DATA EVALUATION RECORD 8

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GUIDELINE165-1

STUDY ID 420197-19

Nichols, R.G. 1990. Confined rotational crop study of MON-13900 herbicide safener. Part I: In-field portion. Project Nos. MSL-9546; EF-99-40; RD 1054. Unpublished study performed by Pan-Agricultural Laboratories, Inc., Madera, CA, and submitted by Monsanto Agricultural Company, Chesterfield, MO.

STUDY ID 420197-20

Bellora, L.K. 1990. Confined rotational crop study of MON-13900 herbicide safener. Part II: Quantitation, characterization, and identification of MON 13900 and its metabolites in rotational crops. Project Nos. MSL-9547; RD 1054. Unpublished study performed and submitted by Monsanto Agricultural Company, Chesterfield, MO.

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CONCLUSIONS:

Confined Accumulation - Rotational Crops

1. This study cannot be used to fulfill data requirements at this time. This study is scientifically sound, but does not meet EFGWB standards for the following reasons:

   complete quantitative data for the day 0 soil samples, including concentrations of MON 13900 and any degradates present, were not reported; and,

   adequate storage stability data were not provided for the plant and soil substrates.

   In order for this study to fulfill the accumulation in confined rotational crops data requirement, the registrant must report the concentrations of MON 13900 and its degradates detected in the day 0 soil samples; provide storage stability data for MON 13900, MON 13900 oxazolidine acid, and MON 13900 oxamic acid from field-spiked and laboratory spiked plant and soil samples; and report the lengths of time the samples were stored prior to analysis.

2. [^{14}C]MON 13900 residues accumulated in lettuce, carrots, and barley planted 30 and 120 days after sandy loam soil was treated with MON 13900 at 0.4 lb ai/A. Accumulation decreased as the length of the rotation increased. [^{14}C]MON 13900 residues were 0.0040-0.0142 ppm in lettuce; 0.0039-0.0173 ppm in carrot tops and 0.0028-0.0095 ppm in roots; and 0.0059-0.1087 ppm in barley straw, 0.0963 ppm in heads, 0.0070 ppm in grain, and 0.0079 ppm in chaff. Attempts to identify [^{14}C]residues in the crops were unsuccessful due to the low concentrations. In the soil, MON 13900 was 0.0010 ppm at 30 days posttreatment and 0.0005 ppm at 120 days; total residues were 0.0536 and 0.0242 ppm, respectively, at the same intervals. MON 13900 oxazolidine acid was also detected.

METHODOLOGY:

Formulated [^{14}C]MON 13900 (1 lb/gallon EC) was prepared by combining oxazolidine ring-labeled [4-^{13}C/^{14}C]MON 13900 (radiochemical purity >98%, specific activity 27.6 mCi/mMol, Monsanto); unlabeled MON 13900 (purity 98.9%, Monsanto); the emulsifiers Witco P1220, Witco CO360, Witco NP330, and monochlorobenzene; and water. The formulated test substance had a radiochemical purity of 98.7% and specific activity of 12.02 mCi/mMol. Formulated [^{14}C]MON 13900 was tank-mixed with alachlor (Lasso at 2.0 lb ai/A) and applied with a backpack sprayer at 0.4 lb ai/A to three plots (each 4 x 10 feet with a 2-foot buffer zone between each plot) of sandy loam soil (62-70% sand, 21-29% silt, 9% clay, 0.3-0.5% organic matter, pH 6.2-7.3, CEC 3.6-4.4 meq/100 g) located in Madera, California on May 11, 1988. The plots were planted to corn 1 day prior to treatment. Three additional plots (each 4 x 16.6 feet) planted to corn were treated with unlabeled formulated MON 13900 tank-mixed with alachlor to serve as controls. Corn was harvested when immature (31 and 66 days postplanting; 30 and 65 days posttreatment) and at maturity (121 days postplanting; 120 days posttreatment).

At 30, 120, and 359 days posttreatment, one of each of the treated and control plots was planted to lettuce, carrots, and barley; each crop covered one-third of a plot (Figure 2). Plots were rototilled to a depth of $\leq$4 inches prior to planting the rotational crops. For the 30- and 120-day rotations, crops were harvested at maturity: 54-152 days postplanting for lettuce, 97-200 days for carrots, and 109-253 days for barley. The 359-day rotation was terminated and the crops were harvested at 63 days postplanting due to low levels of [^{14}C]residues detected (<0.01 ppm) in the 120-day rotational crops. Crop plants were separated into their various components and stored frozen until analysis. Three or six soil cores (1-inch diameter, 0- to 24-inch depth) were taken using a zero-contamination probe prior to treatment, immediately posttreatment, and at 1, 3, 7, 14, 30, 60, 120, 180, and 359 days posttreatment; soil cores were stored frozen until analysis.

Plant samples were ground to a fine powder with dry ice, then subsamples were analyzed for total radioactivity by LSC following combustion. Additional subsamples were extracted twice with 20% aqueous acetonitrile first for 5-10 minutes using a homogenizer or blender, followed by an overnight extraction using a wrist-action shaker. Extracts were separated from the extracted plant tissue by filtration, analyzed for total radioactivity using LSC, and combined if the second extract contained significant levels of [^{14}C]residues. The aqueous acetonitrile extract was concentrated and analyzed for MON 13900 and its degradates by reverse phase HPLC (Program 1) using UV (wavelength unspecified) and radioactivity detection on a Brownlee Polymer RP precolumn followed by a

Hamilton PRP-1 semi-prep polymeric RP column eluted with linear step gradients of acetonitrile and 1% acetic acid ("HPLC Program 1"); fractions were collected at 0.5-minute intervals and analyzed for radioactivity using LSC. The extracted plant tissue was further extracted with 0.4 N ammonium hydroxide; extracts were analyzed for total radioactivity using LSC. Unextracted [^{14}C]residues remaining in the extracted plant tissue were quantified by LSC following combustion.

Subsamples of 30-day rotational barley straw were used for the majority of the degradate isolations and characterizations. Subsamples of ground straw were extracted, purified, and analyzed according to the schematic presented in Figure 13. Purification techniques included ultrafiltration (Amicon molecular weight 5000), HPLC using an Amberchrom-161 polymeric resin column, and anion (Bio-Rad AG-1) and cation exchange (AG 50W-X8 H+ form) column chromatography. Analyses were performed using reverse phase HPLC with a PRP-1 column as described above and high voltage electrophoresis. Derivatives of degradates were produced, as needed, using diazomethane to form methyl esters of carboxylic acids and acetic anhydride plus pyridine for acetylations of neutral compounds; derivatized compounds were analyzed by reverse phase HPLC.

The aqueous acetonitrile extracts from 30-day rotational carrot tops and roots were determined to contain primarily neutral compounds following anion and cation exchange column chromatography, reverse phase HPLC, and HPLC using a Bio-Rad organic acids column to separate organic acids and monosaccharides. Ammonium hydroxide extracts from the carrot tops and roots were acidified with concentrated HCl to precipitate proteins. Precipitated solids were separated from the extract by filtration, then redissolved with 5% sodium dodecyl sulfate solution; filtrates and redissolved proteins were analyzed for total radioactivity using LSC. The degradate, 2-[5-(2-furanyl)-2,2-dimethyl-3-oxazolidinyl]-2-oxoacetic acid (MON 13900 oxamic acid), was initially identified by coelution of reference standard MON 13900 oxamic acid with the 127-day carrot top aqueous acetonitrile extract using reverse phase HPLC; however, attempts to isolate the degradate from the carrot top extract for positive identification were unsuccessful.

To further characterize unextracted [^{14}C]residues in 373-day barley grain, subsamples of ground grain were extracted with chloroform:methanol:water (30:60:19.5, v:v:v) using a blender. Following filtration, the organic and aqueous phases were analyzed for total radioactivity using LSC with no significant levels of [^{14}C]residues detected (quantitative data not provided) in either phase. The extracted plant tissue was pulverized using a mortar and pestle, then extracted with 50% aqueous acetonitrile overnight using a wrist-action shaker. Following filtration, the extract was concentrated and analyzed by LSC, again with no significant levels of [^{14}C]residues extracted. The extracted plant tissue was blended with 0.4 N ammonium hydroxide for 3-5 minutes to remove proteins; following centrifugation, the extract was filtered and analyzed by LSC. The extracted tissue was blended with dimethyl sulfoxide (DMSO):water (90:10, v:v) for 5 minutes to remove starch. Following centrifugation, ethanol was added to the DMSO extract; the resulting precipitate was air-dried and analyzed for [^{14}C]residues by LSC following combustion.

Each soil core was divided into four 6-inch segments; the segments were individually homogenized and subsamples were analyzed for total radioactivity by LSC following combustion. The limit of detection for total [^{14}C]residues was 0.0002 ppm. A composite 0- to 6-inch soil sample was prepared for each sampling interval and extracted two or three times with 50% aqueous acetonitrile.

Following centrifugation, extracts were combined and analyzed for total radioactivity using LSC. Extracts were then filtered (0.2 um), if necessary, prior to being concentrated and analyzed for MON 13900 and its degradates by reverse phase HPLC as described above for the plant extracts. Aliquots of the 180- and 359-day combined acetonitrile extracts were also partitioned with ethyl acetate; organic and aqueous phases were analyzed by HPLC. The 180- and 359-day extracted soil samples were further extracted with 0.4 N ammonium hydroxide; extracts were analyzed for total radioactivity using LSC. Unextracted [^{14}C]residues remaining in the extracted soil were quantified by LSC following combustion. In 14-day soil extracts, the degradate Photolyte II was identified as 3-(dichloroacetyl)-2,2-dimethyl-5-oxazolidinylcarboxylic acid (MON 13900 oxazolidine acid) using the procedures diagrammed in Figure 10. MON 13900 oxazolidine acid was isolated using solvent/solvent partitioning and anion exchange column chromatography, derivatized with diazomethane, and identified by reverse phase HPLC and GC/MS with chemical ionization. Attempts to isolate and characterize [^{14}C]residues in 180-day soil extracts using solvent/solvent partitioning, anion exchange column chromatography, reverse phase HPLC, and HPLC using an organic acids column were unsuccessful.

DATA SUMMARY:

[^{14}C]MON 13900 residues accumulated in lettuce, carrots, and barley planted 30 and 120 days after formulated (1 lb/gallon) oxazolidine ring-labeled [4-^{14}C]MON 13900 (final radiochemical purity >98% and specific activity 12.02 mCi/mMol), tank-mixed with alachlor (Lasso at 2.0 lb ai/A), was applied at 0.4 lb ai/A to sandy loam soil located in Madera, California. The plots were planted to corn 1 day prior to treatment. Accumulation decreased as the length of the rotation increased; the concentration of [^{14}C]residues in crops from the 30-day rotation was approximately 2 to 18x greater than the concentration in crops from the 120-day rotation (Table 6).

In crops planted at 30 days posttreatment, [^{14}C]residues at harvest were 0.0100-0.0142 ppm in lettuce, 0.0173 and 0.0095 ppm in carrot tops and roots, respectively, and 0.0963 and 0.1087 ppm in barley heads and straw, respectively (Table 6).

In crops planted at 120 days posttreatment, [^{14}C]residues at harvest were 0.0040-0.0057 ppm in lettuce, 0.0039 and 0.0028 ppm in carrot tops and roots, respectively, and 0.0070, 0.0079, and 0.0059 ppm in barley grain, chaff, and straw, respectively (Table 6).

In the 30-day rotational crops, aqueous acetonitrile-extractable residues ranged from 36.6 to 72.5% of the recovered radioactivity and unextracted residues ranged from 32.8 to 53.6%. In the 120-day rotational crops, aqueous acetonitrile-extractable residues decreased to 9.3-49.3% of the recovered radioactivity and unextracted residues ranged from 44.9 to 66.8% (Table 7). Although extracted [^{14}C]residues were separated using HPLC, degradates were not identified due to the low concentrations (Table 8 and Figures 8 and 9). In the aqueous acetonitrile-extractable fraction, MON 13900 coeluted with [^{14}C]residues in lettuce, carrot top, and barley (heads and straw) extracts. The degradate

2-[5-(2-furanyl)-2,2-dimethyl-3-oxazolidinyl]-2-oxoacetic acid (MON 13900 oxamic acid) also coeluted with [^{14}C]residues in extracts from all crops (Table 8, Fraction 5).

[^{14}C]Residues associated with glucose/fructose components were also tentatively identified in the crops.

In the 0- to 6-inch soil depth, mean total [^{14}C]residues were 0.0321-0.0542 ppm at 0-3 days posttreatment, increased to 0.1120 ppm at 14 days, then decreased to 0.0536 ppm at 30 days, 0.0242 ppm at 120 days, and were 0.0195 ppm at 359 days (Table 3). In the 6- to 12-inch depth, [^{14}C]residues were 0.0021 ppm at 1 day posttreatment, 0.0012 ppm at 3 days, and ≤0.0009 ppm at all other sampling intervals (Table 9). In the 12- to 18- and 18- to 24-inch depths, [^{14}C]residues were ≤0.0011 ppm at any sampling interval. [^{14}C]Residues were identified only in the 0- to 6-inch soil depth; adequate day 0 soil residue data were not reported. MON 13900 decreased from 0.0110 ppm at 1 day posttreatment to 0.0010 ppm at 30 days and was 0.0005 ppm at 120 days (Table 5). The primary degradate was

3-(dichloroacetyl)-2,2-dimethyl-5-oxazolidinylcarboxylic acid (MON 13900 oxazolidine acid, Photolyte II), detected at a maximum of 0.0367 ppm at 14 days posttreatment, 0.0141 ppm at 30 days, and 0.0002 ppm at 120 days.

During the study (5/11/88 to 7/7/89), rainfall plus irrigation totaled 175.98 inches, air temperatures ranged from 24 to 105 F, and soil temperatures ranged from 35 to 119 F at the 2-inch depth and 39 to 104 F at the 8-inch depth.

COMMENTS:

1. Quantitative data for the day 0 soil samples were not provided. Two weeks after receipt at the analytical lab, the 0- to 6-inch soil samples were analyzed by reverse phase HPLC using a Brownlee RP-18 precolumn followed by a Waters C-18 u-Bondapak column eluted with acetonitrile and pH 3.6 5% acetic acid/KOH ("HPLC Program 2"). Parent MON 13900 comprised 77.5% of the extractable radioactivity in the day 0 soil sample (reported in text). The percent of total [^{14}C]residues that were extractable and the concentrations of degradates from the day 0 soil sample were not reported. At a later time in the study (unspecified), all of the soil extracts except those from the day 0 soil were reanalyzed using the improved HPLC method described in the Methodology section of this review ("HPLC Program 1"). It was not explained why the extracts from the day 0 soil were not reanalyzed.

2. Appropriate methods were not used to determine the storage stability of MON 13900 and its degradate, MON 13900 oxazolidine acid, in the various plant matrices and soil. The 0- to 14-day soil samples were shipped within 1 week after collection, and the 30- to 359-day soil samples were shipped between 2 and 27 days after collection. The actual length of time between sampling and extraction/analysis for each soil and crop sample was not reported.

   As an attempt at a storage stability study, soil and ground foliage (crop type and sample size unspecified) were fortified with parent MON 13900 (concentration unspecified) and stored frozen at -20 to -30 C for 2 weeks. The study author reported that only MON 13900 was detected in the foliage after 1 week of storage and in the soil after 2 weeks of storage; quantitative data were not provided. The study author reported that this storage stability study was terminated at 2 weeks because (1) low levels of radioactivity created inherent problems in the analyses, (2) since 0 and 1 day soil samples were analyzed within 2 weeks of receipt, reanalysis of these samples after freezing would provide storage

stability data, and (3) little to no parent MON 13900 was detected in corn and sorghum from a field uptake study being conducted at another site.

Analysis of the 0- to 6-inch soil samples 2 weeks after receipt by "HPLC Program 2" (see comment 1) indicated that parent MON 13900 comprised 77.5% of the extractable radioactivity in the day 0 sample and 28.7% of the extractable in the day 1 sample (reported in text). After 9 months of storage, the 0- and 1-day soil extracts were reanalyzed using "HPLC Program 2" and compared to subsamples of the 0- and 1-day soil which were re-extracted and analyzed. It was reported that the respective HPLC profiles were nearly identical, but quantitative data were not provided (Figure 24). At some later unspecified time, all of the soil extracts except those from the day 0 soil were reanalyzed using "HPLC Program 1" (described in the Methodology section of this review). In the 1-day 0- to 6-inch soil sample, MON 13900 had decreased from 28.7% to 21.8% of the extractable; the study author presented these data to show that MON 13900 and its degradates did not degrade during frozen storage. It was not explained why only the extracts from the day 0 soil were not reanalyzed using the improved HPLC method, and the concentrations of MON 13900 degradates detected in the day 0 soil extracts were not reported.

For storage stability of MON 13900 degradates in crop matrices, 30-day rotational lettuce harvested at 54 days postplanting (84 days posttreatment) was extracted after 1 and 10 months of frozen storage and analyzed by "HPLC Program 2" (described above). The registrant provided HPLC profiles of the extracts and reported that the profiles were "nearly identical"; quantitative data were not provided (Figure 6).

Acceptable storage stability data should be submitted for the length of time that soil and crop samples were stored. Since incomplete residue data were reported for the day 0 soil samples and there appeared to be a significant concentration of degradate residues in the day 1 soil samples, a freezer storage stability study should be conducted using field spiked samples in addition to laboratory-spiked samples to determine if significant MON 13900 degradation occurred between field sample collection and frozen storage. In addition, the registrant must explain what problems in the analyses were caused by low levels of radioactivity in the initial 2-week storage stability study.

3. Analysis of the 0- to 6-inch day 0 soil samples determined that only 35.5% (range 18.2 to 44.1%) of the total expected radioactivity was applied to the test plots; individual data were not provided. However, analysis of weigh boats used to determine the amount of test solution that intercepted the soil indicated that 82.9% of the total expected radioactivity was applied to the test plots (see Comment 4). HPLC analysis of one aliquot of the tank mixture at 12 days posttreatment found that the purity of the [^{14}C]MON 13900 was 98.66%, indicating that the test substance was not degraded prior to application; supporting raw data, such as the HPLC chromatogram of this analysis with the distribution of radioactivity, were not provided.

4. Plastic weighing boats (3-3/16 x 3-3/16 inches) were used to determine the amount of spray solution that intercepted the soil. The weigh boats were collected immediately posttreatment, sealed in jars, and stored frozen for an unspecified length of time until analysis. For analysis, toluene was added to each jar to dissolve the weigh boat, then aliquots were analyzed for total radioactivity using LSC. It was reported that an average of 82.9% (range 46.9 to 130.3%) of

the total expected radioactivity was applied to the test plots; individual data were not provided.

5. The 359-day rotation was terminated and the crops harvested at 63 days postplanting due to low levels of [^{14}C]residues detected (<0.01 ppm) in the 120-day rotational crops. Also at application, the area for the 359-day rotation did not receive the same application as the 30- and 120-day rotation areas because the "spray solution ran out halfway through the second pass".

6. In the Summary section of the study document, it was reported that extracts from the 30-day rotational crops were purified using an Amicon molecular weight 5000 filter prior to HPLC analysis; however, in the Sample Analysis section under 3.5.3 Extraction and Quantification of Metabolites/Crop Metabolites, it was reported that only extracts from plant samples analyzed in the latter part of the study were purified using ultrafiltration. Except for the 139-day barley straw and 180-day lettuce, it was not specified which other plant sample extracts were subjected to the ultrafiltration. The registrant should specify which plant extracts were purified by ultrafiltration and what effect, such as loss of [^{14}C]residues, the purification process had on the results.

7. The 30-day rotational barley harvested at 109 days postplanting (139 days posttreatment) did not form mature grain; the barley heads were ground and analyzed intact. The 120-day rotational barley harvested at 253 days postplanting (373 days posttreatment) did contain solid grain; grain and chaff were analyzed separately.

8. Immature lettuce, carrots, and barley were not analyzed. Although lettuce was harvested at three intervals for the 30- and 120-day rotations, the registrant reported that the lettuce was always harvested in the mature state.

9. It was reported that monochlorobenzene plus Witco P1220, Witco C0360, and Witco NP330 were used to suspend MON 13900 in water to produce the EC formulation; the Witco products were not further described.

10. Diazinon was applied at 15 lb/A on June 23, 1988 to the 30- and 120-day plots for ant control. Nudrin (methomyl, 1.8 EC) was applied at 2 pints/A on June 30, 1988 to the 30-day plots for lepidoptera control.

11. Data concerning residues in the primary crop (corn) were not reviewed as they are not pertinent to Subdivision N guidelines.

12. At 169 days posttreatment, the treated plot area was accidentally flooded with 65 inches of irrigation water. It was reported that analysis of soil cores collected from the buffer zones indicated that no lateral migration of [^{14}C]residues occurred; quantitative data were not provided.

13. In the Part I study (MRID 42019719), it was reported that the control plot was located 228 feet west of the treated plot with a 0.03% slope declining to the east. The depth to the water table was 95 feet, and the area contained no subsurface drainage system. In the Part II study (MRID 42019720), it was reported that the control plot was located 266 feet northwest of the treated plot.

14. It was reported that the test site had been fallow for approximately 8 years, and that no known pesticides had ever been applied to this site.

15. 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.

16. 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 3. ^{14}C Uptake and Percent Moisture in 0-6" Soil, Expressed in Dry Weight PPM MON 13900 Equivalents

Sample	% Moisture	Dry PPM
0 DAT	5.8	0.0542
1 DAT	5.1	0.0321
3 DAT	4.9	0.0502
7 DAT	5.9	0.0690
14 DAT	7.5	0.1120
30 DAT	8.7	0.0536
60 DAT	ND*	0.0409
120 DAT	4.9	0.0242
180 DAT	4.7	0.0235
359 DAT	6.6	0.0195

* Not Determined. Dry weight PPM were determined using 6.0% moisture content (based upon the approximate average of all other samples.)

Table 5. Metabolite Distribution in MON 13900 Soil Extracts*

Sample	Wet Weight PPM	Extractability (%)	Fraction**	Percent Dist.	Fraction PPM
Day 1	0.0306	164.4***	1	9.55	0.0048
			2(104-105)	8.01	0.0040
			3	21.78	0.0110
Day 3	0.0478	84.1	1	35.78	0.0144
			2	6.74	0.0027
			3	11.85	0.0048
Day 7	0.0649	54.5	1	50.18	0.0177
			2	2.6	0.0009
			3	11.85	0.0042
Day 14	0.1042	66.6	1	52.91	0.0367
			2	1.45	0.0010
			3	10.17	0.0071
Day 30	0.0491	54.0	1	53.36	0.0141
			2	1.26	0.0003
			3	3.83	0.0010
Day 60	0.0386	29.2	(6-8)	13.27	0.0015
			(9-11)	19.67	0.0022
			(64-65)	6.4	0.0007
			1	5.66	0.0006
			2(104-107)	10.92	0.0012
			3	3.46	0.0004
Day 120	0.0230	25.3	(6-9)	24.88	0.0014
			(28-29)	3.76	0.0002
			1	4.19	0.0002
			2 (105-107)	9.92	0.0006
			3	8.65	0.0005

* Sample extracts are 50% aqueous ACN. Samples past Day 120 are not shown due to low level dpms and loss of volatiles in sample work-up. Day 0 soil extract was not analyzed with program 1 and is not shown on this table.

**Fractions were determined by summing the radioactivity contained in the appropriate vials (see key below), unless otherwise indicated in the table (i.e. 2(104-105). This indicates that fraction 2 was collected in vials 104-105, as opposed to vial 105 only). Values expressed in parentheses are vial numbers for peaks not indicated.

***Due to sample inhomogeneity, this is most likely an accurate representation of the 14C contained in the sample, thus, fractions were determined as indicated.

Key:

fraction 1	Photolyte II	vial 68-71
fraction 2	?	vial 105
fraction 3	MON 13900	vial 108-109

Table 6. ^{14}C-Uptake and Percent Moisture in Rotational Crops, Expressed in Wet Weight PPM MON 13900 Equivalents

Sample	Moisture %	Wet PPM
30 DAT subplot		
84 day lettuce	90.7	0.0142
92 day lettuce	91.1	0.0116
111 day lettuce	91.5	0.0100
127 day carrot root	84.0	0.0095
127 day carrot tops	80.3	0.0173
139 day barley straw	34.6	0.1087
139 day barley heads*	17.1	0.0963
120 DAT subplot		
180 day lettuce	89.5	0.0048
209 day lettuce	87.8	0.0057
272 day lettuce	87.1	0.0040
320 day carrot root	90.2	0.0028
320 day carrot tops	80.8	0.0039
373 day barley straw	53.5	0.0059
373 day barley chaff	15.7	0.0079
373 day barley grain	15.3	0.0070

* Day 139 barley heads did not form mature grain, and thus the heads were ground and combusted as one unit.

Table 7. MON 13900 Rotational Crop Extractabilities and Material Balance *

Sample	Wet PPM	20 % aq. ACN Extract (% PCA)	0.4N NH_4OH Extract	End Pellet	Total Recovery
30 DAT subplot					
84 DAT lettuce	0.0142	36.6	2.1	32.8	71.5
92 DAT lettuce	0.0116	43.4	2.3	41.5	88.6
111 DAT lettuce	0.0100	49.8	5.1	49.4	104.3
127 DAT carrot root	0.0095	72.5	8.0	41.7	122.2
127 DAT carrot tops	0.0173	46.8	4.1	53.6	104.5
139 DAT barley straw	0.1087	57.1	8.1	42.0	108.9**
139 DAT barley heads	0.0963	50.2	7.3	38.9	98.9**
120 DAT subplot					
180 DAT lettuce	0.0048	37.9	0	63.9	101.8
209 DAT lettuce	0.0057	28.2	0	44.9	73.1
272 DAT lettuce	0.0040	34.6	NA	NA	NA
320 DAT carrot root	0.0028	-22.0	NA	NA	NA
320 DAT carrot tops	0.0039	20.8	NA	NA	NA
373 DAT barley straw	0.0059	49.3	15.7	48.8	113.8
373 DAT barley chaff	0.0079	18.8	10.4	66.8	96.0
373 DAT barley grain	0.0070	9.3***	8.5	56.9	81.2

PCA = Plant Contained Activity
NA = Not Analyzed

*Extractability shown is expressed as percent of plant contained activity based upon combustion of the wet plant material.

**Total Recovery for barley samples was based upon extracts, pellet, and the radioactivity remaining on the filter paper used. Filter paper trapped an additional 1-3% radioactivity which is not shown in the above table.

***Barley grain was extracted with other solvent combinations which only afforded an additional 6.5% maximum.

Table 8. Metabolite Distribution in MON 13900 Rotational Crop Extracts*

Sample	Sample PPM	Extractability	Fraction**	Percent Dist.	Fraction PPM
Day 84 lettuce	0.0142	36.6	1	52.72	0.0027
			5	5.30	0.0003
			6(67-68)	4.87	0.0003
Day 92 lettuce	0.0116	43.4	1(5-9)	51.70	0.0026
			3(27-29)	5.27	0.0003
			5(58-64)	13.49	0.0007
			6	3.44	0.0002
Day 111 lettuce	0.0100	49.8	1	35.10	0.0017
			3	5.29	0.0003
			5(58-61)	9.46	0.0005
			6(64-65)	4.74	0.0002
Day 127 carrot roots	0.0095	72.5	1(6-9)	82.67	0.0057
			3	4.38	0.0003
Day 127 carrot tops	0.0173	46.8	1	34.74	0.0028
			3(28-31)	9.64	0.0008
			5(59-63)	14.07	0.0011
Day 139 barley straw	0.1087	57.1	1(6-9)	22.29	0.0138***
			(11-12)	21.46	0.0133
			3(28-30)	6.79	0.0042
			5(56-63)	14.19	0.0088
			8(95-98)	3.54	0.0022
Day 139 barley heads	0.0963	50.2	1	19.67	0.0095
			(12-15)	17.98	0.0087
			3	8.78	0.0042
			(31-32)	4.05	0.0020
			5(57-61)	10.10	0.0049
			(66-69)	3.92	0.0019
			(94-98)	4.91	0.0024

*Extractions were performed with 20% aq. ACN (2 X). Metabolite fractions for barley and carrots are only shown for extract 1; barley extracts were profiled separately prior to pooling.

**Fractions were determined by summing the radioactivity contained in the appropriate vials (see key on next page), unless otherwise indicated in the table (i.e. 1(5-9). This indicates that fraction 1 was collected in vials 5-9, as opposed to vial 6-8). Values expressed in parentheses are vial numbers for peaks not indicated.

***Barley straw HPLC metabolite fractions were shown to contain more than one component.

Table 8. Continued

Key:

fraction 1	vial 6-8
fraction 2	vial 18-23
fraction 3	vial 28-29
fraction 4	vial 37-38
fraction 5	vial 56-62
fraction 6	vial 65-66
fraction 7	vial 84-85
fraction 8	vial 94-95

Table 9. MON 13900 Rotational Crop Soil; Dry Weight PPM for Soil 0-24"

Sample	Dry PPM	LOD (PPM)	LOQ (PPM)
Day 0 0-6"	0.0542	0.0002	0.0006
6-12"	ND	0.0002	0.0006
12-18"	ND	0.0002	0.0006
18-24"	< 0.0006	0.0002	0.0006
Day 1 0-6"	0.0321	0.0002	0.0008
6-12"	0.0021	0.0002	0.0008
12-18"	< 0.0008	0.0002	0.0008
18-24"	< 0.0008	0.0002	0.0008
Day 3 0-6"	0.0502	0.0002	0.0008
6-12"	0.0012	0.0002	0.0008
12-18"	< 0.0008	0.0002	0.0008
18-24"	< 0.0008	0.0002	0.0008
Day 7 0-6"	0.0690	0.0002	0.0008
6-12"	ND	0.0002	0.0008
12-18"	< 0.0008	0.0002	0.0008
18-24"	ND	0.0002	0.0008
Day 14 0-6"	0.1120	0.0002	0.0008
6-12"	ND	0.0002	0.0008
12-18"	< 0.0008	0.0002	0.0008
18-24"	ND	0.0002	0.0008
Day 30 0-6"	0.0536	0.0002	0.0008
6-12"	ND	0.0002	0.0008
12-18"	< 0.0008	0.0002	0.0008
18-24"	ND	0.0002	0.0008
Day 60 0-6"	0.0409	0.0002	0.0008
6-12"	< 0.0008	0.0002	0.0008
12-18"	< 0.0008	0.0002	0.0008
18-24"	ND	0.0002	0.0008
Day 120 0-6"	0.0242	0.0002	0.0008
6-12"	0.0009	0.0002	0.0008
12-18"	0.0008	0.0002	0.0008
18-24"	< 0.0008	0.0002	0.0008
Day 180 0-6"	0.0235	0.0002	0.0008
6-12"	< 0.0008	0.0002	0.0008
12-18"	0.0011	0.0002	0.0008
18-24"	< 0.0008	0.0002	0.0008
Day 359 0-6"	0.0195	0.0002	0.0008
6-12"	< 0.0008	0.0002	0.0008
12-18"	< 0.0008	0.0002	0.0008
18-24"	< 0.0008	0.0002	0.0008

LOD and LOQ are the Limit of Detection and the Limit of Quantitation, respectively. Each was determined using Formula (11) and (18) shown in Appendix A. (ND = not detected).

Figure 2. MON 13900 Rotational Crop Plot Locations

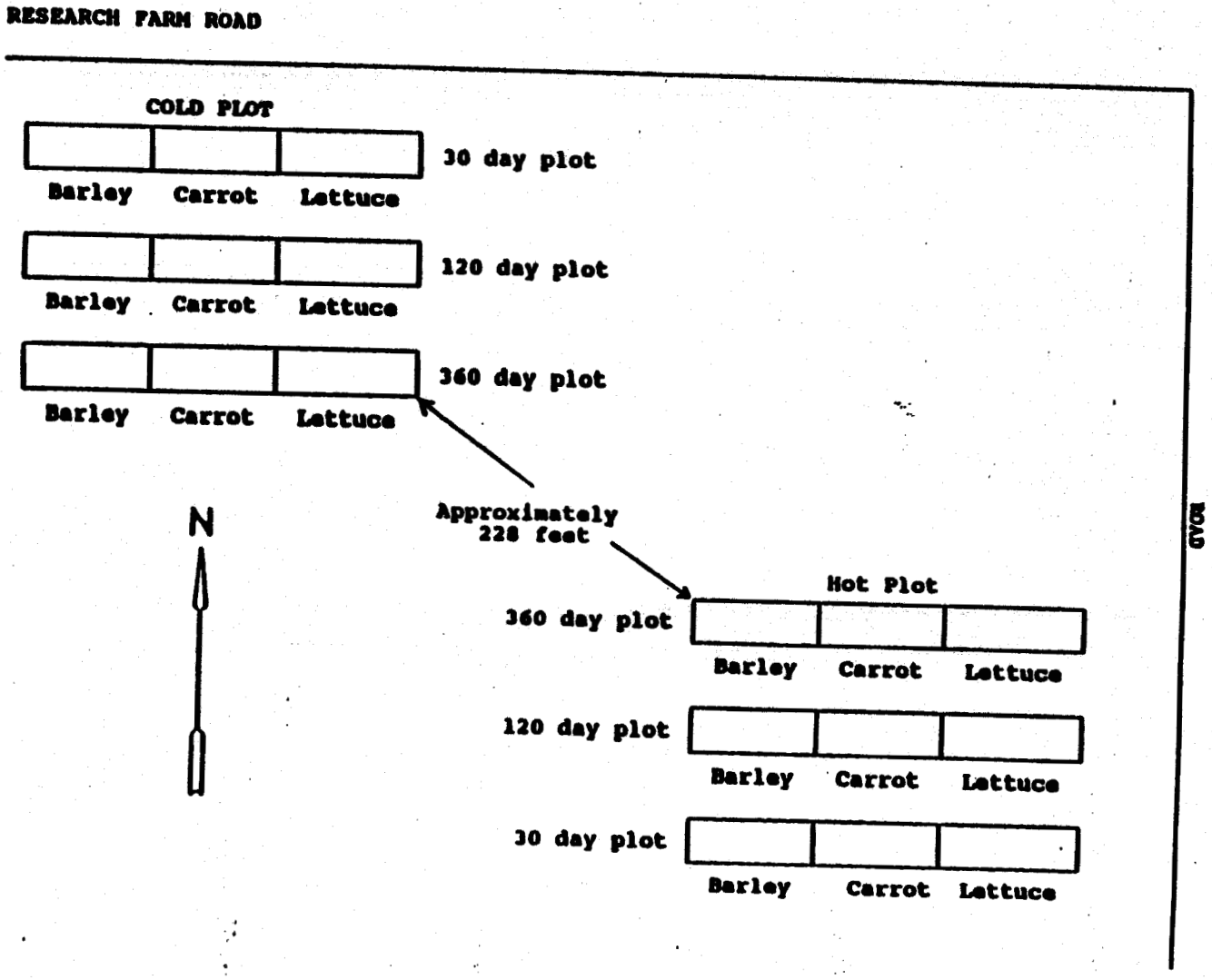


Figure 6. Storage Stability Studies of Day 84 Lettuce Extracts

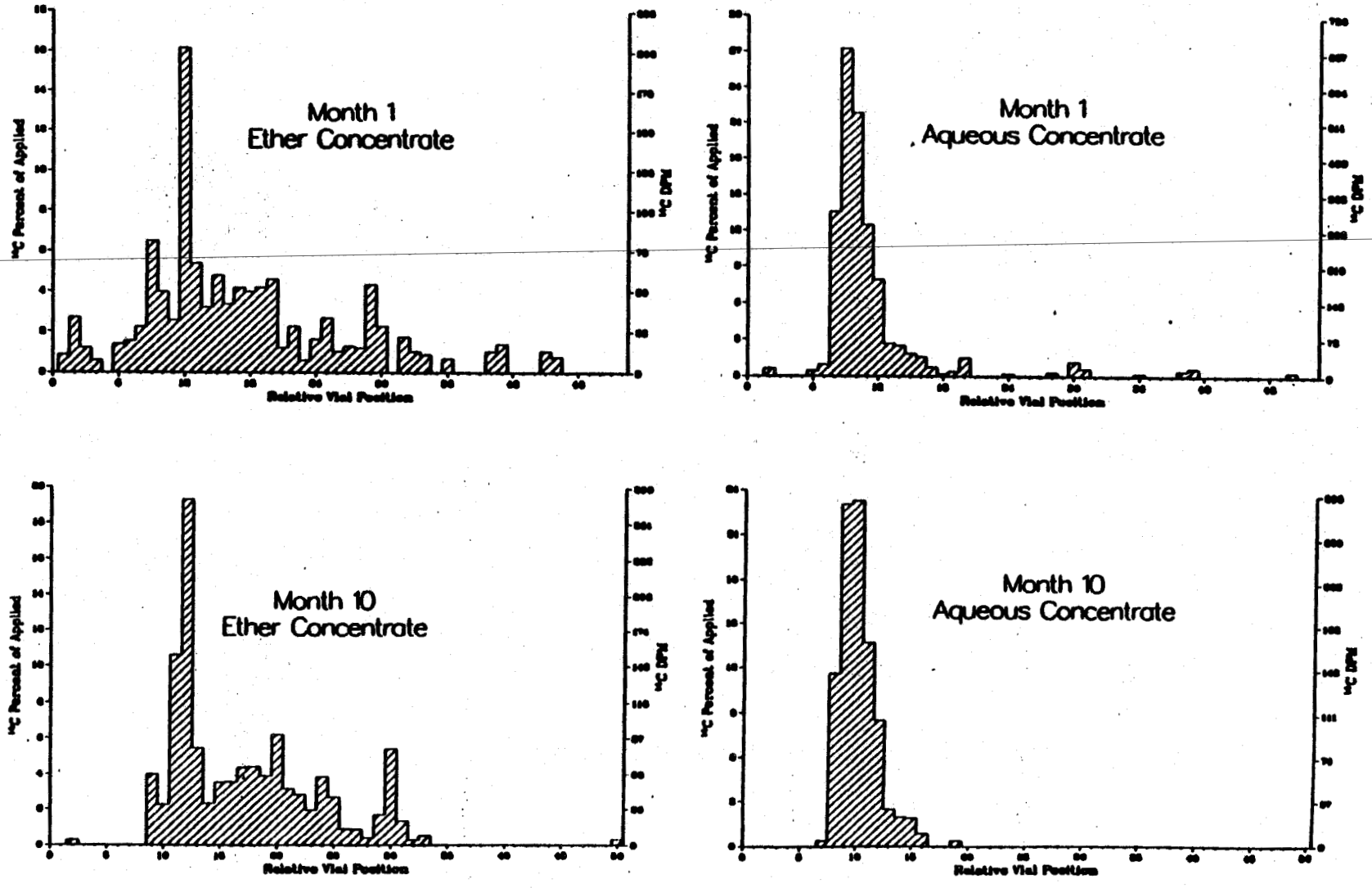


Figure 8. HPLC/LSC Analysis of Day 65 Corn Foliage Extract (Primary Crop) and 30 DAT Rotational Crop Extracts[a]

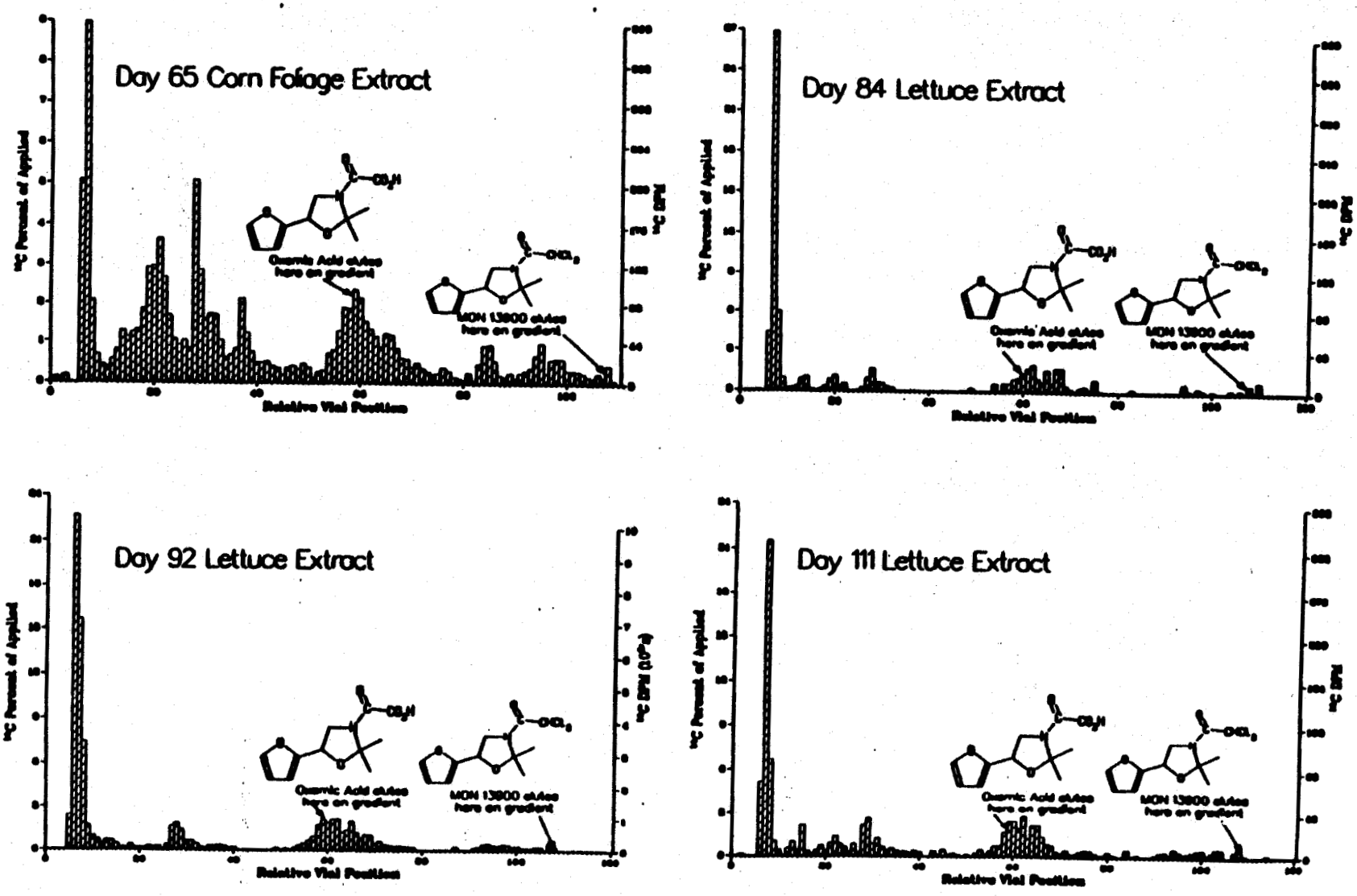
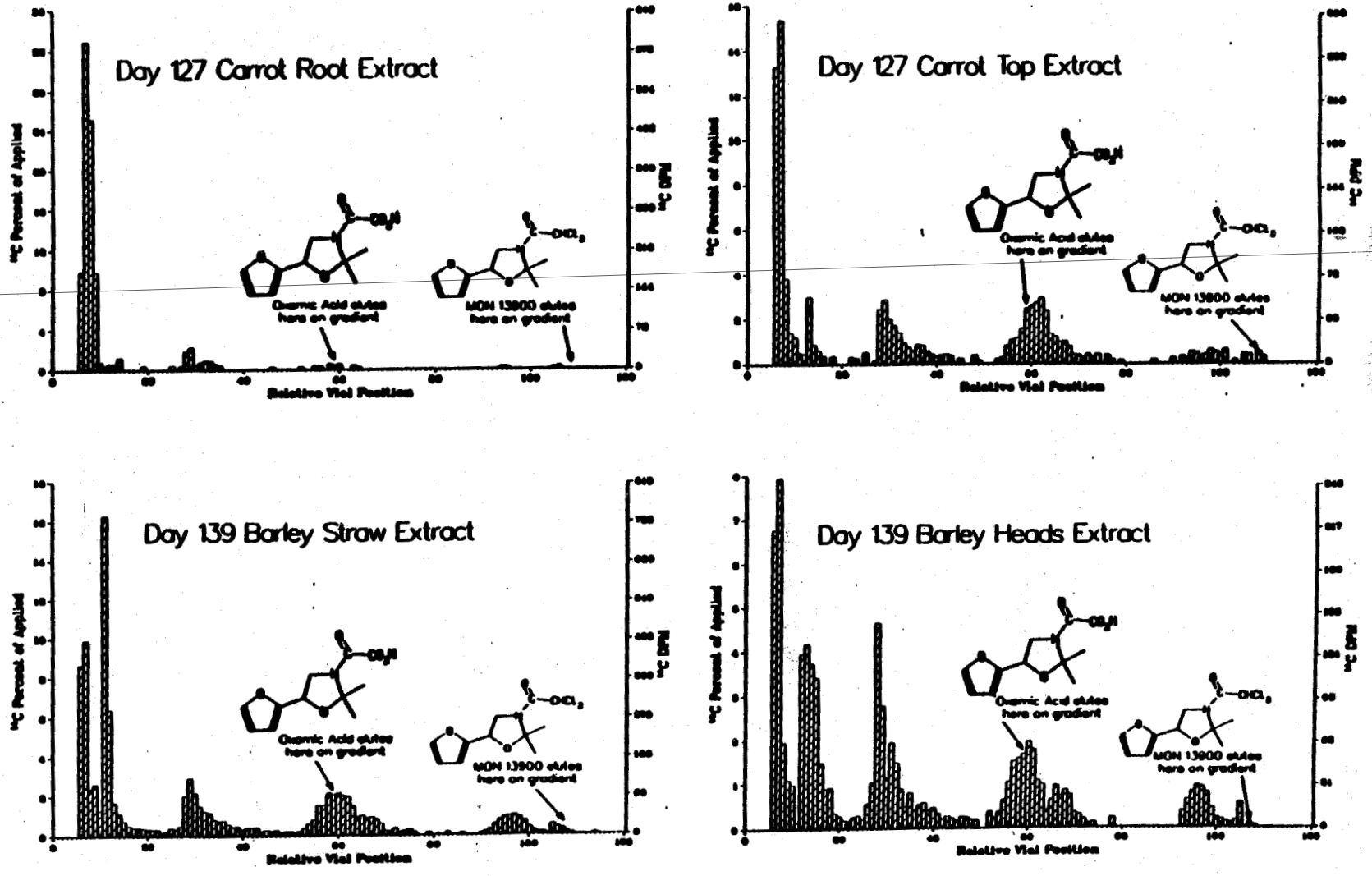


Figure 8. Continued.



*** Barley HPLC/LSC profiles are shown for extract 1 only.**

Figure 9. HPLC/LSC Analysis of 120 DAT Rotational Crop Extracts

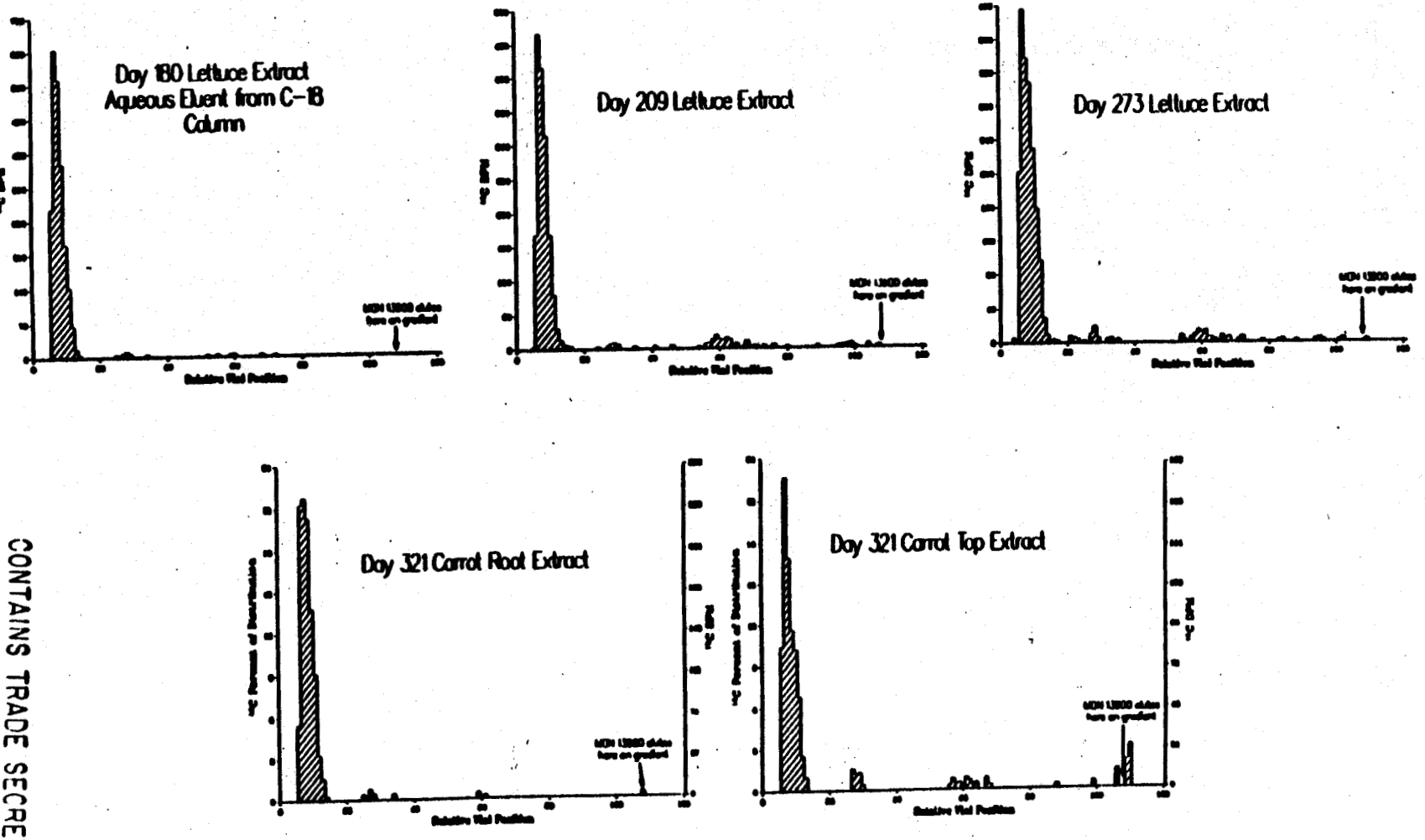


Figure 10. Flow Scheme for the Isolation and Identification of Photolyte (II) from a Day 14 Soil Extract

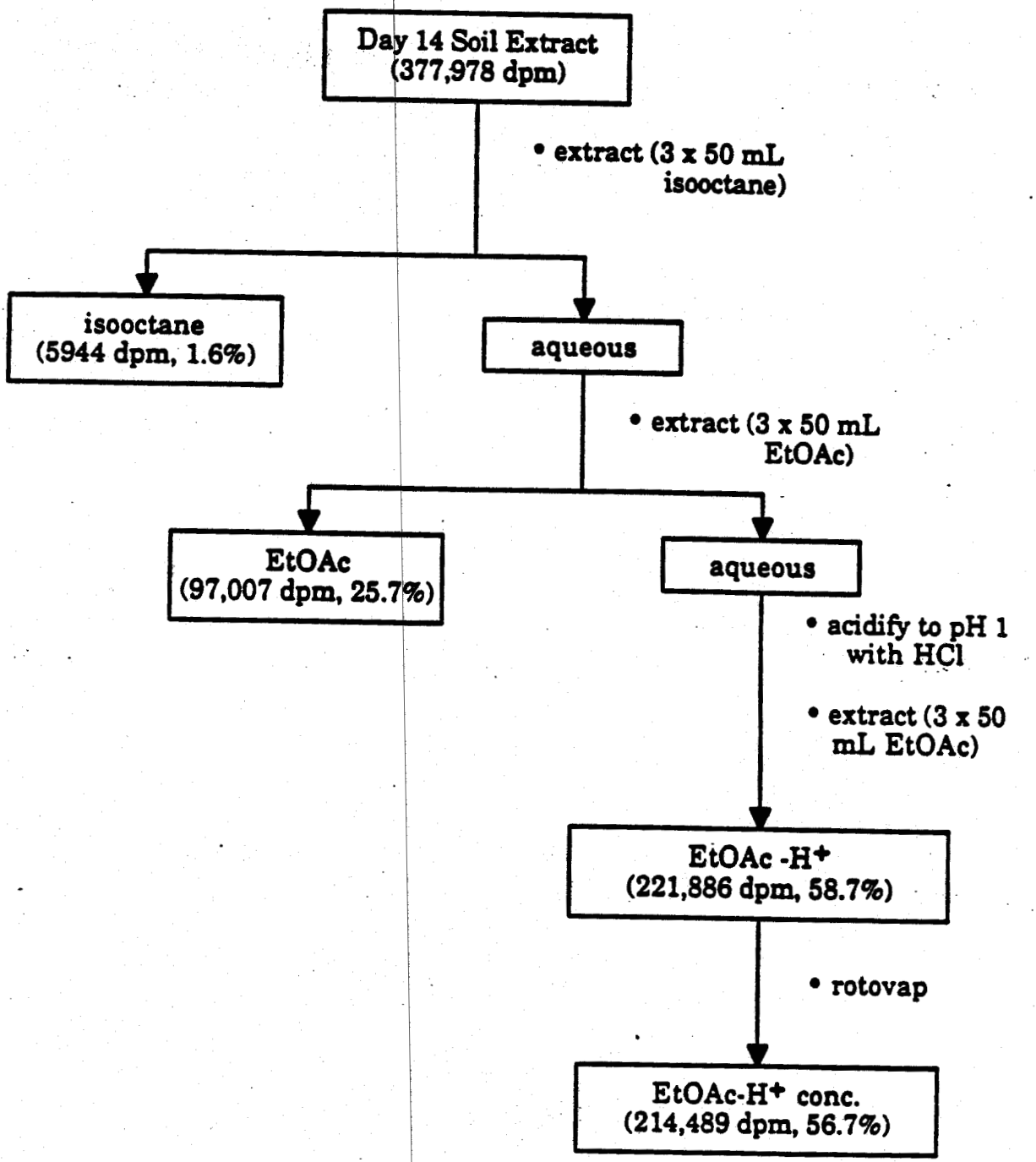


Figure 10 continued.

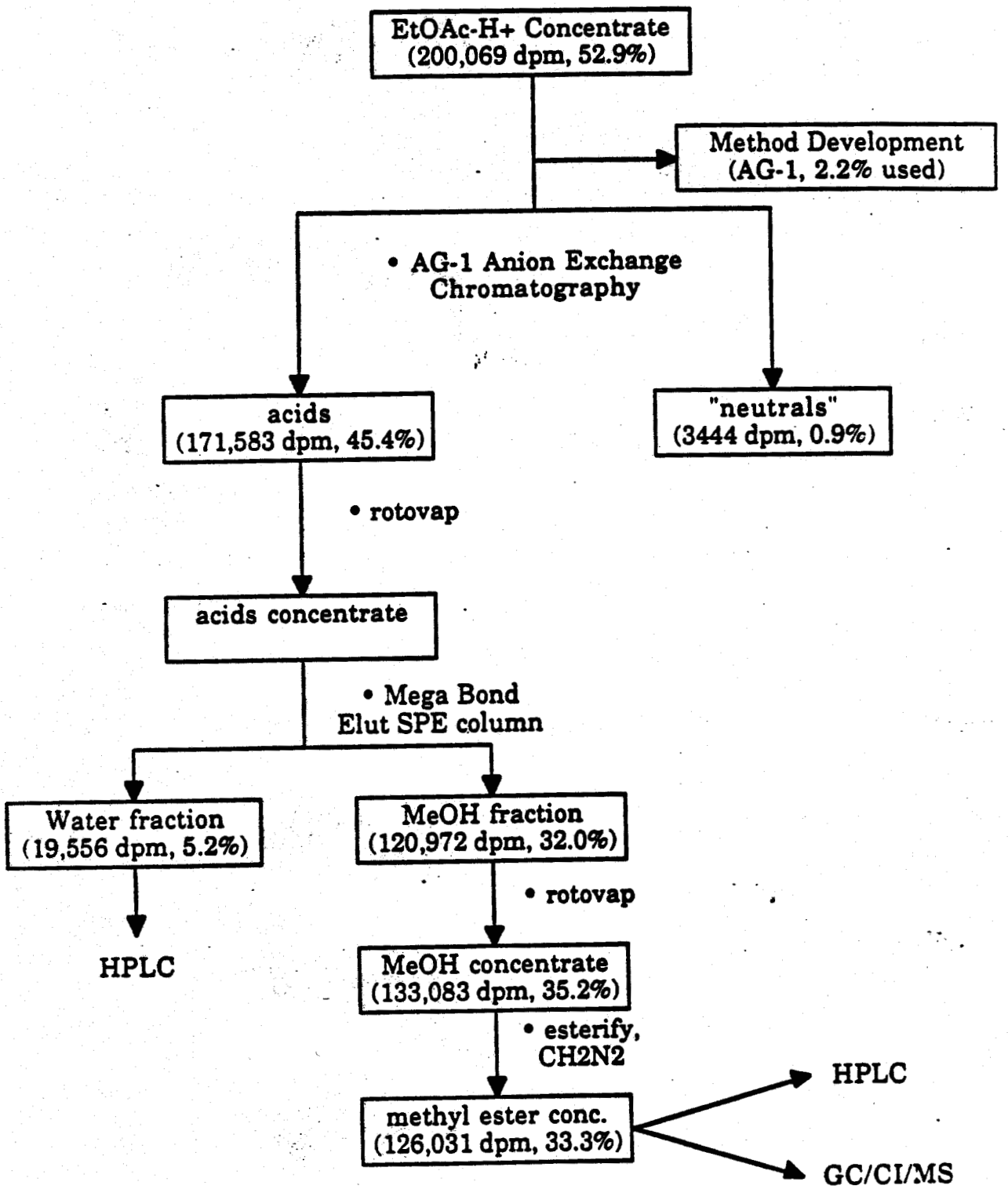


Figure 13. Flow Scheme for the Isolation and Characterization of Barley Straw (Day 139) Metabolites

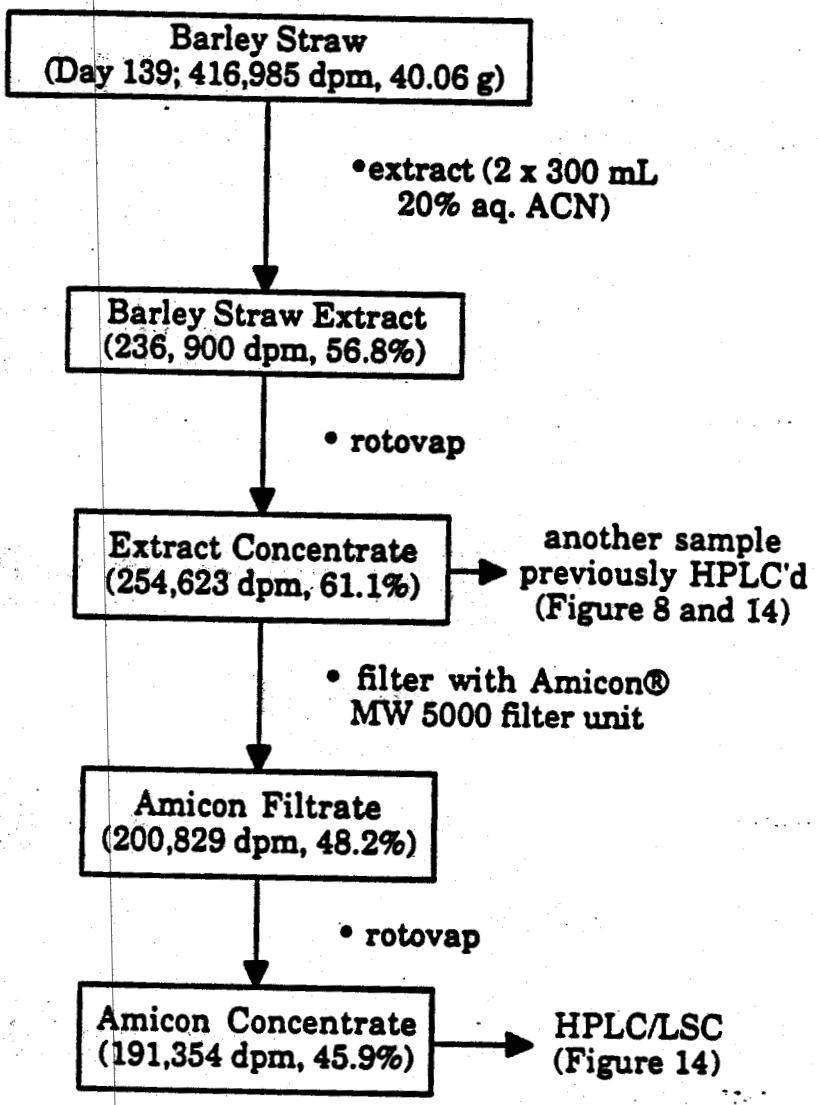


Figure 13. continued

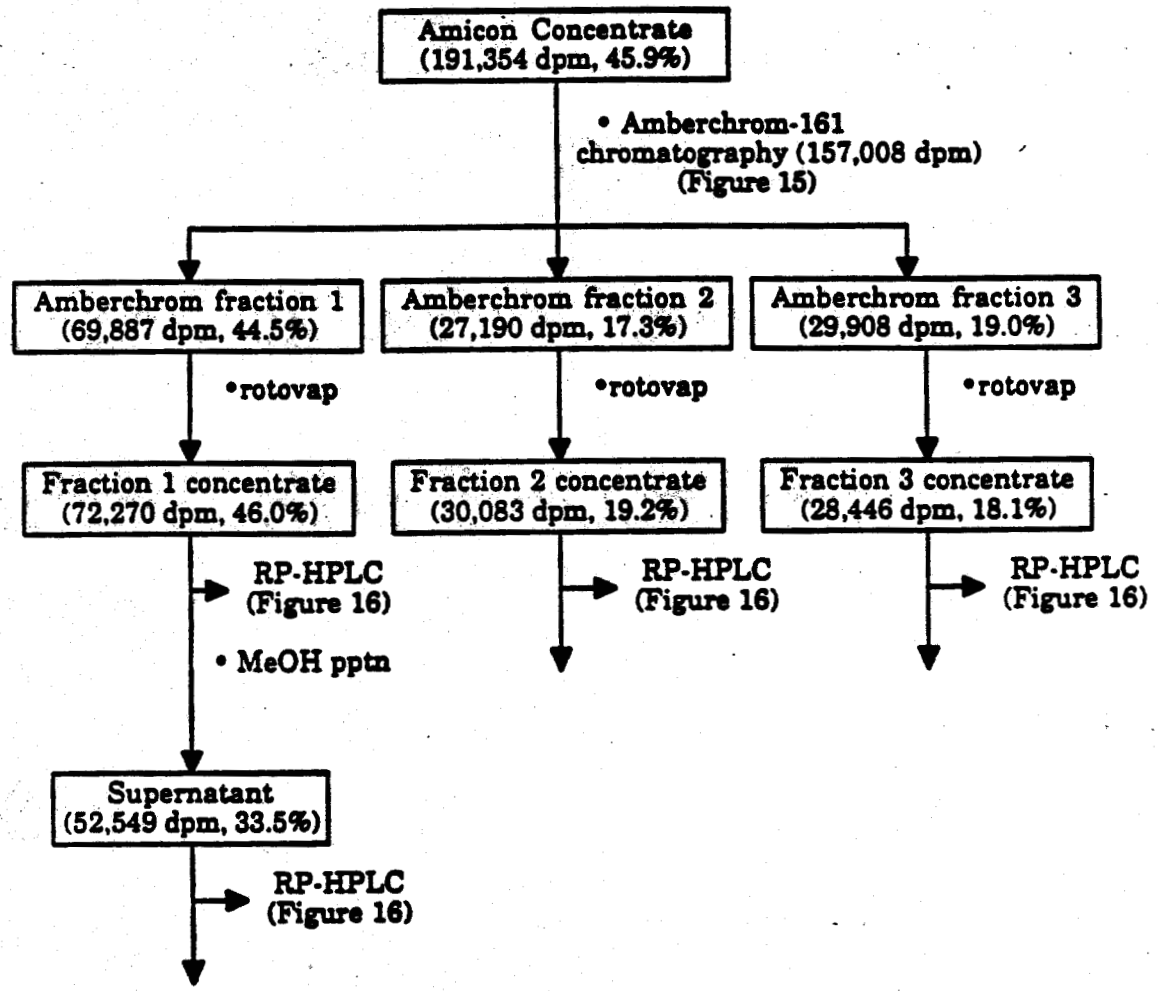


Figure 13. continued

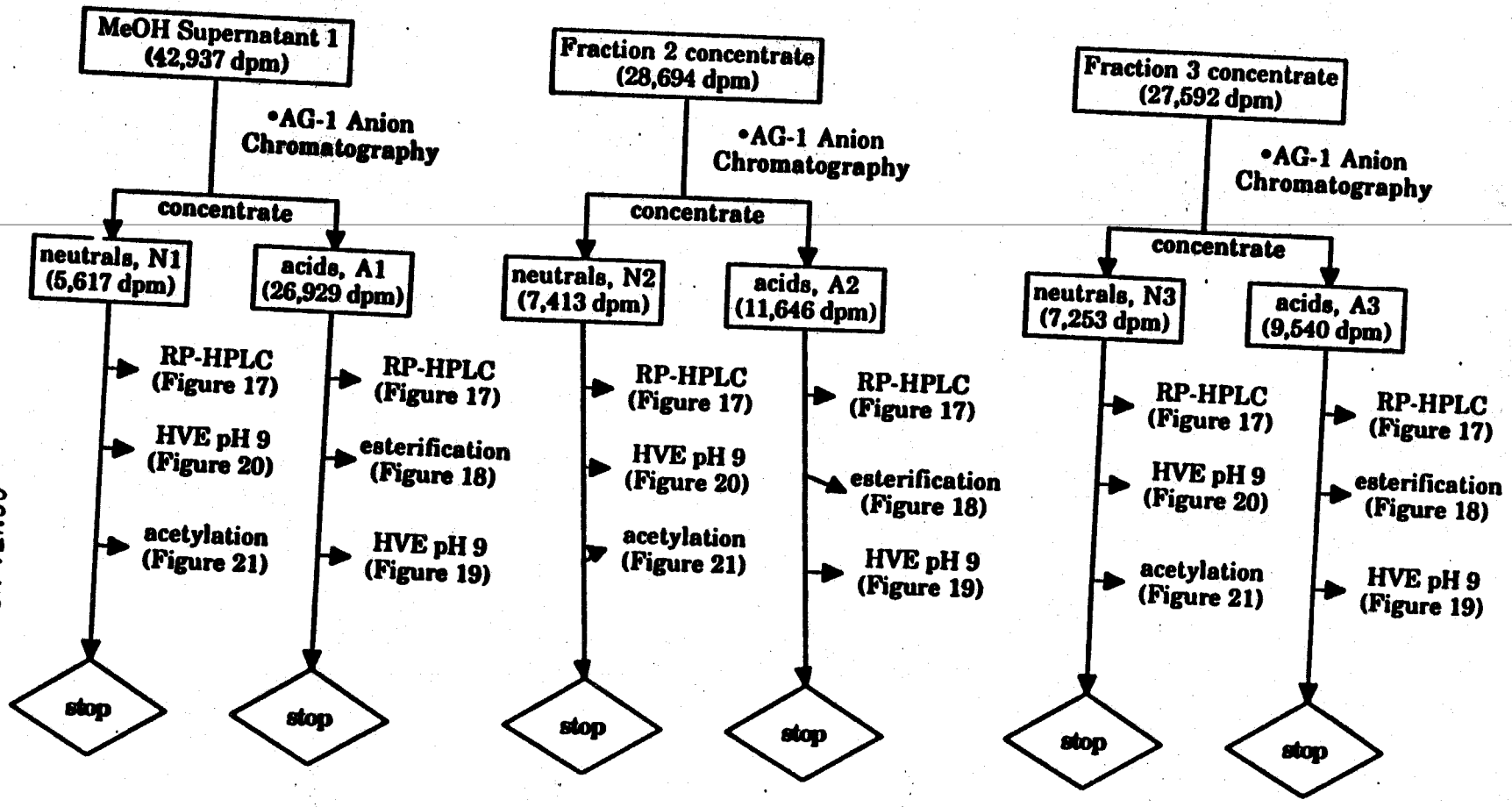


Figure 24. Storage Stability Study on Day 0 and Day 1 Soil; HPLC/LSC Analyses of Storage Stability Samples