TEXT SEARCHABLE DOCUMENT

PC: 900497

DATA EVALUATION RECORD 4

Dohn, D.R. 1990. Aerobic soil metabolism of R-25788. Laboratory Study No. PMS 293. Report No. RR 90-014B. Unpublished study performed by ICI Americas Inc., Richmond, CA, and Pharmacology and Toxicology Research Laboratory, Lexington, KY; and submitted by ICI Americas Inc., Richmond, CA. MRID# 415614-12

REVIEWED BY: M. Dillman TITLE: Staff Scientist

EDITED BY: K. Ferguson TITLE: Task Leader
W. Hurtt Staff Scientist

APPROVED BY: W. Spangler TITLE: Project Manager

ORG: Dynamac Corporation
Rockville, MD
TEL: 301-417-9800

APPROVED BY: E.B. Conerly-Perks
TITLE: Chemist
ORG: EFGWB/EFED/OPP
TEL: 703-305-5245

E.B. Conerly-Perks 1/8/93

SIGNATURE:

CONCLUSIONS:

Metabolism - Aerobic Soil

1. This study is acceptable and fulfills EPA Data Requirements for Registering Pesticides by providing information on the aerobic soil metabolism of R-25788 in silty clay loam soil. No additional information on the aerobic soil metabolism of R-25788 in silty clay loam soil is required at this time.

2. R-25788 degraded with a half-life of 7.5 days in silty clay loam soil that was incubated in the dark at approximately 25 C and 75% of field moisture capacity. CO_2 was the only metabolite identified.

METHODOLOGY:

Portions (250 g dry weight) of sieved (2 mm) silty clay loam soil (10.2% sand, 59.0% silt, 30.8% clay, 3.6% organic matter, pH 6.0, CEC 27.2 meq/100 g) were weighed into biometer flasks, then treated at a nominal concentration of 0.403 ppm with carbonyl-labeled [^{14}C]R-25788 (2,2-dichloro-N,N-di-2-propenylacetamide; radiochemical purity ≥98.4%, specific activity 25.9 mCi/mMol, ICI Americas) dissolved in acetone. The treated soil was moistened with water to 75% of field capacity. Each biometer sidearm was filled with a NaOH solution, a polyurethane foam plug was placed in the bridge between the chambers, and the biometer flasks were attached in series to a continuous air-flow system (Figure 2). The study

2078910

4.3 Rate of Degradation

The xenon exposure time was converted to equivalent solar days (see Appendix A). A tabulation of R-25788 remaining vs. time is given in Table V. The logarithm of the R-25788 concentration vs. time was fitted with least-squares analysis to yield pseudo first-order rate constants. The data and least-squares lines are plotted in Figure 6. Half-lives of 70 days for the irradiated samples and 130 days for the dark controls were calculated. The rate constants for the photolysis and dark control studies are 1.03×10^{-2} and 5.2×10^{-3} day^{-1}, respectively. The overall observed rate constant for the photolysis study consists of two components, which are the rate constant associated with photolysis alone and the rate constant associated with all other processes. The rate constant for photolysis of R-25788 on a soil surface can be calculated as follows:

$$k_p = k_t - k_d$$

where k_t is the observed rate constant for degradation of irradiated R-25788, k_d is that observed for the dark controls, and k_p is the photochemical component of the observed degradation in the irradiated samples. When expressed in actual irradiation time, the photolysis half-life is therefore 135 days (a rate constant of 5.1×10^{-3} day^{-1}). Since one day of continuous irradiation with the xenon lamp is equivalent to 2.2 days of summer sunlight at latitude 37° 56′ N (see Appendix A), the photolysis half-life (in solar days) is 2.2 times the observed half-life. The photolysis half-life is about 300 solar days (k_p = 2.3×10^{-3} $(solar\ day)^{-1}$). Since R-25788 absorbs minimally in the wavelength region relevant to terrestrial sunlight, direct photolysis of R-25788 is expected to be slow. The UV spectrum of R-25788 in water is shown in Figure 7.

5. CONCLUSIONS

Photolysis of R-25788 on a soil surface is slow. The major photolysis product formed represents 7% of the initial R-25788 after the equivalent of 33 days of solar exposure. The photolysis half-life is 300 solar days (k_p = 2.3×10^{-3} $(solar\ day)^{-1}$).

-318-

author stated that humidified oxygen "...was drawn through the sodium hydroxide solution into the main compartment of the biometer flasks to replace the oxygen used by microbial respiration. This arrangement allows the flasks to remain aerobic without a steady stream of gas flowing through or over the treated soil." The treated samples were incubated in the dark at 20-27 C (average 25 ± 0.7 C), and the samples were weighed "periodically" and remoistened if necessary. Duplicate samples, with their respective polyurethane plugs and trapping solutions, were collected at 0, 1, 3, 7, 14, 30, 59, 91, 120, 179, 270, and 360 days posttreatment. At each sampling interval, the NaOH trapping solutions for all remaining samples were collected and replaced with fresh solutions. Soil samples were stored at -20 C until analysis.

Subsamples of the soil samples were analyzed by LSC following combustion. Additional soil subsamples from 0 through 179 days were Soxhlet-extracted in cellulose thimbles, twice with methylene chloride, and then twice with methanol; each extraction was for approximately 20 hours. Similar extracts were combined and rotary-evaporated "at or near room temperature". The concentrated methylene chloride extracts were diluted with additional methylene chloride, and aliquots were analyzed by LSC. The concentrated methanol extracts, which contained solids after rotary evaporation, were sequentially shaken with methanol and water. After each shaking, the mixture was centrifuged, and the supernatant removed and analyzed using LSC. The solids remaining were shaken with scintillation cocktail until a homogenous solution was obtained; this mixture was analyzed by LSC. Aliquots of all extracts were further analyzed by HPLC using an ODS2 (C-18) phase-separation column eluted with water:methanol (gradient delivery) with UV (220 nm) and radioisotopic detection; HPLC eluent fractions were quantitated by LSC. The samples were cochromatographed with reference standards, which were visualized using UV (220 nm) absorbance. The presence of R-25788 was confirmed in select methylene chloride extracts from 0-, 1-, 3-, 7-, 14-, and 30-day sampling intervals by one-dimensional TLC on normal phase silica gel plates developed in hexane:acetone (60:40, v:v) and quantitated by radioscanning. The extracted soil was analyzed for unextracted radioactivity using LSC following combustion. Subsamples of extracted soil from the 3-, 7-, 14-, and 30-day posttreatment sampling intervals were further analyzed for non-organoextractable ^{14}C by sequential extraction with water (twice), 0.01 M calcium chloride, 1.0 M HCl, water, and 5% (w:v) sodium bicarbonate, each time on a wrist action shaker at 25 C. Aliquots of each extract were analyzed using LSC.

Aliquots of NaOH trapping solutions were analyzed for total radioactivity using LSC; additional aliquots of the NaOH trapping solutions were precipitated with barium chloride to confirm the presence of CO_2. The polyurethane plugs were extracted with acetone, and the acetone extracts were analyzed by LSC.

DATA SUMMARY:

Carbonyl-labeled [^{14}C]R-25788 (2,2-dichloro-N,N-di-2-propenyl-acetamide; radiochemical purity >98.4%), at approximately 0.40 ppm, degraded with a registrant-calculated half-life of 7.5 days in silty clay loam soil that was incubated in the dark at 20-27 C and 75% of field moisture capacity for 360 days (Table 9). [^{14}C]R-25788 decreased from 0.387 and 0.416 ppm in duplicate samples immediately posttreatment to 0.303-0.304 ppm (75.2-75.4% of the applied) at 7

days, 0.095-0.101 ppm (23.6-25.1%) at 14 days, 0.029-0.030 ppm (7.2-7.4%) at 30 days, and 0.009 ppm at 179 days (Table 4). $^{14}CO_2$ was the major degradate, totaling 4.7-5.8% of the applied at 7 days posttreatment, 34.8-36.4% at 14 days, 51.6-53.3% at 30 days, and 66.8-72.2% at 179 through 360 days; volatile organic [^{14}C]residues totaled ≤3.1% of the applied at all intervals (Table III). A nonvolatile [^{14}C]degradate eluting (HPLC) with N,N-diallyacetamide was isolated from the soil at a maximum of 0.0039 ppm (7 days); a second degradate was isolated at a maximum of 0.0047 ppm (Table 5). Other methylene chloride-extractable degradates (number not specified) totaled ≤0.0034 ppm at all sampling intervals. [^{14}C]Residues that were not extracted from the soil with organic solvents totaled 0.060-0.064 ppm at 7 days, 0.107-0.112 ppm at 14 and 30 days, and 0.080-0.102 ppm at 59 through 79 days (Table 3). During the study, material balances were >100% of the applied at 0 and 1 days posttreatment, 93.1-99.5% at 3 through 91 days, 90.5-93.6% at 120 through 270 days, and 86.8-90.1% at 360 days (Table III).

COMMENTS:

1. The registrant-calculated half-life of 7.5 days for R-25788 was based only on the 0- through 30-day sampling intervals; use of data through 59 days resulted in a calculated half-life of 11.9 days (Table 9). The study author suggested that the decrease in rate of decline of R-25788 residues and the decrease in CO_2 evolution was "probably due to a decrease in microbial viability caused by depletion of organic matter in the biometer flasks".

2. The soil from one of the 0-day flasks was Soxhlet-extracted only with methanol. However, upon evaporation, much of the radioactivity was lost from the concentrate and recovered in the methanol distillate. Therefore, all remaining soil aliquots were extracted with methylene chloride prior to methanol.

3. Because very low amounts of radioactive residues were extracted from the soil samples collected at 120 and 179 days posttreatment, no attempt was made to extract the soil samples collected at 270 and 360 days.

4. Subsamples of select extracted soil from the 3-, 7-, 14-, and 30-day posttreatment sampling intervals were further extracted for characterization of non-organoextractable ^{14}C. Radioactivity solubilized by aqueous solvents decreased from 57.3% of the non-organoextractable radioactivity at 3 days posttreatment to 29.8% and 29.3% at 14 and 30 days, respectively (Table 8).

5. The study author stated that humidified oxygen "...was drawn through the sodium hydroxide solution into the main compartment of the biometer flasks to replace the oxygen used by microbial respiration." The rate of air flow was not reported. From Figure 2, it is not certain that air exchange occurred as described by the study author; however, the soil samples should have received adequate aeration when the soil was moistened and the trapping solutions were changed.

Table III. Distribution and Recovery of Radiocarbon From Biometer Flasks Containing Soil Treated with [^{14}C]R-25788.

Time	Flask	CO_2 Traps (dpm)	% of Applied	Foam Plug (dpm)	% of Applied	Intact Soil (dpm)		Total Recovery (dpm)	Percent Recovery[1]
						Per Gram (Mean ± S.D.)	Total (295 g)		
	3	N.D.		N.D.		52[2]			
	4	N.D.		N.D.		52			
Day 0	5	5,000	<0.1	60,300	0.2	116,171 ± 3,179	34,270,445	34,335,745	104.1
	6	7,900	<0.1	47,700	0.1	113,918 ± 7,236	33,605,810	33,661,410	102.0
Day 1	7	310,500	0.9	218,850	0.7	115,663 ± 1,916	34,120,585	34,649,935	105.0
	8	304,400	0.9	220,750	0.7	115,134 + 2,069	33,964,530	34,489,680	104.5
Day 3	9	898,600	2.7	693,450	2.1	103,035 ± 2,060	30,395,325	31,987,375	96.9
	10	899,500	2.7	529,800	1.6	106,457 ± 1,308	31,404,315	32,834,115	99.5
Day 7	11	1,909,050	5.8	953,700	2.9	98,656 ± 2,384	29,103,520	31,966,270	96.9
	12	1,560,200	4.7	1,035,800	3.1	98,288 ± 2,934	28,994,960	31,590,960	95.7
Day 14	13	11,487,150	34.8	659,250	2.0	63,533 ± 1,432	18,742,235	30,888,635	93.6
	14	12,014,150	36.4	732,550	2.2	64,035 ± 2,653	18,890,325	31,637,025	95.9
Day 30	15	17,028,100	51.6	513,600	1.6	45,315 ± 1,992	13,367,925	30,909,625	93.7
	16	17,601,600	53.3	507,200	1.5	44,959 ± 1,743	13,262,905	31,371,705	95.1
Day 59	17	20,337,100	61.6	318,350	1.0	35,354 ± 1,715	10,429,430	31,084,880	94.2
	18	19,647,850	59.5	318,000	1.0	36,424 ± 1,333	10,745,080	30,710,930	93.1
Day 91	19	20,834,350	63.1	205,750	0.6	35,597 ± 1,280	10,501,115	31,541,215	95.6
	20	20,619,350	62.5	380,550	1.2	33,506 ± 565	9,884,270	30,784,170	93.3

068078

Table III (Continued). Distribution and Recovery of Radiocarbon From Biometer Flasks Containing Soil Treated with [^{14}C]R-25788.

Time	Flask	CO_2 Traps (dpm)	% of Applied	Foam Plug (dpm)	% of Applied	Intact Soil (dpm)		Total Recovery (dpm)	Percent Recovery[1]
						Per Gram (Mean ± S.D.)	Total (295 g)		
Day 120	21	21,513,500	65.2	206,400	0.6	31,114 ± 1,408	9,178,630	30,898,530	93.6
	22	21,151,700	64.1	210,250	0.6	31,361 ± 398	9,251,495	30,613,445	92.8
Day 179	23	22,051,850	66.8	133,150	0.4	27,438 ± 1,424	8,094,210	30,279,210	91.8
	24	22,684,300	68.7	88,600	0.3	27,257 ± 290	8,040,815	30,813,715	93.4
Day 270	25	23,831,050	72.2	13,800	<0.1	22,890 ± 1,449	6,752,550	30,597,400	92.7
	26	22,773,650	69.0	18,900	<0.1	23,936 ± 485	7,061,120	29,853,670	90.5
Day 360	27	23,569,400	71.4	9,400	<0.1	20,833 ± 804	6,145,735	29,724,535	90.1
	28	22,774,800	69.0	18,000	<0.1	19,797 ± 597	5,840,115	28,632,915	86.8

1 Percent recovery based on application of 32,996,800 dpm per flask.

2 Background value derived from average of oxidation values of five 1 g samples from each control flask.

Table 3. Recovery of ^{14}C from soil after Soxhlet extractions with dichloromethane and methanol.

Day	Flask #	% Recovery of ^{14}C in the soil.[+]				ppm equivalents		
		dichloro-methane extract	methanol extract	not extractable	total	dichloro-methane extract	methanol extract	not extractable
0	5	*	93.4	1.1	94.5	*	0.393	0.005
0	6	113.5	1.4	2.2	117.1	0.468	0.006	0.009
1	7	102.3	0.5	3.1	105.9	0.429	0.002	0.013
1	8	105.6	0.6	3.5	109.6	0.440	0.002	0.015
3	9	110.5	1.4	6.6	118.5	0.412	0.005	0.025
3	10	105.1	1.3	6.8	113.2	0.405	0.005	0.026
7	11	90.6	2.8	17.8	111.2	0.324	0.010	0.064
7	12	91.1	3.4	16.8	111.4	0.325	0.012	0.060
14	13	47.7	4.7	46.8	99.2	0.110	0.011	0.108
14	14	49.9	4.4	48.3	102.6	0.116	0.010	0.112
30	15	23.5	5.4	66.7	95.6	0.039	0.009	0.109
30	16	24.5	2.7	65.4	92.7	0.040	0.004	0.107
59	17	19.0	3.8	78.8	101.6	0.024	0.005	0.101
59	18	16.7	4.3	77.6	98.6	0.022	0.006	0.102
91	19	10.3	4.4	67.9	82.7	0.013	0.006	0.088
91	20	14.4	3.2	68.4	86.0	0.018	0.004	0.083
20	21	11.7	3.3	82.6	97.6	0.013	0.004	0.093
20	22	12.8	4.6	77.9	95.3	0.015	0.005	0.089
79	23	11.1	3.8	80.1	95.0	0.011	0.004	0.080
79	24	11.1	3.2	83.6	97.9	0.011	0.003	0.083

[+] Recovery based on ^{14}C content of soil determined by combustion analysis before extractions.

* Soil from flask 5 was not extracted with dichloromethane.

Table 4. Quantitation of R-25788 in the dichloromethane extracts by HPLC.

Day	Flask #	ppm R-25788[+]	% of CH_2Cl_2 extractable	% of starting R-25788*	Recovery of ^{14}C from the HPLC column[+]
0	6	0.416 ± 0.022	88.8	103.2	94.6 ± 5.1
	7	0.387 ± 0.011	90.3	95.8	93.9 ± 3.3
1	8	0.369 ± 0.013	83.8	91.6	85.5 ± 3.4
3	9	0.362 ± 0.009	87.8	89.8	90.3 ± 2.6
3	10	0.372 ± 0.001	91.8	92.3	94.5 ± 0.2
7	11	0.303 ± 0.013	93.6	75.2	98.4 ± 4.2
7	12	0.304 ± 0.011	93.7	75.4	98.6 ± 3.8
14	13	0.101 ± 0.003	92.0	25.1	103.8 ± 1.9
14	14	0.095 ± 0.003	82.1	23.6	93.7 ± 2.9
30	15	0.029 ± 0.001	75.2	7.2	97.7 ± 1.6
30	16	0.030 ± 0.001	75.0	7.4	98.0 ± 1.8
59	17	0.018 ± 0.000	73.9	4.5	91.1 ± 1.5
59	18	0.016 ± 0.001	72.5	4.0	91.3 ± 4.5
91	19	0.011 ± 0.001	82.5	2.7	103.3 ± 15.4
91	20	0.012 ± 0.000	68.5	3.0	90.9 ± 2.1
120	21	0.011 ± 0.000	83.4	2.7	96.7 ± 1.0
120	22	0.012 ± 0.000	82.4	3.0	94.8 ± 3.6
179	23	0.009 ± 0.001	81.5	2.2	94.3 ± 11.8
179	24	0.009 ± 0.000	82.0	2.2	98.4 ± 2.2

[+] means of three injections

* starting R-25788 = 0.404 ppm.

Table 5. Concentrations of R-25788 degradates in dichloromethane extracts of treated soil.

Day	Flask #	PEAK 1* (2.0-8.0 min) ppm	PEAK 2 (8-12 min) ppm	PEAK 3 ** (18-22 min) ppm
0	6	0.0027	0.0006	0.0006
1	7	+	+	+
1	8	0.0013	0.0008	0.0007
3	9	0.0020	0.0019	0.0014
3	10	0.0020	0.0021	0.0017
7	11	0.0027	0.0034	0.0039
7	12	0.0026	0.0032	0.0031
14	13	0.0034	0.0037	0.0031
14	14	0.0026	0.0047	0.0026
30	15	0.0018	0.0041	0.0010
30	16	0.0022	0.0042	0.0009
59	17	0.0010	0.0021	0.0003
59	18	0.0009	0.0020	0.0002
91	19	0.0007	0.0008	0.0001
91	20	0.0010	0.0017	0.0002
120	21	0.0006	0.0008	0.0001
120	22	0.0008	0.0005	0.0001
179	23	0.0005	0.0004	0.0001
179	24	0.0006	0.0003	0.0001

* Sum of several components that eluted between 2.0 and 8.0 min.

** This region encompasses the retention time of N,N-diallyacetamide.

\+ This extract was analyzed using a different mobile phase composition. Therefore, retention times of the degradates were different. No degradates were detected at 0.01 ppm or greater.

Table 8. Extraction of non-organoextractable ^{14}C from soil with aqueous solvents.

Day	Flask #	Water*	ppm equivalents extracted by			% of non-organoextractable ^{14}C solubilized by aqueous solvents
			$CaCl_2$ (0.01M)	HCl (1M)	$NaHCO_3$ (5%)	
3	10	0.0085	0.0008	0.0014	0.0044	57.3
7	12	0.0143	0.0013	0.0023	0.0096	46.0
14	14	0.0131	0.0017	0.0036	0.0150	29.8
30	16	0.0100	0.0013	0.0032	0.0168	29.3

* Sum of two extractions.

Table 9. Calculation of the half-life of R-25788 in soil using first-order kinetics.

Flask	Day	R-25788 ppm	ln[C]	Data points used	Linear Regression r^2	intercept	slope	t(½)* (days)	C(0)** (ppm)
6	0	0.416	-0.887	0-30 days	0.974	-0.793	-0.0926	7.5	0.453
7	1	0.387	-0.949						
8	1	0.369	-0.997	1-30 days	0.973	-0.775	-0.0934	7.4	0.461
9	3	0.362	-1.016						
10	3	0.372	-0.989	3-30 days	0.972	-0.707	-0.0965	7.2	0.493
11	7	0.303	-1.194						
12	7	0.304	-1.191	0-59 days	0.894	-1.059	-0.0583	11.9	0.347
13	14	0.101	-2.293						
14	14	0.095	-2.354	1-59 days	0.887	-1.087	-0.0576	12.0	0.337
15	30	0.029	-3.540						
16	30	0.030	-3.507						
17	59	0.018	-4.017						
18	59	0.016	-4.135						
19	91	0.011	-4.510						
20	91	0.012	-4.423						
21	120	0.011	-4.510						
22	120	0.012	-4.423						
23	179	0.009	-4.711						
24	179	0.009	-4.711						

Calculation of R-25788 half-life based on HPLC analysis of the dichloromethane extracts.

* $t(\frac{1}{2}) = -[\ln (0.5)]/k$

** Co = exp (intercept)

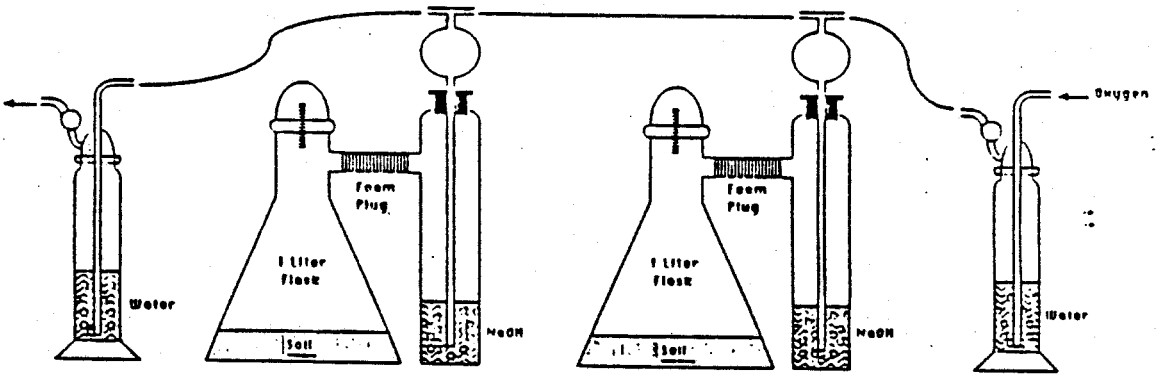

Figure 2. Biometer Flask Assembly Used for Aerobic Metabolism of R-25788.

4. RESULTS AND DISCUSSION

4.1 Relationship Between the Experimental Treatment Rate and the Field Use Rate.

The maximum field use rate of R-25788 is 0.5 lb/acre, which is equivalent to;

$$\frac{0.5\ \text{lb}}{\text{acre}} \times \frac{453.6\ \text{g}}{\text{lb}} \times \frac{1\ \text{acre}}{4{,}840\ \text{yd}^2} \times \frac{(1\ \text{yd})^2}{(91.44\ \text{cm})^2}$$

$= 5.60\ \mu\text{g/cm}^2$

The dry bulk density of the Champaign silty clay loam soil was 2,209 lbs/yd^3 (Appendix 1), which is;

$$\frac{2{,}209\ \text{lbs}}{\text{yd}^3} \times \frac{453.6\ \text{g}}{\text{lb}} \times \frac{(1\ \text{yd})^3}{(91.44\ \text{cm})^3}$$

$= 1.31\ \text{g/cm}^3$

This value can be regarded as an upper limit for the density of this soil under field conditions. A more realistic value can be estimated by taking into account the water content of the soil. An estimate of the actual density of the soil in the biometer flasks is (based on 250 g of soil and 45 g of water with a density of 1.0 g/cm^3);

$$\frac{[(250\ \text{g})\ (1.31\ \text{g/cm}^3)] + [(45\ \text{g})\ (1.0\ \text{g/cm}^3)}{295\ \text{g}}$$

$= 1.26\ \text{g/cm}^3$

Using the values of 5.60 μg R-25788/cm^2, a soil density of 1.26 g cm^3 (both values calculated above), and assuming that the chemical is distributed in the top 11 cm (4.33 in) of soil, the 0.5 lb/acre field rate yields an

initial soil concentration of:

$$\frac{5.60\ \mu g}{cm^2} \times \frac{1\ cm^3}{1.26 g} \times \frac{1}{11\ cm}$$

= 0.404 ppm.

Therefore, the experimental treatment rate of 0.403 ppm (119 µg/295 g soil) duplicates the maximum field rate.

4.2 Evolution of $^{14}CO_2$, Volatiles, and Initial Mass Balance:

An initial mass balance was obtained at PTRL as the sum of the ^{14}C in the carbon dioxide traps, foam plugs, and soil (determined by combustion analysis). These results are summarized in Table 3 of Appendix 3, the recovery ranging from 86.8 to 105.0 %. The formation and decline of $^{14}CO_2$, volatile organic radiocarbon, and soil-associated radioactivity, expressed as % of applied radioactivity, are shown in Figure 3 of Appendix 3. The same data, expressed as ppm of R-25788 equivalents, are shown in Figure 1.

The time course of $^{14}CO_2$ evolution was similar for all flasks (Table 4 of Appendix 3), and the data for a 360 day period are shown graphically for Flasks 27 and 28 (Figure 2). A rapid increase in the rate of $^{14}CO_2$ production is apparent between 7 and 14 days. Approximately 52 % of the applied radiocarbon was released as $^{14}CO_2$ in 30 days, and $^{14}CO_2$ evolution continued at a slow rate thereafter.

The release of total carbon dioxide (determined by titration) is a measure of the microbial activity of the soil. All flasks produced carbon dioxide

at equivalent rates (Table 2 of Appendix 3), and the data for a 360 day period are depicted graphically for Flasks 27 and 28, and Flasks 3 and 4 (control flasks to which 1 ml of acetone was added) in Figure 3. Control and treated flasks produced CO_2 at the same rate, indicating that R-25788 had no effect on the soil microflora's ability to metabolize acetone or soil organic matter. Note the difference in scales between Figs. 2 and 3; at 14 days the total carbon dioxide evolution exceeded $^{14}CO_2$ by a factor of approximately 14,000.

The amount of volatile organic ^{14}C produced reached a maximum after 7 days and then declined (Table 3 and Figure 3 of Appendix 3). This decline in volatile organic ^{14}C after 7 days is probably due to an equilibration between the foam plug and the soil, with soil metabolism serving as a sink. Figure 1 shows the volatile organic ^{14}C expressed as ppm. Only in the flasks harvested at 7 days was the volatile organic ^{14}C equivalent to a residue of 0.01 ppm or greater. Therefore, this material was not further characterized.

4.3 <u>Characterization of the ^{14}C in Soil:</u>

The soil from Flask 5 (0 day) was extracted in a Soxhlet extractor with methanol. On concentration by rotary evaporation, much radioactivity was lost from the concentrate and was recovered in the methanol distillate. It is likely that R-25788 formed an azeotropic mixture with methanol. Therefore, soils from flasks 6-24 (0 to 178 days) were first extracted twice with dichloromethane to remove R-25788, and then with methanol to remove polar degradates. The amounts of ^{14}C recovered in the dichloromethane and methanol extracts, and the amounts of ^{14}C remaining in

the soil after Soxhlet extraction (determined by combustion analysis), are summarized in Table 3 and shown graphically in Figure 4. Because of the very low amounts of parent and extractible degradates in the 120 and 179 day samples, soils from Flasks 25-28 (9 and 12 months) were not extracted or analyzed further.

Results of HPLC analysis of the dichloromethane extracts is shown in Table 4. Recovery of injected radiocarbon was 91.1 to 103.8 %. A representative radiochromatogram is shown in Figure 5, along with a histogram showing the distribution of ^{14}C determined by LSC of the collected fractions and a chromatogram showing the location of R-25788 and the various analytical standards. Most of the ^{14}C cochromatographed with parent in this and all other chromatograms. The concentration of R-25788 determined by HPLC is summarized in Table 4. (along with the fraction of dichloromethane-extractible radioactivity accounted for as parent) and depicted graphically in Figure 6. A number of minor peaks of radioactivity are present (Figure 5). However, in this and all other HPLC analyses of the dichloromethane extracts there were no degradates found at a level of 0.01 ppm or greater. The approximate retention times and concentrations of degradates detected by HPLC are summarized in Table 5 and Figure 7.

Analysis of selected dichloromethane extracts by normal phase TLC confirmed that most of the radiocarbon in these extracts was R-25788. The concentrations of R-25788 in these samples determined by TLC are summarized in Table 6, and are in agreement with the values determined by HPLC (Table 4). Figure 8 shows an image obtained with a radioscanner of a TLC plate on which the dichloromethane extracts of Flasks 14 and 16 were

analyzed. Figure 9 shows profiles of a lane which contained a sample of the flask 14 extract as well as a lane in which 414 dpm (0.0015 μg) of [^{14}C] R-25788 was applied to evaluate the limit of detection. Since each sample lane contained extract equivalent to 0.25 g of soil and it is evident that a discrete spot containing 414 dpm is easily detectable, the method is able to detect a degradate present at 0.006 ppm (0.0015 μg/0.25 g). Although the TLC analysis did detect the presence of degradates of [^{14}C] R-25788 (Figures 8 and 9), none were present at 0.01 ppm or greater. The extract of Flask 14 contained a radiolabeled compound with the same Rf (0.30) as N,N-diallylacetamide.

The radiocarbon content of the methanol extracts was very low, and only in the samples from days 7 and 14 did this fraction contain residues equivalent to 0.01 ppm (Table 3). The methanol extracts from Flasks 13 and 14 (Day 14) were analyzed by reverse-phase HPLC, and the residue was found to consist primarily of three components (Table 7). The most abundant residue cochromatographed with N,N-diallyaminooxoacetic acid, and the least polar component cochromatographed with parent R-25788. The intractable material obtained by concentration of the methanol extracts contained negligible radioactivity (<0.005 ppm)).

The results of extraction of selected soil samples with aqueous solvents is summarized in Table 8. The initial extractions with water removed radiocarbon equivalent to 0.008-0.014 ppm. Further treatment with 0.01 M calcium chloride solution (to simulate a batch equilibrium experiment) and with 1 N hydrochloric acid removed negligible quantities of radioactivity. The final treatment with 5% sodium bicarbonate removed residues equivalent

to 0.004-0.017 ppm. Concentration of the initial water extracts by lyophilization and analysis by reverse-phase HPLC revealed the presence of multiple components present at low levels (data not shown). The other extracts were not analyzed further.

4.4 Calculation of the Half-life of R-25788 in Soil:

The concentrations of R-25788 in soil determined by HPLC were used to calculate the half-life of R-25788 in soil. The rate of decline of R-25788 soil residues decreased dramatically after 30 days (Fig 1), as did the rates of carbon dioxide and $^{14}CO_2$ evolution (Figs. 2 and 3). This was probably due to a decrease in microbial viability caused by depletion of organic matter in the biometer flasks. Therefore, in an attempt to fit the data on the decline of R-25788 to a first order kinetic model, several calculations employing data from various sampling periods were carried out, but no data obtained after Day 59 was used. These calculations are summarized in Table 9. Depending upon the choice of sampling period, the calculated half-life varies from 7.2 to 12 days. A value of 7.5 days, obtained from the 0-30 day sampling periods has been calculated as the experimental half-life in the Abstract of this report. Regardless of the mathematics employed, it is obvious from the analytical data of Table 4 that the "true" half-life lies between 7 and 14 days.

The half-lives of the R-25788 degradates were not calculated because of their very low levels. However, it is evident from the data presented in Table 5 and Figure 7 that these degradates are not persistent.