

Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name: Kresoxim Methyl

MRID #: 443410-25

Matrix: Soil

Analysis: GC/ECD

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**METHOD FOR THE ANALYSIS OF BAS 490 F AND ITS HYDROLYSIS
PRODUCT, BF 490-1 AN N-PROPYL DERIVATIVE, IN SOILS, BASF ANALYTICAL
METHOD NO. D9603**

by

Susan C. Artz

ABSTRACT:

BASF Analytical Method No. D9603, "Method for the Analysis of BAS 490 F and Its Hydrolysis Product, BF 490-1 an n-propyl Derivative, in Soils", was used in the determination of BAS 490 F residues in a soil dislodgeable residue study¹ (BASF report No. ER 96009, BASF Reg. Doc. No. 96/5195, BASF Study 94151). Method development and method validation were conducted at Horizon Laboratories. Soil samples were spiked at 0.5, 1 and 10 ug/ml with analytical standard to validate the method. Recoveries ranged from an average of 101% \pm 7.15% (n=4), 103% \pm 2.38% (n=4) and 102% \pm 2.51% (n=4) for each of the respective levels. The soil extracts were analyzed by GC/ECD.

The average procedural recoveries in the field dislodgeable study¹ using soil were performed at the 0.5 ug/ml, 1 ug/ml and 10 ug/ml levels. The recoveries averaged 106% \pm 9.26% (n=13), 93.4% \pm 8.63 (n=4) and 100% \pm 5.53% (n=17) over the three levels, respectfully. This study has shown that analytical method No. D9603 is suitable for measuring BAS 490 F in soil media used for soil dislodgeable residues.

The independent laboratory validation was done at 0.5 ug/ml and 5 ug/ml. The average recoveries were 79.1% \pm 3.2% (n=6) and 73.1% \pm 2.68 (n=6), respectfully. The soil extracts were analyzed directly by GC/ECD. (BASF Reg. Doc. No. 96/5163²).

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The EPA Worker Re-entry Guidelines, Subdivision K, use an analytical method for the determination of the concentration of test substance residues in soil that has been exposed to test substance from the application of the test substance to the foliage of a particular crop. The measurement of the concentration of test substance represents the potential amount of compound available for physical removal from a passively treated soil.

1.1 SCOPE OF THE METHOD

BASF method D9603 can be used for the determination of BAS 490 F and BF 490-1 applied to soil. In this write-up, however, only the part of the method used for determination of parent, BAS 490 F, will be described in detail since the method was only used for the determination of BAS 490 F residues in soil in an actual field study (BASF Study 94151).

This method can be used for determining the level of BAS 490 F in residue-containing surface soil samples collected from vineyard soil that has been exposed to test substance.

1.2 SOURCE OF THE METHOD

Method D9603 which can be used to determine BAS 490 F residues in soil media was developed and validated by Horizon Laboratories and independently validated by Morse Laboratories for BASF Corporation.

1.3 PRINCIPLE OF THE METHOD

BAS 490 F was extracted with acetonitrile/water, the acetonitrile removed from the water and the water then partitioned with hexane. The hexane was concentrated to dryness and the residue brought up in acetonitrile. An aliquot of acetonitrile was removed and purified by passing the solution through a florisil column. Detection and quantification were achieved by using a gas chromatograph equipped with an electron capture detector. The limit of quantification is 0.5 ug/ml.

1.4 PROJECT HISTORY

The method was initially developed at Horizon Laboratories for the analysis of soil samples produced in a soil dislodgeable residue study (SDR) that was being done to fulfill the FIFRA requirements for worker re-entry. The method was also independently validated by Morse Labs as per the latest Subdivision K guidelines (6/18/96). BAS 490 02 F was the test substance used to develop the method and to

2.0 MATERIALS

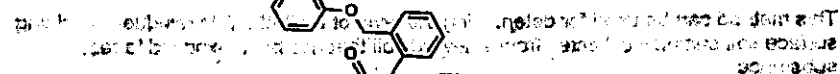
127 b5, c4

10-10-1964

Chemical name: methyl-(E)-methoximino [α-(o-tolyloxy)-o-tolyl]acetate

PH

1101 W. 14th St. 7248



White solid; mp 90-100°C.

Solubility (g/l solvent, 20°C) in water: 0.08 mg/l. (The purity statement can be found in Appendix 2)

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Suggested Sizes/Manufacturer: 604 3AC

Balance with 0.01 g capacity Sartorius, Type 1409

Balance with 0.0001 g capacity, Mettler, Model AE160

Omni Mixer, Model 17150 — Omni International, Model 17150 — Rotary evaporatory with Dewar — Hecke Buncher Inst. and Int'l.

condenser and temperature Buchli

controlled water bath

Mason jars pint

Filter flask 500 ml
Buchner funnel 2 cm

Glass funnel

Separatory funnel 1000 ml

Erlenmeyer flask 250 ml, 500 ml

Flask	125 ml, 500 ml
Chromatographic column	10, 20, 30

Chromatographic column 10 mm i.d. x 30 cm
with stopcock and 250 ml

with stopcock and 250 ml.

reservoir	
Graduated cylinder	50 ml, 100 ml, 250 ml, 500 ml, 1000 ml
Graduated pipet	10 ml
Volumetric pipet	0.5 ml, 1.0 ml, 2.0 ml, 5.0 ml, 20.0 ml
Volumetric flask	10 ml, 25 ml, 50 ml
Culture tubes with Teflon-lined screw caps	13-415
Disposable pasteur pipets	9"
Filter paper	Whatman #4, 9 cm
Cotton balls	100% cotton
Glass wool	
Autosampler vial	2ml, Hewlett-Packard, 5181-3399
Crimp cap, PTFE lined	11 mm, Hewlett-Packard, 5182-0552
Flat-bottom vial insert	6 x 30 mm, Alltech, 98139

2.3 REAGENTS AND CHEMICALS

Acetonitrile	EM Science, EM-AX0142-1, lot# 36010
Hexane	J.T. Baker, 9262-03, lot #'s K06269 and K08306
Sodium chloride	J.T. Baker, 4058-01, lot# K02635
Deionized water	Morse Laboratories, Inc. Polymetric DI water system
Ethyl ether	Burdick & Jackson, 107-1, lot #'s BL096 and BM450
Alcohol, anhydrous	J.T. Baker, 9401-03, lot# K01529
Toluene	EM Science, EM-TX0737-1, lot# 35306
Florisil, 60-100 Mesh, PR Grade	U.S. Silica, 325356, lot# P1182

Note: Equivalent reagents and chemicals from other suppliers may be substituted. It is suggested that HPLC-grade solvents be used in the performing of this method.

3.0 ANALYTICAL METHOD

The general analytical procedure is described in Section 1.3 Principal of the Method and a flow chart can be found in Appendix 3. Details of the method follow.

3.1 STANDARD SOLUTIONS

3.1.1 Stock Solution of BAS 490 F (1.0 mg/ml):

Prepare a 1.0 mg/ml BAS 490 F stock solution by weighing an appropriate amount of analytical grade BAS 490 F (ai) into a volumetric flask. Dissolve with acetonitrile and

dilute to the mark by manual shaking. Further dilutions are made in acetonitrile. Stock solutions are made fresh every six months. BAS 490 F is soluble in acetonitrile at a concentration of 1mg/ml.

3.1.2 Fortification Solutions Using Analytical Standard

The fortification of analytical standard, BAS 490 F, in soil media at three different concentrations is done at room temperature. The spiking solutions at 50, 100 and 500 µg/ml are prepared by further dilution of stock solution with acetonitrile. As an example, to make the 500 µg/ml standard from a 1000 µg/ml stock solution (1 mg/ml), the standard should be diluted 1:2, 1 ml of 1000 µg/ml stock plus 1 ml acetonitrile. This should be done volumetrically. Standard solutions of BAS 490 F are kept frozen during use.

3.1.3 GC Injection Standard Solutions

The standard solutions which can range from 7.5 ng/ml to 200 ng/ml are prepared in acetonitrile and stored in the freezer when not in use. To make a 200 ng/ml standard, a stock solution containing 1000 ng/ml BAS 490 F would be diluted 5 times by taking 1 ml of 1000 ng/ml stock, placing it in a 5 ml volumetric flask and diluting to the mark with acetonitrile.

3.1.4 Solubility of BAS 490 F in Acetonitrile

BAS 490 F is soluble in acetonitrile at a concentration of 1mg/ml.

3.2 CONTROL MATRICES

Soil is the control matrix used in this method. The characterization of the soil is located in the report in Appendix 4.

3.3 SAMPLE WORK-UP

Soil from the test site where the field study was done, (BASF Study 94151, Washington state), was used in the method. The soil was taken from the top 1 cm and sieved so that only those particles 150 µm or less were used as the soil sample for testing. 50 grams of sample was used for analysis. No soil work-up was performed on the samples prior to analysis.

3.4 METHOD PROCEDURE

I. Extraction

A. Weigh out 50.0 g of soil.

B. Spike a control soil with analytical standard as appropriate. A volume of no less than 0.5 ml and no more than 1.0 ml should be used.

C. In a blender add 100 ml of 2:1 ACN/H₂O (v/v) and two Whatmen #4 filter papers (to aid in filtering soil).

D. Blend for 3 minutes, then filter into a 500 ml flask.

E. Rotoevaporate to remove the acetonitrile.

II. Hexane and Methylene Chloride Partition

A. Transfer to a 1L separatory funnel with water.

B. Add 200 ml water.

C. Add 10.0 g NaCl.

D. Add 75 ml of hexane to the original 500 ml flask, rinsing it, then transfer the hexane to the sample in the separatory funnel, and mix the contents gently for 30 seconds.

E. After the phases have separated, drain the lower aqueous layer into a 500 ml flask.

F. Drain the upper hexane layer through a cotton ball into a 500 ml flask (this is the BAS 490 F fraction).

G. Return the aqueous layer to the separatory funnel.

H. Repeat the hexane partitioning twice.

I. Remove the hexane from the BAS 490 F fraction under vacuum on a rotary evaporator.

III. Florisil® Cleanup

NOTE: The Florisil® step may present problems unless the Florisil® is properly profiled prior to running the extract through the florisil column. The exact percentages and volumes of ethyl ether must be determined for each freshly prepared batch of Florisil® in order for the proper recovery of analyte from the column to be achieved. This is a critical step.

A. Dissolve and transfer the sample onto a 1 g, 2% deactivated Florisil® column

with a total of 12 ml of 6% ethyl ether in hexane (v/v). Discard the wash.

NOTE: To make 2% deactivated Florisil®, place Florisil® in an oven overnight at 110°C to 120°C and, cool in a desiccator. Once cool, add 2 ml H₂O to 100 g dried Florisil® and shake for at least 30 minutes.

B. Wash the column with 5 ml of 6% ethyl ether in hexane (v/v). Discard wash.

C. Elute the compounds with 30 ml of 15% ethyl ether in hexane (v/v).

D. Evaporate the sample to dryness. Bring to appropriate final volume in acetonitrile. Inject on a GC/ECD.

3.4 GC INSTRUMENTATION

The quantification of BAS 490 F in soil samples is determined by GC/ECD analysis using the following conditions:

Description of Equipment/Operating Conditions:

Liquid Chromatograph:	Hewlett Packard 5890 A gas chromatograph equipped with a Hewlett-Packard ⁶³ Ni-ECD
HPLC Column:	Fused silica megabore, 15 m x 0.53 mm id, 0.5 µm film thickness, Sup-Herb (Supelco)
Carrier Gas:	Helium at 8 ml/min
Split Flow:	27 ml/min, on at 0.75 minutes
Detector Make-up Gas:	Nitrogen at 65 ml/min
Temperature:	Column: Initial: 95 °C, hold 1.0 minutes Rate 1: 40 °C/min to 240 °C, hold 3.0 minutes Rate 2: 40 °C/min to 280 °C, hold 3.0 minutes Detector: 300 °C Injector: 230 °C
Injection Volume:	2.0 µl
Retention Time:	6.5 min.

Note: The preceding specifications are suggested and may be altered as needed. Actual use conditions and any changes must be documented in the raw data.

3.6 GC CALIBRATION PROCEDURES

Inject several standards (from 7.5 ng/ml to 200 ng/ml) of BAS 490 F until stable responses are observed. Different standard concentrations may be used as appropriate.

Calculation of results is based on peak height measurements using a standard calibration curve (either a linear or a mathematical representation that produces a linear curve). The detector response (peak height) versus weight of standard injected is plotted. The standards should give a linear response. The calibration curve is obtained by direct injection of 2 μ l aliquots of the mixed standards during the course of sample analysis.

3.7 SAMPLE ANALYSIS

2 μ l aliquots of soil extracts are injected into the gas chromatograph.

The peak heights of unknown samples are directly compared with the standard calibration curve to obtain ug/ml BAS 490 F in the final extract solution.

Every 2-3 samples are bracketed with standards to check for shifts in sensitivity or retention time. To do this, an injection sequence including standards and samples must be planned.

If the peak height(s) of the unknown is (are) larger than the highest standard, dilution of an appropriate unknown must be made and the sample re-injected.

3.8 COLLECTION OF DATA

The retention time and peak-height (or peak-area) are measured using computerized chromatography data reduction. The collected raw data are organized using a "Master Sheet" format. The examples of this "Master Sheet" format and pertinent information are given in Appendix 5.

3.9 RECOVERY TEST

The validity of the analytical method is demonstrated by recovery tests before actual analysis of unknown samples is attempted. An untreated sample (control) and fortified (spiked control) samples will also be processed with each set of samples to be analyzed. Typically, one fortification sample is run at the limit of quantification and another is run at a higher concentration. For each fortified sample, an appropriate volume, 0.5 ml or more, but not so much that the consistency of the sample is compromised, of BAS 490 F standard solution is added to a control soil sample.

3.10 CONFIRMATORY TECHNIQUES

Other possible methods of analysis for confirmation purposes include the use of another type of detector, like a nitrogen detector or the use of GC/MS. These have not been tested. One method that has been tested is the use of LC/MSMS where BAS 490 F's parent ion is M/Z 314.1. (BASF Study 94028, Reg. Doc. No. 96/5172).

3.11 TIME REQUIRED FOR ANALYSIS

Analysis of a set of 8 soil samples requires 1.8 working days, including sample extraction, clean-up and GC analysis. The method break point is after the partitioning steps are completed (Part II, Section I.) before the Florisil® step (Part III).

3.12 METHOD INTERFERENCES

1. Sample Matrix

Soils always pose potential problems with interferences. However, in the analyses done with the method at the levels of fortification (0.5 ug/g, 1.0 ug/g and 10.0 ug/g), no insurmountable problems with interferences were encountered. If interfering peaks from the matrix occur in the chromatogram, the GC temperature program could be changed or an alternative GC column such as a Supelco SPB-5 could be used.

2. Other Sources

No problems with interferences or questionable peak identity from labware, solvent or other chemicals, have been encountered to date at the levels at which the analyses were done. No tests were done on other registered pesticides for the purposes of this study.

3.13 POTENTIAL PROBLEMS

The Florisil® step may present problems unless the Florisil® is properly profiled prior to running the extract through the florisisil column. The exact percentages and volumes of ethyl ether must be determined for each freshly prepared batch of Florisil® in order for the proper recovery of analyte from the column to be achieved. This is a critical step.

4.0 METHOD OF CALCULATION

4.1 STANDARD CALIBRATION CURVE

One way to plot a calibration curve is to plot peak height vs concentration of standard injected. Construct a linear-least squares calibration curve in the form $y = bx + c$.

4.2 CALCULATION OF ANALYTE IN SAMPLES

The distinct peak of BAS 490 F seen by GC/ECD from soil media is characterized by a retention time and a peak height. The retention time is used qualitatively to identify an analyte and peak height is used to quantitatively determine the amount of BAS 490 F in soil media. The retention time and height of the peak (or peak-area) are measured using computerized chromatography data reduction software.

The calculation of results are based on peak height measurements. Using the peak height measurements for BAS 490 F in the samples, the amount of the analyte in ng/ml is determined from the appropriate least squares calibration curve. Use of full computer/calculator precision in any intermediate calculations is necessary. Only the final value is to be rounded.

PPM (ug/g) values are calculated using the equation below.

$$\text{ppm (ug/g)} = \frac{(\text{ng/ml Final Extract}) \times (\text{ml Final Extract}) \times (\text{GC Dilution factor})}{50 \text{ g Sample Weight} \times 1000 \text{ ng} \times \% \text{ Dryness}}$$

4.3 CALCULATION OF PROCEDURAL RECOVERIES

Correct fortification results for residues found in the control sample as follows:

$$\text{ppm (corrected)} = \text{ppm in fortified control} - \text{ppm in control}$$

Determine percent recovery from the fortification results as follows:

$$\% \text{ Recovery} = \frac{\text{ppm(corrected)} \times 100}{\text{ppm BAS 490 F added}}$$

5.0 VALIDATION

The validation study was carried out as part of BASF study 94151. The control soil media was collected in Washington state and was used for fortification at three different levels (0.5, 1.0 and 10.0 ug/ml) with analytical grade standard of BAS 490 F. Recoveries averaged 101% +/- 7.15% (n=4), 103% +/- 2.38 % (n=4) and 102% +/- 4.19% (n=4) for the 0.5, 1.0 and 10.0 ug/ml levels, respectively.

6.0 RESULTS AND DISCUSSION

The laboratory fortification recoveries from BASF Study 94151 are given in Table 1. The percent recovery results from the validation at Horizon Laboratories are given in Table 2. the percent recovery results from the ILV performed at Morse Laboratories are given in Table 3.

Representative GC chromatograms of BAS 490 F analyses of selected control, fortified samples, typical standard chromatograms and a standard curve for BAS 490 F are given in Appendix 6.

6.1 Accuracy and Precision:

The accuracy and precision for the method was 101% \pm 7.15% (n=4), relative standard deviation of 7.04% with a 95% confidence interval of 101% \pm 11.4% at the 0.5 ug/ml level (LOQ) as determined in the method validation in BASF Study 94151m (filed).

6.2 Limit of Quantification:

The lowest level at which these media samples containing BAS 490 F in soil could be quantified confidently was 0.5 ug/ml. At this level, the control samples were relatively clean and good recoveries were obtained.

6.3 Limit of Detection:

The detection limit for BAS 490 F was 0.038 ug/ml as determined by visual inspection of the chromatograms at the 7.5 pg level.

6.4 Method Ruggedness:

No testing other than the laboratory validation done at Horizon Laboratories and the independent laboratory validation performed at Morse Laboratories using one soil from a single source was done with the method. Therefore, its general ruggedness is not known.

6.5 Method Limitations:

None known to date.

7.0 CONCLUSIONS

Analytical Method No. D9603 has been shown to be suitable for measuring levels of BAS 490 F at levels of 0.5 ug/ml and higher in soil. The recoveries from soil are satisfactory, even when the method is performed by an outside, inexperienced independent laboratory.

8.0 SAFETY AND HEALTH CONSIDERATIONS

8.1 General

The following practices are highly recommended. Use personal protective equipment such as hoods, lab coats, safety glasses and gloves (nitrile/latex gloves are recommended) when performing the operations described in this method. Take care to avoid exposure to solvents. Dispose of hazardous wastes in an environmentally acceptable manner, in compliance with applicable laws (local, state and federal) and regulations.

8.2 Solvents, Reagents and Standards

All analysts must be familiar with the potential hazards of each of the reagents, solvents, and products used in this method before any laboratory work is done. It is recommended that the Material Safety Data Sheets (MSDSs) be reviewed for all solvents and reagents used in this method. A MSDS for BAS 490 F is attached to this report. (Appendix 6) The laboratory safety manual, product information and other

related materials should be consulted. The exposure to all chemicals should be reduced to the lowest possible level. Analysts should also be aware of OSHA regulations regarding the safe handling of the chemicals specified in this method.

9.0 REFERENCES

1. Artz, S. C., BASF, Clark, J. R., Ph.D., BASF, Jensen, M. P., Agriseach, Inc., Merricks, D. L., Ph. D., Agriseach, Inc. and Howell, R. E., Horizon Laboratories, Inc., "Dissipation of Dislodgeable Soil Residues of BAS 490 02 F Applied to Grapes", BASF Reg. Doc. No. 96/5195, BASF Report No. ER 96009, BASF Study No. 94151. Issued Sept. 18, 1996.
2. Clark, S. E., "Independent Method Validation of BASF Analytical Method D9603, "Method for the Analysis of BAS 490 F and Its Hydrolysis Produce, BF 490-1 as an n-propyl Derivative, in Soils". BASF Report No. 96/5163. BASF Study 96037. Issued Sept. 24, 1996.
3. BASF Study 95050, "Storage Stability Study of BAS 490 F in Soil in Support of a Soil Dislodgeable Residue (SDR) Study in Grapes", in Progress. To be completed December 1996.
4. Jackson, S., Jordan, J., and Wesley, D. S., "Soil Dissipation of BAS 490 F in Orchard/ Vineyard use Patterns," BASF Study 94028, Reg. Doc. No. 96/5172, issued Sept. 20, 1996.

TABLE 1.1

TABLE 1.2

TABLES

TABLE 1.1. Data for the first two years of the study. The data are presented in the following order: (1) the number of subjects who completed the study; (2) the number of subjects who dropped out of the study; (3) the number of subjects who were lost to follow-up; (4) the number of subjects who were excluded from the study; (5) the number of subjects who were included in the study.

TABLE 1.2. Data for the last two years of the study.

TABLE 1.3. Data for the last two years of the study. The data are presented in the following order: (1) the number of subjects who completed the study; (2) the number of subjects who dropped out of the study; (3) the number of subjects who were lost to follow-up; (4) the number of subjects who were excluded from the study; (5) the number of subjects who were included in the study.

TABLE 1.4. Data for the last two years of the study. The data are presented in the following order: (1) the number of subjects who completed the study; (2) the number of subjects who dropped out of the study; (3) the number of subjects who were lost to follow-up; (4) the number of subjects who were excluded from the study; (5) the number of subjects who were included in the study.

TABLE 1.5

TABLE 1.6. Data for the last two years of the study. The data are presented in the following order: (1) the number of subjects who completed the study; (2) the number of subjects who dropped out of the study; (3) the number of subjects who were lost to follow-up; (4) the number of subjects who were excluded from the study; (5) the number of subjects who were included in the study.

TABLE 1.7. Data for the last two years of the study. The data are presented in the following order: (1) the number of subjects who completed the study; (2) the number of subjects who dropped out of the study; (3) the number of subjects who were lost to follow-up; (4) the number of subjects who were excluded from the study; (5) the number of subjects who were included in the study.

TABLE 1.8. Data for the last two years of the study. The data are presented in the following order: (1) the number of subjects who completed the study; (2) the number of subjects who dropped out of the study; (3) the number of subjects who were lost to follow-up; (4) the number of subjects who were excluded from the study; (5) the number of subjects who were included in the study.

TABLE 1.9. Data for the last two years of the study. The data are presented in the following order: (1) the number of subjects who completed the study; (2) the number of subjects who dropped out of the study; (3) the number of subjects who were lost to follow-up; (4) the number of subjects who were excluded from the study; (5) the number of subjects who were included in the study.