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Determination of Chlorpyrifos in Soil by Gas Chromatography

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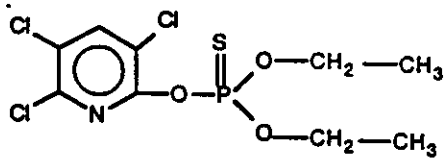
A. Scope

This method is applicable for the quantitative determination of chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl)phosphorothioate] in soil at a validated lower level of quantitation of 0.01 µg/g.



Chlorpyrifos
CAS No. 2921-88-2

B. Principle

Residues of chlorpyrifos are extracted from soil with acidic acetone. A portion of the acetone solution is concentrated, acidified water is added, and chlorpyrifos is partitioned into a known volume of hexane. A portion of the hexane extract is analyzed by gas chromatography using flame photometric detection.

C. Safety Precautions

1. Each analyst should be acquainted with potential hazards of the reagents, products, and solvents before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information on non-DowElanco products should be requested from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

2. Exercise normal laboratory precautions. Acetone and hexane are flammable and should be used in well ventilated areas away from an ignition source.

3. Concentrated phosphoric acid is corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be worn when handling this reagent.

D. Equipment (note K.6.a.)

1. Balance, Analytical, Model AE 200, Mettler Instrument Corporation, Hightstown, NJ 08520.

2. Balance, Pan, Model BB2440, Mettler Instrument Corporation.

3. Centrifuge, with head to accommodate 11-dram vials, Centra-8, International Equipment Company, Needham Heights, MA 02194.

4. Crimper, catalog no. 8710-0979, Hewlett-Packard, Avondale, PA 19311.

5. Evaporator, TurboVap, Model LV, Zymark Corp., Hopkinton, MA 01748.

6. Gas chromatograph, Model 5890, Hewlett Packard, equipped with a flame photometric detector with a phosphorous filter and a split/splitless capillary inlet system, Hewlett-Packard.

7. Micropipetter, 200-µL, Drummond, catalog no. 21-176F, Fisher Scientific, Itasca, IL 60143.

8. Sampler, automatic, Model 7673B, Hewlett-Packard.

9. Shaker, variable-speed reciprocating, with box carrier, Model 6000, Eberbach Corp., Ann Arbor, MI 48106.

10. Ultrasonic bath, Model 3200, Branson Cleaning Equipment Company, Shelton, CT 06484.

E. Glassware and Materials (note K.6.a.)

1. Caps, 24-mm phenolic with poly-seal liner, catalog no. 02-883-5G, Fisher Scientific.

2. Column, capillary gas chromatography, J&W Scientific, DB-5, 10 m x 0.25 mm, 1.0 µm film thickness, catalog no. 122-5033 (30 m column cut to 10 m), J&W Scientific, Folsom, CA 95630-4714.

3. Column (for structure confirmation), capillary gas chromatography, J&W Scientific, DB-1, 20 m x 0.18 mm, 0.4 µm film thickness, catalog no. 121-1023, J&W Scientific.

4. Filter, oxygen, catalog no. 7970, Chrompack, Inc., Raritan, NJ 08869 (note K.6.b.).

5. Gases, hydrogen (99.99%), oxygen (99.6% Extra Dry), helium (99.995%), and nitrogen (99.998%), Scott Specialty Gases, 1290 Combermere Street, Troy, MI 48083.

6. Inlet liner, deactivated, catalog no. 5181-3315, Hewlett Packard.

7. Test tube, culture, 16 x 100 mm, catalog no. 14-923H, Fisher Scientific (note K.6.c.).

8. Vials, 11-dram, catalog no. 03-339-5D, Fisher Scientific.

9. Vials, injection, 2-mL with sealing caps for automatic injection, catalog no. C4011-1 and C4011-1A, National Scientific Company, Lawrenceville, GA 30245.

10. Vial inserts, catalog no. 4011-631, National Scientific Company.

F. Reagents and Chemicals (note K.6.a.)

1. Acetone (Optima Grade), and hexane (Optima Grade), Fisher Scientific.

2. o-Phosphoric acid, 85% (ACS reagent grade), Fisher Scientific.

3. Water, ultra-pure, Model Milli-Q UV plus, Millipore Corp., Bedford, MA 01730.

4. 98% Acetone/1% phosphoric acid/1% water:

   Prepare by adding 10.0 mL of water followed by 10.0 mL of concentrated phosphoric acid into a 1000-mL volumetric flask. Add approximately 800 mL of acetone, swirl the flask, and bring to volume with acetone.

5. 1% Phosphoric acid/99% water:

   Prepare by adding approximately 500 mL of water to a 1000-mL volumetric flask. Add 10.0 mL of concentrated phosphoric acid to the flask, swirl the flask, and bring to volume with water.

6. Standard:

   Chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] analytical standard. Obtain from Sample Coordinator, DowElanco, 9410 Zionsville Road, Indianapolis, Indiana, 46268-1053.

G. Preparation of Calibration Standards/Spiking Solutions

1. Preparation of Chlorpyrifos Spiking Solutions

   a. Stock Solution - dissolve 0.1000 g of chlorpyrifos analytical standard in acetone in a 100-mL volumetric flask to obtain a 1000 µg/mL solution.

b. Dilute the stock solution with acetone in the following manner:

1	2	3	4
Concentration of Initial Solution µg/mL	Aliquot of Initial Solution mL	Final Solution Volume mL	Spiking Solution Final Concentration µg/mL
1000	5.0	100	50
50	50.0	100	25
25	10.0	100	2.50
2.50	25.0	50	1.25
2.50	10.0	100	0.25

200 µL of each solution listed in Column (4) per 5.0-g sample is equivalent to 2.0, 1.0, 0.10, 0.05, and 0.01 µg/g, respectively.

1 mL of each solution listed in Column (4) per 5.0-g sample is equivalent to 10.0, 5.0, 0.50, 0.25, and 0.05 µg/g, respectively.

2. Preparation of Chlorpyrifos Calibration Solutions

Dilute the 1000 µg/mL stock solution from Section G.1.a. with hexane in the following manner:

Concentration of Initial Solution µg/mL	Aliquot of Initial Solution mL	Final Solution Volume mL	Calibration Solution Final Concentration µg/mL
1000	2.0	100	20
20	5.0	100	1.0
1.0	50.0	100	0.50
1.0	25.0	100	0.25
0.50	25.0	100	0.125
0.50	5.0	100	0.025

H. Gas Chromatographic Instrumentation and Conditions

1. Column

Install the splitless liner (Section E.6.) and the capillary column (Section E.2.) on the split/splitless injection port of the gas chromatograph following the manufacturer's recommended procedure.

2. Typical operating conditions for the determination of chlorpyrifos by gas chromatography (GC) with flame photometric detection (conditions should be optimized for specific instruments):

Instrumentation: Hewlett Packard Model 5890 Gas Chromatograph with an FP detector

Column:	J&W Scientific fused silica capillary DB-5 liquid phase, 10 m x 0.25 mm I.D., 1.0 µm film thickness
Temperatures:	
Column:	80 °C for 1.0 min 80 °C to 220 °C at 30 °C/min 220 °C for 5 min
Injector:	220 °C
Detector:	245 °C
Gas Flows:	
Column Linear Velocity:	60 cm/sec Helium
Head Pressure:	10 psi
Detector:	20 mL/min Oxygen 75 mL/min Hydrogen 120 mL/min Nitrogen (Auxiliary gas)
Split Flow:	100 mL/min
Septum Purge Flow:	0.75 mL/min
Injection Technique:	Splitless
Purge Delay:	0.8 min
Injection Volume:	1 µL
Range:	1

3. A typical calibration curve is shown in Figure 1.

4. Typical chromatograms of a standard, control sample, and a 0.01 µg/g recovery sample are shown in Figure 2.

5. Column (for structure confirmation)

   Install the splitless liner (Section E.6.) and the capillary column (Section E.2.) on the split/splitless injection port of the gas chromatograph following the manufacturer's recommended procedure.

6. Typical operating conditions for the confirmation of chlorpyrifos by GC with mass spectrometry detection using selected ion monitoring (conditions should be optimized for specific instruments):

Instrumentation:	Hewlett Packard Model 5890A Series II Gas Chromatograph / Model 5971A Mass Selective Detector
Column:	J&W Scientific fused silica capillary, DB-1 liquid phase, 20 m x 0.18 mm I.D., 0.4 µm film thickness

Temperatures:

Column:	80 °C for 1.0 min 80 °C to 250 °C at 30 °C/min 250 °C for 5 min
Injector:	250 °C
Detector:	285 °C

Gas Flows:

Column Linear Velocity:	30 cm/sec Helium
Head Pressure:	15 psi
Split Flow:	27 mL/min
Septum Purge Flow:	1.25 mL/min

Injection Technique:	Splitless
Purge Delay:	0.8 min
Injection Volume:	1 µL
Monitored Ions:	m/z 258 m/z 314
Electron Multiplier:	1800 volts

7. Typical chromatograms of a standard, control sample, and a fortified sample are shown in Figure 3.

I. Determination of Percent Recovery of Chlorpyrifos from Soil

1. Preparation of Recovery Samples

a. Weigh 5.0 ± 0.05-g portions of thoroughly mixed control or treated soil into a series of 11-dram vials.

b. Use part of the weighed control samples as controls, and fortify the remaining control samples by adding appropriate aliquots of the chlorpyrifos spiking solutions given in the table in Section G.1.b. to obtain concentrations ranging from 0.01 to 10.0 µg/g.

c. Treat each of the above samples as follows:

1) Add 20.0 mL of 98% acetone/1% phosphoric acid/1% water to the samples (19 mL if 1 mL of spiking solution was added).

2) Cap and sonicate the sample for 5 minutes (note K.6.c.).

3) Retighten cap after sonication and shake the sample at approximately 280 excursions per minute for a minimum of 30 minutes.

4) Centrifuge the sample at approximately 2500 rpm for 5 minutes.

5) Transfer 10.0 mL of the acetone solution into a 16 x 100 mm culture test tube and evaporate to 3 mL in a water bath (water temperature between 40-45 °C) under a stream of nitrogen at a rate of evaporation no greater than 7 mL/10 minutes (note K.6.d.).

6) Add 10 mL of 1% phosphoric acid to the concentrated acetone solution and transfer to an 11-dram vial.

7) Rinse the culture tube with an additional 10 mL of 1% phosphoric acid and combine with the prior solution.

8) Extract the solution with 1.0 mL of hexane by shaking for 15 minutes.

9) Centrifuge at approximately 2500 rpm for 5 minutes.

10) Using a disposable glass pipet, transfer a portion of the hexane solution into an injection vial containing a vial insert and seal with cap using a crimper.

11) Analyze 1-µL aliquots as described in Section H. Obtain peak areas and determine the amount of chlorpyrifos present in each sample in µg/mL by referring to a standard regression curve derived on the same day (Section I.2.). If the peak response exceeds the range of the calibration standards, dilute the hexane solution and re-analyze.

2. Calculations of Percent Recovery of Chlorpyrifos

a. Inject the calibration standard solutions (Section G.2.) covering the concentration range of 0.025 to 0.50 µg/mL or higher into the gas chromatograph and record the resulting peak area.

b. Using the standard's concentration and corresponding peak area, prepare a standard curve by plotting the chlorpyrifos concentration (µg/mL) on the abscissa (x-axis) and the peak area on the ordinate (y-axis). Using regression analysis, determine the equation for the curve with respect to the abscissa. A typical power regression calibration curve is shown in Figure 1.

$$Y = \text{constant} \times X^{(\text{exponent})}$$

$$X = \left[\frac{Y}{\text{constant}}\right]^{1/\text{exponent}}$$

To solve for the exponent and constant, use the following formulas:

$$\text{Exponent} = \frac{\eta[\sum(\ln x_i)(\ln y_i)] - \sum \ln x_i \sum \ln y_i}{\eta\sum(\ln x_i)^2 - [\sum(\ln x_i)]^2}$$

$$\text{Constant} = e^{\left[\frac{\sum \ln y_i - b\sum \ln x_i}{\eta}\right]}$$

Where: η = number of standards run
x = concentration (µg/mL) of chlorpyrifos in the standard
y = peak area from the standard
b = exponent

$$\underset{\text{in sample}}{\mu g/mL \text{ chlorpyrifos}} = \left[\frac{\text{peak area of sample}}{\text{constant}}\right]^{1/\text{exponent}}$$

c. Calculate µg/g of chlorpyrifos in each sample as follows:

$$\text{Gross } \mu g/g = \frac{(\mu g/mL \text{ chlorpyrifos})(20 \text{ mL}/10 \text{ mL})(1 \text{ mL})}{5g}$$

$$= (\mu g/mL \text{ chlorpyrifos}) \times (0.4) \times \begin{bmatrix}\text{any additional}\\ \text{dilution factor}\end{bmatrix}$$

d. Determine the percent recovery of fortified samples by first subtracting the average chlorpyrifos µg/g in the control samples from the gross µg/g of the recovery samples for a net µg/g and divide by the theoretical concentration added.

$$\%\text{ Recovery} = \frac{\text{Gross } \mu g/g \text{ Fortified} - \mu g/g \text{ Control}}{\mu g/g \text{ Added}} \times 100$$

An example of calculations using power regression with the data from Figures 1 and 2:

$$\underset{\text{in sample}}{\mu g/mL \text{ chlorpyrifos}} = \left[\frac{\text{peak area of sample}}{\text{constant}}\right]^{1/\text{exponent}}$$

$$0.0260\ \mu g/mL = \left[\frac{24109}{691142.9}\right]^{1/0.9198}$$

$$\mu g/g \text{ Chlorpyrifos} = 0.0260\ \mu g/mL \times 0.4$$

$$\mu g/g \text{ Chlorpyrifos} = 0.0104\ \mu g/g$$

$$\%\text{ Recovery} = \frac{0.0104\ \mu g/g - 0.00\ \mu g/g}{0.01\ \mu g/g} \times 100$$

$$\%\text{ Recovery} = 104\%$$

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J. Determination of Chlorpyrifos in Soil Samples

1. Prepare control, recovery, and treated samples as described in Section I.1. The sample is analyzed on an "as is" basis but the residue is reported on a dry weight basis.

2. Prepare a standard curve and determine the chlorpyrifos concentration in the recovery samples as described in Section I.2.

3. Determine the concentration of each treated sample by substituting the chlorpyrifos peak area obtained into the equation for the standard curve in Section I.2. and solving for the concentration.

$$\begin{matrix}\mu g/mL \text{ chlorpyrifos} \\ \text{in sample}\end{matrix} = \left[\frac{\text{peak area of sample}}{\text{constant}}\right]^{1/\text{exponent}}$$

4. Calculate µg/g of chlorpyrifos in each sample as in follows:

$$\text{Gross } \mu g/g = \frac{(\mu g/mL \text{ chlorpyrifos})(20 \text{ mL}/10 \text{ mL})(1 \text{ mL})}{5g}$$

$$= (\mu g/mL \text{ chlorpyrifos}) \times (0.4) \times \begin{bmatrix}\text{any additional} \\ \text{dilution factor}\end{bmatrix}$$

5. Calculate the percent moisture in the soil.

The water content of a wet soil sample is determined by oven drying at least 10 g of the wet soil for a minimum of 16 hours at 130 °C.

Calculate percent moisture (dry weight basis) as follows:

$$\begin{matrix}\% \text{ Moisture} \\ \text{(dry weight basis)}\end{matrix} = \frac{\text{Water (g)}}{\text{Dry soil (g)}} \times 100$$

$$= \frac{\begin{matrix}\text{sample weight} \\ \text{before drying}\end{matrix} - \begin{matrix}\text{sample weight} \\ \text{after drying}\end{matrix}}{\begin{matrix}\text{sample weight} \\ \text{after drying}\end{matrix}} \times 100$$

6. Correct the gross µg/g (Section J.4.) of chlorpyrifos in each sample for the average percent recovery of the fortified samples and the percent moisture as follows:

$$\begin{matrix}\text{Chlorpyrifos Conc.} \\ \text{(corrected } \mu g/g)\end{matrix} = \begin{matrix}\text{Chlorpyrifos Conc.} \\ \text{(Gross } \mu g/g)\end{matrix} \times \frac{100}{\% \text{ Recovery}} \times \left[1 + \frac{\% \text{Moisture}}{100}\right]$$