Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Halosulfuron (RH-0345)

MRID #: 443527-02

Matrix: Soil

Analysis: GC/NPD

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APPENDIX A

American Cyanamid Company Method M 2509

AMERICAN CYANAMID COMPANY
AGRICULTURAL PRODUCTS RESEARCH DIVISION
HUMAN AND ENVIRONMENTAL SAFETY
P.O. BOX 400
PRINCETON, NEW JERSEY 08543-0400

Recommended Method of Analysis - M 2509

RH-0345 (CL 290,816): GC/NP Method for the Determination of RH-0345 Residues in Soil.

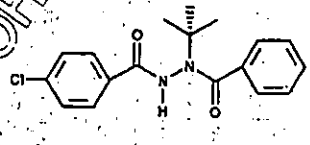
A. **PRINCIPLE:**

Residues of RH-0345 are extracted from soil by shaking with aqueous acid methanol solution. A portion of the extract is then partitioned with methylene chloride after the addition of a 5% sodium bicarbonate aqueous solution. The methylene chloride layer containing the analyte is evaporated to dryness and the extract redissolved in toluene. An aluminum oxide (basic) column clean-up step is performed. The residue fraction from the clean-up column is concentrated to dryness, then dissolved in methanol. The analyte is methylated with 0.2 M trimethylanilinium hydroxide [TMAH] in methanol, the solution is concentrated to dryness, then redissolved in acetone. The quantitation of RH-0345 is accomplished by GC/NP. The results are calculated as RH-0345 by direct comparison of peak heights of samples to those of external standards. The validated sensitivity (LOQ, limit of quantitation) of this method is 10 ppb.

B. **REAGENTS**: (Items from other manufacturers may be used provided they are functionally equivalent.)

1. Analytical Standard: RH-0345 (CL 290,816), analytical grade of known purity, obtained from Rohm and Haas Company, Spring House, PA 19477-0904.

   a. RH-0345: N-4-chlorobenzoyl-N'-benzoyl -N'-tert-butylhydrazine.



2. Solvents: B & J Brand High Purity Solvent, Baxter, Burdick and Jackson, Muskegon, Michigan.

   a. Methylene chloride, Catalog Number 300-4
   b. Methanol, Catalog Number 230-4
   c. Toluene, Catalog Number 347-4
   d. Ethyl acetate, Catalog Number 100-4
   e. Acetone, Catalog Number 010-4

3. Deionized water: Water passed through Millipore's Milli-Q-Plus ultra pure water system. Use this water for all steps.

4. Chemicals:

   a. Aluminum oxide (basic), powder, Brockmann Activity I, Baker Analyzed, Catalog Number 0539-01.

   b. Activated carbon, Darco G-60, 100 mesh, powder, Aldrich Chemical Company, Catalog Number 24,227-6.

   c. Hydrochloric acid, concentrated, 36.5-38.0%, Baker Analyzed, Catalog Number 9535-1.

   d. 0.2 M trimethylanilinium hydroxide, [TMAH], Supelco, Catalog Number 3-3097.

   e. Sodium chloride, crystal, Baker Analyzed, Catalog Number 3624-05.

   f. Sodium bicarbonate, powder, Baker Analyzed, Catalog Number 3506-01.

   g. Sodium sulfate, anhydrous, Baker Analyzed, Catalog Number 5-3891.

5. Solutions:

   a. 0.5 N Hydrochloric acid: Dilute 41.5 mL of concentrated hydrochloric acid to 1 liter with deionized water. Mix well.

   b. Extraction solvent 70% Methanol/30% 0.5N HCL (v/v): Add 30 mL of 0.5 N HCl to 70 mL of methanol and mix well.

   c. 5% Sodium bicarbonate: Dissolve 50 grams of sodium bicarbonate powder in 1 liter of deionized water and mix well.

d. 2% Sodium chloride: Dissolve 20 grams of sodium chloride crystals in 1 liter of deionized water and mix well.

e. 80% Toluene/20% Ethyl Acetate: Add 100 mL of ethyl acetate to 400 mL of toluene (v/v) in a 500-mL graduated mixing cylinder and mix well.

f. 50% Toluene/50% Ethyl Acetate: Add 500 mL of ethyl acetate to 500 mL of toluene (v/v) in a 1-L graduated mixing cylinder and mix well.

g. Purified TMAH: Add 50 mL of methylating reagent (0.2 M TMAH) to a 125-mL Erlenmeyer flask containing 5 grams of activated carbon powder (Darco G-60, 100 mesh). Add stirring bar, stir for 15 minutes and allow to stand for 5 minutes. Filter the mixture through a 100 mm dia., porcelain Buchner filter funnel (Coors No. 60243) containing a double layer of 7-cm Whatman 934-AH, glass microfiber filter paper, which should be thoroughly wet with methanol. Wash the charcoal filter cake with 5 mL of methanol, combining the wash and the initial filtrate. Repeat the filtering process one more time. Transfer the filtrate to a 50-mL graduated cylinder and adjust the volume to 50 mL methanol (See Note 1. in Section O.).

C. APPARATUS: (Items from other manufacturers may be used provided they are functionally equivalent.)

1. Balance, Analytical: Mettler AT261 Delta Range, readable to 0.00001 g.

2. Balance, Pan: Sartorius Model 610 or equivalent, readable to 0.01 g.

3. Chromatography Columns: Glass 250 mL, 250 mm length x 15 mm I.D Teflon stopcock, 250 mL reservoir. Kontes, Article Number 420280-0222.

4. General Laboratory Glassware: Assorted beakers, graduated cylinders, 500-mL filtering flasks, volumetric flasks, and volumetric pipettes (See Note 2. in Section O. for cleaning glassware).

5. Rotary Evaporator: Buchi Model RE-121C, equipped with water bath maintained about 45°C.

6. Vacuum Pump and Controller: Buchi Vacobox B-171. Brinkmann Instruments, Westbury, NY.

7. Separatory Funnels: 500-mL capacity with stoppers.

8. Evaporation Flasks: 25- and 500-mL, round bottom or pear-shaped.

9. Mixing Cylinders: 500-mL capacity with stopper.

10. Gas Chromatograph equipped with an NP Detector: Hewlett Packard Model HP 5890A Gas Chromatograph with NP-Detector interfaced to a Perkin Elmer Data System.

11. GC/NPD Column: 182 cm x 2 mm I.D. glass, packed with 3% OV-17 on 100/120 mesh Supelcoport, Catalog Number 1-1754, Supelco Inc.

12. Centrifuge bottle: A 250-mL canted neck centrifuge bottle (approximately 5.5 cm x 15 cm) with sealing cap, polypropylene, Sorvall, Catalog Number 03069.

13. Reciprocating Lab Shaker: Eberbach Model 6000, Baxter Catalog Number S1105.

14. Centrifuge: Sorvall RC-5B Automatic Superspeed Refrigerated Centrifuge.

15. Filter Funnel: Whatman disposable filter funnel, grade 934-AH, glass microfibre. Whatman Catalog Number 1920-1827.

16. Ultrasonic Bath: Branson Model 2200.

17. Microliter Syringe: 250-µL, Hamilton Company, Reno, Nevada.

18. Disposable Pipets: Pasteur type, 9 inch length, Baxter Catalog Number P5201-2

19. Chromatography Vials: Clear Target Crimp Top Vials, 2 mL (12 x 32 mm) with Target Micro-Serts, 200 mcL, Flat Bottom Inserts and 11 mm Crimp Top Seals with teflon/red rubber septum, National Scientific Company, Catalog Number C4011-1, C4011-631 and C4011-1A, respectively.

20. Glasswool: Pyrex Fiber Glass, Sliver 8 micron, Corning Glass Works Catalog number 3950.

21. PCC-54 Detergent: concentrated, from Pierce Chemical, Rockford, IL

D. **PREPARATION OF STANDARD SOLUTIONS:** (All standards should be stored refrigerated in amber bottles.)

In order to continually monitor the stability of RH-0345 in solution, equivalent concentrations of freshly prepared standard solutions should always be compared chromatographically to the previously prepared standard solutions. Initially, if existing solutions are not available, equivalent concentrations of solutions prepared from duplicate weighings of each standard should be compared.

1. Stock Solution: (Prepare every two months)

Accurately weigh a known amount of RH-0345 (approximately 10 mg) analytical standard into a 100-mL volumetric flask and record the exact weight. Dilute to the mark with methanol, mix well, calculate, and record the exact concentration, correcting for standard purity (approximately 100 mcg/mL). Sonicate solution if necessary to aid dissolution.

2. Non-Methylated Fortification Standard Solutions: (Prepare every two months)

a. Pipet an appropriate amount of the stock solution prepared in D.1. to deliver 1000 mcg of RH-0345 into a 50-mL volumetric flask. Dilute to the mark with methanol and mix well. This solution contains 20.0 mcg/mL RH-0345.

b. Pipet an appropriate amount of the stock solution prepared in D.1. to deliver 1000 mcg of RH-0345 into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. This solution contains 10.0 mcg/mL RH-0345.

c. Pipet 10.0 mL of the 20.0 mcg/mL standard solution prepared in D.2.a. to deliver 200 mcg of RH-0345 into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. This solution contains 2.0 mcg/mL RH-0345.

d. Pipet 10.0 mL of the 10.0 mcg/mL standard solution prepared in D.2.b. to deliver 100 mcg of RH-0345 into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. This solution contains 1.0 mcg/mL RH-0345.

e. Pipet 4.0 mL of the 10.0 mcg/mL standard solution prepared in D.2.b. to deliver 40 mcg of RH-0345 into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. This solution contains 0.40 mcg/mL RH-0345.

f. Pipet 2.0 mL of the 10.0 mcg/mL standard solution prepared in D.2.b. to deliver 20 mcg of RH-0345 into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. This solution contains 0.20 mcg/mL RH-0345.

g. Pipet 1.0 mL of the 10.0 mcg/mL standard solution prepared in D.2.b. to deliver 10 mcg of RH-0345 into a 100-mL volumetric flask. Dilute to the mark with methanol and mix well. This solution contains 0.10 mcg/mL RH-0345.

3. Methylated Linearity and Working Standard Solutions for GC/NP: (Prepare Daily)

   a. Pipet 1.0 mL of each of non-methylated fortification standard solutions prepared in D.2.e. thru D.2.g. into separate 25-mL pear-shaped flasks.

   b. Add 150 µL of 0.2 M trimethylanilinium hydroxide (TMAH) in methanol to each solution and place the flasks in an ultrasonic bath for 15 seconds. Remove the flask from the bath.

   c. Evaporate the methanol solutions to dryness on a rotary evaporator.

   d. Dissolve the residues in 1 mL of acetone and place the flask in an ultrasonic bath for 45 seconds. Remove the flask from the bath. **The concentration of the methylated linearity standards for GC/NP are 0.40, 0.20, and 0.10 mcg/mL, respectively. The 0.20 mcg/mL is also used as working standard for GC/NP analysis.**

   e. Transfer the solutions from step D.3.d. into a GC vials with a Pasteur type pipet. Seal the vials with a cap and crimp. The sample is ready to proceed to the GC/NP analysis.

E. GAS CHROMATOGRAPHIC (GC/NP) CONDITIONS:

1. Instrument:

   a. Gas Chromatograph: Hewlett Packard HP 5890 or equivalent.

   b. Detector: Nitrogen Phosphorous Detector

   c. Data System: Perkin Elmer

2. Column: 182 cm x 2 mm I.D.glass, packed with 3% OV-17 on 100/120 mesh Supelcoport

3. Instrumental Settings:

a.	Injection Temperature	300°C
b.	Detector Temperature	280°C
c.	Oven Temperature	260°C (Oven temperature may vary by 5°C, depending on the column.)

d. Carrier — Helium, at about 25 mL/min

e. Detector Gases — Hydrogen: 3-4 mL/min
Air: 100-120 mL/min

F. LINEARITY CHECK:

The linearity of response of the GC/NP must be checked at least once for each related group of analysis. Linearity must also be confirmed following any change of column, modification of the instrument, or significant alteration of the chromatographic conditions. The linearity of response is checked by injecting all three methylated standards as specified in Section D.3.d. The response ratio (peak response divided by amount of standard injected) for each standard injection is calculated and compared to the average response ratio.

1. Adjust the GC/NP conditions to attain a peak height of 30-50% full-scale deflection for a 1-ng injection of methylated standard.

2. Inject 5-µL aliquots of methylated standard solutions prepared in step D.3.d

3. Determine the peak response of RH-0345 for each standard chromatogram. Calculate the response ratio for the standards by dividing the peak response (height) by the mass (nanograms) of standard injected. Calculate the average response ratio. Significant departure from linearity over this range as indicated by deviation of any response factor from the average response factor greater than 15% indicates instrumental or experimental difficulties which must be corrected before proceeding.

G. SAMPLE PREPARATION:

Samples should be prepared following the current versions of either American Cyanamid Company SOP MREE.R.0508, MREE.R.0509 or MREE.R.0510

H. RECOVERY TEST:

The efficiency of this procedure should always be demonstrated by recovery tests prior to attempting the analysis of any unknown sample. At least two concurrent fortified control samples must be analyzed with every eight samples analyzed. The fortification levels chosen for a study should include the validated sensitivity (LOQ, Limit of Quantitation, 10 ppb) of this method and should bracket the sample concentration range expected or found. If only a single fortified control is run, it should be at the LOQ of the method. The volume of the fortification standard added (in methanol) should be kept to a minimum.

1. Place a 40-g soil sample into a 250-mL canted-neck centrifuge bottle.

2. Add to the soil by pipet or microliter syringe the appropriate volume of the Non-Methylated Fortification Standard Solution appropriate to the fortification level to be tested. Suggested volumes of the Non-Methylated Fortification Standard Solutions (Section D.2) that will yield various levels of fortification on a 40-g soil sample are listed below:

Fortification Level (ppb)	Standard Used	Fortification Solution Volume
1000	20 mcg/mL	2.0 mL
500	20 mcg/mL	1.0 mL
100	2.0 mcg/mL	2.0 mL
50	2.0 mcg/mL	1.0 mL
10	0.40 mcg/mL	1.0 mL

3. Proceed with the Sample Extraction, Sample Partitioning, Aluminum Oxide (Basic) Column Clean-up and Methylation of Sample steps, beginning with step I.1.

I. **SAMPLE EXTRACTION:** (See Note 3. in Section O.)

1. Weigh 40 grams of soil into a 250-mL canted neck centrifuge bottle and add 200 mL of extraction solution (70% methanol/30% 0.5N HCl, v/v).

2. Place the centrifuge bottle on a reciprocating shaker on high speed (approx. 200 cycles per second) for 25 minutes.

3. Remove the centrifuge bottle from the reciprocating shaker and place the bottle into a centrifuge and centrifuge at about 4300 rpm for 5 minutes.

4. Filter the clear supernatant with vacuum through a Whatman disposable filter funnel, grade 934-AH, glass microfibre into a 500-mL filtration flask.

5. Add 200 mL of extraction solution to the centrifuge bottle and resuspend the soil by mixing.

6. Repeat steps I.2., I.3. and I.4. and combine the filtrates.

7. Transfer the combined filtrate into a 500-mL mixing graduated cylinder. Rinse the 500-mL filtration flask with extraction solution and add to the filtrate in the 500-mL mixing graduated cylinder and dilute to 400-mL.

8. Proceed with the Sample Partitioning steps beginning with step J.1.

J. SAMPLE PARTITIONING:

1. Transfer a 200-mL aliquot of the sample filtrate solution from step I.7. to a 500-mL round bottom flask. Concentrate the sample filtrate solution to about 100 mL, using a rotary evaporator equipped with a water bath set at about 45°C. (Watch carefully for "bumping".)

2. Transfer the concentrated sample filtrate solution into a 500-mL separatory funnel. Rinse the 500-mL round bottom flask with 5 mL of methanol and transfer the rinse into the separatory funnel.

3. Add 100 mL of 5% sodium bicarbonate solution to the separatory funnel and gently mix the sample for 30 seconds. Wait for a minute after mixing.

4. Add 150 mL of methylene chloride to the separatory funnel and gently partition the sample for 30 seconds and allow the phases to separate. Any emulsions that form can be broken mechanically with a stirring rod or by adding 80 mL of 2% sodium chloride solution.

5. Drain the lower (methylene chloride) layer into a 500-mL round bottom flask.

6. Partition the upper aqueous phase one additional time with 150 mL of methylene chloride and combine the methylene chloride fractions in the 500-mL round bottom flask.

7. Use a rotary evaporator equipped with a water bath set at about 45°C to evaporate the methylene chloride to dryness.

8. Remove any traces of methylene chloride or moisture by adding 2-3 mL of methanol to the flask and evaporate the sample to dryness.

9. Dissolve the residues in 25 mL of toluene and place the flask in an ultrasonic bath for 30 seconds. Remove the flask from the bath in preparation for Aluminum Oxide (Basic) Column Clean-up (Section K).

K. ALUMINUM OXIDE (BASIC) COLUMN CLEAN-UP:

1. A small glasswool plug is inserted into a 15 mm I.D. x 250 mm chromatographic column with a 250 mL reservoir and the column is dry packed with 15 mL (or 13.2 g) of the aluminum oxide (basic) (See Note 4. in Section O.).

2. Top the column with 3 mL of anhydrous granular sodium sulfate.

3. Condition the column by adding 25 mL of toluene and elute to the top of the sodium sulfate bed. **Do not allow the column to run dry!**

4. Transfer the dissolved residue (Section J.9.) solution to the column and elute to the top of the sodium sulfate. Discard the loading solution.

5. Rinse the flask with 50 mL of toluene/ethyl acetate (80/20; v/v) and then transfer this solution to the column and elute to the top of the sodium sulfate. **Do not allow the column to run dry!**

6. Collect all washes and discard.

7. Elute the analyte by adding 200 mL of ethyl acetate/toluene (50/50; v/v) to the flask, swirl to mix, then transfer to the column.

8. Collect the eluant in a 500-mL round bottom flask and let the column run dry.

9. Evaporate the eluant to dryness on a rotary evaporator equipped with a water bath set at about 45°C.

10. Dissolve the residues with 2 x 5 mL rinses of methanol and transfer to a 25-mL pear-shaped flask. Evaporate the combined methanol rinses to dryness on a rotary evaporator equipped with a water bath set at about 45°C then proceed with the methylation of the sample (Section L).

L. METHYLATION OF SAMPLE:

1. Dissolve the residues in Section K.10. in 1 mL of methanol and place the flask in an ultrasonic bath for 30 seconds. Remove the flask from the bath.

2. Add 150 µL of 0.2 M trimethylanilinium hydroxide (TMAH) in methanol to the solution and place the flask in an ultrasonic bath for 15 seconds. Remove the flask from the bath.

3. Evaporate the combined methanol solutions to dryness on a rotary evaporator equipped with a water bath set at about 45°C.

4. Dissolve the residues in 1 mL of acetone and place the flask in an ultrasonic bath for 45 seconds. Remove the flask from the bath.

5. Transfer the solution from step L.4. into a GC vial with a Pasteur type pipet. Seal the vial with a cap and crimp. The sample is ready to proceed to the GC/NP analysis (Section M.). The remainder of the sample should be stored in a refrigerator.

M. GAS CHROMATOGRAPH WITH NITROGEN PHOSPHOROUS DETECTOR (GC/NP) ANALYSIS:

1. Assess the RH-0345 retention time and response using the GC/NP conditions specified in Section E. If necessary, minor adjustments may be made to the GC/NP conditions to more closely match the chromatographic response shown in Figure 1 to 4.
2. Obtain a stable GC/NP response by injecting 5-µL aliquots of the methylated 0.20 mcg/mL RH-0345 standard to condition the column and the NP detector.
3. Establish the linearity of the RH-0345 response as described in section F.
4. Make a standard injection (methylated 0.20 mcg/mL) after every two 5-µL sample injections and use the average peak height of the standards before and after the sample injections for quantitation of the RH-0345 residues.
5. Compare the peak height of the sample with those obtained from a 5-µL injection of the RH-0345 standard (methylated 0.20 mcg/mL).
6. If a sample peak goes off-scale or the response is above the highest linearity standard, pipet an appropriate aliquot of the sample into a 25-mL pear-shaped flask, using an appropriate syringe. Add 150 mcL of methylating agent and 10 mL of methanol to the flask. Sonicate and evaporate the solution to dryness on a rotary evaporator equipped with a water bath set at about 45°C. Redissolve the residue in 1.0 mL of acetone, sonicate and reinject. The dilution factor (DF) is then included in the calculations (See Section N).

N. CALCULATIONS:

Calculate the apparent RH-0345 residue in ppb in the injected samples from the sample peak height response and the average peak height response of the working standard immediately preceding and immediately following the samples as follows:

$$PPB = \frac{R(SAMP) \times V1 \times V3 \times C(STD) \times V5 \times DF \times 1000}{R(STD) \times V2 \times W \times V4}$$

$$\% \text{ RECOVERY} = \frac{\text{PPB FOUND} \times 100}{\text{FV} \times \text{FC} \times 1000/\text{W}} = \frac{\text{PPB FOUND} \times 100}{\text{PPB ADDED}}$$

Where:

R(SAMP) = Sample Response (chromatographic response in peak height units).

R(STD) = Average Standard Response (average chromatographic response for the peak of interest of the working standard chromatograms in peak height units).

W = Weight of the sample in grams (40 grams).

V1 = Total volume of extraction solvent in mL (400 mL).

V2 = Aliquot of extract taken for analysis in mL (200 mL).

V3 = Volume of solvent added to dissolve residues for final GC/NP analysis (1.0 mL).

V4 = Volume of sample solution injected in µL (5 µL).

V5 = Volume of working standard solution injected in µL (5 µL).

C(STD) = Concentration of working standard solution injected (0.20 mcg/mL).

DF = Dilution factor.

FV = Fortification volume in mL.

FC = Fortification concentration in mcg/mL

1000 = A conversion factor from mcg to ng

Typical chromatograms are shown in Figure 1.

O. NOTES ON THE METHOD:

1. Each lot of methylating reagent must be tested for potential impurities which could interfere with RH-0345 determination. Prepare a reagent blank by adding 150 µL of methylating reagent to 1 mL of methanol in a 25-mL pear shaped flask. Evaporate to dryness and dissolve the residue in 1.0 mL of acetone. The injection of 5 µL of this reagent blank into the GC/NP as described in Section M. will determine if any interfering impurities are present before proceeding with F.1. and L.1..

The purified methylating reagent should be re-tested at reasonable intervals to make sure that impurities do not reappear.

2. The directions for cleaning all glassware used for this method are as follows:

   a. Using PCC-54 detergent concentrate from Pierce Chemical, Rockford, IL; add 20 mL of PCC-54 concentrate to 1-liter of deionized water.

   b. Completely immerse the glassware in the working solution, and soak the glassware from 3 to 24 hours at room temperature.

   c. Remove the glassware from the PCC-54 working solution and immediately rinse well with deionized water. Proper rinsing is needed to insure total removal of detergent, preventing formation of any film. The final rinse should be done with methanol.

   d. Change the PCC-54 working solution often to maintain good cleaning power. The pH of the solution will drop as the solution loses cleaning power (So check the pH of the solution once in a while).

3. Soil samples should be processed through the end of the aluminum oxide (basic) column clean-up (Step K.10.) without interruption.

4. It is very important to evaluate each new lot of aluminum oxide (basic) (used for sample clean-up) consistency by running standards through the elution scheme and determining the percent recovery by GC/NP (after the sample has been methylated.). Recovery should be greater than 80%. Should the recovery be lower than 80%, obtain a new lot and evaluate it.

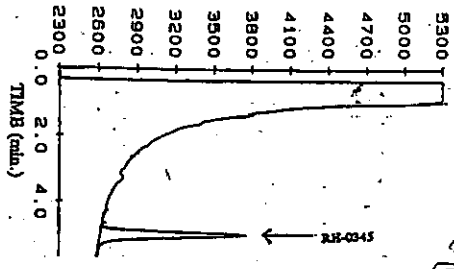
Approvals:

A. Khunachak — 5/16/96
Author — Date

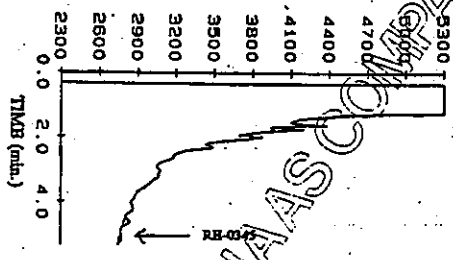
G. L. Picard — 5/16/96
Group Leader — Date

BG/ak/ct

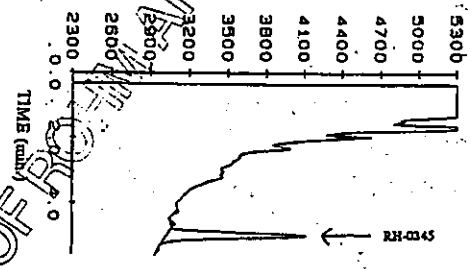
Figure 1: Typical Chromatograms for the Determination of RH-0345 Residues in Tippecanoe Soil



RH-0345 (CL 290,816) Standard, 1 ng injected (5 µL of 0.20 mcg/ mL)

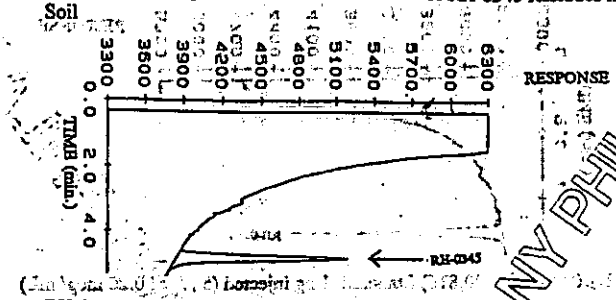


Control Tippecanoe Soil (Sample # AC6105.37A, Maxim Queue # 6110101A), 100 mg equivalent of soil sample injected, <1.01 ppb Apparent RH-0345 (CL 290,816) Found

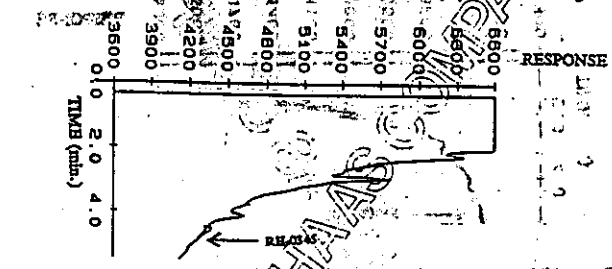


Control Tippecanoe Soil fortified with RH-0345 (CL 290,816) at 10 ppb (Sample # AC6105.37A, Maxim Queue # 6110102A), 100 mg equivalent of soil sample injected, 9.22 ppb RH-0345 Found, 92% Recovered.

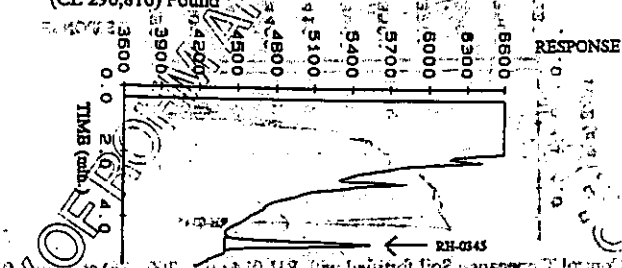
Figure 2: Typical Chromatograms for the Determination of RH-0345 Residues in Beardon Soil



RH-0345 (CL 290,816) Standard, 1 ng injected (5 µL of 0.20 mcg/ mL)

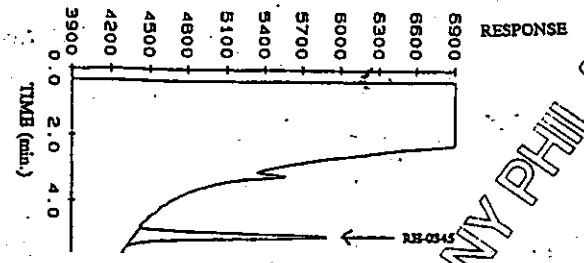


Control Beardon Soil (Sample # AC6105.37B, Maxim Queue # 6110301B), 100 mg equivalent of soil sample injected, <0.867 ppb Apparent RH-0345 (CL 290,816) Found

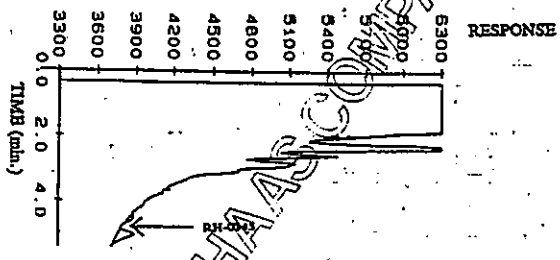


Control Beardon Soil fortified with RH-0345 (CL 290,816) at 10 ppb (Sample # AC6105.37B, Maxim Queue # 6110302B), 100 mg equivalent of soil sample injected, 8.20 ppb RH-0345 Found, 82% Recovered.

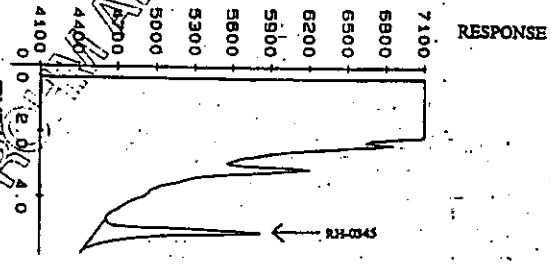
Figure 3: Typical Chromatograms for the Determination of RH-0345 Residues in Sharkey Soil



RH-0345 (CL 290,816) Standard, 1 ng injected (5 µL of 0.20 mcg/ mL)

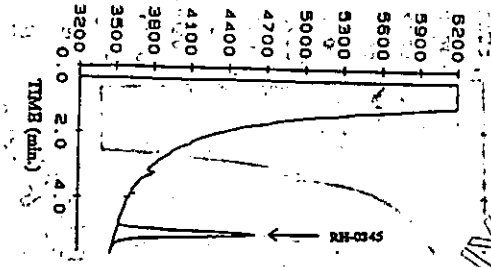


Control Sharkey Soil (Sample # AC6105.37C, Maxim Queue # 6110201A), 100 mg equivalent of soil sample injected, <1.09 ppb Apparent RH-0345 (CL 290,816) Found

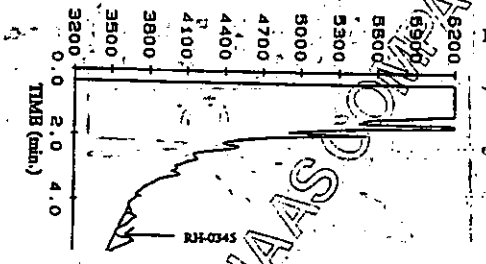


Control Sharkey Soil fortified with RH-0345 (CL 290,816) at 10 ppb (Sample # AC6105.37C, Maxim Queue # 6110202B), 100 mg equivalent of soil sample injected, 8.67 ppb RH-0345 Found, 87% Recovered.

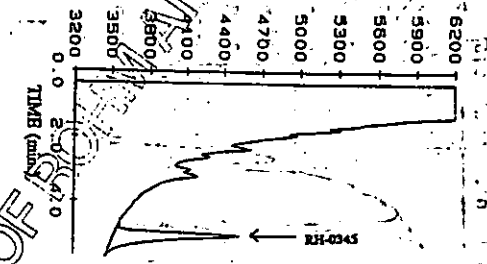
Figure 4: Typical Chromatograms for the Determination of RH-0345 Residues in Buelah Soil



RH-0345 (CL 290,816) Standard, 1 ng injected (5 µL of 0.20 mcg/ mL)



Control Buelah Soil (Sample # AC6105.37D, Maxim Queue # 6110401A), 100 mg equivalent of soil sample injected, <1.11 ppb Apparent RH-0345 (CL 290,816) Found



Control Buelah Soil fortified with RH-0345 (CL 290,816) at 10 ppb (Sample # AC6105.37D, Maxim Queue # 6110402A), 100 mg equivalent of soil sample injected, 9.24 ppb RH-0345 Found, 92% Recovered.

DEVIATIONS:

Any deviation from this protocol, or relevant laboratory SOPs, shall be described by a deviation statement as soon as possible. All SOP or protocol deviations will be signed and dated by the Principal Analyst and/or the Study Director. All protocol deviations must be maintained with the protocol. All SOP deviations must be maintained with the raw data files. Any deviations must include the reason for change and the effect of the change on the outcome of the study.

QUALITY ASSURANCE:

Quality assurance shall be the responsibility of the Test Site and shall be carried out in accordance with applicable GLP regulations and Standard Operating Procedures of the Test Site. The Quality assurance Unit of the Test Site must provide timely written reports of all inspections to the Study Director and American Cyanamid Company Management. A statement signed by the Quality Assurance manager or designee, listing the phases inspected and the inspection dates, will be included in the analytical phase report. The American Cyanamid US Quality Assurance Group will review the protocol and final report and will provide an additional Statement of Quality Assurance.

GLP COMPLIANCE:

The study will be performed in compliance with the Good Laboratory Practice Standards as specified in 40 CFR Part 160. A Statement of Compliance or noncompliance with Good Laboratory Practices will be signed by the Study Director at the conclusion of the study and included in the final report.

APPROVALS:

Residue Chemistry Group Leader:

Gerald L. Picard — 10/3/95 Date

Study Director:

Alisa Khmelnitsky — 10/3/95 Date

CYANAMID

RESIDUE SUPPORT STUDY PROTOCOL AMENDMENT

Copy To: MREE File
US Quality Assurance Group
Laboratory Personnel
Those Signing Protocol
Manager, Residue Chemistry

PROTOCOL NUMBER: RH95PT04

AMENDMENT NUMBER: 01

TITLE:

Validation of GC/MSD Method M 2509 for the Determination of Residues of RH-0345 (CL 290,816) Residues in Soil

AMENDMENT(S) TO BE MADE:

The new control Sharkey soil (Sample # AC6105.37C) and control Tippeecanoe soil (Sample # AC6105.37A) are assigned to be used inplace of the control Sharkey soil (Sample # AC6794.117A) and control Tippeecanoe soil (Sample # AC6794.117B) stated in the protocol.

REASON FOR AMENDMENT(S):

These two control soil samples are no longer available.

IMPACT ON STUDY:

None

APPROVALS:

Residue Chemistry II Group Leader: Gerald L. Picard 10/4/95 Date

Study Director: Alisa Khunachak 10/4/95 Date