RESIDUE METHOD FOR DETERMINATION OF FORTRESS® INSECTICIDE ACTIVE INGREDIENT DPX-43898 AND ITS OXON ANALOG IN-34158 IN SOIL BY ELECTRON-CAPTURE GAS CHROMATOGRAPHY

Walter A. Babicki, George F. Barber, and Robert V. Slates

INTRODUCTION AND SUMMARY

Scope

An analytical method is described for simultaneously determining DPX-43898 (the active ingredient in Fortress® insecticide) and its oxon analog IN-34158 in soil. Chemical names and structural formulas for DPX-43898 and IN-34158 are given in Figure 1. The method is based on extraction of the analytes from soil in a hexane/acetone mixture, removal of the acetone from the extract by two water washes, and determination of the analytes in the hexane solution by capillary gas chromatography using an electron-capture detector. The lower limit of quantitation for each analyte in soil is 0.01 ug/g.

SAFETY AND HANDLING CONSIDERATIONS

DPX-43898 and IN-34158 are highly toxic by either oral or dermal exposure. All contact of these compounds with skin, eyes, clothing, or the respiratory system (through inhalation of

the vapors) should be avoided. All work involving DPX-43898 and IN-34158 should be conducted in a hood while wearing eye protection and protective gloves. Based on permeability studies, nitrile rubber gloves should provide acceptable protection for up to 8 hours. Neither butyl nor neoprene rubber gloves provide adequate protection. Wash thoroughly with soap and water after handling these compounds and before eating or smoking. If DPX-43898 or IN-34158 does come into contact with skin, immediately remove contaminated clothing and wash the contaminated skin or hair with plenty of soap and water.

MATERIALS AND METHODS

The following sections include a list of suggested equipment, reagents, and recommendations for preparing stock solutions and standards.

Equipment

Composite soil samples using a Hobart commercial food chopper.

During soil extraction, tumble samples using a mechanical tumbler driven by a variable-speed motor. The tumbler should hold several bottles or separatory funnels, turning them end-over-end to mix their contents.

After extraction, separate soils and extracts on an IEC Centrifuge Model K (International Equipment Co., Needham Heights,

MA) with 250-mL Nalgene^{R} wide-mouth centrifuge bottles (VWR Scientific, San Francisco, CA, Cat. No. 21020-367).

Use Millex^{R}-SR 0.5-μm disposable filter units (Millipore Corporation, Bedford, MA, Cat. No. SLSR025NB) to remove particulate matter from sample solutions prior to gas chromatographic analysis.

Perform analyses on a Varian Model 3700 gas chromatograph (Walnut Creek, CA) equipped with a Varian Ni-63 electron-capture detector. Perform chromatographic separations on a 25-m x 0.32-mm i.d. Ultra 1 capillary column (Hewlett Packard, Avondale, PA, Cat. No. 19091A-112) with a 0.52-μm film thickness of crosslinked methyl silicone gum.

Reagents and Standards

The reference standard of DPX-43898 (compound number SD208304, Code 6-1-0-0) was synthesized and assayed at the Shell Agricultural Chemical Company, Biological Sciences Research Center in Modesto, California. Its chemical purity was 99.8%. The reference standard of IN-34158 was synthesized and assayed at the Du Pont Agricultural Products Department, Research and Development Division, Wilmington, Delaware. Its chemical purity was 92 $\pm$ 5%.

Use HPLC-grade organic solvents (hexane and acetone) from Fisher Scientific Company (Pittsburgh, PA). Use deionized water for solvent partitioning.

Prepare separate stock solutions (100 µg/mL) of DPX-43898 and IN-34158 by dissolving 0.100 g of each analyte in 1000 mL of acetone.

For control sample fortifications up to and including 0.40 µg/g, combine equal amounts of the stock solutions and dilute with hexane. Prepare solutions containing equal concentrations of DPX-43898 and IN-34158 at 0.5, 1.0, 2.0, 5.0, and 10.0 µg/mL. For fortifications of 1.0 µg/g and higher, separately pipet the analytes into the samples from the 100 µg/mL stock standards.

For gas chromatographic analyses, prepare mixed standards containing equal concentrations of each analyte at 0.005, 0.010. 0.020, and 0.03 µg/mL by serial dilution of the stock 100 µg/mL standards in hexane. Because the analytes are relatively volatile, do not evaporate the acetone from aliquots of stock standard solutions prior to dilution. When not in use, all solutions of standards and samples should be stored in a refrigerator at 6 °C or colder.

Analytical Procedure

Sample Preparation

To minimize contamination, begin compositing segments from the untreated plots and proceed to those having increasingly higher treatment rates. Composite the deepest core segments

first, because they should contain the lowest concentration of analyte residues.

To composite soil samples, lay all of the frozen soil tubes for one sampling from a single test on a clean surface in a hood. Accurately measure each tube from the soil surface, marking it at depths of 3, 6, and 12 inches. If the tube is longer than 18 inches, continue marking it in six-inch increments. In some instances, the soil may have been compressed during sampling, resulting in soil cores that are shorter than the actual sampling depth. If the deepest segments of the tubes have soil cores less than 5.5 inches long, significant compression has occurred, and the length of the deepest soil segments must be recorded in the study records.

While the soil tubes are still frozen, cut them with a sharp heavy knife, using the marks as guides to provide segments of 0-3, 3-6, 6-12, 12-18, 18-24, 24-30, and 30-36 inches. Begin compositing the soil segments by transferring the soil for the deepest segment of each core to the bowl of a Hobart chopper. Add sufficient dry ice to keep the soil frozen while operating the chopper until the soil mixture is thoroughly homogenized. Divide the homogenous mixture into three approximately equal portions and transfer each to appropriately labeled sample bottles or bags. Close the sample container loosely so dry ice can sublime without introducing moisture. After sublimation is complete, seal containers tightly and freeze the samples until analyzed.

Repeat the compositing procedure for each soil sample depth. Be sure the Hobart chopper bowl and blades are thoroughly washed with soap and water and dried before compositing each segment. Complete the compositing process with minimal delay, being sure to keep the soil frozen to minimize volatilization of analytes.

Determination of Sample Dry Weight

Prior to analysis of each composited sample, determine the sample dry weight as follows: Weigh a 50.0-g portion of the sample into a preweighed disposable metal foil pan and allow the sample to air-dry at ambient temperature to a constant weight. Record the final weight as variable "SDW" for use in calculating analyte concentrations.

Soil Extraction and Fortification

To determine DPX-43898 and IN-34158 simultaneously in composited soil samples, weigh 50.0 g of undried soil into a 250-mL Nalgene® centrifuge bottle. If the sample dry weight is greater than 47.5 g, add 10 mL of deionized water to the sample. To prepare a fortified control to determine recovery efficiency, pipet into the sample 1.0 mL of a 0.50 µg/mL (or a higher concentration standard depending on the fortification level) mixed standard solution containing equal concentrations of DPX-43898 and IN-34158 in hexane. Do not evaporate the solvent because the analytes are volatile. Add 200 mL of a

hexane/acetone mixture (1:1 by volume) to the centrifuge bottle, cap it, and tumble it on a mechanical tumbler for 2 hours at medium speed. Centrifuge the sample at 3000 rpm for 5 minutes, and decant the extract into a 500-mL glass separatory funnel.

Clean-up by Solvent Partitioning

To remove acetone from the extract, add 200 mL of deionized water to the extract in the separatory funnel. Shake or tumble the mixture for 2 minutes and allow the phases to separate. Drain off the lower acetone-water phase and discard it. Add another 200 mL of deionized water to the extract and repeat the water wash of the extract. Drain and discard the lower aqueous phase, and transfer the upper hexane phase to a 100-mL volumetric flask. Add enough hexane to the flask to make exactly 100 mL of sample solution. Mix the solution well and filter 3-5 mL of the sample solution through a 0.5-μm Millex^{R} filter for analysis. Analyze the sample solution by electron-capture gas chromatography as described in the following section.

Instrumentation

Descriptions and Operating Conditions for Gas Chromatographic Analysis

Simultaneously determine DPX-43898 and IN-34158 in the sample solutions by capillary gas chromatography (GC) using a

Ni-63 electron capture detector. Analyze samples and mixed standards containing 0.005 ug/mL (or higher depending on the expected residue levels) of each analyte, using the following conditions:

Inlet: Capillary splitter system with a short section of packed precolumn for mixing.
Split ratio: 6
Temperature 250 °C

Column: Hewlett Packard Ultra 1
(Crosslinked methyl silicone gum)
Length: 25 m
I.D.: 0.32 mm
Film Thickness: 0.52 μm
Temperature: 165 °C

Detector: Ni-63 electron capture
Temperature: 340 °C
Make up gas: Nitrogen

Carrier Gas: Helium
Inlet Pressure: 30 psi

Injection Volume: 1 μL

Retention Times: IN-34158 2.2 min.
DPX-43898 2.64 min.

Calibration Procedures

Prepare several mixed standard solutions in hexane at concentrations that span the concentration ranges expected for the analytes in the sample solutions. Analyze the standards frequently during a series of sample analyses to provide data for standard curves. If the analyte peak height for a sample falls outside the working range of the standard curve, quantitatively dilute the sample and reinject it to keep the detector response within the working range of the standard curve.

To determine recovery efficiency, prepare and analyze at least one control sample and one control sample fortified with known quantities of the analytes before sample extraction. Follow this procedure for every 4 or 5 samples analyzed. Fortification levels should approximate the residue concentration of the samples being analyzed.

Confirmatory Techniques

The identity of the chromatographic peaks for DPX-43898 and IN-34158 were confirmed by gas chromatographic analysis of standards with mass spectometric detection. Mass spectra for DPX-43898 and IN-34158 are provided in Appendix I.

Time Required for Analysis

A batch of 6-8 samples can be prepared in an eight-hour day and can be analyzed overnight on an automated gas chromatograph.

Methods of Calculation

Determine residues of DPX-43898 and IN-34158 in soil samples by comparing the chromatographic peak height of each analyte with the corresponding peak heights for standards of known concentration. Calculate µg/g (on a dry-weight basis) for each analyte in soil samples using Equation 1. For fortified control samples, calculate the percent recovery for each analyte using Equation 2. For these calculations, separate standard curves must be prepared for DPX-43898 and IN-34158. The response factor (R_f) for each analyte is simply the slope of its standard curve through the origin.

(1) $$\text{µg/g of Analyte in Soils} = \frac{[PK]_s \times VS \times DF}{R_f \times VI \times SDW}$$

(2) $$\text{Percent Recovery of Analyte from Soil} = \frac{100\% \times [PK]_R \times VS \times DF}{R_f \times VI \times SP}$$

Variables for Equations 1 and 2 are defined as follows:

$$R_f = \frac{[PK]_{STD}}{VI \times C}$$ = Instrument reponse factor for the analyte in μvolts/μg.

C = Concentration of DPX-43898 or IN-34158 in the standard solution in μg/mL.

DF = Dilution factor for samples requiring sample dilution. DF =1.0 if no dilution is required.

$[PK]_R$ = Chromatographic peak height (in μvolts) for the analyte in a fortified recovery sample.

$[PK]_s$ = Chromatographic peak height (in μvolts) for the analyte in an unknown sample.

$[PK]_{STD}$ = Chromatographic peak height (in μvolts) for analyte in a standard solution.

SDW = Sample dry weight in g.

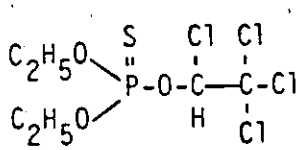
SP = Weight of analyte (in μg) fortified into a sample.

VI = Injection volume (in mL) of the sample or standard solution.

VS = Volume (in mL) of sample solution before injection or dilution.

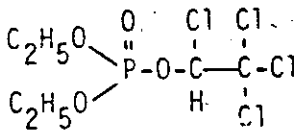
FIGURE 1

CHEMICAL NAMES AND STRUCTURAL FORMULAS FOR DPX-43898 AND IN-34158



DPX-43898

Phosphorothioic acid,
O,O,diethyl-O-(1,2,2,2-tetrachloroethyl)ester



IN-34158

Phosphoric acid, diethyl(1,2,2,2-tetrachloroethyl)ester