ANALYTICAL METHOD FOR THE DETERMINATION OF PROPACHLOR AND ITS MAJOR METABOLITES IN SOIL

I. SUMMARY/INTRODUCTION

A. SCOPE

The analytical procedure described here is for the determination of parent propachlor and the three major acid metabolites in soil: Oxanilic, sulfonic, and sulfinyl acetic acids. The method is also capable of determination three minor neutral soil metabolites: Norchloropropachlor, hydroxypropachlor, and propachlor methylsulfone. The structures of these compounds are shown in Figure 1. The sections in this document list the equipment and chemicals used and describe the preparation of standard solutions for analyzing these analytes in soil. This document also provides typical instrument conditions, chromatograms and validation data for this analytical method.

B. PRINCIPLES

The procedure consists of overnight Soxhlet exhaustive extraction of a soil sample with 50% acetonitrile in water. The extract is rotary evaporated to remove acetonitrile followed by partitioning with methylene chloride to give an aqueous phase containing the three acids and an organic phase containing parent propachlor and the three neutral metabolites. The three acids in the aqueous phase are cleaned up via a disposable SAX column, eluted with a buffer solution, evaporated to dryness, and taken up in a 10% acetonitrile-potassium monophosphate buffer. Quantitation is by HPLC using a C18 analytical column with UV detection at 210 nm.

Parent propachlor and neutral metabolites in methylene chloride are evaporated to dryness, redissolved in ethyl acetate, and cleaned up via a disposable NH_2 column containing a small bed of a mixture of activated carbon and NH_2 packing material. The eluate is rotary evaporated to approximately 1 ml and adjusted to a final volume of 10 ml with 20% ethyl acetate in iso-octane. Quantitation is by GC using a DB-5 capillary column with nitrogen specific detection. The lower limit of method validation (LLMV) was 0.01 ppm each for parent propachlor, the three neutral metabolites, oxanilic and sulfonic acids and 0.05 ppm for the sulfinyl acetic acid. The LLMV is the lowest fortification level tested that yielded acceptable recoveries (average >70%).

II. MATERIALS AND METHODS

The following materials, equipment, and reagents are required to perform the

analysis. Appropriate substitution for certain items is left to the discretion of the analyst. Cleaning of the glassware and other equipment should be carried out so as to minimize contamination of future samples. The cleaning procedure should be checked to verify appropriate cleanliness. Analysis of reagents and solvents should be carried out to assure a minimum contribution of interferences to actual samples.

A. EQUIPMENT

Top loading balance, Mettler Model PC 440 or equivalent.

Analytical balance, Mettler Model AE163 or equivalent.

Filling funnels, 75 mm, Fisher No. 10-346B.

Rotary Evaporator Units:

- Vacuum trap, Kontes No. 926910-1000
- Adapters, Kontes No. 570200-2524
- Evaporator motor, Fisher No. 09-548-100
- Hot plate, Baxter No. H2156-1
- Stainless bowls, Cole-Parmer No. L-07274-00
- Vacuum pump, Baxter No. P8408-1
- Vacuum tubing, Baxter No. T6500-4
- Ball valve, Supelco No. 2-2140M
- Metering valve, Supelco No. 2-2116M
- T-connectors, Fisher No. 15-315-12

(Note: Use one vacuum pump capable of generating pressures of approx. 1×10^{-4} torr for every two rotary evaporator units.)

Shaker, Baxter No. S1063-1.

Shaker head, Baxter No. S1063-10.

Separatory funnel holders, Baxter No. S1063-20.

Hemispherical heating mantles, Fisher No. 11-472-10F.

Lab jacks, Fisher No. 14-673-10.

Magnetic stirring motors, Scientific Products No. S9302-3.

Variable transformers, Fisher No. 09-521-22.

Teflon egg shaped stir bars, Fisher No. 14-511-58AS.

Soxhlet extraction units, Fisher No. 09-551-10C and 09-553C (see Figure 2).

Whatman 43 mm x 123 mm cellulose extraction thimbles, Fisher No. 09-656H.

500 mL Round bottom flasks, Fisher No. 10-067-2F.

250 mL Round bottom flasks, Fisher No. 10-067-2D.

100 mL Round bottom flasks, Fisher No. 10-067-2B.

250 mL Separatory funnels, Fisher No. 10-437-10C.

250 mL Graduate cylinders, Fisher No. 08-556E.

4000 mL Graduated cylinders, Fisher No. 08-556H.

10 mL Graduated centrifuge tubes, Fisher No. 05-538-38A.

100 mL Volumetric flasks, Fisher No. 10-210C.

2000 mL Volumetric flasks, Fisher No. 10-210H.

Pasteur pipettes 5 3/4" length, Fisher No. 13-678-6A.

Pasteur pipettes 9" length, Fisher No. 13-678-6B.

Serological pipettes from 0.1 through 5 mL, Fisher No.'s 13-676-28A,B,C,D,E,F.

Disposable 0.22 µm Teflon disk filters, Fisher No. DDF-02T-2550.

Disposable 3 mL syringes, Fisher No. 14-823-40.

HPLC solvent clean-up assembly, 47 mm Ultra-ware, Kontes No. 953845-0000.

Membrane filters, 0.22 µm, Millipore No. GSWP 047-00.

Varian Model 3600 or 3700 Gas Chromatograph equipped with a Thermionic Specific Detector (TSD).

Perkin Elmer Series 4 LC equipped with a LC-95 UV detector.

Alltech Alltima C18 (250 mm x 4.6 mm I.D., 5µm) LC analytical column, Cat. No. 88057.

Alltech Alltima C18 (0.40 x 1.25 cm) guard column, Cat. No. 88058.

J&W Scientific DB-5 (30 m x 0.25 mm I.D., 0.25 μm) capillary column, Part No. 122-5032.

Fisher Recordall Series 5000 strip chart recorders.

Whatman #2 filter papers, 12.5 cm diameter, Fisher No. 09-810F.

Burdick & Jackson 24-port vacuum manifolds, Baxter No. 9401-DK.

Fisher Accumet model 915 pH meter or equivalent.

Assorted standard laboratory equipment.

B. REAGENTS AND STANDARDS

The following reagents are used in this analytical method. Specific brands are listed to aid the chemist in finding these items. In some cases equivalent reagents can be used which are obtained from other vendors. As with the equipment list, it is recommended that, if at all possible, the items listed be obtained for use in this method.

Acetonitrile, Fisher No. A996-4.

Methylene chloride, Fisher No. D-142.

Ethyl acetate, Fisher No. E-195.

Iso-octane, Fisher No. 0301-4.

Methanol, Optima grade, Fisher No. A454-4.

Potassium hydroxide pellets, Fisher No. P251-500

Potassium phosphate monobasic, Fisher No. P284-500.

Anhydrous sodium sulfate, Fisher No. S421-500.

Deionized water (d-H_2O) from a Milli-Q water purification system (Millipore Co.), or equivalent. This system consists of an activated carbon cartridge for the removal of organics in series with two mixed-bed ion exchange cartridges for removal of ionic species.

10% Potassium hydroxide solution (W/V): Prepare by adding 10 gm of KOH to 90 mL of d-H_2O and mix well.

50% Acetonitrile in water (V/V): Prepare by mixing equal volumes of acetonitrile and d-H_2O and mix well.

20% Ethyl acetate in iso-octane (V/V): Prepare by adding 200 mL of ethyl acetate to 800 mL of iso-octane and mix well.

10% Acetonitrile in 0.02 M pH 5 KH_2PO_4 buffer: First prepare 0.02 M pH 5 buffer by dissolving 10.88 gm KH_2PO_4 in 4000 mL of d-H_2O and adjust the pH to 5 with 10% KOH. Filter the resulting solution through a 0.22 µm membrane filter. Add 200 mL acetonitrile to a 2000 mL volumetric flask, dilute to the mark with the 0.02 M pH 5 buffer and mix well.

50% Acetonitrile in 0.05 M pH 5 KH_2PO_4 buffer: First prepare 0.05 M pH 5 buffer by dissolving 27.2 gm of KH_2PO_4 in 4000 ml of d-H_2O and adjust the pH to 5 with 10% KOH. Filter the resulting solution through a 0.22 µm membrane filter. Obtain a 50% (V/V) solution by mixing equal volumes of the buffer and acetonitrile and mix well.

Solid phase extraction columns - 20 mL NH_2 aminopropyl, Varian No. AI-122560-28.

Solid phase extraction columns - 60 mL SAX quarternary amine, Varian No. AI-122560-37.

NH_2 packing material from Varian (not listed on catalog, ordered through phone # 1-800-538-1735).

Darco G-60 activated carbon, Aldrich No. 24227-6.

20% Carbon in NH_2 packing (W/W): Prepare by adding 15 gm of Darco activated carbon to 60 gm of Varian NH_2 packing and mix well by mechanical shaking for 1 hour, turning bottle after half an hour.

Propachlor: 2-Chloro-N-(1-methylethyl)-N-phenylacetamide, >99% (CP 31393, synthesized by Monsanto, see Figure 1).

Norchloropropachlor: N-(1-methylethyl)-N-phenylacetamide, 100% (CP 58297, synthesized by Monsanto, see Figure 1).

2-Hydroxypropachlor: 2-Hydroxy-N-(1-methylethyl)-N-phenylacetamide, 100% (CP 47742, synthesized by Monsanto, see Figure 1).

Propachlor Methylsulfone: N-(1-methylethyl)-2-(methylsulfonyl)-N-phenylacetamide, 99% (CP 172326, synthesized by Monsanto, see

Figure 1).

Propachlor Oxanilate: [(1-Methylethyl)phenylamino]oxoacetic acid, sodium salt, >95% (CP 118700, synthesized by Monsanto, see Figure 1).

Propachlor Sulfonate: 2-[(1-Methylethyl)phenylamino]-2-oxoethanesulfonic acid, sodium salt, 99% (CP118702, synthesized by Monsanto, see Figure 1).

Propachlor Sulfinyl Acetate: [([(1-Methylethyl)phenylamino]-acetyl)sulfinyl]acetic acid, sodium salt, 95% (CP 147935, synthesized by Monsanto, see Figure 1).

Propachlor Acid Metabolite Stock Solutions

A 1000 µg/mL stock solution of propachlor oxanilate (CP 118700) is prepared by weighing 0.1000 gm of analytical grade oxanilate into a 100 mL volumetric flask. Add 10 mL d-H_2O and mix well to insure complete dissolution. Dilute to mark with acetonitrile.

A 1000 µg/mL stock solution of propachlor sulfonate (CP 118702) is prepared by weighing 0.1000 gm of analytical grade sulfonate into a 100 mL volumetric flask. Add 10 mL d-H_2O and mix well to insure complete dissolution. Dilute to mark with acetonitrile.

A 1000 µg/mL stock solution of propachlor sulfinyl acetate (CP 147935) is prepared by weighing 0.1000 gm of analytical grade sulfinyl acetate into a 100 mL volumetric flask. Add 10 mL d-H_2O and mix well to insure complete dissolution. Dilute to mark with acetonitrile.

Parent Propachlor and Neutral Metabolite Stock Solutions

The following 1000 µg/mL stock solutions are prepared by weighing 0.1000 gm of the individual analytes into separate 100 mL volumetric flasks and dilute to mark with the solvents as indicated. Mix well to insure complete dissolution.

CP No.	Analyte	Weight	Dilution	Solvent	Conc. µg/mL
31393	Propachlor	0.1000 gm	100.0 mL	ACN	1000.0
31393	Propachlor	0.1000 gm	100.0 mL	EtOAc	1000.0
58297	Norchloropropachlor	0.1000 gm	100.0 mL	ACN	1000.0
58297	Norchloropropachlor	0.1000 gm	100.0 mL	EtOAc	1000.0

47742	Hydroxypropachlor	0.1000 gm	100.0 mL	ACN	1000.0
47742	Hydroxypropachlor	0.1000 gm	100.0 mL	EtOAc	1000.0
172362	Methyl Sulfone	0.1000 gm	100.0 mL	ACN	1000.0
172362	Methyl Sulfone	0.1000 gm	100.0 mL	EtOAc	1000.0

Note: ACN = Acetonitrile; EtOAc = Ethyl acetate.
All solutions are transferred to amber glass bottles and stored at 0-6 °C.

HPLC External Calibration Standards for Acid Metabolites

Pipet 10.0 mL each of the 1000.0 μg/mL stock solutions of CP 118700, 118702, and 147935 into a 100 mL volumetric flask. Dilute to mark with 10% acetonitrile in 0.02 M pH 5 KH_2PO_4 buffer and mix well. This solution contains 100 μg/mL of each acid metabolites.

The following standards are made by diluting with 10% acetonitrile in 0.02 M pH 5 KH_2PO_4 buffer.

Milliliters of 100 μg/mL Used	Final Volume After Dilution	Final Concentration of Acid Metabolites, μg/mL
10.0	100.0 mL	10.00
5.0	100.0	5.00
2.0	100.0	2.00

Milliliters of 10.0 μg/mL Used	Final Volume After Dilution	Final Concentration of Acid Metabolites, μg/mL
10.0	100.0 mL	1.00
5.0	100.0	0.50
2.0	100.0	0.20

Milliliters of 1.0 μg/mL Used	Final Volume After Dilution	Final Concentration of Acid Metabolites, μg/mL
10.0	100.0 mL	0.10
5.0	100.0	0.05

Note: All solutions are transferred to amber glass bottles and stored at 0-6 °C.

GC External Calibration Standards for Propachlor and Neutral Metabolites

Pipet 10.0 mL each of the 1000 μg/mL stock solutions in ethyl acetate containing CP 31393, CP 58297, CP 47742, and CP 172362 into a 100 mL volumetric flask. Dilute to mark with ethyl acetate and mix well. This

solution contains 100 μg/mL each of propachlor, norchloropropachlor, hydroxypropachlor, and propachlor methylsulfone.

Pipet 10.0 mL from the 100 μg/mL into a 100 mL volumetric flask and dilute to the mark with ethyl acetate and mix well. This solution contains 10.0 μg/mL each of the four analytes.
The following dilutions are made with ethyl acetate.

Milliliters of 10.0 μg/mL Used	Final Volume After Dilution	Final Concentration of Propachlor and Neutral Metabolites, μg/mL
0.25	100 mL	0.025
0.50	100	0.050
1.00	100	0.100
2.00	100	0.200
4.00	100	0.400
6.00	100	0.600
8.00	100	0.800
10.00	100	1.000

Note: All solutions are transferred to amber glass bottles and stored at 0-6 °C.

Preparation of Fortification Solutions

Pipet 10.0 mL each of the 1000.0 μg/mL stock solutions of CP 31393, CP 58297, CP 47742, CP 172362, CP 118700, 118702, and 147935 into a 100 mL volumetric flask. Dilute to mark with acetonitrile and mix well. This solution contains 100 μg/mL of each analyte.

Pipet 5.0 mL each of the 1000.0 μg/mL stock solutions of CP 31393, CP 58297, CP 47742, CP 172362, CP 118700, 118702, and 147935 into a 100 mL volumetric flask. Dilute to mark with acetonitrile and mix well. This solution contains 50.0 μg/mL of each analyte.

From the 100 μg/mL fortification solution pipet 10.0 mL into a 100 mL volumetric flask. Dilute to the mark with acetonitrile and mix well. This solution contains 10.0 μg/mL of each analyte.

From the 50.0 μg/mL fortification solution pipet 10.0 mL into a 100 mL volumetric flask. Dilute to the mark with acetonitrile and mix well. This solution contains 5.0 μg/mL of each analyte.

From the 10.0 μg/mL fortification solution pipet 10.0 mL into a 100 mL volumetric flask. Dilute to the mark with acetonitrile and mix well. This solution contains 1.0 μg/mL of each analyte.

Note: All solutions are transferred to amber glass bottles and stored at 0-6 °C.

C. ANALYTICAL PROCEDURE

This analytical procedure requires approximately 3 working days for a set of 12 samples for two analysts. Day one extracts the samples. Day two concentrates the extracts and partitions the extracts into aqueous and organic phases followed by clean up via SAX and NH_2 columns. Day three make final preparations of the samples and quantitation by GC-TSD and HPLC-UV. This three-day approach does not include sample preparation.

SAMPLE PREPARATION

Soil samples are taken in the field with a soil probe which uses a plastic liner to hold the soil core in place. The soil sample in the plastic liner is properly labeled and frozen as soon as practical for shipment to Monsanto. The frozen soil core is then cut into appropriate depth segments. Multiple samples for each sampling interval are combined and mixed thoroughly to obtain a homogeneous sample. After mixing, the samples are stored frozen until analyzed.

DAY 1

EXTRACTION

1. Weigh 50.0 ± 0.04 gm of soil into a Whatman cellulose extraction thimble. Fortify at this stage. Example: Pipet 0.5 mL of the 5.0 µg/mL fortification solution directly onto the soil for a 0.05 ppm fortification of each analyte.

2. Add 300 mL of 50% ACN in water and a magnetic stirrer to a 500 mL round bottom flask and connect to the Soxhlet extractor as shown in Figure 2.

3. Set a variable-transformer controlled heating mantle under the flask. The heating mantle should be located on top of a magnetic stirring motor unit mounted on a lab jack.

4. Turn on the cooling water in the reflux condenser, the variac for heating mantle, and the magnetic stirrer. The variac should be set at about 90 volts to maintain a moderate reflux.

5. Soxhlet extract the soil overnight for about 16 hours. Example: Start the extraction at about 4 PM and terminate at about 8 AM the next morning.

DAY 2

EVAPORATION

6. Allow the Soxhlet apparatus to cool to ambient temperature. Drain all extract in the extraction tube and the thimble to the 500 mL round bottom flask.

7. Rinse the soil in the thimble with about 20 mL of 50% ACN in water and combine the rinse with the extract.

8. Rotary evaporate the extract to about 100-120 mL with an ambient temperature water bath. Do not heat the water bath. Loss of volatile neutral analytes may occur if heat is applied. It is also important that the final volume is in the 100-120 mL range to ensure complete removal of acetonitrile and minimal loss of neutral analytes. This step usually takes about 2-3 hours depending on the efficiency of the vacuum pump.

PARTITIONING

9. Transfer the extract to a 250 mL graduated cylinder.

10. Rinse the 500 mL flask with 2 x 10 mL water and add to the graduated cylinder.

11. Adjust the volume of the extract in the graduated cylinder to 150 mL with water.

12. Transfer the extract to a 250 mL separatory funnel. Rinse the cylinder with about 10 mL of water and add to the separatory funnel.

13. Rinse the 500 mL flask and the graduated cylinder with 50 mL of methylene chloride and transfer to the separatory funnel. Extract the neutral analytes by shaking for 5 minutes on a mechanical shaker. Allow 15 minutes for phase separation after shaking.

14. Fold and fit a Whatman #2 filter paper onto a filling funnel. Add 15-20 gm anhydrous sodium sulfate onto the filter paper. Wash the sodium sulfate with about 25 mL of methylene chloride. Discard the methylene chloride wash.

15. Drain the lower methylene chloride layer from Step 13 directly onto the prewashed sodium sulfate to remove any moisture and collect in a 250 mL round bottom flask. Save the aqueous phase.

16. Add another 50 mL methylene choride to the separatory funnel and

repeat Steps 13 and 15.

17. Rinse the sodium sulfate with 25 mL of methylene choride and combine to give a total of about 125 mL methylene chloride in the flask.

18. Rotary evaporate the methylene chloride to just dryness under reduced pressure with an ambient temperature water bath. Do not apply heat or allow the extract to foam or "bump" out of the flask during this step. It is critical to release the vacuum immediately when all methylene chloride is gone to minimize volatility loss of the analytes.

19. Proceed to NH_2 Column Cleanup, Step 21, below.

20. Save the aqueous phase in the separatory funnel as it contains the acid metabolites. Proceed to SAX Column Cleanup, Step 29, below.

DAY 3

NH_2 COLUMN CLEANUP

21. Add 1.0 ± 0.01 gm of 20 wt% carbon in NH_2 packing onto a 20 mL NH_2 solid phase extraction (SPE) column.

22. Wash the column with 20 mL of methanol and then 20 mL of ethyl acetate under gravity flow. Do not allow the column to dry. Add additional 5 mL of ethyl acetate to wet the column if necessary. Discard the wash.

23. Dissolve the residues in the 250 mL flask from Step 18 with 5 mL of ethyl acetate. Apply the sample to the column. Allow to elute dropwise and collect in a 100 mL round bottom flask.

24. Rinse the 250 mL flask with 5 mL ethyl acetate and apply to column.

25. Repeat Step 24 with another 10 mL of ethyl acetate.

26. After the column is completely drained, apply another 10 mL of ethyl acetate directly onto the column to give a total of 30 mL eluate.

27. Rotary evaporate to about 1 mL under reduced pressure with an ambient temperature bath. Do not apply heat or allow to go to dryness.

28. Transfer the sample to a 10 mL graduated centrifuge tube. Rinse the

flask twice with 3 to 4 mL of 20% ethyl acetate in iso-octane and add to the centrifuge tube. Adjust the final volume to 10 mL with 20% ethyl acetate in iso-octane. The sample is now ready for GC quantitation.

SAX COLUMN CLEANUP

29. Connect the Burdick and Jackson Vavuum Manifold to a vacuum source not to exceed 20" Hg. Place a 1 L liquid trap between the vacuum source and the manifold.

30. To use the manifold, remove cover and place a Luer hub solvent guide needle on the male Luer fitting of each flow control valve. Replace the cover and close all flow control valves.

31. Place desired number of 60 mL SAX columns into the female Luer receptacles on the cover. Turn vacuum on at the source and set vacuum to about 5" Hg using the bleed valve of the manifold by aligning the two holes.

32. Wash each column with 50 mL of methanol and then 50 mL of water. If the column goes dry, add additional 5 mL of water. Turn the control valve off when the water level in the column just reaches the top of the packing bed.

33. Apply the aqueous phase from Step 20 to the column and elute under a pressure of about 5" Hg. After all aqueous phase has been through the column, rinse the separatory funnel with 50 mL of 50% acetonitrile in water and apply to the column. Discard all aqueous eluate at this point.

34. Wash the column with an additional 100 mL of 50% acetonitrile in water and discard the eluate.

35. Elute the three acid metabolites with 125 mL of 50% acetonitrile in 0.05 M pH 5 KH_2PO_4 buffer under a pressure of about 5" Hg and collect in a 8 oz glass jar.

36. Transfer the eluate to a 250 mL round bottom flask. Rinse the jar with about 10 mL of 50% acetonitrile in water and add to the flask.

37. Rotary evaporate to dryness at a temperature up to 40 °C.

38. Dissolve residue in exactly 3 mL of 10% acetonitrile in 0.02 M pH 5 KH_2PO_4 buffer.

39. Filter sample through a 0.22 µm disk filter before injecting into the HPLC for separation and quantitation.

III. INSTRUMENT PARAMETERS

A Varian 3600 GC-TSD has been used for the quantitation of parent propachlor and its neutral metabolites, whereas a Perkin Elmer HPLC equipped with UV detector has been used for the quantitation of the three acid metabolites. Be aware that different instruments may require modification of these parameters for optimum separation and sensitivity of these analytes.

A. GC OPERATING CONDITIONS

Instrument:	Varian 3600 GC equipped with a Thermionic Specific Detector (TSD)
Column:	J&W Scientific DB-5 (30 m x 0.25 mm I.D., 0.25 µm) capillary column
Temperature:	100 °C hold for 1 min, then program at 3 °C/ min to 200 °C and hold for 1 min, then program at 10 °C/min to 240 °C and hold for 2 min. Total run time is approximately 42 min
Injector Temp:	250 °C
Detector Temp:	300 °C
TSD Bias Voltage:	2.8 - 3.5 mV
Attenuation:	4
Range:	10^{-12}
Injection Vol:	5 µL
Hydrogen Flow:	approx. 4.5 mL/min
Air Flow:	approx. 175 mL/min
Nitrogen Flow:	approx. 2 mL/min
N_2 Make-up:	approx. 28 mL/min
Split Ratio:	1 to 10

B. HPLC OPERATING CONDITIONS

Instrument:	Perkin Elmer Series 4 LC equipped with a LC-95 UV detector
Column:	Alltech Alltima C18 (250 mm x 4.6 mm I.D., 5µm) analytical column with Alltima C18 guard column
Temperature:	Ambient
Detection:	UV detector at 210 nm
Flow Rate:	1.0 mL/min
Mobile Phase:	10% Acetonitrile in 0.02 M pH 5 KH_2PO_4 buffer
Response Time:	2000 msec
Sample Size:	100 µL
Recorder Range:	AUFS 0.1

IV. INTERFERENCES

A. SOIL SAMPLES

Using the analytical method described here, very little or no background has been observed. If observed, the background concentration is subtracted from fortified samples prior to calculating recovery.

B. SOLVENT

Ethyl acetate was found to contain a trace unknown co-eluting with hydroxypropachlor. This interference was minimized by evaporating the ethyl acetate eluate to about 1 mL and then adjust the final volume to 10 mL with 20% ethyl acetate in iso-octane as described in Steps 27 and 28.

C. LABWARE

The glassware cleaning procedure consists of washing in a mechanical washer with hot soapy water followed by deionized water rinse and final acetone rinse. No interferences have been observed.

V. CONFIRMATORY METHOD

A confirmatory method for parent propachlor and its three acid metabolites can be found in MSL-6815. The previous method utilizes HPLC cleanup as opposed to the more convenient solid phase extraction cleanup described here.

VI. TIME REQUIRED FOR ANALYSIS

A set of 12 samples requires approximately 3 days per two analysts from initial extraction to final determination.

VII. MODIFICATION OR POTENTIAL PROBLEMS

Propachlor and its neutral metabolites are volatile. During rotary evaporation of the extract in 50% acetonitrile-water, in methylene chloride and in ethyl acetate, heat MUST not be applied and should follow the specific instructions as indicated in Steps 8, 18 and 27.

VIII. METHOD OF CALCULATION

Calculations of amounts of the analytes found in each analytical sample, the estimated accuracy of the set, the percent soil moisture, and the ppm concentration of the analytes are calculated as described.

A. QUANTITATION OF ANALYTES

A linear calibration curve is generated for every set of samples run. Eight levels of standards are prepared on the range of 0.025 µg/mL to 1.00 µg/mL for parent propachlor and the neutrals, and 0.05 µg/mL to 10.0 µg/mL for the acid metabolites. These standards are interspersed among the samples. The calibration curves are generated by plotting the peak area of the detector response against the concentration of each calibration standard for each of the analytes. The response of any given sample must not exceed the response of the most concentrated standard. If this occurs, dilution of the sample will be necessary with the appropriate HPLC mobile phase or GC solvent.

The concentration of the analytes in the soil extract is determined based upon the peak area of the elution peak of each analytes. The concentration is determined by comparison of peak areas to to a calibration curve generated from concurrently run external standards.

Percent recovery is calculated as follows:

$$\% \text{ Recovery} = \frac{\text{PPM Found}}{\text{PPM Fortified}} \times 100$$

If the check sample was found to contain interference with one of the analytes, then this amount must be substracted from the amount found in order to calculate recovery for that sample.

B. MOISTURE DETERMINATION

The percent soil moisture is determined for a given sample in order to calculate the residue level based on dry soil weight and is determined as follows:

Weigh a 60 or 100 mL beaker and record this weight to a hundredth of a gram. Next, weigh out an aliquot of 20.0 ± 10% of the soil sample analyzed and record this weight to a hundredth of a gram. Place the beaker containing the soil in a dry heat oven set to at least 120 °C for at least 12 hours. After this period, remove the beaker and allow to cool. Reweigh the beaker plus the dry soil and record this weight to a hundredth of a gram. The amount of moisture contained in that soil is the difference between the combined (beaker + soil) weight before drying and the combined weight after drying. The percent moisture is calculated using the following formula.

$$\% \text{ Moisture} = \frac{\text{Combined Wet Wt} - \text{Combined Dry Wt}}{\text{Wt of Wet Soil}} \times 100$$

C. RESIDUE DETERMINATION

After determining the concentration of each analyte in the sample extract, the total amount of residues in the 50 gm soil sample (ppm) is determined. To do this, the concentration (µg/mL) of the analytes in the extract is multipied by the total dilution volume of the extract to obtain the total amount (µg) of each analyte in the sample analyzed. The total amount of each analyte is then divided by the dry soil weight (gm) of the sample analyzed resulting in µg/gm or parts per million (ppm). The equation is shown below:

$$\frac{\mu\text{g of Analyte}}{\text{mL}} \times \frac{\text{Dilution Volume}}{\text{Dry Soil Weight}} = \text{PPM Analyte}$$

Where: µg of Analyte/mL = Concentration of analyte in the extract.

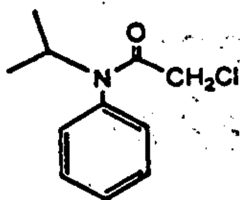
Dilution volume = The total dilution volume (mL) of the extract taking into account any further dilution necessary to maintain peak area of the analyte within the highest standard of the calibration curve.

IX. LIMIT OF QUANTITATION (LOQ)

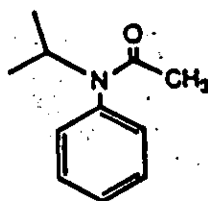
The limit of quantitation has not been defined. For this SOP, the lower limit of method validation (LLMV) is 0.01 ppm each for all analytes except the sulfinyl acetic acid metabolite which is 0.05 ppm. The LLMV is defined as the lowest level of fortification where acceptable recovery was obtained. An example of method validation data is presented in Tables 1 and 2 and example chromatograms are shown in Figure 3.

Figure 1

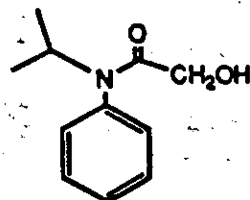
Structures of Propachlor and its Soil Metabolites



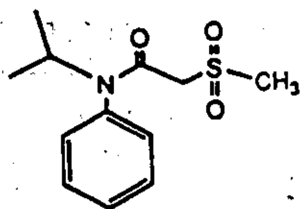
Propachlor
CP 31393



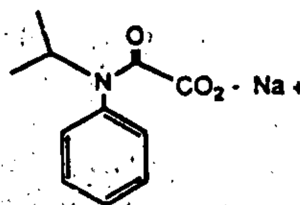
Norchloropropachlor
CP 58297



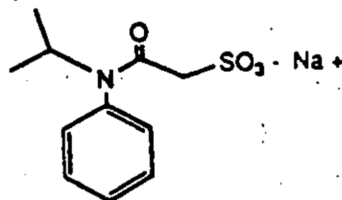
2-Hydroxypropachlor
CP 47742



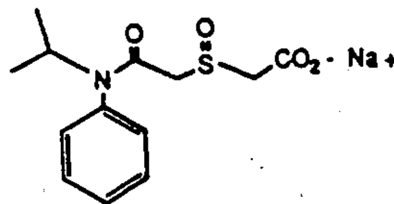
Propachlor Methylsulfone
CP 172326



Propachlor Oxanilate
CP 118700



Propachlor Sulfonate
CP 118702



Propachlor Sulfinyl Acetate
CP 147935

A. Theory and Equations

The amount of analyte in an unknown sample was determined from the detector response using linear calibration eqn. (1):

$$\text{Amount Injected} = A \times (\text{Detector Response}) + B \quad (1)$$

The calibration standards within each set were used to determine the parameters A and B. A linear least squares regression was first performed to estimate the slope, M, and intercept, K, of eqn. (2):

$$\text{Response} = K + M \times \text{Amount Injected} \quad (2)$$

The parameters A and B were then calculated from eqns. (3) and (4):

$$A = \frac{1}{M} \quad (3); \qquad B = -\frac{K}{M} \quad (4)$$

The residue in the unknown sample was calculated using eqn. (5):

$$\text{Residue (ppm)} = \frac{[(A \times \text{response}) + B] \times \text{concn. vol.}}{\text{sample size}} \quad (5)$$

where concn. = concentration; vol. = volume

B. Sample Calculation

Norchloropropachlor residues in Sample 4-S (0-6 inch soil layer)

Using eqn. (5), with response = 156.1810 (mV-secs), conc. vol. = 10 mL, sample wt. = 49.99, A = 3.2056E-3, and B = 1.3862E-3, residue (ppm) can be calculated as:

$$\text{Residue} = \frac{[(3.2056E-3 \times 156.1810) + 1.3862E-3] \times 10}{49.99} \ \mu g/g$$

Residue = 0.10042717 ppm, or

Residue = 0.10043 ppm (to five decimal places)