SUMMARY

Propargite and its metabolite 2-(p-t-butylphenoxy)-cyclohexanol [TBPC] were extracted from soil with acetone. The extract was freed from water and then analyzed by GC using a flame photometric detector in sulfur mode for propargite and a flame ionization detector for TBPC.

A. MATERIALS

A.1 Equipment

Balance
Glass jars (1 liter)
#4 filter paper
Graduated cylinder (500 ml)
Separatory funnel (500 ml)
Round Bottom flask (100 ml)
Rotovap
Assorted test tubes
Steam bath
Glass vials with Teflon lined lids.
Pasteur pipette (9 inch)
Glass wool

A.2 Reagents/Supplies

All solvents should be pesticide residue grade.

Propargite analytical standard	Obtain from Uniroyal Chemical Co., Inc.
TBPC analytical standard	Obtain from Uniroyal Chemical Co., Inc.
Acetone	Various suppliers.
Toluene	Various suppliers.
Chloroform	Various suppliers.
Decanol	Various suppliers.
Hexane	Various suppliers.
Dichloromethane	Various suppliers.
Activated Alumina (80-200 mesh)	Alcoa Type F-20.

A.3 Analytical Standards

Analytical standards of propargite and TBPC are available from Uniroyal Chemical Company, Inc. division of CK Witco. Standards are kept frozen. Certificates of Analysis (COAs) for these two standards are shown in Appendix 1. The COAs also show the structures of the standards and their typical purities. Appendix 1 also contains MSDS sheets for the standards. One should obtain MSDS sheets for the solvents directly from their suppliers.

B. SAFETY AND HEALTH

This method should be performed by trained chemical personnel. Hazards associated with the chemicals used in this analytical method are shown in the MSDS sheets in Appendix 1.

C. ANALYTICAL METHODS

C.1 Principle of the Methods

Soil samples are extracted with acetone. Chloroform is then added to allow separation of any water that may have been present in the soil sample. The chloroform-acetone layer (lower) is separated and a small amount of decanol "keeper" is added to it. The solvents are removed by evaporation on a rotovap and finally on a steam bath. For the analysis of propargite, hexane is added to bring up to volume and analysis is done by GC with a flame photometric detector in sulfur mode. For TBPC dichloromethane is added to bring up to volume and analysis is done by GC with a flame ionization detector.

C.2 Types of Soils

This method is predicted to be applicable to most soil types. A number of propargite field dissipation studies have been done using this method (*see title page for related studies*). In Uniroyal Chemical Co., Inc. Study 8845 soil from a Georgia, USA location was analyzed to a depth of 36 inches. The soil composition varied depending upon the depth from sandy loam (0-1 ft depth), sandy clay loam (1-2 ft. depth), and clay (2-4 ft depth). The analytical method worked equally well on all these types of soil.

C.3 Sample Processing

The soil samples are normally received frozen and are stored frozen at -20°±2°C. Before analysis they are thawed and hand mixed.

C.4 Extraction Method

GENERAL NOTE: Weigh out and extract the soils on an "as received" (wet) sample basis. Conduct an air dry moisture determination on a separate portion of each sample. Calculate residues on both the "as received" and on the air dried soil basis.

1. Weigh out a 200 g sample of soil into a quart jar. Spike at this point if applicable. (*Note:* The weighed sample is "as received" or wet sample basis. Do not pre-dry the soil.)
2. Add 200 mL acetone and shake, by hand every 10 minutes for the next two hours.
3. Decant the acetone through #4 filter paper into a 500 mL graduated cylinder.
4. Add 100 mL acetone to the soil remaining in the jar. Shake twice at 5-minute intervals. Decant the acetone again into the graduated cylinder through the same filter paper.
5. Transfer the soil into the filter paper. Rinse the jar with 75 mL of acetone and pour both washings and the remaining soil into the filter paper.
6. Rinse the soil in the filter paper with 25 mL of acetone. Continue rinsing the jar and the soil with additional acetone until the volume in the cylinder reaches the 400 mL mark. Mix well.
7. Transfer a 200 mL aliquot of the extract to a 500 mL separatory funnel. Add 200 mL chloroform and shake for 1 minute. Let stand for 5 minutes.
8. Drain the chloroform-acetone layer into a 1000 mL round bottom flask through #4 filter paper. Discard the water layer. (*Note*: Amount of water layer depends on the moisture content of the soil.)
9. Add 0.1 mL of 1% decanol in acetone as a keeper, and evaporate using a rotovap to 2-3 mL. Transfer to a test tube with chloroform.
10. Adjust the volume to 4 mL, mix well and divide the extract into two equal parts of 2 mL each in test tubes (one tube for propargite and one tube for TBPC). Add 1 drop of 1% decanol to each tube.

11. Evaporate each tube to dryness using a steambath, then bring up to a final volume of 2 mL each: one tube for propargite in hexane; the other tube for TBPC in dichloromethane. (For each fraction, the final volume = 2 mL) (1 mL = 25.0 g)
12. Submit for GC analysis.
13. To determine the air dry soil moisture, proceed as follows:
    - Using a top loader balance, weigh a disposable aluminum weighing dish to one decimal accuracy.
    - Add 10.0 g ± 1.0 g of "as received" wet soil and record the weight.
    - Place the dish, uncovered on a counter or cabinet top for 24 hours. Temperature is to be "room temperature" (usual range is 60-75° F).
    - Reweigh the dish plus dry soil.

C.5 Fortifications

During the course of analyzing the samples for Uniroyal study 8845, a number of method spikes were used ranging from 0.1 ppm to 2.0 ppm. Fortifications were carried out by adding the appropriate standard solution directly onto a weighed portion of an untreated soil sample check in the extraction container and allowing it to dry prior to adding the extraction solvent. The checks and fortified (spiked) samples were extracted along with each set of treated samples.

A minimum of one spike was run for each sample set. This is approximately one spike for every ten treated samples.

Fortifications were made using diluted stock solutions of propargite or TBPC dissolved in acetone. Stock solutions containing 1040 µg/ml of propargite and 1000 µg/ml of TBPC in acetone were prepared for study 8845. Diluted solutions containing 100 µg/ml were then made from the stock solutions and were used to spike the soil samples. The number of µg used to spike the soil samples is shown in the residue raw data sheets in Appendices 3 and 4.

C.6 Clean-up

Interferences in soil often require a special clean-up procedure after step #11, (see C.4-extraction method) in order to do the analysis of TBPC. This clean-up was not required for analysis of propargite and was not always required for TBPC. When required, the following clean-up procedure is used:

REAGENTS: Alumina, Activated (80-200 mesh, Alcoa Type F-20) MCB AXO612-3 kept at 130°C for a minimum of 12 hours then deactivated by adding 4% water and equilibrating in a glass vial with a teflon lined lid for a minimum of 4 hours and a maximum of 8 hours. All solvents are pesticide residue grade.

PROCEDURE:

1. Evaporate the dichloromethane (from Extraction procedure, Step #11, Section C.4) to dryness using a steambath.
2. Redissolve the extract with 2 mL of toluene (1 mL = 25.0 g).
3. Prepare a column by inserting a small glass wool plug into a 9-inch disposable Pasteur pipette. Add prepared alumina to a height of 2-inches.
4. Pipet 1 mL of toluene extract onto the column.
5. As soon as the extract has just passed into the alumina start washing the column with toluene until a total of 5 mL has been collected. (This is a combination of sample extract plus clean toluene.)
6. Move the column to a clean test tube having a minimum capacity of 10 mL.
7. Elute the TBPC from the column with a mixture of 10% acetone in hexane. Elute into the test tube until a total of 8 mL has been collected.
8. Add one drop of 1% decanol in acetone to the eluate, and evaporate just to dryness using a steambath.
9. Pipet 1 mL of dichloromethane into the tube to redissolve the extract (1 mL = 25.0 g)
10. Submit for GC analysis.

C.7 Derivatization

No derivatization is required for the methods described in this report.

D. INSTRUMENTATION

The instrumentation and operating conditions for the analysis of propargite and TBPC are shown below. As shown, it is possible to use different columns for TBPC.

PROPARGITE:

Instrument:	MicroTek 220, FPD-S
Column:	15 m x 0.53 mm, DB-1, 5.0 um (J&W)
Temperatures:	Column – 255°C Detector – 200°C Sampler – 240°C
Carrier Gas and Flow:	N_2 at 32 to 35 mL/min

TBPC:

Instrument:	Varian 1400 equipped with FID
Column:	25 m x 0.53 mm, Methyl Silicone, 5.0 um (Quadrex)
Temperatures:	Column – 210°C Detector – 200°C Sampler – 230°C
Carrier Gas:	N_2 at 25 psi
Makeup Gas:	N_2 at 15 psi

Instrument:	Varian 1400 equipped with FID
Column:	30 m x 0.53 mm, DB-17, 1.0 um (J&W)
Temperatures:	Column – 190°C Detector – 220°C Sampler – 230°C
Carrier Gas:	N_2 at 30 psi
Makeup Gas:	N_2 at 10 psi

Instrument:	Varian 1400 equipped with FID
Column:	30 m x 0.53 mm, DB-1701, 1.0 um (J&W)
Temperatures:	Column – 187°C Detector – 200°C Sampler – 230°C
Carrier Gas:	N_2 at 25 psi
Makeup Gas:	N_2 at 10 psi

E. POTENTIAL INTERFERENCES

It is possible that some soil samples may have interferences. While the columns and conditions shown in Section D have worked well historically alternate columns may have to be used for certain soil samples or the GC conditions may have to be changed somewhat to improve resolution. As mentioned in section C.6, a special clean-up of some soil samples may be necessary before analyzing for TBPC. A control soil should always be run along with the samples to determine if interfering substances are present in the soil.

F. CONFIRMATORY TECHNIQUES

No confirmatory techniques were performed in report 8845. Identification depended solely on retention time compared to standards.

G. TIME REQUIRED FOR ANALYSIS

Analyses in report 8845 consisted of one control soil, one method spike, and nine study samples. Preparation and extraction were usually done on one day and analyses on the following day. Thus, it is expected that a set of ten to twelve samples could be completed in two days.

H. MODIFICATIONS OR POTENTIAL PROBLEMS

There is some variance allowed in the soil sample size used for extraction. The original method (Sisken (1973) see Appendix 2) used a 400 g samples and 400 ml of acetone for extraction. The method described in this report (based on Uniroyal study 8845) generally used 200 g of soil and 200 ml of acetone. If smaller or larger acetone extracts are used, it is important to adjust the amount of chloroform used to separate the water so the acetone/chloroform ratio remains at 1:1. The original method also mentions that the rotovap used to remove the acetone/chloroform is run at 40°C under mild vacuum. The conditions used in Uniroyal study 8845 are not mentioned.

There are also some variations allowed in the column type, carrier gas, and flow rates. Additionally, the original method used an increasing oven temperature technique, whereas, the method in report 8845 was performed isothermally.

The methods mentioned in this report were developed ten or more years ago. It is expected that today's practitioner will use electronic integration methods rather than the peak height method described herein.

I. METHODS OF CALCULATION

Calculations are done separately for propargite and TBPC as shown below.

I.1 Calculations for Propargite

1. A four point standard curve, plotted on log-log graph paper is required for each chromatogram. Peak height x attenuation vs nanograms injected is used to calculate nanograms found.
2. All method spikes are corrected by subtracting the amount of analyte found in the control (check) from that found in the fortified control (spike).
3. Residues found in "treated" samples are not corrected for spike recovery.
4. In study 8845, three significant figures were used for calculating residues on the "as received" soil moisture basis.

EXAMPLE FROM STUDY 8845: Sample #74, Rep 2, 0-6" (M.L. #49341) with 14-day Post 3rd Application

Standard Curve:

ng injected	peak height	attenuation	peak height x attenuation
5	15 mm	16x	240 mm
10	51 mm	16x	816 mm
15	103 mm	16x	1648 mm
20	172 mm	16x	2752 mm

Sample:

1. Peak height x attenuation = corrected peak height

   148 mm x 16 = 2368 mm

2. From Standard curve find nanograms (FOUND)

   2368 mm = 18.2 ng

3. $\frac{\text{Nanograms Found}}{\text{Amount of Sample Injected (mg)}}$ = ppm $\qquad \frac{18.2 \text{ ng}}{50.0 \text{ mg}} = 0.364$ ppm

I.2 Calculations for TBPC

1. Averaged standard sensitivity (mm/ng) is used to calculate nanograms found.
2. A standard curve (peak height in mm vs nanograms injected) is generated on a regular basis to show linearity for the compound. This standard curve is not used for calculating residues.
3. All method spikes are corrected by subtracting the amount of analyte found in the control (check) from that found in the fortified control (spike).
4. Residues found in treated samples are not corrected for spike recovery.
5. In study 8845, three significant figures were used for calculating residues on the "as received" soil moisture basis.

EXAMPLE FROM STUDY 8845: Sample #74, Rep 2, 0-6" (M.L. #49341) with 14-day Post 3rd Application.

Average Standard Sensitivity

ng injected	peak height	sensitivity
50	97.5 mm	1.95 mm/ng
50	98.5 mm	1.97 mm/ng
50	95.5 mm	1.91 mm/ng
50	91 mm	1.82 mm/ng
		7.65 mm/ng

$$\text{AVERAGE SENSITITY} = \frac{7.65 \text{ mm/ng}}{4} = 1.91 \text{ mm/ng}$$

Sample:

1. Peak height divided by average standard sensitivity = nanograms found

$$\frac{11.5 \text{ mm}}{1.91 \text{ mm/ng}} = 6.02 \text{ ng}$$

2. $$\frac{\text{Nanograms Found}}{\text{Amount of Sample Injected (mg)}} = \text{ppm} \quad \frac{6.02 \text{ ng}}{50.0 \text{ ng}} = 0.120 \text{ ppm}$$

As mentioned above for propargite, a four point curve plotted on log-log graph paper was used to calculate the nanograms found. For TBPC a similar standard curve is used to show linearity for TBPC but is not used in calculating the residues. A typical standard curve is shown in Appendix 3.

I.3 Calculation of Soil Moistures

Weight of wet soil plus dish – weight of dish = weight of wet soil

Weight of wet soil plus dish – weight of dry soil + dish = moisture lost

$$\% \text{ Moisture} = \frac{\text{moisture lost (g)}}{\text{weight of wet soil (g)}} \times 100$$

I.4 Calculation of Soil Residues From an "As Received" Basis to an Air Dry Basis

Residues (in ppm) on air dry basis =

$$\frac{\text{Residue (in ppm) on an "as received" basis}}{\text{g air dry soil/100 g "as received" soil}}$$

where: g air dry soil =

(100% – % moisture to air dry basis) x 100 g

I.5 Calculation of Recoveries

As an example the 0.1 ppm spike from the propargite stability study residue raw data sheet is used. (See Appendix 3).

The weight of soil used was 200 g. The propargite and TBPC from this soil were extracted into 400 ml of solvent (original extract, plus washes and dilutions)—see Section C.4. One-half this volume of extract or 200 ml was used for the analysis. This is equivalent to using:

$$\frac{200 \text{ ml}}{400 \text{ ml}} \times 200 \text{ g soil} = 100 \text{ g of soil}$$

After further work-up, the analytes are contained in 4 ml of solvent. One-half of this 4 ml is used to analyze for propargite and one-half is used to analyze for TBPC. This is equivalent to using:

$$\frac{2 \text{ ml}}{4 \text{ ml}} \times 100 \text{ g of soil} = 50 \text{ g of soil}$$

In the method, 4 µl is injected (note: this amount may vary, but is the actual amount as shown on the raw data sheet). This is equivalent to using:

$$\frac{4 \text{ µl}}{2 \text{ ml}} \times 50 \text{ g of soil}$$

$$= \frac{4 \text{ µl}}{2000 \text{ µl}} \times 50{,}000 \text{ mg of soil}$$

$$= 100 \text{ mg of soil}$$

This is the number in column 17 of the raw data work sheet labeled (rather ambiguously) "mg inject".

To calculate the ppm found, the following calculation is used:

8.5 ng were found/100 mg of soil (*see above*)

Therefore,
$$\frac{8.5 \text{ ng}}{100 \text{ mg soil}} = \frac{8.5 \times 10^{-9} \text{ g}}{100 \times 10^{-3} \text{ g soil}}$$

$$= \frac{8.5 \times 10^{-9} \text{ g}}{0.1 \text{ g soil}} = \frac{8.5 \times 10^{-9} \times 10^{7} \text{ g}}{10^{6} \text{ g soil}}$$

$$= 0.085 \text{ ppm}$$

The recovery is:

$$\frac{\text{ppm found}}{\text{ppm added}} \times 100\%$$

$$= \frac{0.085 \text{ ppm}}{0.100 \text{ ppm}} \times 100\%$$

$$= 85\%$$