Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Pyriproxyfen

MRID #: 440369-16

Matrix: Soil

Analysis: GC/NPD

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APPENDIX 3

ANALYTICAL METHOD

VALENT U.S.A. CORPORATION
VALENT TECHNICAL CENTER
DUBLIN, CALIFORNIA

DETERMINATION OF PYRIPROXYFEN AND PYPAC RESIDUES IN SOIL AND SOIL SEDIMENT METHOD RM-33S-1-5

DATE: AUGUST 5, 1994
REVISED: OCTOBER 26, 1995

INTRODUCTION

This method determines residues of pyriproxyfen [V-71639, 4-phenoxyphenyl(RS)-2-(2-pyridyloxy) propyl ether] and one of its metabolites, PYPAC [(RS)-2-(2-pyridyloxy) propionic acid], in soil and soil sediment. Pyriproxyfen and PYPAC are simultaneously extracted from soil using methanol/0.1 N NaOH but are separated by partitioning with dichloromethane. The pyriproxyfen, which is extracted into the dichloromethane phase, is cleaned-up with column chromatography and analyzed by gas chromatography using a nitrogen-phosphorus specific flame-ionization detector (NPD). The basic aqueous phase, containing the PYPAC residues, is acidified and extracted with ethyl acetate and methylated with methyl iodide. After cleanup by silica gel solid phase extraction, the sample extract is analyzed by gas chromatography using a NPD.

This method is a modification of methods PYR793 and PAC893, developed by Analytical Bio-Chemistry Laboratories, Inc.[1,2]. The method was revised on June 20, 1995 to change the sample weight extracted and to modify the evaporation and derivatization steps in the PYPAC procedure. The method was further revised on August 3, 1995 and August 8, 1995 to change the partitioning procedures to improve PYPAC recoveries. The October 9, 1995 revision was made to add confirmatory column parameters. The October 26, 1995 revision was made to add the solid phase extraction cleanup step to the PYPAC procedure.

REAGENTS

Acetone - Pesticide quality or equivalent.

Aluminum oxide, activated, neutral, Brockmann I, Aldrich Cat. # 19,997-4 or equivalent.

Dichloromethane - Pesticide quality or equivalent.

Ethyl acetate - Pesticide quality or equivalent.

Hexane - Pesticide quality or equivalent.

REAGENTS (CONTINUED)

Hastings-Sendroy buffer - combine 611 mL of 1/15 M disodium hydrogen phosphate with 389 mL of 1/15 M potassium phosphate. Mix well.

Hydrochloric Acid - concentrated, 36.5-38.0% in water. Prepare a 1.0 N solution by carefully adding 100 mL of the concentrated acid to a 1 liter volumetric flask, partially filled with deionized water. Dilute to volume with deionized water, stopper and shake.

Methanol - Pesticide quality or equivalent.

Methyl iodide (Iodomethane) - 99%, Aldrich Cat# I-850-7 or equivalent.

1-Octanol - 99+%, Aldrich Cat #29,324-5 or equivalent.

Phosphoric acid, 85%, Analytical Reagent Grade or equivalent. Prepare a 1 M solution by carefully adding 98 mL of the 85% solution to a 1L flask, partially filled with deionized water. Dilute to volume with deionized water, stopper and shake.

Potassium phosphate, monobasic, reagent grade or equivalent. Prepare a 1/15 M solution by dissolving 9.07 grams in 1 liter of deionized water. Mix well.

Sodium chloride - reagent grade or equivalent. Prepare a 5% (w/v) aqueous solution by adding 50 grams to a 1L flask and diluting to volume with deionized water. Stopper and shake.

Sodium hydroxide - reagent grade, 50% (w/w) solution. Prepare a 0.1 N solution by carefully adding 8 grams of the 50% solution to a 1L flask, partially filled with deionized water. Dilute to volume with deionized water, stopper and shake.

Sodium phosphate, dibasic, heptahydrate ($Na_2HPO_4 \cdot 7H_2O$), reagent grade or equivalent. Prepare a 1/15 M solution by dissolving 17.9 grams in 1 liter of deionized water. Mix well.

Sodium sulfate - anhydrous, granular, AR grade or equivalent.

Tetrabutylammonium hydroxide (TBAH)- 1.0 M solution in methanol, Aldrich Cat# 23,018-9 or equivalent.

Toluene - Pesticide quality or equivalent.

Water - deionized.

REFERENCE STANDARDS

Pyriproxyfen - analytical standard of known purity, available from Valent U.S.A. Corporation. Prepare a stock solution containing 1 mg/mL in acetone. Prepare calibrating/linearity standards by diluting this stock solution to 0.10, 0.50, 1.0, and 2.0 μg/mL with toluene. Prepare a fortifying solution by diluting the stock solution to 1.0 μg/mL with acetone.

PYPAC - analytical standard of known purity, available from Valent U.S.A. Corporation.. Prepare a stock solution containing 1 mg/mL in acetone. Prepare a fortifying solution by diluting the stock solution to 1.0 μg/mL with acetone.

PYPAC-Methyl Ester - analytical standard of known purity, available from Valent U.S.A. Corporation. Prepare a stock solution containing 1 mg/mL in acetone. Prepare calibrating/ linearity standards by diluting this stock solution to 0.02, 0.05, 0.10, 0.20, and 0.50 μg/mL with hexane:ethyl acetate (5:2, v/v).

All reference standard solutions should be stored at ≤0°C when not in use.

EQUIPMENT

Baker SPE®-12G Column Processor (12-port vacuum manifold) - J.T. Baker Product Number 7018-00 or equivalent system.

BakerBond SPE® silica gel disposable columns - 3 mL, J.T. Baker Procuct Number 7086-03 or equivalent.

Büchner funnels - 9 cm diameter.

Dri-Bath®, Type 17600, Thermolyne or equivalent.

Filter flasks - 500 mL.

Filter funnels - glass, 10 cm diameter.

Filter paper - Whatman GF/A glass fiber or equivalent.

EQUIPMENT (CONTINUED)

Gas Chromatograph - Hewlett-Packard Model 5890, equipped with a packed column glass insert for splitless injection (HP Part No. 5080-8732), an NP detector, automatic sampler, and an HP ChemStation or equivalent system.

Glass chromatography column - 10.5 mm ID x 250 mm with 200 mL reservoir, Kontes Cat. # K-420280-0213 or equivalent.

Graduated cylinders - glass, 10 mL.

Linear shaker - Eberbach Cat. # 6000 or equivalent.

Mason jars - 1 pint with plastic screw cap lids or equivalent.

N-Evap - Organomation Model 111 or equivalent.

Pasteur pipets - 5¾" and 9".

pH paper - range 0-14, EM Science or equivalent.

Reacti-Vials® - 5.0 mL, with open top screw cap with Teflon® laminated disc, Pierce Cat# 13223, or equivalent.

Rotary evaporator - Büchi (Brinkman) or equivalent, equipped with a temperature controlled water bath.

Round-bottom flasks - 50 mL, 100 mL, 500 mL.

Separatory funnels - 500 mL.

Volumetric flasks - 10 mL and 1 liter.

Vortex mixer - Vortex Genie 2, VWR or equivalent.

Ultrasonic cleaner, Branson 3200 or equivalent.

ANALYTICAL PROCEDURES I - ANALYSIS FOR PYRIPROXYFEN

Extraction

Weigh 20 grams (± 0.1 grams) of soil into a one pint Mason jar. At this point, if required by the testing facility, control samples to be used for method recoveries may be fortified with pyriproxyfen and PYPAC (See Note 1). Add 40 mL of methanol/0.1 N NaOH (4:1, v/v) to the sample, cap and place on the linear shaker. Shake on HIGH for 15 minutes.

Filter the sample into a 500 mL filter flask using a 9 cm Büchner funnel and 9 cm Whatman GF/A glass fiber filter paper. Rinse the Mason jar with 20 mL of extraction solvent and add to the Büchner funnel. Transfer the filter cake back into the Mason jar and re-extract with a second 40 mL portion of methanol/ 0.1 N NaOH (4:1, v/v). Filter as above, combining this extract with the first extract. Rinse the Mason jar with 20 mL of extraction solvent and add to the Büchner funnel.

Transfer the combined filtrates to a 500 mL round-bottom flask. Rinse the filter flask with two 20 mL portions of methanol/0.1 N NaOH (4:1, v/v) and add to the 500 mL round-bottom flask. Evaporate to approximately 20 mL using a rotary-evaporator and water bath set to <40°C.

Dichloromethane/Water Partitioning

Add 100 mL of a deionized water to the round-bottom flask and swirl. Transfer the sample to a 500 mL separatory funnel, add 1 mL of Hastings-Sendroy buffer, and adjust the pH to 7 with 1 M phosphoric acid (approximately 0.75 mL). Check the pH with pH paper to ensure that the pH is approximately 7. Add 100 mL of dichloromethane to the separatory funnel using portions of this dichloromethane to rinse the round-bottom flask. Shake for approximately 1 minute and drain the lower dichloromethane layer through a 10 cm filter funnel containing approximately 50 grams of sodium sulfate, suspended on a glass wool plug, into a 500 mL round-bottom flask.

Add 100 mL of dichloromethane to the separatory funnel and partition as above. Drain the lower dichloromethane layer through the filter funnel containing the sodium sulfate into the 500 mL round-bottom flask containing the first extract. Rinse the sodium sulfate with two 10 mL of dichloromethane. **SAVE THE UPPER AQUEOUS LAYER FOR PYPAC ANALYSIS (REFER TO ANALYTICAL PROCEDURES II - ANALYSIS OF PYPAC).** Evaporate the combined dichloromethane layers, containing the pyriproxyfen residues, to dryness using a rotary-evaporator and water bath set to <40°C.

Alumina Column Cleanup (See Note 2)

Place a glass wool plug at the bottom of the glass chromatography column. Close the column stopcock and add 25 mL of hexane:ethyl acetate (10:1, v/v) to the column. Measure 10 mL of alumina in a 10 mL graduated cylinder and slowly add to the column while gently tapping the side of the column. Rinse the alumina down the column walls with hexane:ethyl acetate (10:1, v/v). Measure 1-3 mL of sodium sulfate in the 10 mL graduated cylinder and add to the top of the column. Open the column stopcock and allow the solvent to drain to the top of the column. Discard this eluant.

Add 3 mL of hexane:ethyl acetate (10:1, v/v) to the round-bo..m flask containing the sample extract. Swirl and sonicate for 15 seconds to dissolve the residue and transfer to the top of the column using a Pasteur pipet. Rinse the flask with three 3 mL portions of hexane:ethyl acetate (10:1, v/v) and transfer each rinse to the top of the column. Drain the solvent to the top of the sodium sulfate layer after each rinse. Discard this eluant (a total of 12 mL).

Place a 100 mL round-bottom flask under the column and elute the pyriproxyfen with 28 mL of hexane:ethyl acetate (10:1, v/v). Evaporate the eluate to dryness using a rotary-evaporator and water bath set to <40°C. Add 2.0 mL of toluene to the flask containing the dried sample residue. Swirl and sonicate for 15 seconds to completely dissolve the residue. Transfer the extract to a screw cap vial using a Pasteur pipet and store at ≤0°C until GC analysis.

Gas Chromatography Measurement (See Notes 3 and 4)

Transfer a portion of the sample extract to an autosampler vial using a Pasteur pipet and analyze, along with calibrating standard solutions, using the following operating conditions:

Column: DB-17 (30 M x 530 μm) wide bore capillary (1.0 μm film thickness), J & W Scientific Cat # 125-1732 or equivalent.

Column Oven Temperature Program:

Initial Temp:	265°C
Hold Time:	2.5 minutes
Prog Rate:	10°C/minute
Final Temp:	280°C
Hold Time:	6 minutes

Detector Temperature: 300°C
Injector Temperature: 250°C
Carrier Gas: Helium at 10 mL/min
Detector Makeup Gas: Helium at 20 mL/min
Air: 110 mL/min
Hydrogen: 3.6 mL/min
Injection Size: 1.0 μl
Retention Time: 4.1 minutes (See Figure 1)

The GC parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography or to resolve matrix interferences. Each set of chromatograms must be clearly labelled with the GC parameters used. See Note 6 for alternate GC parameters.

The recommended sequence of samples and standards for analysis is: calibrating standard, sample, sample, sample, calibrating standard, etc. The calibrating standard vials contain 1.0 µg/mL of pyriproxyfen in toluene. This sequence may, however, be modified if the reproducibility requirement is met. See Note 4.

Calculations

The amount of pyriproxyfen in each sample is calculated using the following formula:

$$ppm\ Pyriproxyfen = \frac{B \times C \times V \times DF}{A \times W}$$

Where:

B = integration counts for pyriproxyfen in the sample.
C = concentration of the pyriproxyfen calibrating standard (1.0 µg/mL).
V = final volume of the sample extract (2.0 mL).
DF = dilution factor, used if the sample extract is diluted prior to analysis.
A = mean integration counts for the pyriproxyfen standards.
W = sample weight (20 grams)

ANALYTICAL PROCEDURES II - ANALYSIS FOR PYPAC

Extraction

Add 3.0 mL of 1 N HCl to the aqueous phase in the separatory funnel containing the PYPAC residues (from the dichloromethane/water partitioning step, ANALYTICAL PROCEDURES I-ANALYSIS FOR PYRIPROXYFEN). Stopper the funnel and shake gently to mix completely. Check the pH with pH paper to ensure that the pH is <3.

Add 100 mL of ethyl acetate to the separatory funnel and shake for approximately one minute. Drain the lower aqueous layer into the original 500 mL round-bottom flask. Pour the upper ethyl acetate layer into a clean 500 mL round-bottom flask through a 10 cm filter funnel containing approximately 20 grams of sodium sulfate, suspended on a plug of glass wool..

Return the aqueous phase to the separatory funnel, add 100 mL of ethyl acetate and partition as above. Discard the lower aqueous layer. Pour the upper ethyl acetate layer through the 10 cm filter funnel containing the sodium sulfate into the 500 mL round-bottom flask containing the first extract. The sample extract may be stored overnight at ≤0°C.

Methylation

CAUTION: METHYL IODIDE IS HIGHLY TOXIC AND A CANCER SUSPECT AGENT. WEAR GLOVES AND WORK UNDER A HOOD.

Evaporate the combined ethyl acetate layers to approximately 5 mL using a rotary-evaporator and water bath set to <30°C. Transfer the extract to a 50 mL round-bottom flask using three 5 mL portions of acetone and a Pasteur pipet. Evaporate the acetone just to dryness using a rotary-evaporator and water bath set to <30°C. Remove the flask immediately from the evaporator and transfer the extract to a 5 mL Reacti-Vial® using three 1 mL portions of acetone and a Pasteur pipet. Evaporate to approximately 1 mL using the N-Evap and a gentle stream of nitrogen (water bath set to <30°C). Adjust the volume to approximately 1 mL with acetone if necessary.

Add 50 µl of TBAH followed by 100 µl of methyl iodide to the Reacti-Vial®. Cap the Reacti-Vial®, vortex briefly, and heat to 45-50°C in the Dri-Bath for 2 hours. At the end of the reaction period, remove the vial from the Dri-Bath and cool to room temperature. Add 50 µl of 1-octanol to the vial, vortex briefly, and carefully evaporate the acetone to approximately 0.3 mL under a gentle stream of nitrogen. Do not immerse the vial in the water bath. **(DO NOT ALLOW THE ACETONE TO COMPLETELY EVAPORATE-SEE NOTE 5)**. Add 1 mL of hexane:ethyl acetate (5:2, v/v) and vortex for approximately 15 seconds.

Silica Gel SPE Cleanup

Attach a Bakerbond SPE® silica gel disposable column to the vacuum manifold. Attach a 10 mL glass syringe (plunger removed) to the column and pre-condition the column with 10 mL of hexane. **Do not exceed a flow rate of 5 mL/minute. Do not allow the column to dry before the sample is applied.**

Remove the glass syringe from the column and place a 10 mL volumetric flask under the column. Transfer the sample extract to the column using a Pasteur pipet and apply vacuum until the solvent reaches the top of the packing. Rinse the Reacti-Vial® with two 1 mL portions of hexane:ethyl acetate (5:2, v/v) and transfer each portion to the column using a Pasteur pipet, allowing each portion to reach the top of the packing before adding the next. Dilute the extract to volume with hexane:ethyl acetate (5:2, v/v). The extract may be stored a ≤0°C until analysis.

Gas Chromatography Measurement (See Notes 3 and 4)

Transfer a portion of the sample extract to an autosampler vial with a Pasteur pipet and analyze, along with calibrating standard solutions, using the following operating conditions:

Column: DB-17 (30 M x 530 μm) wide bore capillary (1.0 μm film thickness)
J & W Scientific Cat # 125-1732 or equivalent.

Column Oven Temperature Program:

Initial Temp:	130°C
Hold Time:	2.0 minutes
Prog Rate:	5°C/minute
Final Temp:	150°C
Hold Time:	2 minutes
Prog Rate A:	25°C/minute
Final Temp A:	200°C
Final Time A:	2 minutes

Detector Temperature: 300°C
Injector Temperature: 250°C
Carrier Gas: Helium at 10 mL/min
Detector Makeup Gas: Helium at 20 mL/min
Air: 110 mL/min
Hydrogen: 3.6 mL/min
Injection Size: 2.0 μl
Retention Time: 4.0 minutes (See Figure 4)

The GC parameters shown above are given only as a guide. They may be modified as needed to optimize the chromatography or to resolve matrix interferences. Each set of chromatograms must be clearly labelled with the GC parameters used. See Note 7 for alternate GC parameters.

The recommended sequence of samples and standards for analysis is: calibrating standard, sample, sample, sample, calibrating standard, etc. The calibrating standard vials contain 0.20 μg/mL of PYPAC-ME in hexane:ethyl acetate. This sequence may, however, be modified if the reproducibility requirement is met. See Note 4.

Calculations

The amount of PYPAC in each sample is calculated using the following formula:

$$ppm\ PYPAC = \frac{B \times C \times V \times MW \times DF}{A \times W}$$

where:

- B = integration counts for PYPAC-ME in the sample.
- C = concentration of the PYPAC-ME calibrating standard (0.20 µg/mL).
- V = final volume of the sample extract (10.0 mL).
- MW = molecular weight factor (167/181 = 0.923) to convert PYPAC-ME to PYPAC.
- DF = dilution factor, used if the sample extract is diluted prior to analysis.
- A = mean integration counts for the PYPAC-ME standards.
- W = sample weight (20 grams)

LIMITS OF DETECTION AND QUANTITATION

The limit of detection (LOD) of pyriproxyfen and PYPAC in soil and soil sediment analyzed by this method is 0.01 ppm. The validated limit of quantitation (LOQ) for both analytes is 0.02 ppm.

ANALYSIS TIME

A trained analyst can complete the analysis of a set of eight samples for pyriproxyfen and PYPAC in approximately 16 hours. The results are available within 48 hours of initiating the analysis.

NOTES

1. At Valent, a standard operating procedure (SOP# VR-002) requires that fortified control samples be analyzed with each set of samples. If the testing facility does not require concurrent analysis of fortified control samples, or if a UTC sample is not available, this method requirement may be waived.

   The level of fortification is generally 0.02 ppm (the LOQ of the method) and/or 0.1 ppm of analyte. These fortifications are made by adding 0.40 mL and 2.0 mL, respectively, of the 1.0 µg/mL fortifying solutions to a 20 gram sample. Method recoveries must be 70% to 120% to be acceptable unless approved by the chemist responsible for the analysis.

2. The alumina may be used as received from the manufacturer. Each batch must be checked for recovery of pyriproxyfen as follows: Transfer 1.0 mL of the 1.0 µg/mL pyriproxyfen fortifying solution to a 50 mL round-bottom flask and evaporate just to dryness using a rotary-evaporator and water bath set to <40°C. Transfer the residue to an alumina column and elute the pyriproxyfen as described under Alumina Column Cleanup.

   Evaporate the eluate just to dryness using a rotary-evaporator and water bath set to <40° C. Add 1.0 mL of toluene to the flask and swirl to completely dissolve the residue. Analyze this eluant and the 1.0 µg/mL calibrating standard as described under Gas Chromatography Measurement. If the recovery of pyriproxyfen is less than 90%, then the elution profile of pyriproxyfen must be determined.

3. At Valent, linearity of the gas chromatograph must be determined each day that samples are analyzed (Valent SOP# VR-007). Linearity is determined by analyzing a minimum of four linearity standards containing from 0.10 to 2.0 µg/mL of pyriproxyfen and from 0.02 to 0.5 µg/mL of PYPAC. The response for each standard is normalized to response per 1.0 µg/mL by dividing the response of each standard by its concentration. The coefficient of variation (CV) of these responses must be 10% or less. Sample extracts must be diluted to bring the concentration of each analyte within the range of linearity established.

4. At Valent, reproducibility of an analytical run is determined by calculating the CV from the peak units obtained for the calibrating standards analyzed during the run. For a run to be acceptable, these CV's must be 10% or less unless approved by the chemist responsible for the analysis (Valent SOP# VR-013).

NOTES (CONTINUED)

5. Methyl-PYPAC is quite volatile and can be lost during evaporation. Octanol must be added as a "keeper" to the methylated extracts before evaporating. The evaporation procedure described must be followed exactly as written or significant losses of PYPAC may occur.

6. If matrix interferences are encountered during the analysis of pyriproxyfen, the following GC parameters may be used:

   Column: DB-5 (30 M x 530 μm) wide bore capillary (1.5 μm film thickness).

   Column Oven Temperature: 250°C
   Detector Temperature: 300°C
   Injector Temperature: 300°C

   Carrier Gas: Helium at 20 mL/min
   Make-Up Gas: Helium at 10 mL/min
   Air: 102 mL/min
   Hydrogen: 3.8 mL/min
   Injection Size: 1.0 μl
   Retention Time: 3.8 minutes

7. If matrix interferences are encountered during the analysis of PYPAC, the following GC parameters may be used:

   Column: DB-5 (30 M x 530 μm) wide bore capillary (1.5 μm film thickness).

   Column Oven Temperature: 120°C
   Detector Temperature: 300°C
   Injector Temperature: 300°C

   Carrier Gas: Helium at 20 mL/min
   Make-Up Gas: Helium at 10 mL/min
   Air: 102 mL/min
   Hydrogen: 3.8 mL/min
   Injection Size: 2.0 μl
   Retention Time: 3.8 minutes