

ANALYTICAL METHOD

The analytical method used to analyze soil collected for this study was Chevron method RM-30D. A copy of this method can be found in Appendix II. In this method, flumioxazin is extracted from the soil using a 5:1 mixture of acetone and 0.1 N HCl. The flumioxazin residues are partitioned from the resulting aqueous extract with dichloromethane, cleaned-up with column chromatography (Florisil) and analyzed by gas chromatography using a nitrogen-phosphorus specific flame-ionization detector (NPD).

TEST METHOD (CONTINUED)

RM-30D has also been successfully validated by an independent laboratory⁷.

Detector calibration for this method was accomplished by injecting calibrating standards before, during, and after study samples. The response factor for flumioxazin was calculated by dividing the mean of the peak responses obtained during an analytical run by the concentration of the standard.

Peak integration for all analyses was accomplished using a Hewlett-Packard Model 1000 Laboratory Automation System. The resulting peak units were entered into a Lotus 1-2-3® spreadsheet, which was used to calculate standard response factors and sample results. This spreadsheet was also used to perform all statistical analyses for the run and to generate an analytical data sheet for the sample set. Analytical data sheets for all soil residue data in this report are included in Appendix III. Analytical data sheets for all storage stability data in this report are included in Appendix V.

Soil moistures were determined on each soil sample analyzed for this study. The procedure used is described in Chevron SOP # RE-50/Valent SOP # VR-023, "Moisture Determinations". Samples are weighed into tared aluminum weighing pans and placed in an oven at 135°C for 2 hours. After cooling, the samples are reweighed. The % moisture is then calculated using the following formula:

$$\% \text{ Moisture} = \frac{\text{Gross Wet Weight} - \text{Gross Dry Weight}}{\text{Net Sample Weight}} \times 100$$

QUALITY ASSURANCE

Quality assurance measures taken during the analytical portion of this study included, but were not limited to the following: Prior to study initiation, samples of soil from the proposed untreated control and treated plots were analyzed to ensure that no apparent residue of flumioxazin were present that would interfere with the study.

All analytical standards used in this study were prepared and stored in accordance with Chevron SOP # RE-03, "Analytical Standards", or Valent SOP #VR-003, "Analytical Standard Solutions". These SOP's require that standard solutions be kept refrigerated at all times when not in use. They also require that "working solutions", which are diluted periodically from a stock solution, be calibrated against a "monitoring stock solution". The "monitoring stock solution" must be prepared annually and stored for standard calibration purposes only.

TEST METHOD (CONTINUED)

The linearity of the chromatographic systems was verified each day that study samples were analyzed, in accordance with Chevron SOP # RE-07/Valent SOP #VR-007, "Linearity of Measurements". Linearity is determined by analyzing standards containing a minimum of four concentrations. The response for each standard is normalized to response per 1.0 $\mu\text{g/mL}$ by dividing the response of each standard by its concentration. The coefficient of variation (CV) of these normalized responses was required to be 10% or less. Deviation from this requirement required the approval of the Study Director.

The reproducibility of the chromatographic systems was verified each day that study samples were analyzed, in accordance with Chevron SOP # RE-013/Valent SOP #VR-013, "Reproducibility of Standard Measurements". The coefficient of variation (CV) of the detector responses for calibration standards analyzed with study samples was calculated for each data set. For an analytical run to be acceptable, the CV of these responses was required to be 10% or less. Deviation from this requirement required the approval of the Study Director.

One UTC sample and one laboratory fortified sample were analyzed concurrently with each set of study samples to verify method performance. Fortifications were made at 0.01 ppm (the LOQ of the method) or at 0.05 ppm. For an analytical run to be acceptable, these method recoveries were required by the analytical method to be between 70% and 120%. Deviation from this requirement required the approval of the Study Director. If the recovery of flumioxazin in a data set was unacceptable, the results for the concurrently analyzed samples were not reported.

The temperatures of all freezers used to store samples and standards for this study were continuously monitored by a datalogger. Data from the datalogger are printed on a regular schedule and archived at Valent's Dublin Archive, 6560 Trinity Court, Dublin, CA 94568. During the period of July 4-7, 1991, an equipment failure caused the temperature of the freezer where study samples were being stored to rise to +5°F. Soil samples previously fortified with flumioxazin for the freezer storage stability study were being stored in the same freezer during this period of time. Analysis of the freezer storage stability samples after this freezer failure verified that the failure had no adverse effects on the study.

REFERENCES

1. Fathulla, R. N., *Aerobic Soil Metabolism of ¹⁴C-S-53482*, Hazleton Laboratories America, Inc. Laboratory Identification HLA 6311-104, November 27, 1991. MRID # 42684906.
2. Fathulla, R. N., *Aerobic Soil Metabolism of [THP-¹⁴C]-S-53482*, Hazleton Laboratories America, Inc. Laboratory Identification HLA 6311-156, April 19, 1993. MRID # 42884009.
3. Corbin, B. R., *Soil Dissipation of [Phenyl-¹⁴C]-Flumioxazin Under Actual Field Conditions In North Carolina*, Valent Study Number VP-10728, April 16, 1996. MRID # not assigned.
4. Pensyl, J. W., *Half-Life of Flumioxazin in Four Soils Under Laboratory Conditions*, Valent Study Number VP-11579. MRID # not assigned.
5. Pensyl, J. W., *Field Dissipation Study with V-53482 WDG Herbicide In Mississippi*, Valent Study Number VP-1013. MRID # not assigned.
6. Pensyl, J. W., *Terrestrial Field Dissipation Study with V-53482 WP Herbicide On No-Till Ground In Illinois*, Valent Study Number VP-11278. MRID # not assigned.
7. Kruplac, J. F., *Independent Laboratory Validation of the Method For Quantitation of Flumioxazin in Soil*, ADC Project Number 1550, Valent Study Number VP-11293, December 29, 1995. MRID # not assigned.

TABLE I

NOMENCLATURE AND STRUCTURE OF REFERENCE STANDARDS

Technical grade flumioxazin was used as the reference standard in this study. Flumioxazin is the active ingredient in Valent's herbicide V-53482 WDG. The reference standard used in this study was provided by Sumitomo Chemical and certified by Sumitomo prior to study initiation.

Common Names: flumioxazin, S-53482, V-53482

Chemical Name: 7-fluoro-6-((3,4,5,6-tetrahydro)phthalimido)-4-(2-propynyl)-1,4-benzoxazin-3(2H)-one

CAS Number: 103361-09-7

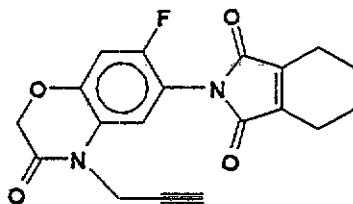
Lot Number: PYG-89021-M

Purity: 94.8%

Lot Number: PPG-90111-M

Purity: 94.7%

Structure:



APPENDIX II
ANALYTICAL METHOD

CHEVRON CHEMICAL COMPANY
AGRICULTURAL CHEMICALS DIVISION
RESIDUE CHEMISTRY LABORATORY
RICHMOND, CALIFORNIA

DETERMINATION OF V-53482
RESIDUES IN SOIL
RESIDUE METHOD RM-30D

FILE NO.: 740.01 / V-53482
DATE: JANUARY 21, 1991
REVISED: AUGUST 5, 1991

INTRODUCTION

This method describes the procedure for the determination of V-53482, [7-fluoro-6-((3,4,5,6-tetrahydro)phthalimide)-4-(2-propynyl)-1,4-benzoxazin-3(2H)-one] in soil. It is a modification of a method¹ developed by Sumitomo Chemical Co., Ltd. Briefly, the method involves extraction with acetone/0.1N HCl (5/1), partition of the residues into dichloromethane, cleanup using Florisil column chromatography, and measurement by gas chromatography utilizing a nitrogen-phosphorus detector.

This method was revised on February 7, 1991 to change the limit of detection from 0.01 ppm to 0.005 ppm, on March 6, 1991 to change the extraction solvent from acetone/H₂O (5/1) to acetone/0.1N HCl (5/1), and on August 5, 1991 to change the GC column parameters.

REAGENTS

Acetone - Pesticide Quality

Dichloromethane - Pesticide Quality

Ethyl acetate - Pesticide Quality

Florisil - PR grade, U.S. Silica Co. Blend 2 parts 100-200 mesh and 3 parts 60-100 mesh and activate overnight at 130°C. Allow to cool overnight before using.

Hexane - Pesticide Quality

Hydrochloric Acid - 36.5 - 38.0%, Baker-Analyzed[®] Reagent, J.T. Baker or equivalent. Prepare a 0.1 N solution by carefully adding 40 ml of acid to 4 liters of deionized water.

Sodium Chloride - Certified ACS grade.

Sodium chloride - 5% (w/v) solution. Prepare by dissolving 5 g analytical grade crystals in 100 ml deionized water.

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Sodium sulfate - Anhydrous, granular, reagent grade.

V-53482 - Reference standard of known purity, available from Sumitomo Chemical Company, Ltd. Prepare a stock solution containing 1.0 mg/ml in acetone. Dilute this stock solution with acetone to prepare a fortifying solution containing 1.0 µg/ml to 2.0 µg/ml of V-53482.

Water - Deionized

SPECIAL EQUIPMENT

Eberbach Reciprocating Shaker or equivalent.

Liquid Chromatography Columns - 300 x 19 mm i.d. glass with Teflon[®] stopcock plugs.

Rotary Evaporators equipped with water bath capable of being heated to 40°C.

Ultrasonic bath.

Büchner Funnels.

Suction Flasks.

Round-bottom Flasks - 50 ml, 250 ml and 500 ml capacity with 24/40 ground glass joints.

Filter paper - Whatman #1.

Separatory Funnels - 500 ml equipped with Teflon[®] stopcocks.

Gas Chromatograph - Hewlett-Packard 5890A or equivalent, equipped with a nitrogen-phosphorus flame ionization detector, autosampler and an integrator. The following parameters are given only as a guide. Changes may be required to optimize the chromatography.

Column: 15 M x 0.53 mm ID, DB-17 (50% phenyl-methyl silicone megabore column, 1.0 µm film (J & W Scientific)

Column Temperature Program:

Initial Temperature: 250°C
Initial Hold Time: 1 minute
Program Rate: 20°C/minute
Final Column Temperature: 280°C
Final Hold Time: 8 minutes

RM-30D

Packed column injector port with megabore adaptor
Carrier Gas Flow Rate: 10 ml/min (He)
Auxillary Gas Flow Rate: 25 ml/min (He)
Detector Gases Flow Rates:
 Hydrogen: 3.5 ml/min
 Air: 110 ml/min

Injector Temperature: 275°C
Detector Temperature: 300°C
Injection volume: 1µl

V-53482 Retention Time: 7.7 min. (See Figure 1)

ANALYTICAL METHOD

Extraction

Thoroughly mix the sample. Transfer 10 g to a 1 pint Mason jar. At this point, a control sample may be fortified for recovery purposes with an appropriate volume of the 1 µg/ml fortifying solution of V-53482 (e.g. 0.1 ml of this solution would fortify the sample with 0.01 ppm).

Add 50 ml of acetone/0.1N HCl (5/1, v/v) and shake for 10 minutes. Allow to soak overnight at room temperature. Shake 10 minutes. Filter the supernatant through a Büchner funnel using Whatman #1 filter paper.

Repeat the extraction and filtration using another 50 ml acetone/0.1N HCl (5/1, v/v). Rinse the filter cake with two 20-ml portions of extraction solvent.

Partition

Transfer the filtrate to a 500 ml separatory funnel and add 150 ml of 5% aqueous sodium chloride solution. Add 80 ml of dichloromethane to the separatory funnel in two portions, using each portion to rinse the suction flask. Shake for 1 minute. (An emulsion may form at this point but usually breaks if allowed to stand. Adding sodium chloride crystals will sometimes assist in breaking the emulsion but may also result in the phases reversing, i.e., the dichloromethane phase will be on top instead of on the bottom. Centrifugation may also be used to break emulsions). Filter the dichloromethane extract through sodium sulfate (pre-washed with 20 ml of dichloromethane) into a 500 ml round bottom flask.

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Repeat the partition and filtration steps with an additional 60 ml portion of dichloromethane. Rinse the sodium sulfate cake with 20 ml of dichloromethane. Evaporate the combined dichloromethane extracts to dryness using a rotary vacuum evaporator equipped with a water bath set $\leq 40^{\circ}\text{C}$. (See note 1).

Florisil Column Cleanup

Place a glass wool plug at the bottom of a 300 mm x 19 mm i.d. glass chromatographic column. Close the column stopcock and add 40 ml hexane/ethyl acetate (2/1, v/v) to the column. Slowly add 15 g of activated Florisil to the column while gently tapping the side of the column. Open the stopcock and allow the solvent to drain to the top of the packing.

Redissolve the concentrated sample residue in 1 ml of ethyl acetate, dilute with 2 ml of hexane, sonicate and transfer to the top of the column. Rinse the round bottom flask with three 3-ml portions of hexane/ethyl acetate (2/1, v/v). Transfer each rinse to the column, allowing each 3-ml portion to drain to the top of the column before adding the next rinse.

Elute the column with an additional 28 ml of hexane/ethyl acetate (2/1, v/v) (total volume 40 ml). Discard this eluate. Place a 250 ml round bottom flask under the column and elute the V-53482 with 70 ml of hexane/ethyl acetate (2/1, v/v).

Evaporate this eluate to dryness using a rotary vacuum evaporator equipped with a water bath set $\leq 40^{\circ}\text{C}$. (See note 1). Transfer the residue to a 50 ml round bottom flask using three 5-ml portions of acetone and evaporate to dryness using a rotary vacuum evaporator equipped with a water bath set $\leq 40^{\circ}\text{C}$ (See note 1).

MEASUREMENT

Redissolve the residue in 0.5 ml acetone. A sample may be dissolved in a larger volume of acetone if the V-53482 concentration is expected to exceed the concentration of the highest linearity standard. Quickly transfer the sample to an autosampler vial using a Pasteur pipet. Seal the vial immediately to minimize evaporation losses.

Load the autosampler with the sample and reference standard vials to be analyzed. A suggested sequence would be: conditioning shot, standard, fortified sample, standard, control sample, standard, sample, standard, etc. The standard vials contain a reference standard containing 1.0 $\mu\text{g/ml}$ of V-53482 in acetone.

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CALCULATION

Use the following formula to calculate the amount of V-53482 present in the samples. Use the dilution factor if a sample was dissolved in more than 0.5 ml.

$$\text{ppm} = \frac{\text{Sample Pk Units}}{\text{Avg. Std Pk Units}} \times \frac{0.5 \text{ ml}}{10 \text{ g sample}} \times 1.0 \mu\text{g/ml} \times \text{Dil Factor}$$

LIMIT OF DETECTION

The limit of detection for V-53482 is 0.005 ppm when 10 g of soil is extracted and analyzed using the described method.

NOTES

1. Samples must be removed from the rotary evaporator immediately after the solvent has evaporated to avoid loss of V-53482.
2. The linearity of the gas chromatographic system should be verified each day data are generated by analyzing at least four concentrations encompassing the concentration range of the samples and including the reference standard solution (1.0 $\mu\text{g/ml}$ V-53482 in acetone) and a lower limit of 10% of the reference solution. The mean of the response factors (response equivalent to 1 $\mu\text{g/ml}$) should have a coefficient of variation of $\pm 10\%$ or less. Exceptions will be allowed only with approval by supervisory personnel.
3. A fortified control sample must be analyzed concurrently with each set of samples. Method recovery must be between 70% to 120% to be acceptable. All samples analyzed concurrently with a fortified sample which does not have an acceptable method recovery must be reanalyzed. Exceptions will be allowed only with supervisory approval.

REFERENCE

1. Ohnishi, J., Hirota, M. and Yamada, H., Report No. ER-MT-8939, "Residue Analytical Method for S-53482 in Soil," November 24, 1989.

J. W. Pensyl 8/7/91
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Reviewed by *J. W. Pensyl* 8-7-91

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RM-30D

Sodium chloride - 5% (w/v) solution. Prepare by dissolving 5 g analytical grade crystals in 100 ml deionized water.

Sodium sulfate - Anhydrous, granular, reagent grade.

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Water - Deionized

SPECIAL EQUIPMENT

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Liquid Chromatography Columns - 300 x 19 mm i.d. glass with Teflon[®] stopcock plugs.

Rotary Evaporators equipped with water bath capable of being heated to 40°C.

Ultrasonic bath.

Büchner Funnels.

Suction Flasks.

Round-bottom Flasks - 50 ml, 250 ml and 500 ml capacity with 24/40 ground glass joints.

Filter paper - Whatman #1.

Separatory Funnels - 500 ml equipped with Teflon[®] stopcocks.

Gas Chromatograph - Hewlett-Packard 5890A or equivalent, equipped with a nitrogen-phosphorus flame ionization detector, autosampler and an integrator. The following parameters are given only as a guide. Changes may be required to optimize the chromatography.

Column: 30 M x 0.53 mm ID, DB-17 (50% phenyl-methyl silicone) megabore column, 1.0 µm film (J & W Scientific)

Packed column injector port with megabore adaptor

Carrier Gas Flow Rate: 15 ml/min (He)

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Auxillary Gas Flow Rate: 15 ml/min (He)

Detector Gases Flow Rates:

Hydrogen: 3.5 ml/min

Air: 100 ml/min

Injector Temperature: 300°C

Detector Temperature: 300°C

Column Oven Temperature: 280°C, isothermal

Injection volume: 2µl

V-53482 Retention Time: 7.9 min. (See Figure 1)

ANALYTICAL METHOD

Extraction

Thoroughly mix the sample. Transfer 10 g to a 1 pint Mason jar. (For recovery purposes, fortify a control sample with an aliquot of an acetone solution of V-53482). Add 50 ml of acetone/0.1N HCl (5/1, v/v) and shake for 10 minutes. Allow to soak overnight at room temperature. Shake 10 minutes. Filter the supernatant through a Büchner funnel using Whatman #1 filter paper. Repeat the extraction and filtration using another 50 ml acetone/0.1N HCl (5/1, v/v). Rinse the filter cake with two 20-ml portions of extraction solvent.

Partition

Transfer the filtrate to a 500 ml separatory funnel and add 150 ml of 5% aqueous sodium chloride solution. Add 80 ml of dichloromethane to the separatory funnel in two portions, using each portion to rinse the suction flask. Shake for 1 minute. (An emulsion may form at this point but usually breaks if allowed to stand. Adding sodium chloride crystals will sometimes assist in breaking the emulsion but may also result in the phases reversing, i.e., the dichloromethane phase will be on top instead of on the bottom. Centrifugation may also be used to break emulsions). Filter the dichloromethane extract through sodium sulfate (pre-washed with 20 ml of dichloromethane) into a 500 ml round bottom flask.

Repeat the partition and filtration steps with an additional 60 ml portion of dichloromethane. Rinse the sodium sulfate cake with 20 ml of dichloromethane. Evaporate the combined dichloromethane extracts to dryness using a rotary vacuum evaporator equipped with a water bath set $\leq 40^{\circ}\text{C}$. (See note 1).

RM-30D

Florisil Column Cleanup

Place a glass wool plug at the bottom of a 300 mm x 19 mm i.d. glass chromatographic column. Close the column stopcock and add 40 ml hexane/ethyl acetate (2/1, v/v) to the column. Slowly add 15 g of activated Florisil to the column while gently tapping the side of the column. Open the stopcock and allow the solvent to drain to the top of the packing.

Redissolve the concentrated sample residue in 1 ml of ethyl acetate, dilute with 2 ml of hexane, sonicate and transfer to the top of the column. Rinse the round bottom flask with three 3-ml portions of hexane/ethyl acetate (2/1, v/v). Transfer each rinse to the column, allowing each 3-ml portion to drain to the top of the column before adding the next rinse. Elute the column with an additional 28 ml of hexane/ethyl acetate (2/1, v/v) (total volume 40 ml). Discard this eluate. Place a 250 ml round bottom flask under the column and elute the V-53482 with 70 ml of hexane/ethyl acetate (2/1, v/v).

Evaporate this eluate to dryness using a rotary vacuum evaporator equipped with a water bath set $\leq 40^{\circ}\text{C}$ (See note 1). Transfer the residue to a 50 ml round bottom flask using three 5-ml portions of acetone and evaporate to dryness using a rotary vacuum evaporator equipped with a water bath set $\leq 40^{\circ}\text{C}$ (See note 1).

MEASUREMENT

Redissolve the residue in 0.5 ml acetone. A sample may be dissolved in a larger volume of acetone if the V-53482 concentration is expected to exceed the concentration of the highest linearity standard.

Transfer the solutions to be measured to vials for use on the automatic liquid sampler and load on the autosampler. A suggested sequence would be: conditioning shot, standard, fortified sample, standard, control sample, standard, sample, standard, etc. The standard vials contain a reference standard containing 1.0 $\mu\text{g/ml}$ of V-53482 in acetone.

CALCULATION

Use the following formula to calculate the amount of V-53482 present in the samples. Use the dilution factor if a sample was dissolved in more than 0.5 ml.

$$\text{ppm} = \frac{\text{Sample Pk Units}}{\text{Avg. Std Pk Units}} \times \frac{0.5 \text{ ml}}{10 \text{ g sample}} \times 1.0 \mu\text{g/ml} \times \text{Dil Factor}$$

RM-30D

LIMIT OF DETECTION

The limit of detection for V-53482 is 0.005 ppm when 10 g of soil is extracted and analyzed using the described method.

NOTES

1. Samples must be removed from the rotary evaporator immediately after the solvent has evaporated to avoid loss of V-53482.
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J. W. Pensyl

Reviewed by *JW Fujie* 3-26-91

cc G. H. Fujie
Residue Files