability test should be performed on the solutions for as long as they were stored before extraction and/or analysis. This is needed to show that the samples did not degrade in storage.

3. Two degradates (Unknowns 1 and 2), present at up to 0.04 ppm, were not identified. Subdivision N guidelines specify that all degradates present at >0.01 ppm should be identified.

4. The half-life of pebulate in the static system was ≈35 days. In the experiment employing a flow-through system, the calculated half-life of pebulate was only ≈10 days. The shorter half-life may have been a result of increased volatilization.

FIGURE 7

Aerobic Soil Metabolism Apparatus for Static Incubation



1. U-tube
2. Soil (250 g)
3. Foam Plug (trap for volatiles)
4. 1 N KOH (75 ml)
5. H_2O Saturation Bottle
6. Oxygen

Table II. TLC Analysis of the Acetone-Extractable ^{14}C From Soil Treated With [Butyl-^{14}C] Tillam and Incubated Aerobically.

Product Distribution 3/ 4/

1/ 2/	0-TIME				1-DAY				3-DAYS				7-DAYS			
	REP. I		REP. II		REP. I		REP. II		REP. I		REP. II		REP. I		REP. II	
PRODUCT	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM
Tillam	95.59	4.90	97.90	4.71	97.51	4.25	97.03	4.33	96.03	4.53	95.83	4.49	93.08	3.84	87.57	3.63
Tillam-Sulfoxide	0.45	0.02	0.29	0.01	0.90	0.04	1.19	0.05	2.03	0.1	2.8	0.13	5.11	0.21	8.83	0.37
Unknown 1	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01
Unknown 2	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01
Unknown 3	0.61	0.03	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01
Unknowns 4,5,6	1.63	0.08	1.59	0.08	1.11	0.05	1.41	0.06	0.89	0.04	0.74	0.03	0.72	0.03	1.61	0.07
Origin 5/	1.69	0.09	0.22	0.01	0.47	0.02	0.36	0.02	1.06	0.05	0.62	0.03	1.09	0.04	1.99	0.08
TOTAL	100.00	5.12	100.00	4.81	99.99	4.36	99.99	4.46	100.01	4.72	99.99	4.68	100.00	4.12	100.00	4.15

1/ Analyses were conducted by two-dimensional TLC using solvent system A in the first direction and solvent system B in the second direction. See Figure 1 for a representative separation of products in System AxB.

2/ In MRC laboratory notebook 1150, unknowns 1, 2, 3, 4, 5 and 6 were labeled, respectively, as unknowns 4, 5, 7, 8, 9, and 10.

3/ PPM values are expressed in terms of Tillam equivalents. See Table XXII of ABC Final Report No. 33875.

4/ ND denotes Not Detected. No ^{14}C spot was observable on the autoradiogram of the TLC plate.

5/ The polar ^{14}C which remained at the origin was largely butylethylamine and butylamine (See Table III).

Data Sources: [1150:62-66, 145-148]

Table II (Continued). TLC Analysis of the Acetone-Extractable ^{14}C From Soil Treated With [Butyl-^{14}C]Tillam and Incubated Aerobically.

Product Distribution 3/ 4/

1/ 2/	14-DAYS				1-MONTH				2-MONTHS				3-MONTHS			
	REP. I		REP. II		REP. I		REP. II		REP. I		REP. II		REP. I		REP. II	
PRODUCT	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM
Tillam	82.30	3.27	78.07	3.04	69.10	2.23	62.71	2.04	65.20	1.47	68.27	1.46	71.75	0.95	54.43	0.59
Tillam-Sulfoxide	13.71	0.54	16.19	0.63	24.34	0.79	28.87	0.94	24.15	0.54	21.50	0.46	17.52	0.23	37.28	0.41
Unknown 1	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	1.14	0.03	1.36	0.03	1.22	0.02	2.06	0.02
Unknown 2	ND	<0.01	ND	<0.01	1.32	0.04	ND	<0.01	1.74	0.04	ND	<0.01	1.77	0.02	1.33	0.01
Unknown 3	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01
Unknowns 4,5,6	1.49	0.06	1.58	0.06	0.41	0.01	0.82	0.03	0.38	0.01	0.65	0.01	ND	<0.01	ND	<0.01
Origin 5/	2.49	0.10	4.16	0.16	4.82	0.16	7.60	0.25	7.40	0.17	8.22	0.18	7.75	0.10	4.90	0.05
TOTAL	99.99	3.97	100.00	3.89	99.99	3.23	100.00	3.26	100.01	2.26	100.00	2.14	100.01	1.32	100.00	1.08

1/ Analyses were conducted by two-dimensional TLC using solvent system A in the first direction and solvent system B in the second direction. See Figure 1 for a representative separation of products in System AxB.

2/ In MRC laboratory notebook 1150, unknowns 1, 2, 3, 4, 5 and 6 were labeled, respectively, as unknowns 4, 5, 7, 8, 9, and 10.

3/ PPM values are expressed in terms of Tillam equivalents. See Table XXII of ABC Final Report No. 33875.

4/ ND denotes Not Detected. No ^{14}C spot was observable on the autoradiogram of the TLC plate.

5/ The polar ^{14}C which remained at the origin was largely butylethylamine and butylamine (See Table III).

Data Sources: [1150-62-66, 145-148]

Table 11 (Continued). TLC Analysis of the Acetone-Extractable ^{14}C From Soil Treated With [Butyl-^{14}C]Tillam and Incubated Aerobically.

Prnduct Distribution 3/ 4/

1/ 2/ PRODUCT	4-MONTHS				6-MONTHS				9-MONTHS				12-MONTHS			
	REP. I		REP. II		REP. I		REP. II		REP. I		REP. II		REP. I		REP. II	
	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM
Tillam	66.05	0.75	64.55	0.79	50.97	0.38	56.93	0.36	24.40	0.08	42.61	0.16	23.34	0.05	51.20	0.14
Tillam-Sulfoxide	21.63	0.25	22.37	0.28	30.14	0.22	28.57	0.18	35.65	0.12	34.91	0.13	42.49	0.08	32.03	0.09
Unknown 1	1.18	0.01	3.39	0.04	3.71	0.03	1.89	0.01	4.85	0.02	3.70	0.01	ND	<0.01	ND	<0.01
Unknown 2	1.40	0.02	1.85	0.02	2.27	0.02	1.90	0.01	4.83	0.02	3.11	0.01	ND	<0.01	ND	<0.01
Unknown 3	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01
Unknowns 4,5,6	ND	<0.01	ND	<0.01	ND	<0.01	0.65	<0.01	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01
Origin 5/	9.73	0.11	7.85	0.10	12.92	0.10	10.07	0.06	30.26	0.10	15.68	0.06	34.17	0.07	16.77	0.05
TOTAL	99.99	1.14	100.01	1.23	100.01	0.75	100.01	0.62	99.99	0.34	100.01	0.37	100.00	0.20	100.00	0.28

1/ Analyses were conducted by two-dimensional TLC using solvent system A in the first direction and solvent system B in the second direction. See Figure 1 for a representative separation of products in System AxB.

2/ In MRC laboratory notebook 115D, unknowns 1, 2, 3, 4, 5 and 6 were labeled, respectively, as unknowns 4, 5, 7, 8, 9, and 10.

3/ PPM values are expressed in terms of Tillam equivalents. See Table XXII of ABC Final Report No. 33875.

4/ ND denotes Not Detected. No ^{14}C spot was observable on the autoradiogram of the TLC plate.

5/ The polar ^{14}C which remained at the origin was largely butylethylamine and butylamine (See Table III).

Data Sources: [1150-62-66, 145-148]

TABLE XXII (Continued)

^{14}C-Extractable, ^{14}C-Nonextractable, ^{14}C-Volatile and Total ^{14}C-Residues of Tillam in Aerobic Soil Metabolism Study Incubated Under Static Conditions

	2 Month		3 Month		4 Month		6 Month		9 Month		12 Month	
AS1	PPM	%	PPM	%	PPM	%	PPM	%	PPM	%	PPM	%
Acetone/Methanol Soluble	2.25	47.7	1.33	28.2	1.14	36.5	0.736	15.6	0.341	7.22	0.196	4.15
Soil-Bound	1.15	24.4	1.11	23.5	1.37	43.9	1.30	27.5	1.23	26.1	1.02	21.6
$^{14}CO_2$	0.358	7.58	0.238	8.72	0.555	17.8	1.03	21.8	1.82	38.6	1.66	35.2
Foam Plug Trap	0.135	2.86	0.0523	1.92	0.0508	1.63	0.0492	1.04	0.0349	0.739	0.00837	0.17
Total	3.89	82.4	2.73	57.8	3.12	66.1	3.12	66.1	3.43	72.7	2.88	61.0
AS11												
Acetone/Methanol Soluble	2.14	45.3	1.09	26.8	1.23	36.7	0.630	13.3	0.385	6.46	0.282	5.97
Soil-Bound	1.08	22.9	1.49	36.7	1.40	41.8	1.23	26.1	1.33	28.2	1.14	24.2
$^{14}CO_2$	0.0168	0.356	1.37	33.7	0.641	19.1	1.21	25.6	1.91	40.5	0.692	14.7
Foam Plug Trap	0.0158	0.335	0.114	2.81	0.0830	2.48	0.0588	1.25	0.0285	0.604	0.0126	0.267
Total	3.25	68.9	4.06	86.0	3.35	71.0	3.13	66.3	3.65	77.3	2.13	45.1

[1] ppm = µg ^{14}C-Tillam equivalents/gram of study soil.

[2] % = Percent of initial concentration as determined by day 0 combustions = 4.72 µg/g.

Table IV. TLC Analysis of the Acetone-Extractable ^{14}C From Sterile Soil Treated With [8uty1-^{14}C]Tillam and Incubated Aerobically.

Product Distribution 3/ 4/

1/ 2/ PRODUCT	1-MONTH				3-MONTH			
	REP. I		REP. II		REP. I		REP. II	
	%	PPM	%	PPM	%	PPM	%	PPM
Tillam	94.02	3.84	92.61	4.13	93.35	2.93	93.6	3.02
Tillam-Sulfoxide	2.97	0.12	3.03	0.14	3.53	0.11	3.61	0.12
Unknown 1	ND	<0.01	ND	<0.01	0.7	0.02	ND	<0.01
Unknown 2	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01
Unknown 3	ND	<0.01	ND	<0.01	ND	<0.01	ND	<0.01
Unknowns 4,5,6	2.34	0.10	3.71	0.17	ND	<0.01	ND	<0.01
Origin 5/	0.66	0.03	0.65	0.03	2.43	0.08	2.8	0.09
TOTAL	99.99	4.09	100.00	4.47	100.01	3.14	100.01	3.23

1/ Analyses were conducted by two-dimensional TLC using solvent system A in the first direction and solvent system B in the second direction. See Figure 1 for a representative separation of products in System Ax8.

2/ In MRC laboratory notebook 1150, unknowns 1, 2, 3, 4, 5 and 6 were labeled, respectively, as unknowns 4, 5, 7, 8, 9, and 10.

3/ PPM values are expressed in terms of Tillam equivalents.

4/ ND denotes Not Detected. No ^{14}C spot was observable on the autoradiogram of the TLC plate.

5/ The polar ^{14}C which remained at the origin was largely butylethylamine and butylamine (See Table III).

Data Sources: [1150:65, 146]

STUDY AUTHOR(S)'S RESULTS AND/OR CONCLUSIONS

SUMMARY

[1-^{14}C-Butyl]Tillam was applied at a 5 ppm rate to a sandy loam soil and aerobically incubated at 25 °C. Soil samples were periodically analyzed by extracting with acetone and investigating the extracts by TLC for the occurrence of Tillam and its degradates.

The soil half-life of Tillam was shown by regression analysis to be 35.5 days. The acetone-soluble degradates were composed principally of Tillam sulfoxide (0.87 ppm at peak concentration which occurred at one month) and several minor products, including butylethylamine and butylamine. With the exception of Tillam sulfoxide and the amines, all acetone extractable degradates declined in concentration to less than 0.01 ppm Tillam equivalents by the end of the study.

In sterile soils, Tillam was slower to degrade and its principal degradate, Tillam sulfoxide, was formed only to a minor extent (0.1 ppm at one and three months).

The Tillam applied to the soil partially volatilized. TLC analysis of the ethyl acetate extracts of the polyurethane foam traps located within the soil incubation flasks revealed that the trapped ^{14}C was in the form of unchanged Tillam.