Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Dodine

MRID #: 449857-02

Matrix: Soil

Analysis: GC/MS

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Summary Flowchart of Analytical Method

EXTRACTION

(1). Weigh 50 g of soil into a nalgene bottle.
(2). Spike as needed and wait for 10 min.
(3). Extract with 70 mL of 0.05 M KOH in 90:10 MeOH:H_2O mixture twice.
(4). Extract with 70 mL 1% HCl in methanol once.
(5). Combine all filtered extraction solutions and take final volume to 250 mL.

PARTITION

(1). Take a 50 mL aliquot and rotary evaporate to ~10 mL.
(2). Add 7.9 g salt and 25 mL water to a 125 mL sep-funnel, also transfer 10 mL extract to sep-funnel.
(3). Extract three times with 45 mL dichloromethane. Drain each lower phase through a folded 1 PS filter containing 10 g sodium sulfate.
(4). Rinse the sodium sulfate with 10 mL dichloromethane.
(5). Rotovap to dryness.

DERIVATIZATION

(1). Reconstitute in 6 mL of 1-chlorobutane.
(2). Add 100 µL of methanol, 30 µL of hexafluoroacetylacetone.
(3). Heat and stir at 100 °C for 1 hr.
(4). Dry down the solution under nitrogen at 30 - 35°C.
(5). Dilute to appropriate volume with MeOH and sonicate.
(6). Filter sample through a Gelman nylon filter.

Dodine: Method of Analysis for Dodine in Soil

I. Introduction

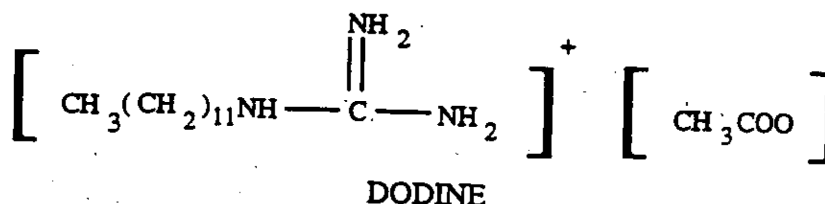
A. Scope

An analytical method is described for the analysis of dodine in soil, as defined in the Pesticide Assessment Guidelines, Subdivision O.

B. Principle

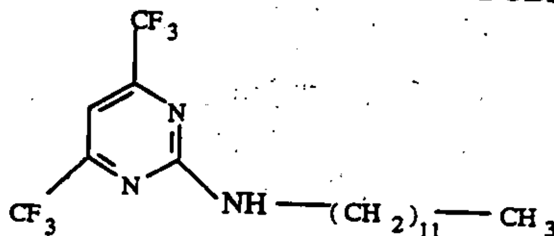
Soil samples are extracted using KOH in methanol/water twice and 1% HCl in methanol once. The extract is filtered and a 50 ml (10 gram equivalent) aliquot is taken. The aliquot is rotary-vap down to ~10 mL, added water and salt and partitioned into Methylene Chloride. A solvent exchange is done and the extract is then derivatized with hexafluoroacetylacetone. Quantification of the derivatized dodine is accomplished by gas chromatography using a mass selective detector.

C. Structures

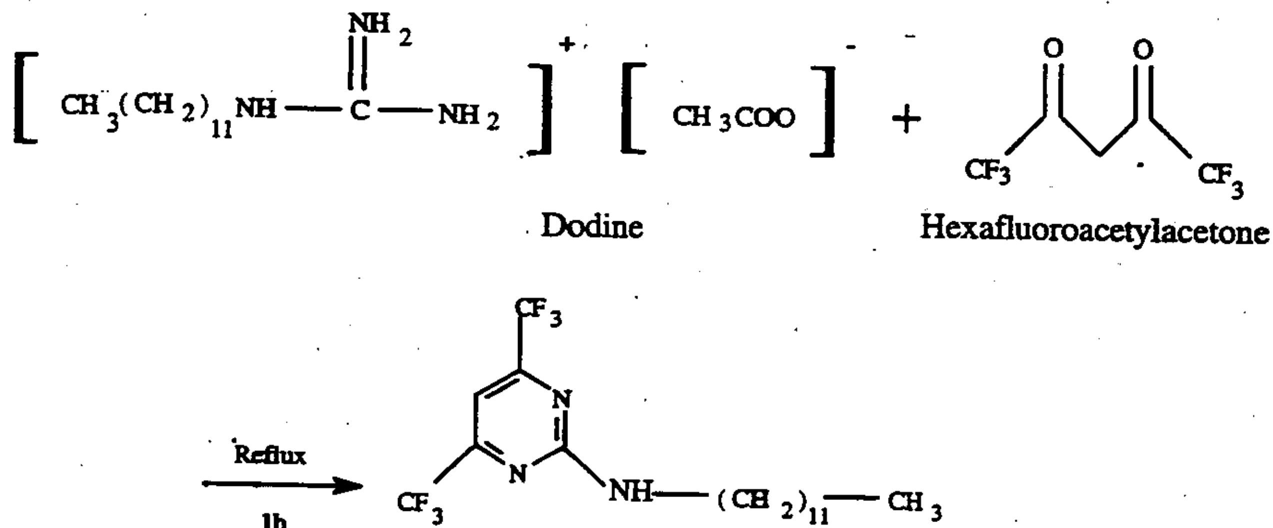


DODINE

DERIVATIZED DODINE



D. **Reaction**



II. Materials

Reagents and Solvents were used as received from supplier, unless otherwise noted. Equivalent reagents and equipment may be substituted where appropriate.

A. Reagents

1. Sodium Sulfate, anhydrous, granular, J.T. Baker, Cat. No. 3375-05
2. Sodium Chloride, Reagent Grade, ACS, VWR Cat. No. VW6430-1 or equivalent
3. Potassium Hydroxide, pellets, Fisher Scientific, Cat. No. P251-500 or equivalent
4. Hydrochloric Acid, 36.5% -38%, GR, EM Cat. No. HX0603-13 or equivalent
5. 1,1,1,5,5,5 - Hexafluoro2,4-Pentanedione, 98%, Aldrich Cat. No. 23,830-9

B. **Solvents**

1. 1-Chlorobutane, 99.5% , Anhydrous, Aldrich Cat. No. 41-425-5
2. Methanol, EM OMNISOLV, VWR Scientific Cat. No EM-MX0484-1 or equivalent
3. Dichloromethane, EM OMNISOLV, VWR Scientific Cat. No EM-DX0831-1 or equivalent
4. Cyclohexane, EM OMNISOLV, VWR Scientific Cat. No EM-CX2286-1 or equivalent
5. Water, EM HPLC Grade, VWR Scientific Cat. No EM-WX0004-1 or equivalent

C. **Equipment**

1. Aluminum Crimp-Top Seal, 11 mm TFE/RUB Septum, Sun Brokers, Inc., Cat. No. 200 100
2. Analytical Balance
3. Autosampler Vials, 1 ml, clear, Wheaton, Cat. No. 223682
4. Disposable Pasteur Pipettes
5. Polypropylene Copolymer Centrifuge Bottle, 250 ml, Nalgene, Cat. No. 3141-0250
6. Graduated Cylinders, 250 ml with #27 stopper joint
7. Vacuum Adapters, S-1110-Special with #27 inner stopper joint Southeastern Lab Apparatus, Inc
8. Boiling Flasks, 125 ml with 24/40 joint and 250 mL with 24/40 joint
9. Separatory Funnels, 125 ml
10. Sonicator, Model 5200, Branson, Cat. No. B5210DTH
11. Volumetric Flasks, 100 ml, class A

12. Volumetric Pipettes, appropriate sizes, class A

13. Hewlett-Packard 5890 Series II GC equipped with Mass Selective Detector (refer to Section V of this document for details)

14. Capillary Column, DB-5, 30 m X 0.25 mm i.d., 0.25 µm film thickness, J & W Scientific, Cat. No. 122-1232 or equivalent

15. 10 mL Reaction Vial, Pierce Cat. No. 13225

16. Teflon Dics, Pierce Cat. No. 12722

17. Open Top Caps, Pierce Cat. No. 13219

18. Magnetic Stir Bars (spin vanes), VWR Cat. 58949-272 or equivalent

19. Rotary Evaporator, Brinkmann model ROTAVAPOR R110

20. Horizontal Shake with Timer, Thomas or equivalent

21. Buchner funnels, Coors porcelain 9 cm

22. GF/A Filter paper, Whatman Cat No. 1820 090, 9 cm

23. Phase Separator paper, Whatman N0. 1 PS, 11 cm, Cat No. 2200 110

24. Funnel, polypropylene, 66 mm top

25. Pipettes, appropriate sizes, Oxford or equivalent

26. Digital Pipettes, appropriate sizes, Eppendorf or equivalent

27. Reaction-Therm III Heating/Stirring Module, Pierce No. 18935 or equivalent

28. Optional: Dispensette Bottle-Top, appropriate sizes, Brinkmann

29. Nitrogen evaporation manifold with moisture trap

30. 3 cc Syringe, B-D N0. 309585 or equivalent

31. Nylon Acrodisc filter (13 mm, 0.45 µm), Gelman No. 4426

D. Analytical Standards

Analytical Standards available from Rhône-Poulenc Ag Company

1. **Dodine:** 1-dodecylguanidinium acetate

2. **Derivatized dodine:** 2-dodecyl-4,6-bis(trifluoromethyl)pyrimidine

III. Standard Solution Preparation

A. General

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

2. After preparation, standards should be transferred from the volumetric flasks into screw-capped amber bottles to prevent possible photodegradation.

3. Store standard solutions in the refrigerator at or below 4 °C when not in use.

B. Fortification and Calibration Standard Solutions

The following is provided as an example of how standard solutions may be prepared. Other concentrations may be used as appropriate.

1. Weigh 0.1000 g (±0.1 mg) of each analytical standard individually into 100 ml volumetric flasks. Dissolve each analytical standards in methanol and mix well. Dilute to final volume with methanol. Concentration of each standard is 1000 µg / ml.

2. Withdraw a 10.0 ml aliquot from each of the 1000 µg / ml individual standards and add to a 100 ml volumetric flask. Dilute to volume with methanol. The concentration of this standard is 100 µg / ml.

3. By further dilution of the 100 µg / ml standard with methanol, prepare a series of standards to serve as fortification standards or calibration standards.

IV. Methods of Analysis

The tilde symbol (~) indicates 'approximately'.

The "•" symbol indicates an appropriate stopping point. Samples may be stored in freezer(< 0° C) overnight and allowed to come to room temperature before continuing.

A. Sample Preparation

Use samples as received from processor.

B. Extraction

1. Weigh ~50.0 grams of soil into a 250 ml nalgene bottle. Spike at appropriate level and allow to sit for ~10 minutes.

2. Add ~70 mL of 0.05 M KOH in 90:10 MeOH:H_2O and shake on horizontal shaker for ~15 minutes. Centrifuge at ~2500 rpm for ~5 minutes.

3. Attach a 9 cm buchner funnel to a 250 ml mixing cylinder using a vacuum adapter. Decant liquid through a GF/A filter paper with slow vacuum.

4. Repeat step 2 and decant supernate liquid into cylinder.

5. Add ~70 mL of 1 % HCL in methanol to soil and shake for ~15 minutes, transfer everything (including soil) into the buchner funnel. Rinse the nalgene bottle and cap with small amount of methanol and transfer into the buchner funnel.

6.• Rinse buchner funnel tip and adapter with methanol and take volume to 250 mL with methanol. Mix this 250 mL extraction solution well. (Extract A)

C. Partition and solvent exchange

1. Take a 50 mL aliquot of Extract A with a class A Volumetric pipette and transfer to a 125 mL flat bottom flask. Rotary evaporate to ~ 10 mL at 40°C .

2. Add 7.90 - 7.94 g of salt to a 125 ml sep funnel, and transfer the 10 ml of extract from the 125 ml flat bottom flask. Add 25 mL of distilled water to the 125 ml flat bottom flask, swirl and transfer into the 125 ml sep funnel. Shake until all of the salt dissolves.(NOTE-1)

3. Add ~45 mL of Dichloromethane to the 125 ml flat bottom flask and rinse, then transfer to 125 ml sep funnel and shake vigorously for ~1 minute. Remember to vent frequently.

4. Fold PS-1 filter paper into 66 mm plastic funnel and add ~10 grams of sodium sulfate onto the filter paper. Drain the dichloromethane through the funnel and into a 250 ml flat bottom flask.

5. Repeat step 3 two times, draining the dichloromethane through the sodium sulfate and into the 250 ml flask each time. (Extract B) (NOTE-2)

6. Rinse sodium sulfate with ~10 ml of dichloromethane twice combining it with Extract B.

7. Concentrate Extract B to dryness using a rotary evaporator at 30 °C. Blow off any remaining dichloromethane with a gentle stream of nitrogen.(NOTE-3)

D. Derivatization

1. Add 3 mL of 1-chlorobutane and sonicate until residue is dissolved. Transfer the 3 mls to a 10 ml reaction vial, rinse the flask with an additional 3 mls and add that to the reaction vial.

100 see 2LV.

2. Add 100 uL of methanol, 30 uL of 1,1,1,5,5,5 Hexafluro-2,4-pentanedione, and a triangle stir magnet to the reaction vial. Seal tightly with teflon seal and place the reaction vials in the heating block. Heat and stir at 100 °C for 1 hour.

3. Remove from heat and allow to cool for ~5 minutes before opening caps. Rinse cap and stir magnet with methanol, into the reaction vial not exceeding the 10ml volume. Then concentrate to dryness at 30°C-35°C with a gentle stream of N_2. (NOTE-4)

4. Dilute to appropriate volume with methanol and sonicate. (NOTE-5)

5. Filter sample through a Gelman nylon filter to eliminate solids before injecting

E. General Method Notes:

1. The salt solution in sep funnel is a saturated solution, It will be difficult to get all the salt into solution.

2. The 125ml flat bottom flask does not need to be rinsed with dichloromethane after the first time.

3. Nitrogen must have a moisture trap attached, the derivitization that follows is highly sensitive to moisture, any moisture will cause poor recoveries.

4. The heating blocks can be used for this if you allow time for the blocks to cool or replace the hot blocks and allow cool blocks to equilibrate. This is preferable to a water bath, as moisture will give recovery problems

5. Dilute to no more than 9 mls at first, sonicate and filter, then make further dilutions if necessary from this volume. Use class A pipettes and class A volumetric flasks to do these further dilutions.

V. Gas Chromatography

A. Instrumentation

1. Gas Chromatograph: Hewlett-Packard 5890 Series II GC, 7673 Autosampler, 18594B Sampler Controller, Split/Splitless Injector, or an equivalent system

2. Detector: Mass Selective Detector, Hewlett-Packard Model 5972 or equivalent

3. Data Acquisition: Hewlett-Packard ChemStation

4. Column: J & W Scientific DB-5 30 m X 0.25 mm i.d., 0.25 μm film thickness (or HP-5 same diminsions)

B. Gas Chromatograph Conditions

1. Carrier Gas: Helium, Head Pressure set at 15 PSI with a 1.5 minute 45psi pulse at injection.

2. Inlet Liner: 4-mm i.d. nominal volume 900 μl, borosilicate glass with single taper on GC end (HP part #5181-3316)

3. Injector Temperature: 250 °C

4. Detector Temperature: 325 °C

5. Oven Temperatures: Initial: 100 °C, hold 1 minute
   Ramp 20 °C / min to 195°C, hold 0 minute
   Ramp 5 °C / min to275 °C, hold 3 minute
   Ramp 30 °C / min to 300 °C, hold 5 minutes

6. Injection Volume: 1.0 μL

7. Splitless injection with split vent off for 60 seconds.

C. **Mass Selective Detector Parameters**

1.	Tune File	Maximum Sensitivity Autotune
2.	Solvent Delay	3.0 or 6.0 minutes
3.	EM voltage	400-600 over tune
4.	Acquisition Mode	SIM
5.	SIM Parameters	GROUP 1 Derivatized dodine High Resolution - Yes Dwell time per ion 100 msec Starting time 8.0 min Quantitation ion 244.00 Qualifier ions 245.00 & 399.20

D. **General Chromatography Notes / Potential Problems**

1. Several standards should be injected prior to actual analysis using a new column or after the GC has set idle for any considerable length of time to condition and/or to remove any contaminants.

2. A gold plated seal is used at the interface of the glass liner and column. Hewlett-Packard Part No. 18740-20885.

3. The GC parameters are guidelines and can be optimized for the instrument and column actually used. Record the actual GC conditions used for data acquisition and include in report.

4. The detector ion amu values are nominal. Exact values should be determined from a scan run after each tune.

VI. Quantification of Residues

A. **Calibration Curves**

1. Linear regression should be used to generate a calibration curve for the analyte. At least four different standard concentrations should be run with each set of samples. Standards

should be interspersed with samples to compensate for any minor change 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.

2. Linear regression coefficients should be calculated from 'peak area' (or 'peak height') versus 'nanogram / ml injected'. Data from the analytical standards should be fit to the linear equation, $y = a + bx$.

where: y = peak area or height
a = calibration line intercept
b = calibration line slope
x = conc of analyte in inj soln

B. Quantification of Residues

1. Derivatized dodine should be quantified by comparison to the standard curves obtained from a linear regression analysis of the data.

2. Equations

2.1 Concentration of analyte in sample in ppb (parts per billion).

$$z = (y - a) / b \times c / d$$

where: y = peak area (or height), response of analyte of interest

a = intercept of calibration line from linear regression (area or height)

b = slope of calibration curve from linear regression (response per ng/ml)

c = final volume of sample (ml)

d = sample weight (g)

z = conc of analyte in sample (ppb)

2.2 Corrected concentration of analyte in sample in ppb.

$$Z' = z \times C$$

where: Z' = corrected concentration

z = concentration found from curve
C = conversion factor

for conversion of

derivatized dodine to dodine C = 0.72

2.3 Percent recovery

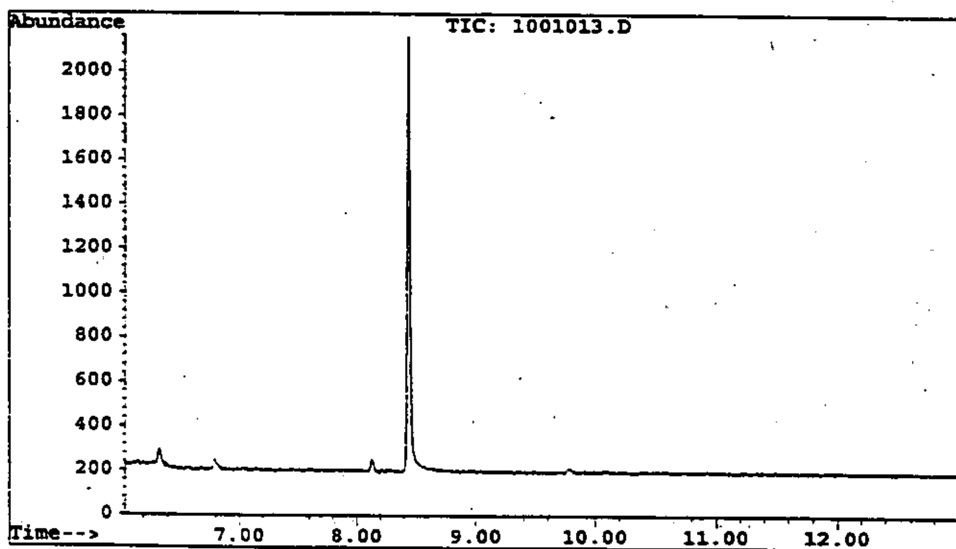
$$\% \text{ recovery} = \frac{\text{(ppb found in fort sample - ppb found in UTC)}}{\text{actual fortification level in ppb}} \times 100\%$$

3. Residues shall be reported as dodine equivalents.

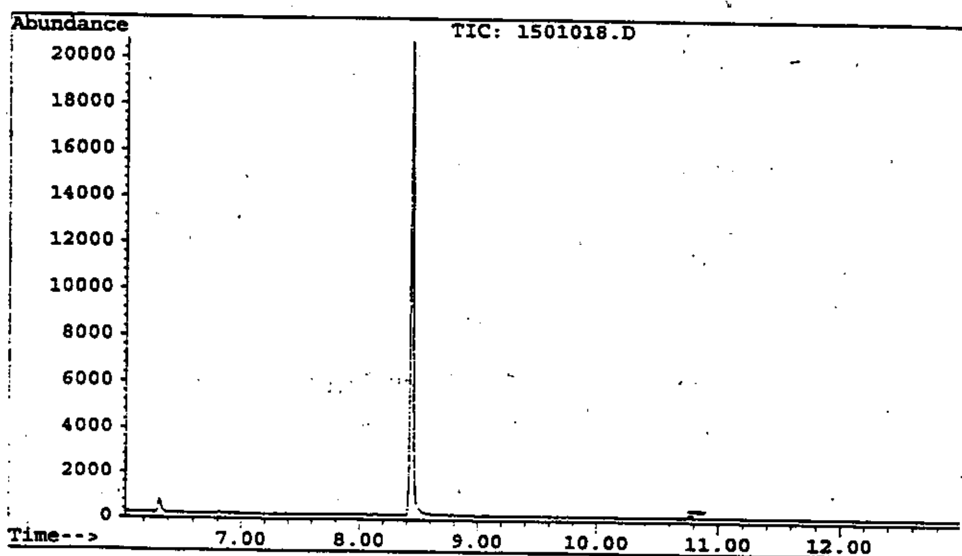
VII. Example Chromatograms

A. Standards

1. Derivatized Dodine Standard, 20 ng/ml in methanol

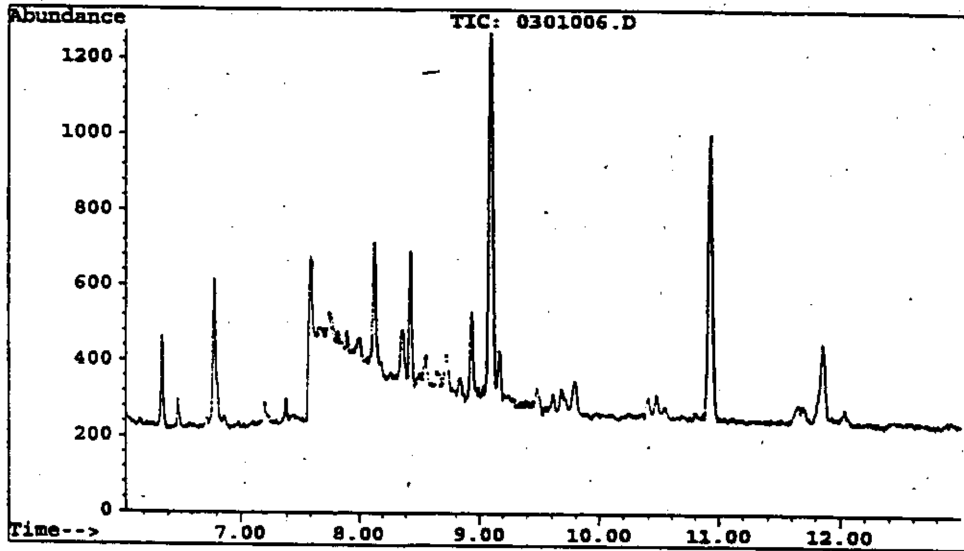


2. Derivatized Dodine Standard, 150 ng/ml in methanol

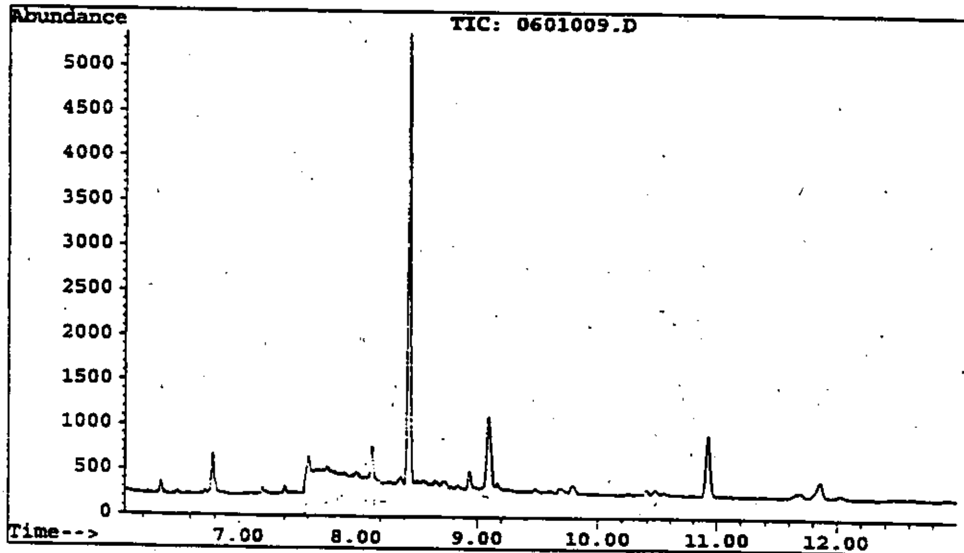


B. California Soil Samples

1. Soil, California, Untreated Control

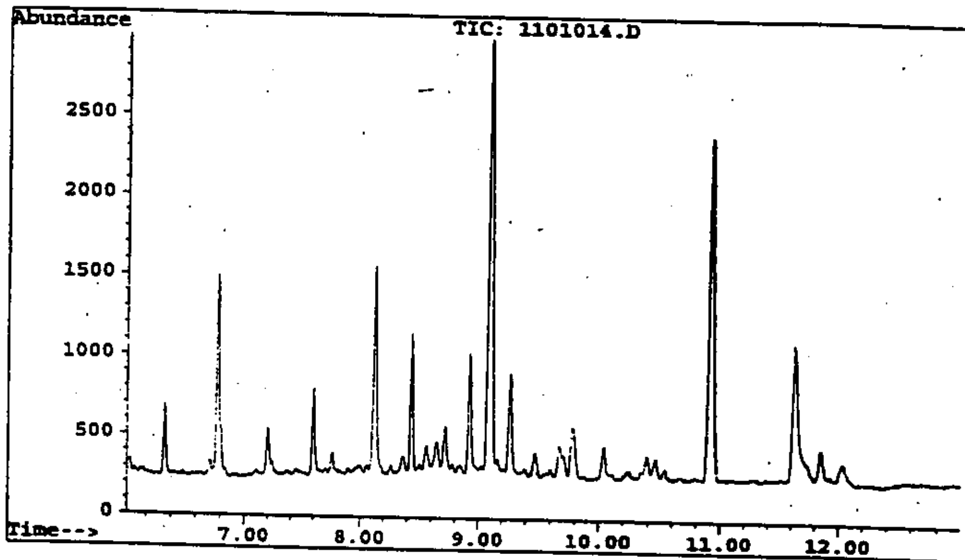


2. Soil, California, Untreated Control Fortified at 10 ppb Level

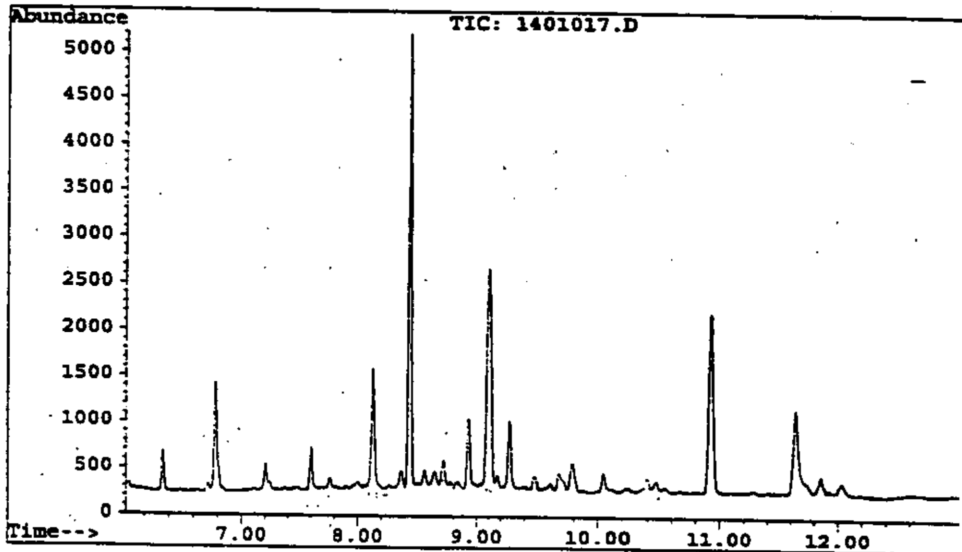


C. Washington Soil Samples

1. Soil, Washington, Untreated Control

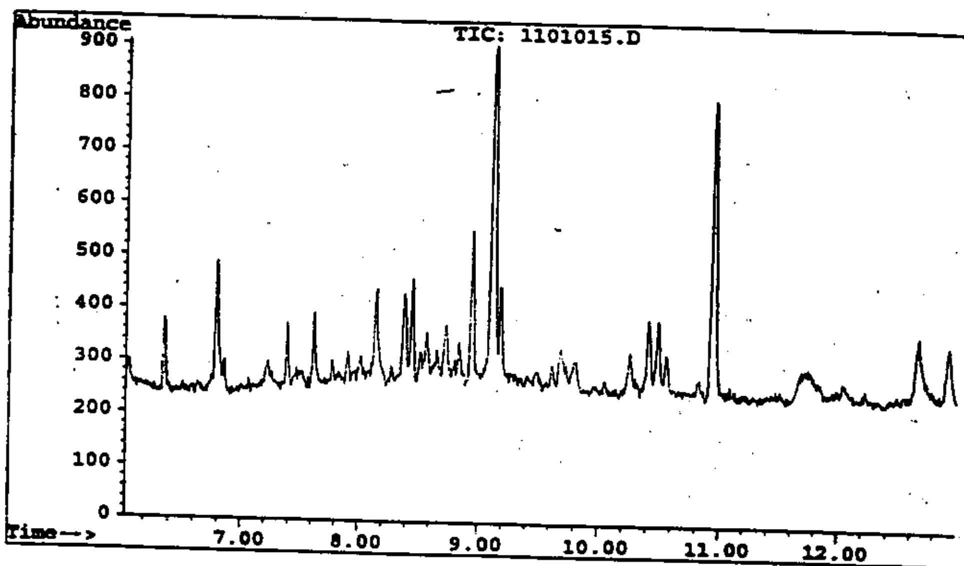


2. Soil, Washington, Untreated Control Fortified at 10 ppb Level

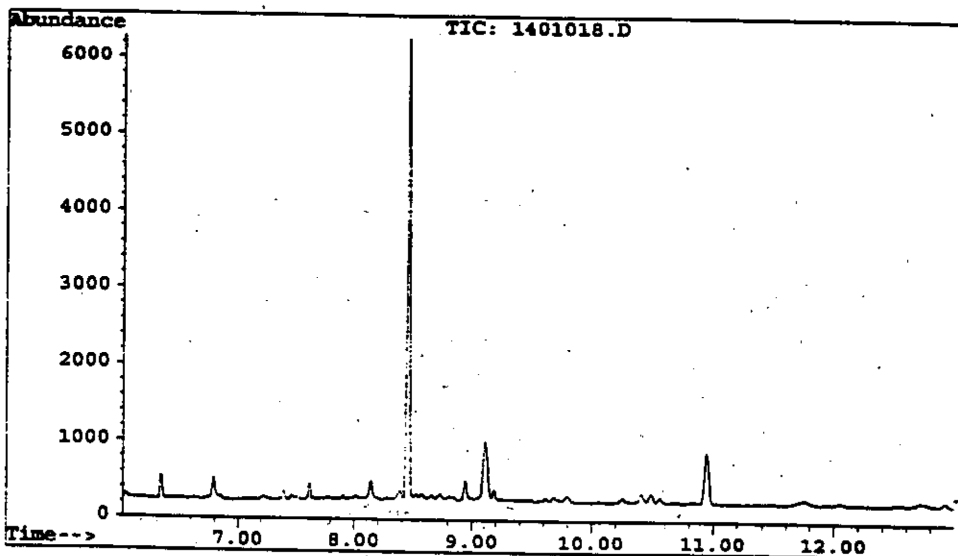


B. **New Jersey Soil Samples**

1. **Soil, New Jersey, Untreated Control**

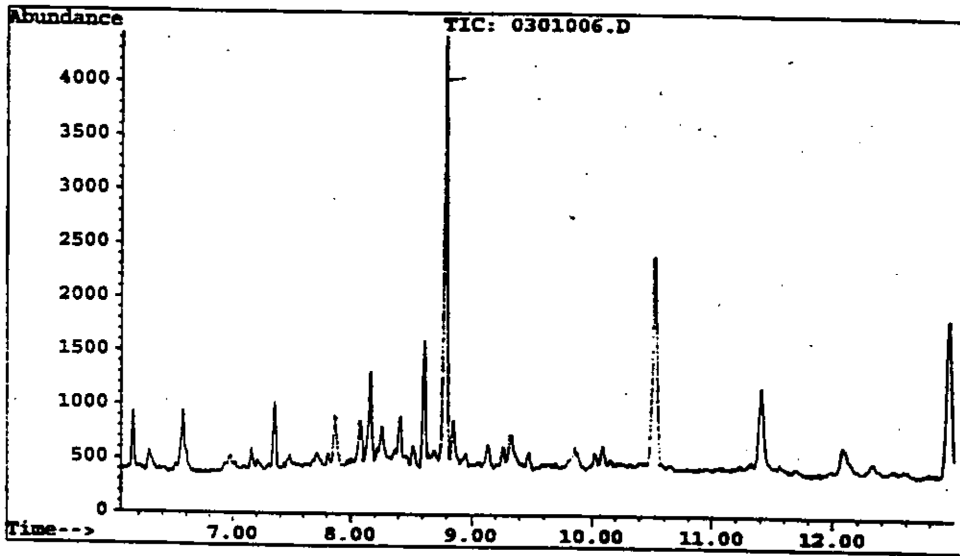


2. **Soil, New Jersey, Untreated Control Fortified at 10 ppb Level**



B. Georgia Soil Samples

1. Soil, Georgia, Untreated Control



2. Soil, Georgia, Untreated Control Fortified at 10 ppb Level