DATA EVALUATION RECORD 3

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GUIDELINE 161-3

STUDY ID 420197-15

Estigoy, L., and K. Shepler. 1991. Photodegradation of [^{14}C]MON-13900 in/on soil by natural sunlight. Project Nos. MSL-10982; PTRL-213W; RD 1054. Unpublished study performed by Pharmacology and Toxicology Research Laboratory, Richmond, CA, and submitted by Monsanto Agricultural Company, Chesterfield, MO.

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CONCLUSIONS:

Degradation - Photodegradation on Soil

1. This study is acceptable and fulfills EPA Data Requirements for Registering Pesticides by providing information on the photodegradation of oxazolidine ring-labeled [4-^{14}C]MON 13900 on soil. No additional data on the photodegradation of MON 13900 on soil are required at this time.

2. MON 13900 photodegraded with a half-life of approximately 8 days on silty clay loam soil that was irradiated outdoors in California during April-May. In contrast, MON 13900 did not significantly degrade during 20 days of incubation in the dark. The only degradate whose identity was determined was 3-(dichloroacetyl)-2,2-dimethyl-5-oxazolidinylcarboxylic acid, although several unidentified minor degradates were also detected.

METHODOLOGY:

Sieved (2 mm) silty clay loam soil (7% sand, 62% silt, 31% clay, 3.5% organic matter, pH 5.8, CEC 22.9 meq/100 g) was added (3.1 g dry weight/dish) to 5-cm Petri dishes and wetted with distilled water (3 mL/dish); the resulting slurry was spread evenly in each dish and air-dried. The air-dried soil layers had an exposed surface area of 19.6 cm^2; the final thickness was not specified. The soil layers were adjusted to 75% of field capacity with deionized water, then treated with oxazolidine ring-labeled [4-^{14}C]MON 13900 (radiochemical purity 98.5%, specific activity 12.94 mCi/mMol, Monsanto), dissolved in acetonitrile, at approximately 99 ug/dish (equivalent to 0.41 lb ai/A). Four treated soil samples were taken for analysis at time 0; the remaining twenty dishes of treated soil were placed in stainless steel photolysis chambers (Figure 4). The samples to be irradiated were in photolysis chambers covered with quartz plates while the dark control samples were in chambers covered with rubber coated glass plates to exclude light. Each chamber was equipped with a circulating coolant jacket that maintained the irradiated soil samples at 22.8 ± 3.1 C and the dark control soil samples at 21.6 ± 1.6 C (Table IV). Humidified air was drawn (flow rate unspecified) through each photolysis chamber, then sequentially through ethylene glycol (one trap) and 10% NaOH (two traps) trapping solutions. The treated soils were incubated outdoors under natural sunlight in Richmond, California (37.45°N, 122.26°W) for 20 days from April 17 through May 7, 1990; the chambers were oriented perpendicular to the sun's path. During the 20-day study, the daily maximum sunlight intensity ranged from 15,553 to 93,268 uW/cm^2 and the total radiant energy received by the irradiated soil samples was 199.95 $W\text{-}min/cm^2$ at 250-700 nm; the average daily radiant energy was determined to be 9.52 $W\text{-}min/cm^2$ (Table V). Duplicate irradiated and dark control soil samples were collected at 0, 1, 2, 3, 8, and 20 days posttreatment; trapping solutions were collected and replaced at each sampling interval after day 0.

Soil samples were sequentially extracted with acetonitrile (2 hours), acetonitrile:water (1:1, v:v; overnight), and water (2 hours) using a wrist-action shaker; extracts were separated from the soil by centrifugation, then decanted and combined. Aliquots of the extract were analyzed for total radioactivity using LSC. Additional aliquots were analyzed by reverse phase HPLC using UV (210 nm) and radioactivity detection on an Ultrasphere C-18 column eluted with linear gradients of acetonitrile and 0.002 M dibasic ammonium phosphate. Radioactive compounds were identified by comparison to retention times of unlabeled reference MON 13900, MON 13900 oxazolidine acid (CP-141957), and MON 13900 oxamic acid sodium salt (CP-133956). Extracts of selected samples were also analyzed using two-dimensional TLC on silica gel plates developed with butanol:acetonitrile:water (6:1:1, v:v:v) followed by toluene (saturated with formic acid):ether (10:3, v:v). Radioactive areas were visualized and quantified by radioscanning; identification was made by comparison with unlabeled reference standards cochromatographed with the samples. Unextracted $[^{14}C]$residues were quantified using LSC following combustion.

To characterize unextracted $[^{14}C]$residues, previously extracted soil samples which contained >10% of the applied radioactivity were further extracted with 0.5 N NaOH for 4 hours at 80 C. Extracts were separated from the soil by centrifugation, decanted, and adjusted to pH 7; aliquots were then analyzed by ion exclusion HPLC using UV (210 nm) and radioactivity detection on a Bio-Rad Aminex (HPX-87H) column eluted with linear gradients of acetonitrile and 0.01 N sulfuric acid. Selected base extracts were also analyzed using one-dimensional TLC on silica gel plates developed with butanol:acetic acid:water (6:1:1, v:v:v); detection, quantitation, and identification were performed as described above. Unextracted $[^{14}C]$residues remaining in the extracted soil were quantified using LSC following combustion.

Triplicate aliquots (0.5 mL) of the trapping solutions were analyzed for total radioactivity using LSC. The presence of $^{14}CO_2$ in the NaOH trapping solutions was confirmed in selected samples using barium chloride precipitation.

DATA SUMMARY:

Oxazolidine ring-labeled [4-^{14}C]MON 13900 (radiochemical purity 98.5%), at 0.41 lb ai/A, photodegraded with an observed half-life of approximately 8 days on silty clay loam soil that was irradiated outdoors in California for up to 20 days during April-May 1990. In contrast, [^{14}C]MON 13900 did not significantly degrade in the dark controls incubated under similar conditions. During the study, the radiant energy received by the irradiated soil totaled 199.95 Watt-minute/cm^2 at 250-700 nm. The only degradate whose identity was determined was

> 3-(dichloroacetyl)-2,2-dimethyl-5-oxazolidinylcarboxylic acid (MON 13900 oxazolidine acid; CP-141957).

HPLC analysis of extracts of soil that had been irradiated for 20 days determined that MON 13900 comprised 41.6-42.9% of the applied radioactivity, MON 13900 oxazolidine acid (CP-141957) yielded 5.5-5.6%, one unidentified organic [^{14}C]compound (Unknown #3) accounted for 4.2-5.4% (maximum 6.4% at 8 days), and several unidentified polar [^{14}C]compounds contributed a total of 4.7-6.5% (maximum 13.7% at 8 days; Table VII). After 20 days of irradiation, $^{14}CO_2$ totaled 3.3% and unextracted [^{14}C]residues yielded 30.4%. Base extraction and analysis of soil-associated [^{14}C]residues detected several unidentified [^{14}C]compounds which amounted to a total of 21.3-23.9% of the applied radioactivity (no single [^{14}C]compound contributed ≥10% of the applied; Table VIII). In extracts from soil that had been incubated in the dark for 20 days after treatment, MON 13900 was the only compound detected, and accounted for 93.3-95.5% of the applied radioactivity. During the study, material balances ranged from 89.0 to 109.5% of the applied (Table VII).

COMMENTS:

1. The observed photodegradation half-life of MON 13900 on soil was approximately 8 days. Using first order kinetics where x = time and y = ln(concentration of MON 13900), the study authors calculated an "initial" half-life of 8.8 days (r^2 = 0.866) (excluding the day 20 data points; Figure 11). Because photodegradation of MON 13900 actually appeared not to follow first-order kinetics, the study authors did not use the day 20 data points, since the process had slowed considerably by that time.

2. Two-dimensional TLC analysis of 2- and 8-day irradiated soil extracts confirmed the presence of MON 13900 and MON 13900 oxazolidine acid and agreed quantitatively with the HPLC analysis data (Tables VII and IX). The TLC analysis detected four unidentified organic [^{14}C]compounds (Unknowns A, B, C, and D) plus a large polar region, each comprising ≤7.6% of the applied radioactivity (Table IX and Figures 9A-C), whereas the HPLC analysis detected one unidentified organic [^{14}C]compound (Unknown #3) plus several unidentified polar [^{14}C]compounds. Since no single photodegradate accounted for ≥10% of the applied, the discrepancy in the number of unidentified organic and polar [^{14}C]compounds detected using the two analytical methods is not significant.

3. The study protocol stated that the test substance would be oxazolidine ring-labeled [4-^{13}C/^{14}C]MON 13900; however, in the study report the test substance was only described as being labeled with ^{14}C.

4. The number of photolysis chambers was not specified.

5. The registrant reported that MON 13900 [3-(dichloroacetyl)-5-(2-furanyl)-2,2-dimethyloxazolidine] is a safener intended for use with chloroacetanilide and sulfonylurea herbicides in corn and sorghum. The maximum projected use rate for MON 13900 is 0.4 lb/A.

6. The registrant reported that for studies conducted using radiolabeled MON 13900, the compound was synthesized with the radiolabel in the carbon atom adjacent to the nitrogen in the oxazolidine ring portion of the molecule. Studies were not conducted with the compound labeled in the furan ring portion of the molecule because degradation of the radiolabeled furan ring would result in radiolabeled ring fragments that would be natural products composed of low numbers of carbon, hydrogen, and oxygen atoms.

Table VII. Product Balance and Unextracted[14 C]MON 13900 in Soil Expressed as a Percent of Applied Radiocarbon Following Acetonitrile/Water Extraction.
Based on HPLC analysis (Method A).

Sample/ Replicate	MON 13900	CP-141957	Polar Unknowns	Unknown #3	CO2	Unextracted Radiocarbon Remaining in Soil	Other Unknowns**	Recovery
DAY 0								
Irradiated (1)	92.6				-	1.7	0.4	94.7
Irradiated (2)	101.2				-	2.0	0.6	103.8
Dark Control (1)	99.2				-	2.4	0.4	102.0
Dark Control (2)	100.1				-	2.8	0.3	103.2
DAY 1 (24 hours)								
Irradiated (1)	76.3	0.8	5.0	1.7	0.5	7.8	0.8	92.9
Irradiated (2)	82.7	1.4	5.1	1.4	0.5	6.4	0.3	97.8
Dark Control (1)	97.3				0.2	3.4	1.1	102.0
Dark Control (2)	93.4				0.2	3.2	0.8	97.6
DAY 2 (48 hours)								
Irradiated (1)	62.1	4.0	11.1*	3.8	0.8	25.5***	2.2	109.5
Irradiated (2)	70.5	1.2	6.6	2.2	0.8	11.7***	0.4	93.4
Dark Control (1)	86.8				0.3	3.5	0.5	91.1
Dark Control (2)	83.5				0.3	5.0	0.2	89.0

* TLC analysis of day 2 and 8 samples indicates the presence of more than one component in this quantitated peak (See Table IX and Figure 9).

** This includes organic volatiles and miscellaneous, low yield random peaks detected in radiochromatogram.

*** Aliquots of these soil samples were re-extracted and analyzed with an additional HPLC method. See Table VI and Table VIII.

Table VII. (cont.) Product Balance and Unextracted[14 C]MON 13900 in Soil Expressed as a Percent of Applied Radiocarbon Following Acetonitrile/Water Extraction
Based on HPLC analysis (Method A).

Sample/ Replicate	MON 13900	CP-141957	Polar Unknowns	Unknown #3	CO2	Unextracted Radiocarbon Remaining in Soil	Other Unknowns**	Recovery
DAY 3 (72 hours)								
Irradiated (1)	65.5	2.1	8.1	3.5	1.0	14.9***	0.7	95.8
Irradiated (2)	57.4	1.3	7.9	2.4	1.0	23.7***	0.4	94.1
Dark Control (1)	93.9				0.3	3.0	0.3	97.5
Dark Control (2)	96.4				0.3	5.7	0.2	102.6
DAY 8 (191.5 hours)								
Irradiated (1)	47.6	2.6	10.4*	4.7	1.8	25.2***	5.3	97.6
Irradiated (2)	48.9	2.4	13.7*	6.4	1.8	23.1***	4.3	100.6
Dark Control (1)	92.1				0.6	5.8	2.1	100.6
Dark Control (2)	86.3				0.6	4.3	1.9	93.1
DAY 20 (479 hours)								
Irradiated (1)	42.9	5.6	6.5	4.2	3.3	30.4***	1.3	94.2
Irradiated (2)	41.6	5.5	4.7	5.4	3.3	30.4***	0.4	91.3
Dark Control (1)	95.5				0.7	8.9	0.3	105.4
Dark Control (2)	93.3				0.7	6.6	0.1	100.7

* TLC analysis of day 2 and 8 samples indicates the presence of more than one component in this quantitated peak (See Table IX and Figure 9).

** This includes organic volatiles and miscellaneous, low yield random peaks detected in radiochromatogram.

*** Aliquots of these soil samples were re-extracted and analyzed with an additional HPLC method. See Table VI and Table VIII.

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Table VIII. Product Balance and Unextracted[14 C]MON 13900 Equivalents in Soil Expressed as a Percent of Applied Radiocarbon Following 0.5N NaOH Extraction
Based on HPLC analysis (Method B).

Sample/ Replicate	Unknown #1	Unknowns* #2	Other Unknowns**
DAY 2 (48 hours)			
Irradiated (1)	4.2	10.3	1.4
Irradiated (2)	3.4	7.5	1.1
DAY 3 (72 hours)			
Irradiated (1)	2.2	4.6	0.9
Irradiated (2)	5.5	9.2	2.0
DAY 8 (191.5 hours)			
Irradiated (1)	***	***	***
Irradiated (2)	5.8	9.2	1.5
DAY 20 (479 hours)			
Irradiated (1)	6.5	10.8	4.0
Irradiated (2)	7.7	11.4	4.8

* Most HPLC analysis indicates Unknown #2 is comprised of at least 2 poorly resolved peaks. This was confirmed by use of a flow-through detector to increase resolution (Appendix A8). Thus it is anticipated that the day 20 unknown #2 is 2 peaks, each <10% of the applied radiocarbon.

** This column includes all other low yield peaks detected in the base extract by HPLC.

*** Following TLC and attempts to develop analysis methods insufficient sample was available for HPLC.

note: HPLC recoveries were low (63.5±7.1%) for all base extracts (both Methods A and B) suggesting the possible presence of yet further unidentified products. The yields stated above would then be an overestimate.

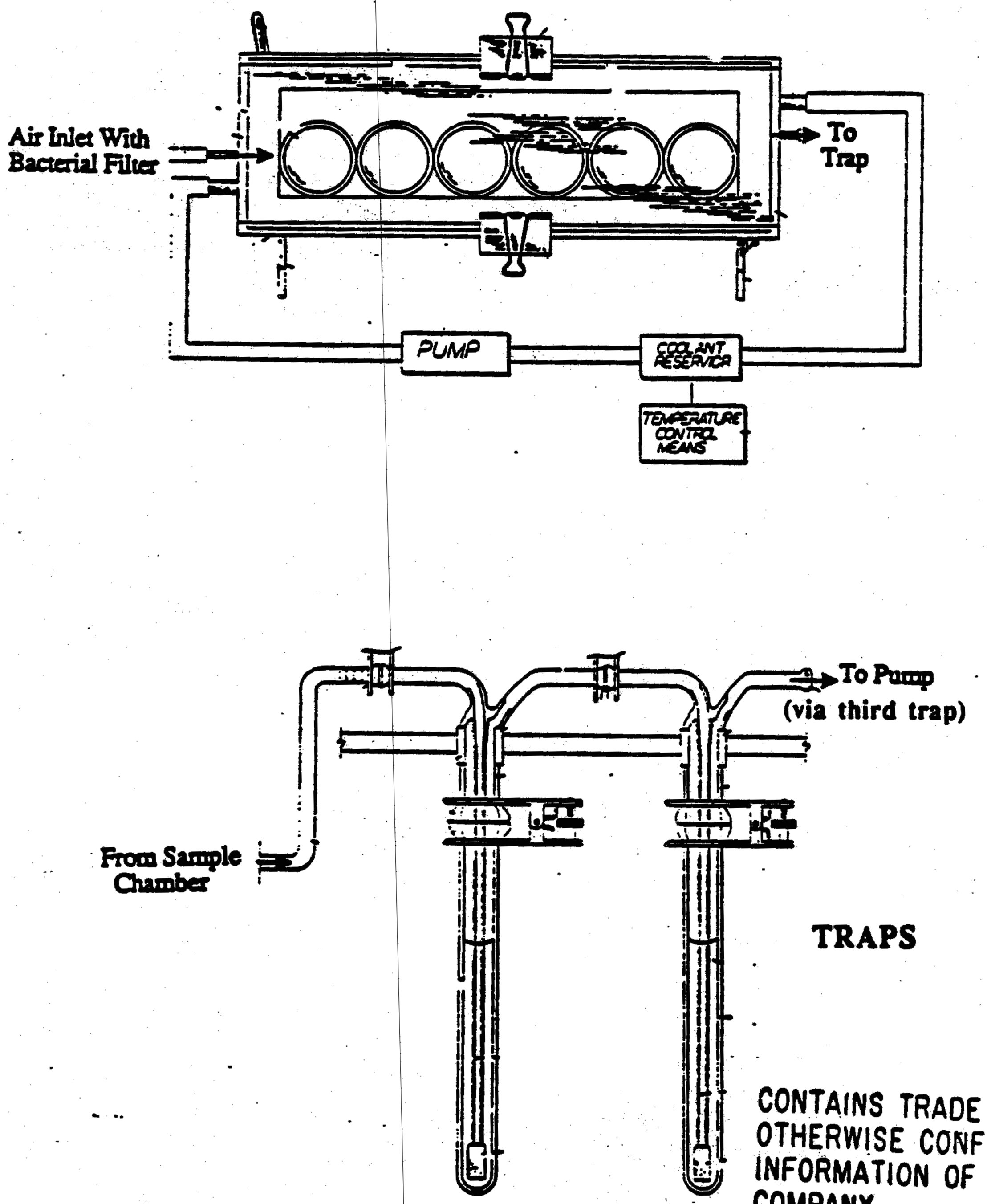
Table IX. Quantitation of Radiographic Scans of 2-dimensional TLC.

Quantitation from Scan - See Figures 9A-C

Sample	Polar Region	Unknowns				CP 141957	MON 13900
		A	B	C	D		
Day 2, Light (1)	9	4	7	6	2	2	76
Day 8, Light (1)	10	4	10	4	2	4	67
Day 8, Light (2)	9	4	10	5	2	4	65

Expressed as Percent of Applied Radiocarbon

Sample	Polar Region	Unknowns				CP 141957	MON 13900
		A	B	C	D		
Day 2, Light (1)	7.5	3.3	5.8	5.0	1.7	1.7	63.2
Day 8, Light (1)	7.0	2.8	7.0	2.8	1.4	2.8	47.1
Day 8, Light (2)	6.8	3.0	7.6	3.8	1.5	3.0	49.1


SOIL CHAMBER

TRAPS

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Figure 4. Apparatus Used to Expose [^{14}C]MON 13900 on Soil Surfaces to Natural Sunlight.

P213W
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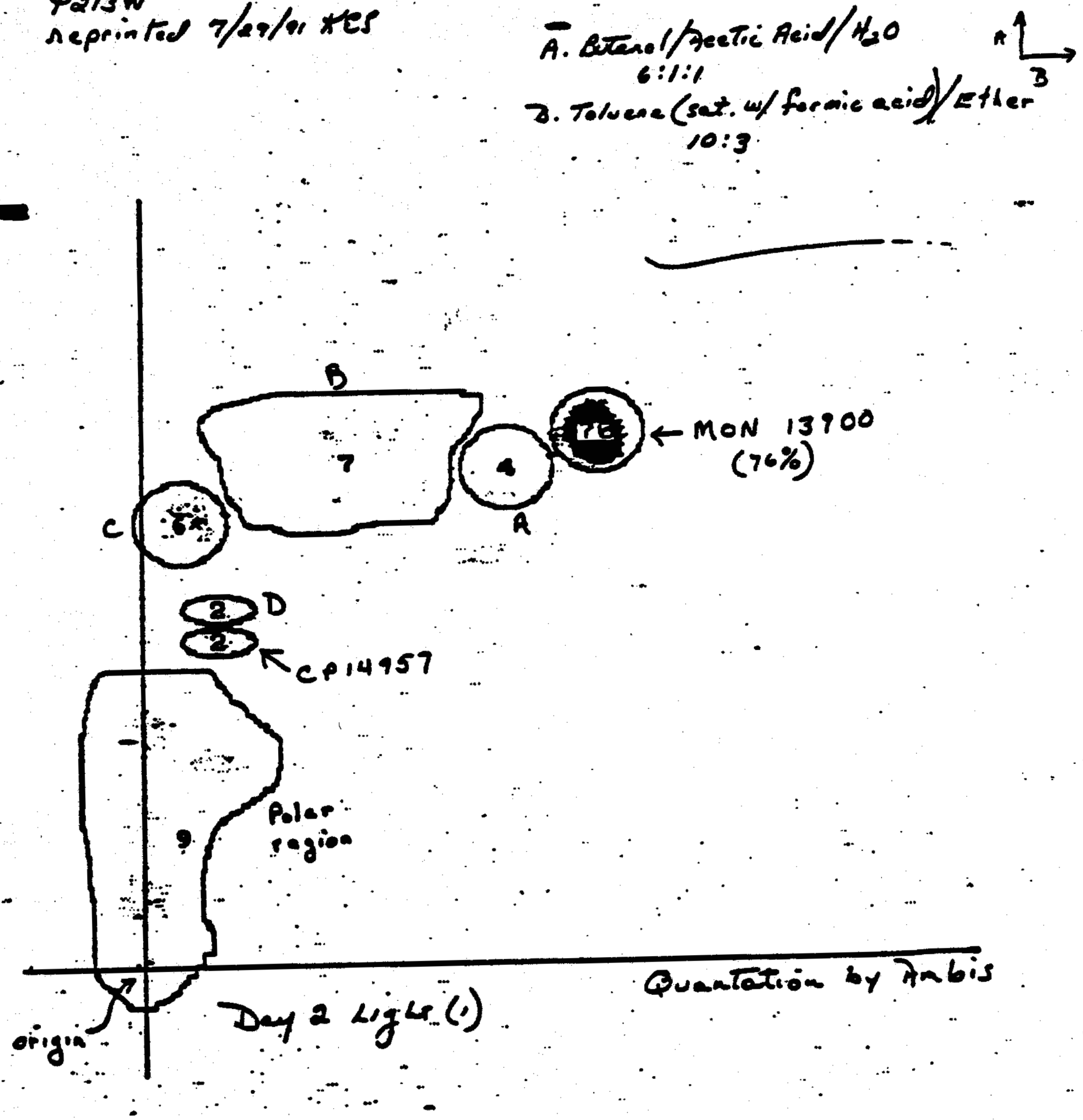
A. Butanol/Acetic Acid/H_2O
6:1:1
B. Toluene (sat. w/ formic acid)/Ether
10:3



Day 2 Light (1)

Quantation by Ambis

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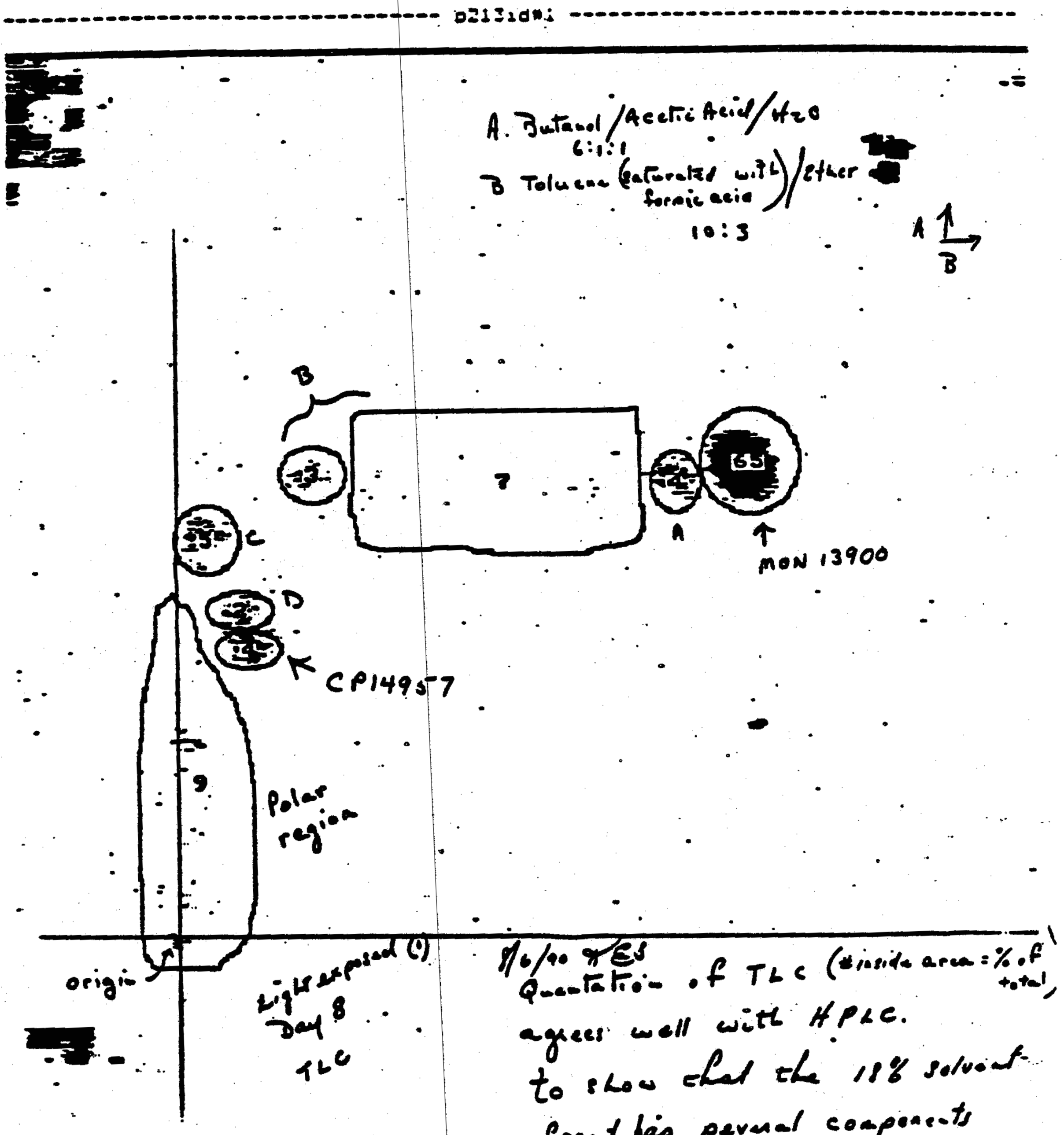
Figure 9A. Two Dimensional TLC Radiographic Scan.
Day 2, Light (1)



[redacted]

A. Butanol/Acetic Acid/H2O
6:1:1

B Toluene (saturated with formic acid)/Ether
10:3

[redacted]

8/6/90 KES
Quantitation of TLC (#inside area = % of total)
agrees well with HPLC.
to show that the 18% solvent
front has several components

[redacted]

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Figure 9B. Two Dimensional TLC Radiographic Scan.
Day 8, Light (1)

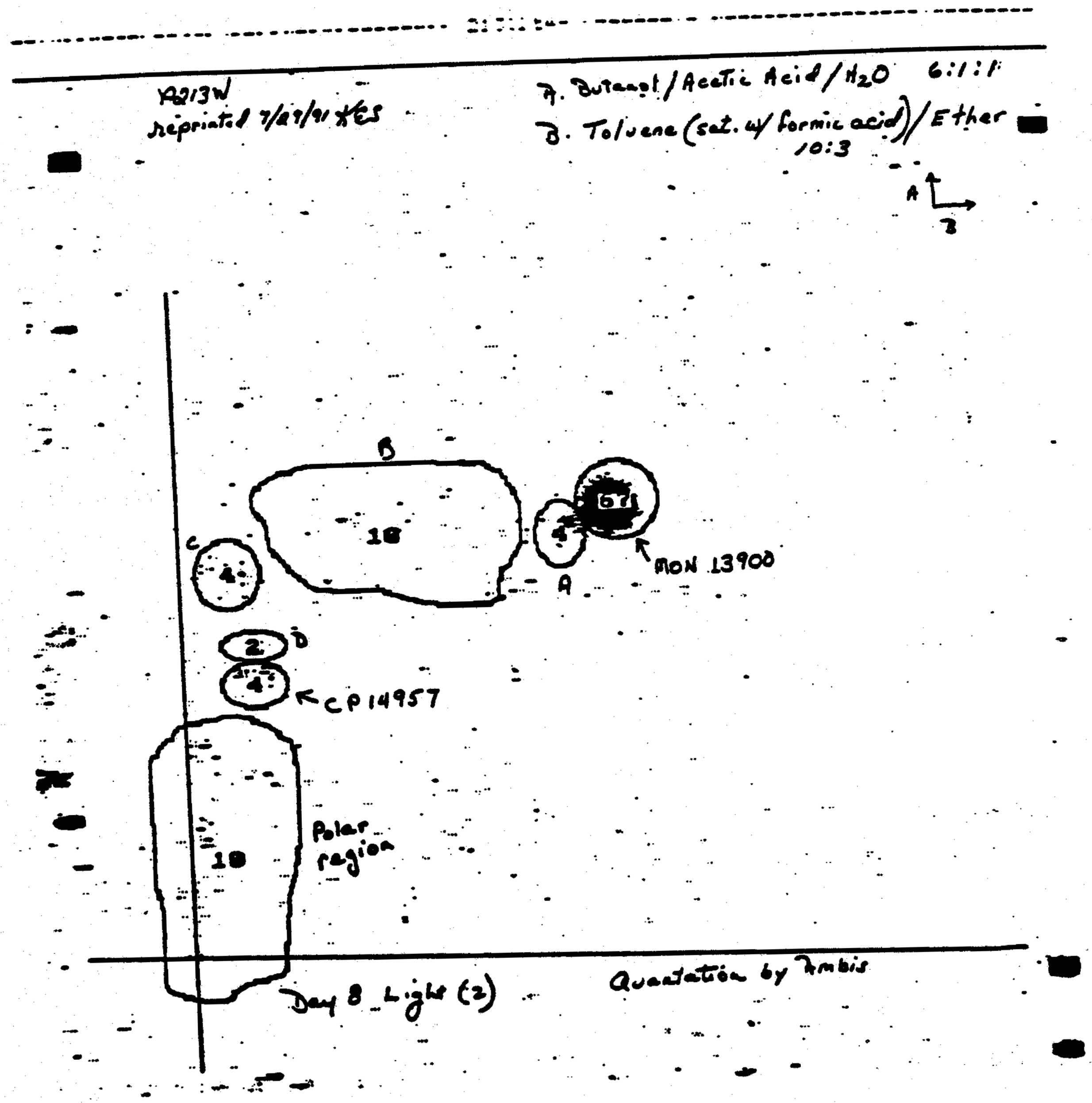

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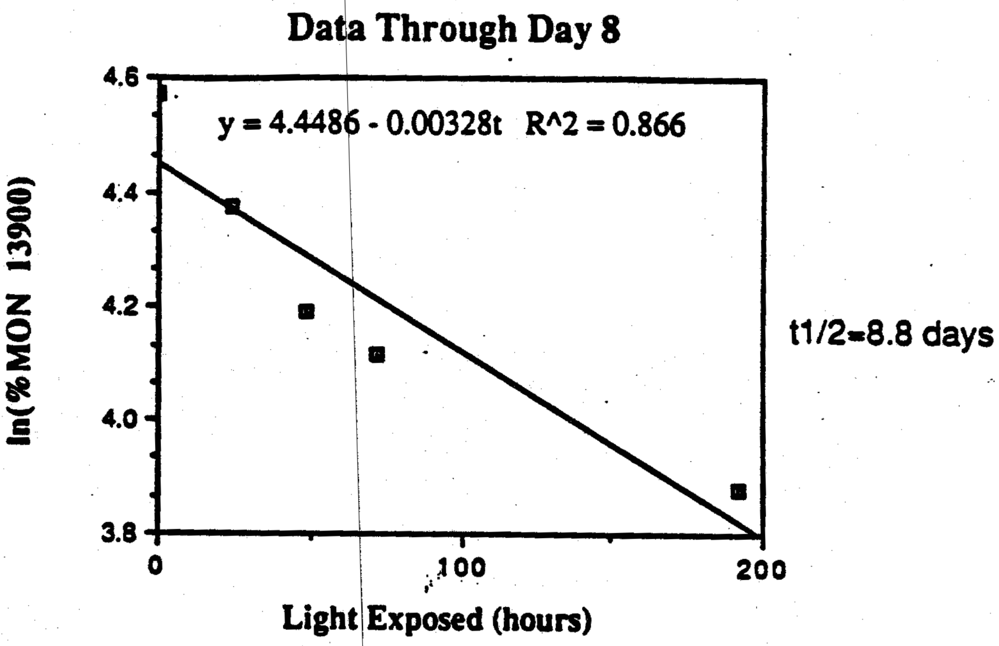
Figure 9C. Two Dimensional TLC Radiographic Scan.
Day 8, Light (2)



Calculations

The photodegradation rate constant and half-life of MON 13900 were calculated assuming pseudo-first order kinetics. Relatively little degradation of MON 13900 occured after 8 days, therefore the 20 day value was not included in the calculation for light exposed samples. The degradation rate constants were calculated from the following equation:

$$\ln C = -kt + \ln C_0 \quad (y = mx + b)$$

where:
k = rate constant
C = chemical concentration
t = time
C_0 = initial chemical concentration

The half-life of MON 13900 was calculated using the following equation:

$$t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k}$$

Figure 11. Degradation Rates of [^{14}C]MON 13900 Light Exposed (Based on Table VII).

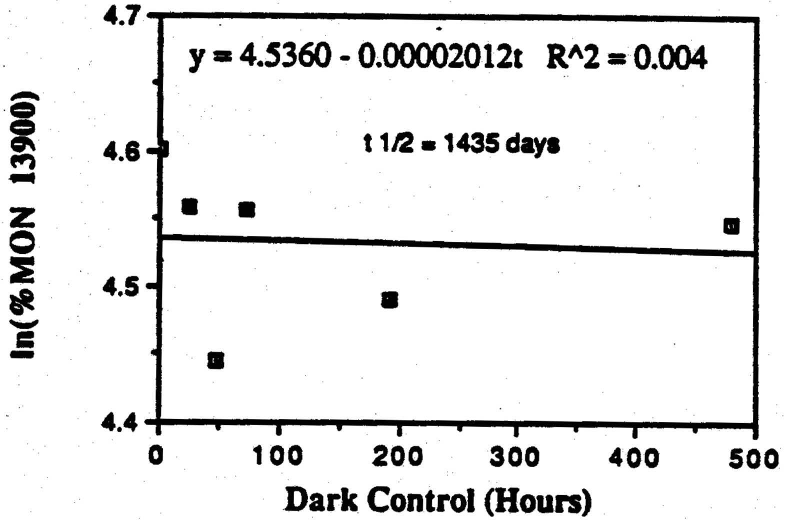


Figure 11 (cont.) Degradation Rates of [^{14}C]MON 13900 Dark Control (Based on Table VII).

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Table IV. Soil Surface Temperatures Throughout the Study Period.

		Minimum (°C)		Maximum (°C)		Mean (°C)±S.D.	
Day	Date	Light	Dark	Light	Dark	Light	Dark
0	4/17/90	18.6	14.3	33.7	25.5	22.9±7.0	23.6±4.0
1	4/18/90	18.4	13.1	28.5	27.8	24.5±12.3	18.5±5.0
2	4/19/90	21.6	23.8	27.5	25.6	*	*
3	4/20/90	*	*	*	*	*	*
4	4/21/90	21.3	15.6	35.7	32.8	*	*
5	4/22/90	13.1	15.6	29.4	23.8	21.4±3.2	19.6±3.5
6	4/23/90	21.8	20.7	32.3	26.5	25.3±2.5	23.3±1.7
7	4/24/90	15.5	13.7	32.2	31.2	23.9±4.7	22.3±4.4
8	4/25/90	15.4	12.3	32.9	30.1	20.7±5.4	20.8±6.7
9	4/26/90	11.8	14.1	34.1	31.7	21.3±7.5	21.6±5.8
10	4/27/90	12.2	17.1	30.5	31.0	19.7±6.4	22.8±4.3
11	4/28/90	13.9	13.2	33.2	27.6	17.7±4.3	20.4±4.4
12	4/29/90	10.3	10.7	27.6	26.0	18.9±5.0	18.4±5.5
13	4/30/90	*	*	*	*	*	*
14	5/1/90	19.7	15.3	27.3	31.1	19.2±4.4	22.5±6.4
15	5/2/90	19.7	12.8	27.9	31.0	22.7±2.8	21.6±6.6
16	5/3/90	21.0	13.2	30.1	29.4	24.0±2.0	21.0±4.9
17	5/4/90	20.9	12.0	28.7	36.4	24.1±2.6	20.6±7.2
18	5/5/90	26.8	19.3	35.9	26.1	30.1±3.2	22.6±2.7
19	5/6/90	18.0	20.9	36.8	28.0	27.7±4.2	23.7±2.3
20	5/7/90	16.2	13.9	29.5	31.6	23.6±4.9	23.2±4.6
Mean±S.D.		17.4±4.2	15.0±3.7	31.2±3.1	29.1±3.2	22.8±3.1	21.6±1.6

* Data not available due to computer malfunction.

Table V. Light Intensity and Total Energy for the Study Period.

		Light Intensity (µW/cm2)			Total Light Energy (W. min/cm2)	
Day	Date	Minimum	Maximum	Mean + S.D.	daily	cumulative
0	4/17/90	67	29385	13755.5±9597.1	11.04	11.04
1	4/18/90	66	28464	12157.6±9683.6	5.08	16.12
2	4/19/90	67	15553	*	9.52	25.64
3	4/20/90	*	*	*	9.52	35.16
4	4/21/90	68	22856	12282.5±7945.5	9.52	44.68
5	4/22/90	67	17307	6294.2±4606.7	5.08	49.76
6	4/23/90	67	23241	12351.8±7853.4	10.05	59.81
7	4/24/90	66	24050	13808.5±8754.0	11.36	71.17
8	4/25/90	67	24280	13062.0±8685.2	10.76	81.93
9	4/26/90	68	24006	12779.6±8602.8	10.52	92.45
10	4/27/90	67	23827	12491.7±8454.5	10.28	102.73
11	4/28/90	66	22467	11738.2±7653.6	9.79	112.52
12	4/29/90	66	24050	12883.7±8815.1	10.73	123.25
13	4/30/90	*	*	*	9.52	132.77
14	5/1/90	70	24171	26808.7±20117.0	11.68	144.45
15	5/2/90	68	90656	25390.8±19417.1	9.31	153.76
16	5/3/90	63	22850	25318.1±19616.8	9.32	163.08
17	5/4/90	62	91967	25267.8±19854.1	9.09	172.17
18	5/5/90	67	93268	25306.0±19526.0	9.33	181.50
19	5/6/90	67	83516	25892.0±20342.3	9.31	190.81
20	5/7/90	68	90691	25582.0±19666.0	9.14	199.95

Note: The average daily light energy for the study period was 9.52 W. min/Cm2. This value was added to the cummulative energy totals on the days for which the computer generated data was not available (*).

* Data not available due to computer malfunction.

International Light #1490 Radiometer wavelength interval of integration is 250-700 nm (Appendix A4).