Method for DYLOX and DDVP in SOIL

INTRODUCTION

DYLOX is a trade name for Trichlorfon (common name) an organophosphorous insecticide, Dimethyl (2,2,2 - trichloro - 1 - hydroxyethyl) phosphonate, (also known as DIPTEREX, NEGUVON).

In the presence of dilute alkali, DYLOX undergoes dehydrochlorination and rearrangement to yield the vinyl derivative, Dimethyl 2,2-dichlorovinyl phosphate (DDVP). This process has occurred in both laboratory and field studies.

Previous reports have shown that DYLOX is not chromatographed intact, but may be broken down in the injection port to the compound dimethyl phosphite and a small amount of DDVP. This method isolates the metabolite DDVP for separate analysis then converts DYLOX with Sodium Methoxide to DDVP for separate quantitation.

This is a modification of the residue analysis method for DYLOX in soil as described in MOBAY Report 30993. Modifications involve the addition of aqueous acid to the Soxhlet flask preceding extraction, the partitioning of DDVP from the aqueous acid using toluene, the isolation of DYLOX with the addition of NaCl and chloroform/acetone extraction, and the conversion of DYLOX to DDVP with Sodium Methoxide. The parent DYLOX is measured as DDVP and the injection port pyrolysis is eliminated. The residues are measured versus a DDVP standard.

This method is applicable to soil residues with method quantitation limits of 0.010 ppm - 2.0 ppm DYLOX and 0.010 ppm - 0.50 ppm DDVP. For samples with higher levels of components, a) the initial sample size can be decreased (maintaining sample homogeneity) or b) the appropriate sample fraction (DYLOX or DDVP) may be quantitatively fractionated by dilution to a known volume and pipetting a known volume for extraction and analysis.

ANALYTICAL PROCEDURE

Apparatus Required

Assorted laboratory glassware
Gas Chromatograph, Perkin-Elmer Model 2000 equipped with Nitrogen - Phosphorous Detector (GC-NPD).
Rotary Vacuum Evaporator
Soxhlet Extraction Assembly

Reagents Required

Acetone, Pesticide Grade or equivalent
Chloroform, Pesticide Grade or equivalent
Methanol, Pesticide Grade or equivalent
Toluene, Pesticide Grade or equivalent
Sodium Methoxide, Alldrich Chemical Co., 25 Wt % soln in Methanol
Decyl Alcohol, Alldrich Chemical Co.
Sodium Sulfate, anhydrous, crystalline

Standards Preparation

DDVP

In a dry stoppered volumetric flask, weigh a known amount of DDVP (normally a liquid) and diluted to volume with Toluene. Dilute subsequent analytical standards in Toluene with the working standards diluted with 0.2 % Decyl Alcohol/Toluene. Dilute spiking standards with 50 % Chloroform/Methanol.

DYLOX

In a dry stoppered volumetric flask, weigh a known amount of DYLOX (normally a solid) and diluted to volume with Toluene. Dilute subsequent analytical standards in Toluene with the working standards diluted with 0.2 % Decyl Alcohol/Toluene. Dilute spiking standards with 50 % Chloroform/Methanol.

Retain original standards and subsequent dilutions in a freezer maintained at - 20 degrees Centigrade.

Sample Preparation for DYLOX and DDVP

Store all original samples in a freezer maintained at -20 degrees Centrigrade. Transport samples in dry ice. When working with the initial samples, maintain temperatures as cold as feasible to minimized component degradation. Store incomplete extractions or final extracts in a refrigerator/freezer at 2 degrees Centrigrade or lower.

1. Weigh 25 grams of soil in a Soxhlet Thimble (Use a small cotton ball to retain sample in thimble).

2. Place thimble in Soxhlet extractor, add 150 ml. of 1:1 mixture Chloroform/Methanol, add 2 ml of 0.1M HCl to solvent flask, and extract 4 hours at approximately 5 cycles/hour.

3. Allow the extract to cool to room temperature, add 100 ul Decyl Alcohol (keeper solution), and concentrate the extract using a rotary vacuum evaporator and a 40 degree Centrigrade water bath. Water from the sample will fog the evaporator when

complete. Approximately 20 mls of aqueous extract may remain.

4. Transfer the aqueous residue (DYLOX and DDVP) to a 250 ml separatory funnel using 80 mls of 0.01M HCl, followed by 20 mls Toluene.

5. Shake the separatory funnel, allow the phases to separate, and drain the lower aqueous phase into a second 250 ml separatory funnel containing 15 mls Toluene.

6. Shake the second separatory funnel, allow the phases to separate. Drain the aqueous phase into a beaker, combine with additional aqueous phases from the first extraction. Combine the Toluene phase (second separatory funnel) with the first separatory funnel Toluene. DDVP is in the Toluene phase and DYLOX is in the aqueous phase.

7. Drain the Toluene through 3 - 5 gram Sodium Sulfate into a 250 ml standard taper 24/20 erlynmeyer. Rinse the container and Sodium Sulfate with an additional 10 mls Toluene and combine.

8. Transfer the Aqueous extract back to the second separatory funnel. Add 28 grams Sodium Chloride and shake until the salt dissolves.

9. Rinse the beaker with 100 mls of 3:1 Chloroform/Acetone and add to the separatory funnel. Shake and allow the phases to separate.

10. Drain the Chloroform/Acetone (lower phase) through 25 grams Sodium Sulfate into 250 ml erlynmeyer flask.

11. Repeat Chloroform/Acetone extraction with 50 mls 3:1 Chloroform/Acetone and combine extracts. Rinse Sodium Sulfate with 25 ml chloroform. Add 100 ul Decyl Alcohol.

12. Evaporate the Toluene (DDVP) fraction and the Chloroform (DYLOX) extracts just to dryness using a vacuum rotary evaporator at 40 degrees Centigrade. Reconstitute the DDVP fraction to 5.0 mls with Toluene and retain for GC Analysis. The chloroform (DYLOX) fraction is ready for conversion to DDVP.

Base Catalyzed Conversion of DYLOX to DDVP

1. Dissolve the DYLOX residue (Chloroform fraction) in 10 mls Methanol.

2. Add 1 ml of 0.024M Sodium Methoxide (50 ul/ 10 mls Methanol) and let stand 6 +/- 0.25 minutes. This reaction is run at 22 degrees Centigrade. Terminate the reaction after 6 minutes by adding 1 ml 0.24M methalonic HCl.

3. Flash evaporate the methanol residue to dryness (Decyl alcohol residue).

4. Dissolve the residue with 5.0 mls Toluene and retain for GC Analysis. Note: The Toluene may be turbid due to colloidal NaCl. Allow the solution to clarify by standing overnight or centrifuging.

Sample Analysis

Instrumental Conditions

The following instrumental conditions (Gas Chromatography / Nitrogen - Phosphorus Detector) are used for the analysis of DYLOX (converted to DDVP) and DDVP in the sample extracts.

Column Packing - 2 % DEGS-PS on 80/100 Chrom W
Column Dimensions - 6 ft x 2 mm, glass
Carrier Gas - Helium
Flow Rate Carrier - 35 cc/min
Injection Port Temperature - 250 Centigrade
Detector Temperature - 250 Centigrade
Column Temperatures -
Initial Temp - 120 Centigrade for 8 minutes
Temperature Ramp - 16 degrees Centigrade/min.
Final Temperature - 180 degrees Temperature
Final Temperature Time - 6.6 minutes

The temperature program is used to expedite the elution of co-extractive compounds. Additional cleanup of extracts could eliminate the necessity of the temperature program.

Sample and standard chromatograms with data printout is attached.

Sampling Sequence

The sequence of sample/standard injections is as follows:
1. Inject an intermediate standard to condition the column.
2. Inject a blank solution (with or without Decyl Alcohol)
3. Inject the lowest standard (twice).
4. Inject the remaining standards in order of concentration.
5. Inject a blank solution.
6. Inject the continuing standard (twice).
7. Inject the first sample.
8. Inject the continuing standard.
9. Repeat the sequence of continuing standard, sample,

standard, etc.
10. Randomly inject blanks (0.2% Decyl Alcohol/Toluene) as a sample.
11. Inject the last continuing standard in duplicate.
12. As necessary re-inject, all standards from blank to highest level.

Data Collection

Data collection may be based upon peak area or peak height. Perform statistical analysis on the standard curves. For reporting purposes only the raw data (peak height or area) are used for calculation. The continuing standard must be less than 20 % difference before and after the sample injection. The average continuing standard is used for calculation.

Calculations

Blanks or control sample results are not subtracted from sample results. Control sample results are subtracted from control spike data results for the calculation of percent recovery. Data calculations were performed using the average standard response of standard injections before and after each sample.

Sample results are reported as ppm, DYLOX equivalents on a dry weight basis and total micrograms per sample, DYLOX equivalents on an as received basis. Sample results less than 0.0094 ppm are reported as <0.010 ppm and are not reported as total micrograms.

Control samples and control sample spikes are reported as actual values ppm on an as received basis, DYLOX equivalents. Total micrograms were are not reported on control samples or control sample spikes.

Matrix Equivalent Standards is defined as the level of result that an average sample weight and average final volume would be if that average sample had an equivalent response as the standard (i.e. in this method the average sample weight is 25.0 grams, average final volume is 5.0 mls, therefore a 0.5 ug/ml DDVP standard is a matrix equivalent standard of 0.1 ppm).

Sample Calculations:

Field Treated Samples:

$$ppm = \frac{\text{Spl area}}{\text{Std area}} \times (\text{Std Conc}) \times \frac{25.0}{\text{Spl Wt}} \times \frac{\text{Fin Vol}}{5.0} \times \frac{100}{100 - \%M} D.F. \times CF$$

and

$$ug = \frac{\text{Spl area}}{\text{Std area}} \times (\text{Std Conc}) \times \frac{25.0}{\text{Spl Wt}} \times \frac{\text{Fin Vol}}{5.0} \times DF \times CF \times TCW$$

where
- Spl Area = sample area
- Std Area = average area from before and after standards
- Std Conc = DDVP standard Conc. relative to matrix (ppm)
- Spl Wt = sample weight taken for analysis (g)
- Fin Vol = final volume of sample extract (mL)
- %M = percent moisture of field sample
- DF = dilution factor
- CF = conversion factor to DYLOX equiv (1/0.858)
- TCW = total sample composite weight

Recovery Calculations:

$$\%\ R = \frac{\text{Spl Area}}{\text{Std Area}} \times (\text{Std Conc}) \times \frac{25.0}{\text{Spl Wt}} \times \frac{\text{Fin Vol}}{5.0} \times DF \times CF \times \frac{100}{\text{Spike}}$$

where
- % R = percent Recovery
- Spl Area = sample area
- Std Area = average area from before and after standards
- Std Conc = DDVP standard conc relative to matrix (ppm)
- Spl Wt = sample weight taken for analysis
- Fin Vol = Final volume of sample extract (mL)
- DF = dilution factor
- CF = conversion factor to DYLOX equiv (1/0.858)
- Spike = ppm spike in DYLOX equiv relative to matrix with precorrection to actual sample weight