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

validate it. This is because this is the form of BAS 490 F that was used in the field SDR study.

2.0 MATERIALS

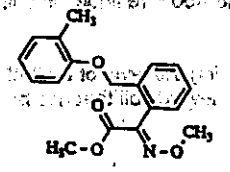
2.1 REFERENCE STANDARD:

Common name: Kresoxim methyl

BASF code: BAS 490 F

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

Structure:



Appearance: White solid

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

2.2 EQUIPMENT

Equipment	Suggested Sizes/Manufacturer
Balance with 0.01 g capacity	Sartorius, Type 1409
Balance with 0.0001 g capacity	Mettler, Model AE160
Omni Mixer	Omni International, Model 17150
Rotary evaporatory with Dewar condenser and temperature controlled water bath	Haake Buchler Instr. and Buchil
Mason jars	pint
Filter flask	500 ml
Buchner funnel	9 cm i.d.
Glass funnel	82 mm o.d.
Separatory funnel	1000 ml
Erlenmeyer flask	250 ml, 500 ml
Flask	125 ml, 500 ml
Chromatographic column with stopcock and 250 ml	10 mm i.d. x 30 cm

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 ug/ml standard from a 1000 ug/ml stock solution (1 mg/ml), the standard should be diluted 1:2, 1 ml of 1000 ug/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 um 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/H_2O (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% deactived Florisil®, place Florisil® in an oven overnight at 110°C to 120°C and, cool in a desicator. Once cool, add 2 ml H_2O 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:	6.5min.	

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 µl aliquots of the mixed standards during the course of sample analysis.

3.7 SAMPLE ANALYSIS

2 µl 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).[4]

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 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.

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.

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

4.3 CALCULATION OF PROCEDURAL RECOVERIES

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

ppm (corrected) = ppm in fortified control - ppm in control
Determine percent recovery from the fortification results as follows:

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