Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Cyclanilide

MRID #: 438683-33

Matrix: Soil

Analysis: HPLC/UV

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RPA 90946

METHOD OF ANALYSIS FOR THE DETERMINATION OF RPA 90946 (cyclanilide) AND ITS METABOLITE 2,4-DICHLOROANILINE FROM SOIL

I. INTRODUCTION

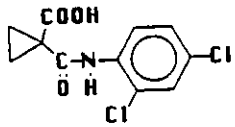
A. Scope

This method describes the procedure for determining the possible residue of RPA 90946 and its major metabolite 2,4-dichloroaniline in soil. The method was verified by fortifying soil samples from Mississippi, Texas, California and North Carolina at 10, 50 and 100 ppb levels and obtaining recoveries at the 70-95% level.

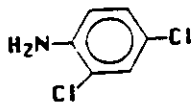
B. Principle

Residues are extracted from soil with methanol:water (1:1), and then partitioned into dichloromethane. The aniline partition is done at ≥ pH10 while the RPA 90946 partition is done at ≤pH3. The fractions are kept separate and no further clean up of RPA 90946 is needed. However the 2,4-dichloroaniline is derivatized to the 2-chloropropionamide (2,4-DCPA) with 2-chloropropionyl chloride in the presence of triethylamine used as an acid scavenger. Further purification of the 2,4-DCPA is accomplished by silica gel chromatography. Following solvent evaporation, quantification of RPA 90946 and 2,4-DCPA is performed by HPLC using UV detection (255nm). The method was verified by fortifying soil from North Carolina, California, Mississippi and Texas at 10, 50 and 100 ppb levels.

C. Structures



RPA090946



2,4-dichloroaniline

II. MATERIALS

A. Equipment

1. Polyethylene, 250mL wide mouthed screw-capped bottles (Nalge Co.) or equivalent
2. Atlab horizontal shaker (Arthur H. Thomas Co.) or equivalent
3. Separatory funnels, 1L
4. Centrifuge, Marathon 10K (Fisher) or equivalent
5. Rotary Evaporator (Buchi) or equivalent
6. Chromatography columns,11 mm. i.d. (Kontes) or equivalent
7. Zymark Turbo Vap II concentration workstation or equivalent.
8. Glass wool
9. 500ml Flat or Round Bottom Flask
10. 500ml Erlenmyer Flask

B. Reagents

1. Methanol, B&J ChromPure HPLC Solvent (Burdick & Jackson) or equivalent
2. Water, OmniSolv (EM Science) or equivalent
3. Dichloromethane, B&J ChromPure HPLC Solvent (Burdick &Jackson) or equivalent
4. 2-Chloropropionyl chloride, 99% (Aldrich) or equivalent
5. Triethylamine, 99+% (Aldrich) or equivalent
6. Hexane, UV B&J Brand High Purity Solvent (Burdick & Jackson) or equivalent
7. Ethyl ether, anhydrous GR (EM Science) or equivalent
8. Silica gel 63-200, 60A (ICN Biomedicals) or equivalent
9. Sodium hydroxide pellets, 'Baker Analyzed' Reagent, (J. T. Baker) or equivalent

10. Hydrochloric acid, 37% (EM Science) or equivalent
11. Glacial acetic acid . (EM Science) or equivalent
12. Milli-Q water or equivalent
13. 2,4-Dichloroaniline (2,4-DCA), 99.4% (Rhône-Poulenc Ag. Co.)
14. Cyclanilide (RPA90946), 99.7% (Rhône-Poulenc Ag. Co.)
15. N-(2,4-dichlorophenyl)-2-chloropropionamide (2,4-DCPA), 100% (Rhône-Poulenc Ag. Co.)

C. Extraction Solution

1. 1:1- approximately equal volumes of methanol and water

D. Sodium Hydroxide Solution

1. Dissolve 1 gram sodium hydroxide pellets per 100ml water.

E. 20µg/ml Solution of Triethylamine in Dichloromethane

1. Add ~0.3mL triethylamine to a 100mL volumetric flask. Dilute to the mark with dichloromethane and invert until thoroughly mixed.
2. Pipet 10ml of the solution from E.1 into a 100mL volumetric flask and dilute to the mark with dichloromethane. Thoroughly mix. This is a 20µg/ml solution.

III. STANDARDS

The stated concentrations of standard solutions should be adjusted to account for the purity of the neat solid standards.

A. Fortification Solution

1. Weigh 0.10XX g. of 2,4-dichloroaniline and RPA 90946 into a 100mL volumetric flask and dilute to the mark with methanol. The concentration of this standard is 10XX µg/mL for each analyte.

2. Withdraw a 1.0mL aliquot from this solution and add to a 100mL volumetric flask. Dilute to the mark with methanol. The concentration of this standard is 10.XX µg/mL for each analyte.
3. By further dilution of the 10µg/mL standard with methanol, prepare a series of standards to serve as fortification solutions.
4. After preparation, standards should be transferred from the volumetric flasks into screw-capped brown bottles to prevent possible photodegradation.
5. Store standards in the refrigerator at ~5°C when not in use.
6. Other dilutions may be used as required.
7. The analyte standards may be weighed out or diluted separately and used separately for fortification.

B. Calibration Standard Solutions

1. Weigh 0.10XXg of N-(2,4-dichlorophenyl)-2-chloropropionamide (2,4-DCPA) into a 100mL volumetric flask and dilute to the mark with methanol. The concentration of this standard is 10XX µg/mL.
2. Withdraw a 1.0mL aliquot from this solution and add to a 100mL volumetric flask. Dilute to the mark with methanol. The concentration of this standard is 10.XX µg/mL.
3. By further dilution of the 10µg/mL standard with MeOH:H_2O:acetic acid (50:50:2), prepare a series of standards to serve as calibration solutions.
4. Repeat the above standard preparation with RPA 90946 using the same volumes, weights and reagents.
5. Other dilutions may be used as required.

C. Prequalification of 2-Chloropropionyl Chloride

The derivatization reagent, 2-chloropropionyl chloride, has shown a tendency to hydrolyze. It should be stored under nitrogen and when the bottle is opened, a nitrogen blanket should be provided. The reagent should be prequalified weekly or as the need arises (when derivatization yields are <70%) according to the following procedure

Note: Dilutions and concentrations other than those listed below may be used as required.

1. Add 1.5mL of a 10µg/mL 2,4-dichloroaniline standard prepared in dichloromethane (prepared as the fortification solutions except using dichloromethane as solvent) to each of two 500mL round-bottomed flasks containing 300mL dichloromethane.
2. Add 5 drops of 2-chloropropionyl chloride to each flask. Swirl, stopper, and let sit for 30 minutes at room temp.
3. Evaporate the solutions to near dryness on a rotary evaporator with the bath temperature not exceeding 30°C and the pressure not exceeding 27 inches of mercury.
4. Dissolve the residues in 50mL MeOH:H_2O:Acetic acid(50:50:2). Swirl.
5. Quantify the residues by HPLC. Compare the areas of the two HPLC analyses with that obtained with two 0.45µg/mL N-(2,4-dichlorophenyl)-2-chloropropionamide standards. If the yield of the derivatization is >85%, then the 2-chloropropionyl chloride is pure enough for use.
6. If the yield of the derivatization is <85%, then the 2-chloropropionyl chloride must be fractionally distilled with one-tenth the volume of quinoline, under nitrogen, using at least a 7 inch Vigreux column. The distillation rate should not exceed 3 drops/sec. The head temperature should be carefully monitored and a forerun collected until the temperature is stable and has reached 106-108°C. More

than one fraction should be collected. The distillation yield will be ~50%. The distillation fractions should be prequalified separately.

IV. METHOD

A. Sample preparation

1. Pulverize soil in a mortar and pestle if necessary.

B. Extraction and Clean Up (see Figure1 for Flowchart of Method)

1. Weigh 50g soil samples into 250mL wide-mouthed polypropylene screw cap bottles.
2. Fortification for the purpose of recovery determination should be done at this point. After fortification, samples should set approximately 10 minutes before addition of the extraction solution.
3. Add 100mL extraction solution (50% MeOH-H_2O) and shake for 10 minutes on the mechanical shaker
4. Centrifuge for 7 minutes at ~3000 rpm. Decant into a 1 L separatory funnel containing 4 ml of 1% NaOH solution.
5. Repeat extraction step (3) one more time with a fresh 100mL 50% MeOH-H_2O. Shake for 10 minutes, centrifuge for 10 minutes at ~4000 rpm. Decant into the 1L separatory funnel.
6. Add 500ml of Milli-Q water to separatory funnel. Swirl. The pH of the solution is confirmed with pH paper. The pH should be ≥ pH10.
7. Extract with 2x100mL dichloromethane. Drain the dichloromethane layers into a 500mL Erlenmeyer flask. (or an appropriate size flask) being careful to avoid the addition of water. The organic and aqueous layers may require ~10 minutes for a complete separation. Save the aqueous layer for further work up later in the method. (step#17)

8. Add 1 mL of the 20 µg/mL solution of triethylamine/DCM from II.E.2 to the dichloromethane extract. Swirl gently. Then add 0.1mL 2-chloropropionyl chloride. Swirl gently to mix, stopper the flask, and let sit 30 minutes at room temperature. The solution can sit overnight if necessary.
9. Decant into a 500mL flat or round-bottomed flask. This is done in order to minimize the transfer of water. Evaporate to ~1mL volume on a rotary evaporator with the bath temperature less than 30°C and the vacuum pressure not greater than 27 inches of mercury vacuum. Some water (~1mL) may be left in the residue.
10. Plug a 25cm high, 11mm i.d. glass chromatography column with glass wool.
11. Slurry pack 3g silica gel into the glass column with hexane.
12. Add 10mL hexane to the residue from step#9 to dissolve the sample. Transfer sample to the silica gel column. Be careful not to add water to the column. Percolate the sample through the column until the liquid level is even with the top of the packing. Discard the eluent. Take care to not allow the column to go dry.
13. Add 10mL more hexane to the sample flask, swirl and transfer to the column. Be careful not to add water to the column. Elute and save the eluent.
14. Elute the 2,4-DCPA from the column with 50ml of ethyl ether. Collect this eluent and combine with the hexane eluent from step 13. Rotary evaporate just to dryness with the bath temperature less than 30°C and the vacuum pressure not greater than 27 inches of mercury vacuum. Remove from the rotary evaporator as soon as the sample is dry. Once the pressure is released a small amount of liquid may recondense in the flask. A *gentle* stream of nitrogen may be applied to complete evaporation.
15. Dilute the residues with mobile phase as necessary to maintain the 2,4-DCPA concentration within the standard curve.
16. Quantify the residues by HPLC.

17. Acidify the aqueous layer from step#7 with 10 drops of concentrated hydrochloric acid. Swirl gently. Confirm by pH paper that the pH is ≤ pH3. Add a few drops more if needed.
18. Extract with 2X100ml dichloromethane. Pass the dichloromethane layer through a small plug of glass wool being held by a funnel. This is done to trap any "rag layer" that might drain from the separatory funnel. Pool the dichloromethane extracts in a 250ml Turbo Vap tube.
19. Evaporate the solvent at 30° C and ~5-10 psi of nitrogen until *just* dry.
20. Dilute the residues with mobile phase as necessary to maintain the RPA 90946 concentration within the standard curve.
21. Quantify the residues by HPLC.

C. Critical Issues

1. Care needs to be taken in steps 7,12 and 13 to eliminate the carryover of water.
2. Loss of recovery may be seen in the 2,4-DCPA fraction due to volatility in evaporation steps 9 and 14 if the sample is allowed to go dry for a long period of time.
3. Steps 16 and 21 are separate injections. Do not combine extracts because interferences in one will affect quantification of the other.

D. HPLC Chromatographic Analysis

1. Waters 717 plus Autosampler (Millipore) complete with Waters 510 HPLC pumps or equivalent
2. Waters 486 Tunable Absorbance Detector (Millipore) or equivalent
3. Waters Data-Capture system, or integrator, or equivalent
4. Column: Zorbax SB-Phenyl 4.6mm x 25cm (MacMod)
5. Guard column: Phenyl Newguard 7 micron 15x3.2mm (Aplied Biosystems) or equivalent
6. In-line filter: Frit 0.062 x 0.25 (Upchurch) or equivalent
7. Oven temperature: ambient

8. Mobile phase: 70:30 methanol/water (H_2O contains 2% acetic acid)
9. Injection volume: 150µL
10. Flow rate: 0.5mL/min
11. Approximate Retention Time: 2,4-DCPA: 14.0 min.
    RPA90946: 19.5 min.
12. Run Time: 45 min.
13. Detector: 255nm.
14. Mobile Phase Gradient:

Time (min)	Methanol (%)	H_2O (%)
0.0	70	30
5.0	70	30
27.0	90	10
28.5	90	10
28.6	70	30

Note: These LC parameters are guidelines and can be optimized for the chromatograph and column actually used.

E. Quantification of Residues

1. Single point calibrations were used to analyze for the 2,4-dichloroaniline (as 2,4-DCPA) in 3 of the 4 soil sites.
2. Linear regression was used to analyze both the RPA 90946 and the 2,4-dichloroaniline (as 2,4-DCPA) in the 4th site.
3. For the single point calibration, residues obtained from soil samples fortified at the 10ppb level were dissolved in 5ml mobile phase and compared with a 0.15µg/mL calibration solution of 2,4-DCPA and a 0.1010µg/ml calibration solution of RPA 90946. Residues obtained from soil samples fortified at the 50ppb level were dissolved in 25ml mobile phase and residues obtained from soil samples fortified at the 100ppb level were dissolved in 50ml mobile phase.
4. The final dilution volume was the same for the linear curve analysis.

5. Other dilutions may be used as required.

From the single point calibration, % recoveries were calculated using the following formula:

$$\% \text{ recovery} = \frac{100tAcd}{sB}$$

where:
t = conc in µg/mL of standard
A = area of fortified sample
B = area of standard
s = µg of analyte fortified
d= final dilution volume, ml
c = conversion factor, 0.642 (this is 1 for RPA 90946 quantification)

The conversion factor (c) corrects for the molecular weight ratio between 2,4-DCPA (252.52) and 2,4-dichloroaniline (162.02). The appropriate factor is 0.642.

A sample calculation follows for 2,4-DCA results obtained at the 50ppb fortification level:

$$\%\text{recovery} = \frac{100(0.15105)(81253)(0.642)(25)}{2.565(87051)}$$

$$\%\text{rec} = 88.2$$

Alternatively, linear regression could be used to generate calibration curves for 2,4-DCPA and RPA 90946. At least four different standard concentrations should be interspersed with samples to compensate for any minor changes in instrument response. Extracts should be diluted such that the peak areas obtained are within the area range between the lowest and highest standards injected.

Linear regression coefficients are calculated on peak area versus concentration of standard (µg/mL). The data from the analytical standards are then fit to the linear model.

$$y=a+bx$$

The coefficient of determination, r^2, is also calculated. The equation that can be used to estimate the residues in the samples is:

$$ppm=cd(y-a)/(gb)$$

where: y = peak area or height
b = slope
d = dilution volume (mL)
g = sample weight, grams
a = intercept
c = conversion factor

The conversion factor (c) corrects for the molecular weight differences between 2,4-DCPA (252.52) and the 2,4-dichloroaniline (162.02). The appropriate factor is 0.642 for the analysis of 2,4-DCA. The factor is 1 for the analysis of RPA 90946.

Percent recovery of spiked control samples can be calculated using the following:

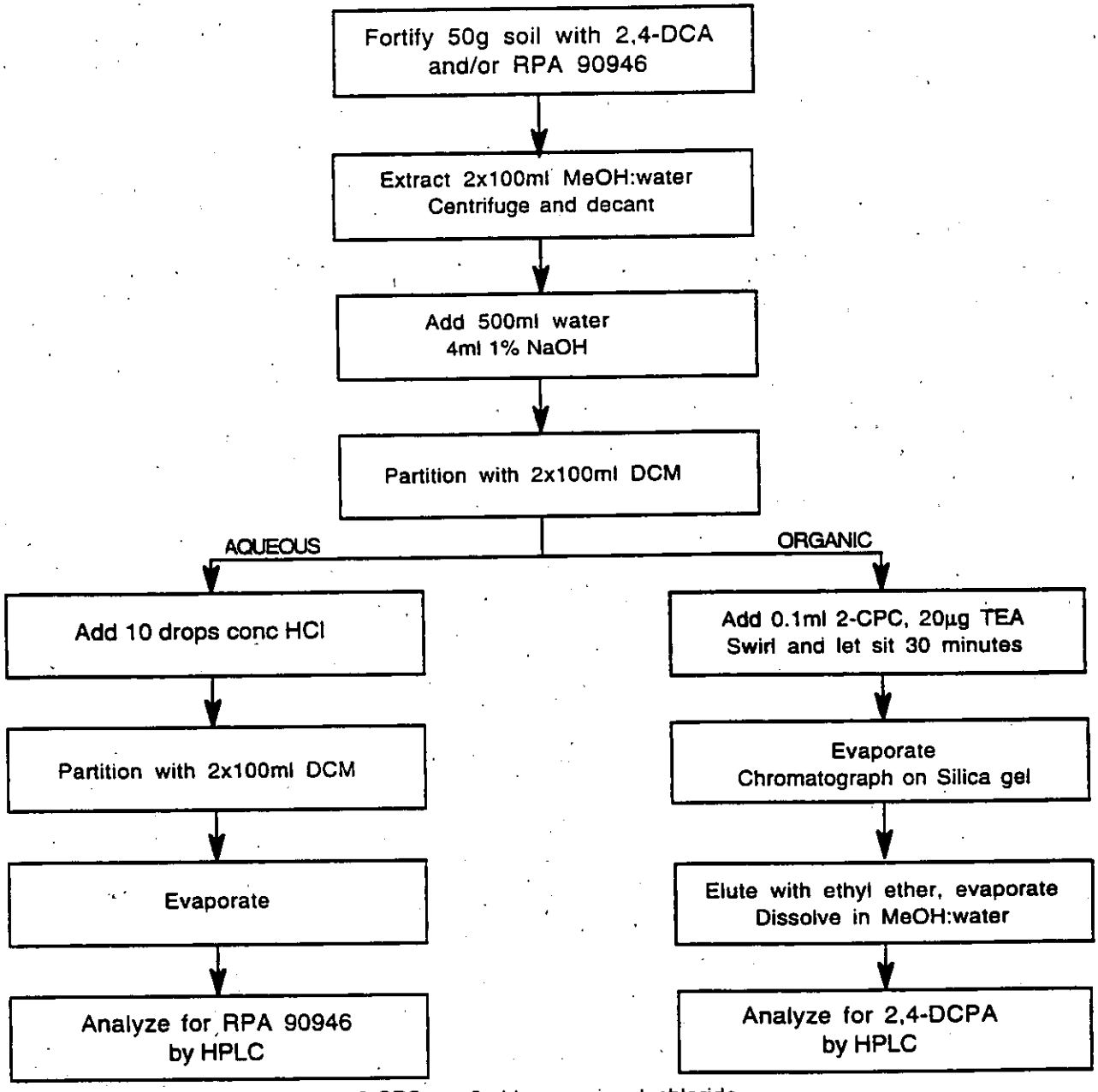
$$\% \text{ recovery} = 100(r-u)/s$$

where: r = µg recovered in spiked untreated control
u = µg found in the untreated control
s = µg added to the untreated control.

BY:	Craig A. Smitley	January 30, 1995 Date
	Anibal Lopes	January 30, 1995 Date
	Joseph R. Hudson	January 30, 1995 Date
APPROVAL:	Alain Y. Chêne, Group Leader	January 30, 1995 Date

FIGURE1

FLOWCHART OF METHOD



2-CPC = 2-chloropropionyl chloride
TEA = triethylamine
DCM = dichloromethane
HCl = hydrochloric acid
NaOH = sodium hydroxide
2,4-DCA = 2,4-dichloroaniline

Rhône-Poulenc Ag Company
2 Alexander Drive, Research Triangle Park, NC 27709

Version: 860/V3.0 Printed: 23-Jan-1995 at 10:25:26 Page 1
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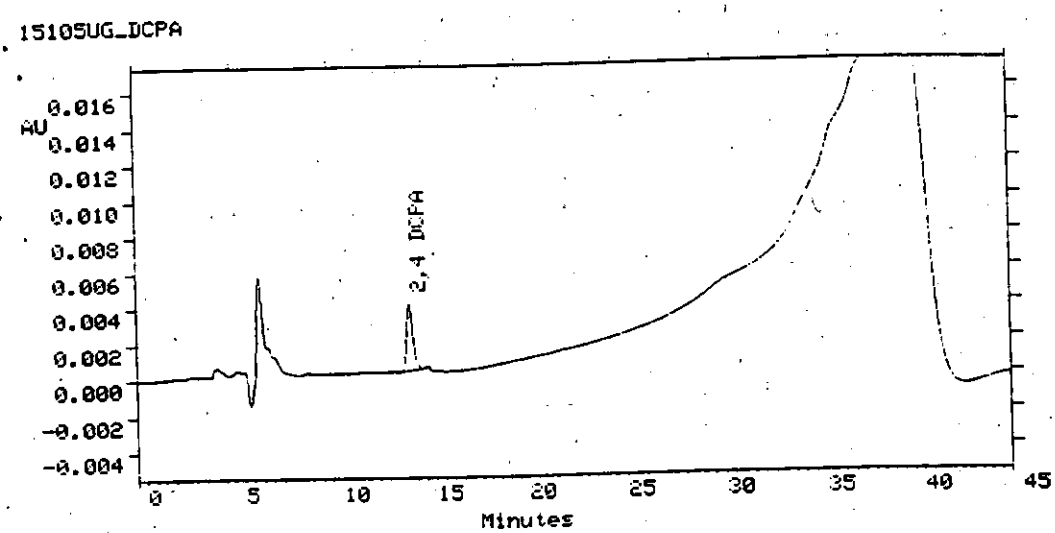

LC Results:

Peak Name	Ret Time	Area	Int	Amount
2,4 DCPA	14.142	85808	BB	CAL
RPA 90946	19.500	-	NF	-

Total Area 85808 Total Amount 0.000 Total Height 3727

2,4 -DCPA STANDARD AT 0.15105 µg/ml, USED FOR 10ppb LEVEL

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Version: 860/V3.0 Printed: 23-Jan-1995 at 10:29:09 Page 1
Node: RTPVX4 LC Project: CAS_90946_METH_DEC_JAN_95 User: CSMITLEY

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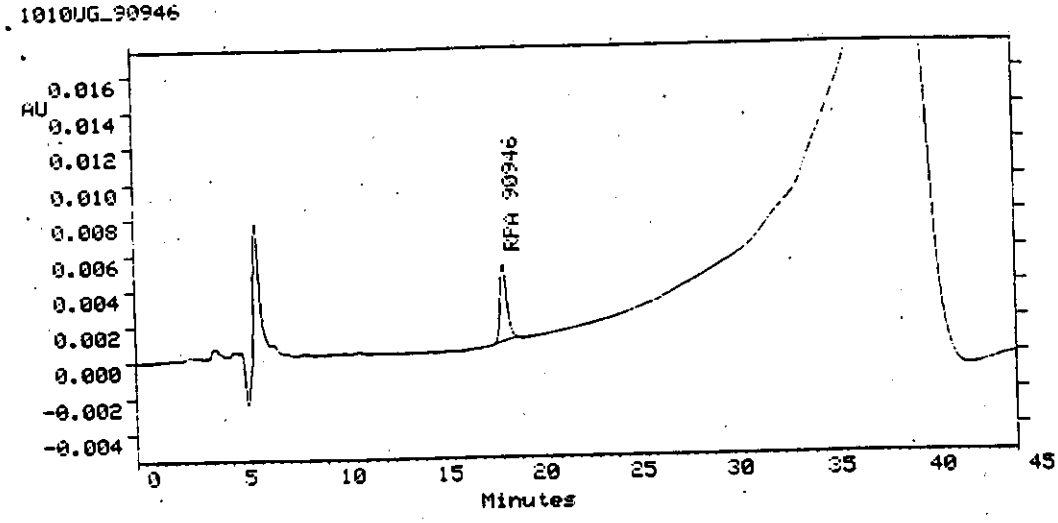

LC Results:

Peak Name	Ret Time	Area	Int	Amount
2,4 DCPA	14.000	-	NF	-
RPA 90946	19.125	109427	BB	CAL

Total Area 109427 Total Amount 0.000 Total Height 4405

RPA 90946 STANDARD AT 0.1010 µg/ml, USED FOR 10ppb LEVEL

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Version: 860/V3.0 Printed: 23-Jan-1995 at 11:27:50 Page 1
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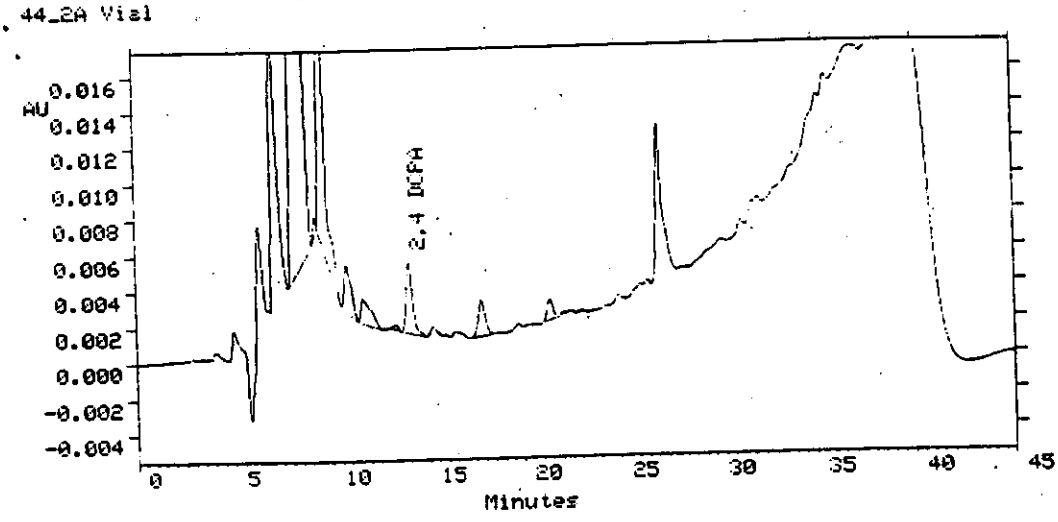

LC Results:

Peak Name	Ret Time	Area	Int	Amount
2,4 DCPA	14.025	88184	BB	0.154
RPA 90946	19.500	-	NF	-

Total Area 88184 Total Amount 0.154 Total Height 4023

RECOVERY OF 2,4-DCPA FROM NORTH CAROLINA SOIL FORTIFIED AT 10ppb LEVEL WITH 2,4-DICHLOROANILINE

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Version: 860/V3.0 Printed: 23-Jan-1995 at 11:30:08 Page 1
Node: RTPVX4 LC Project: CAS_90946_METH_DEC_JAN_95 User: CSMITLEY

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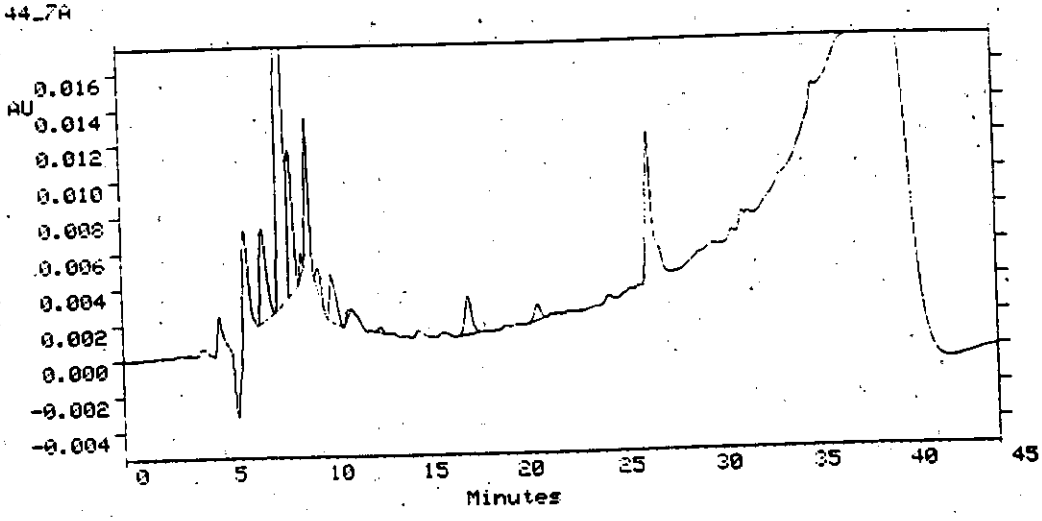

LC Results:

Peak Name	Ret Time	Area Int	Amount
2,4 DCPA	14.000	- NF	-
RPA 90946	19.500	- NF	-

Total Area 0 Total Amount 0.000 Total Height 0

UNTREATED CHECK OF NORTH CAROLINA SOIL FOR 2,4 DCPA FRACTION

Rhône-Poulenc Ag Company
2 Alexander Drive, Research Triangle Park, NC 27709

Version: 860/V3.0 Printed: 23-Jan-1995 at 11:28:28 Page 1
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44_2B 13-Jan-1995 3:25:15

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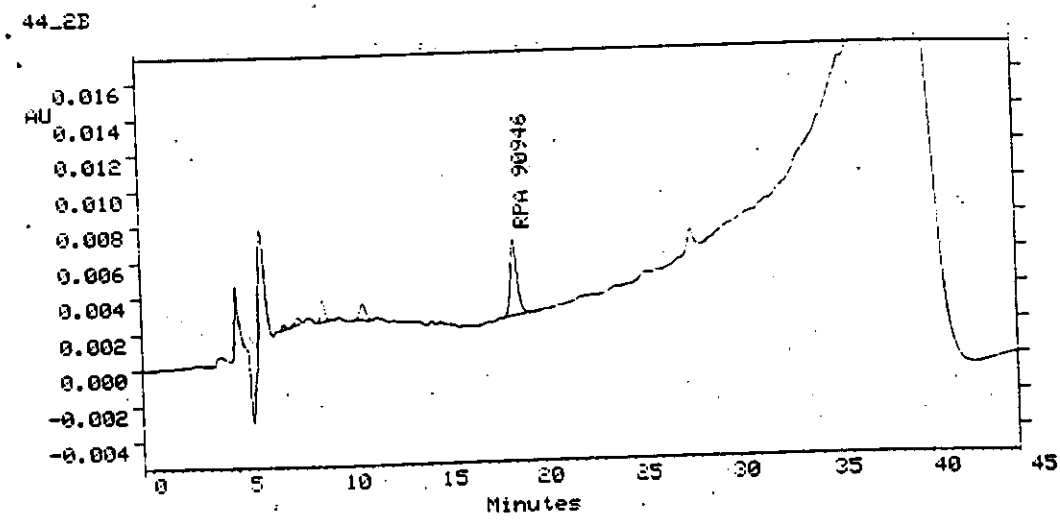

LC Results:

Peak Name	Ret Time	Area	Int	Amount
2,4 DCPA	14.000	-	NF	-
RPA 90946	19.500	102536	BB	0.094

Total Area 102536 Total Amount 0.094 Total Height 4196

RECOVERY OF RPA 90946 FROM NORTH CAROLINA SOIL FORTIFIED AT 10ppb LEVEL

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Version: 860/V3.0 Printed: 23-Jan-1995 at 11:27:35 Page 1
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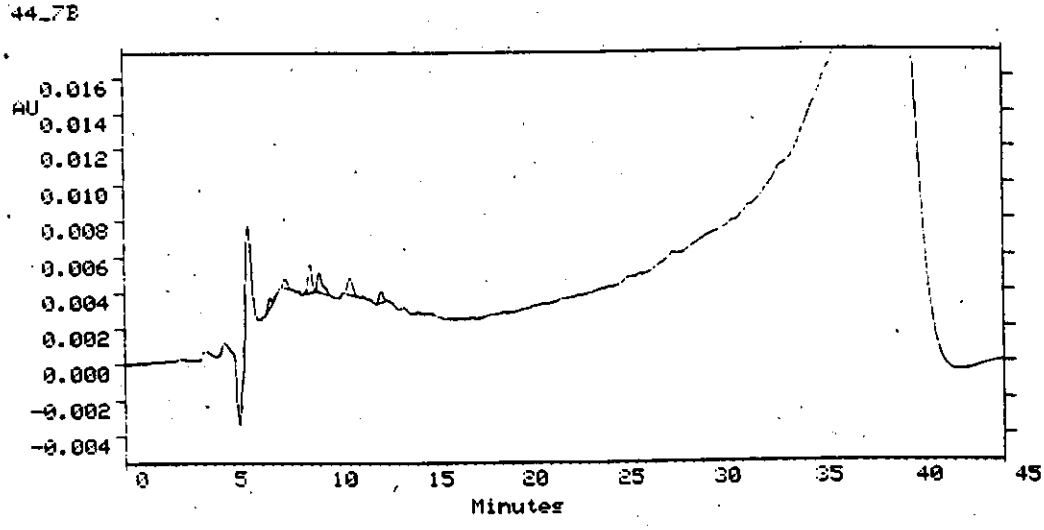

LC Results:

Peak Name	Ret Time	Area Int	Amount
2,4 DCPA	14.000	- NF	-
RPA 90946	19.500	- NF	-

Total Area 0 Total Amount 0.000 Total Height 0

UNTREATED CHECK OF NORTH CAROLINA SOIL FOR RPA 90946 FRACTION