

Cover Sheet for

**ENVIRONMENTAL CHEMISTRY METHOD**

***Pesticide Name:*** Kresoxim Methyl (BAS490 F)

***MRID #:*** 443410-35

***Matrix:*** Soil

***Analysis:*** LC/MS/MS

This method is provided to you by the Environmental Protection Agency's (EPA) Environmental Chemistry Laboratory (ECL). This method *is not* an EPA method but one which was submitted to EPA by the pesticide manufacturer to support product registration. EPA recognizes that the methods may be of some utility to state, tribal, and local authorities, but makes no claim of validity by posting these methods. Although the Agency reviews *all* Environmental Chemistry Methods submitted in support of pesticide registration, the ECL evaluates only about 30% of the currently available methods. Most methods perform satisfactorily but some, particularly the older methods, have deficiencies. Moreover, the print quality of the methods varies considerably because the methods originate from different sources. Therefore, the methods offered represent the best available copies.

If you have difficulties in downloading the method, or further questions concerning the methods, you may contact Elizabeth Flynt at 228-688-2410 or via e-mail at [flynt.elizabeth@epa.gov](mailto:flynt.elizabeth@epa.gov).

*Handwritten initials*

BASF CORPORATION  
AGRICULTURAL PRODUCTS GROUP  
Agricultural Research Center  
Post Office Box 13528  
Research Triangle Park, NC 27709

443410-35

**Study Title:**  
BASF METHOD FOR RESIDUE DETERMINATIONS OF BAS 490 F,  
AND METABOLITES BF 490-1 AND BF 490-5 IN SOIL USING LC/MS/MS

Method No. D9503

Study 95088

**Data Requirement:**

Guideline 164-1

**Study Completion Date:**

10-10-96

**Authors:**

Jeannine Jordan, BASF

John Perkins, Steve Lowes, David Anderson, ABS

Jennifer Q. Jiang, Jack Henion, ABS

**Developers:**

John R. Perkins, Steve Lowes, David J. Anderson, Jennifer Q. Jiang, Jack D. Henion, Advanced  
BioAnalytical Services

Jeannine Jordan, Nelson Delgado, Steve McGown, Robert D. Zehr and Ira Moss, BASF

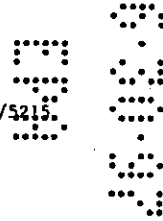
**Performing Laboratories:**

BASF Corporation  
Agricultural Research Center  
P.O. Box 13528  
Research Triangle Park, NC 27709

Advanced BioAnalytical Services  
15 Catherwood Road  
Ithaca, NY 14850

BASF Registration Document No. 96/5215

This report consists of 121 pages



96VIRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

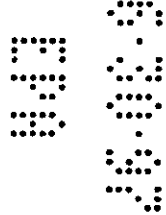
**PR 86-5 DATA CONFIDENTIALITY CLAIM**

No claim is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10 (d) (1) (A), (B), or (C).

**COMPANY** BASF CORPORATION / AGRICULTURAL PRODUCTS GROUP

**COMPANY AGENT** Karen R. Blundell **DATE** 10/16/96

Sr. Registration Specialist Karen R. Blundell  
**Title** **Signature**



46VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

**GOOD LABORATORY PRACTICES STATEMENT**

The study described in this report was conducted in compliance with the Good Laboratory Practice Standards as described in United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 160, Federal Register, issued 17 August 1989 with the following exception.

Results of in-process inspections by the QAU of the performing laboratory were reported on a timely basis to the Project Coordinator and Project Coordinator Management. Results of all inspections were reported to the BASF Study Director and BASF Management at the completion of review of the draft study report.

Study Director: *Garrine Jordan*

Date: 10-10-96

Sponsor: *A. M. J.*

Date: 10/10/86

Submitter: *Karen R. Blundell*

Date: 10/16/96

Project Coordinator: *AKC*

Date: 10/7/96

96VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

BASF CORPORATION  
AGRICULTURAL PRODUCTS GROUP  
Agricultural Research Center  
Post Office Box 13528  
Research Triangle Park, NC 27709

BASF METHOD FOR RESIDUE DETERMINATIONS OF BAS 490 F,  
AND METABOLITES BF 490-1 AND BF 490-5 IN SOIL USING LC/MS/MS

**AUTHORS:** Jeannine Jordan, BASF (919)547-2249  
John Perkins, Steve Lowes, David Anderson,  
Jennifer Jiang, Jack Henion, ABS

**SUPERVISORY PERSONNEL:** Arno J. Krotzky

**STUDY DIRECTOR:** Jeannine Jordan

**WORK DONE BY:** John Perkins, Jennifer Jiang, Jack Henion, ABS  
Jeannine Jordan, Nelson Delgado, Steve McGown,  
Ira Moss and Robert D. Zehr, BASF

Method Number: D9503

Report Date: October 8 1996

**ABSTRACT:**

BAS 490 F is an experimental fungicide for the primary control of Apple Scab and Powdery Mildew, developed by BASF Corporation. To understand the fate of this compound in the environment, it is important to analyze soil samples for BAS 490 F and its metabolites BF 490-1 and BF 490-5. The method for determining residues of BAS 490 F and its metabolites is summarized as follows: Soil samples are first extracted twice with methanol on a mechanical shaker. The samples are centrifuged, then decanted into 50 mL polypropylene centrifuge tubes. The soil marc is resuspended in 0.1 N sodium hydroxide, extracted on the mechanical shaker, centrifuged and decanted, then rinsed with NANOpure water, centrifuged and decanted. The combined basic extract and aqueous rinse is then acidified and partitioned with 9:1 dichloromethane/ethyl acetate. The methanol extract and dichloromethane extract are concentrated and combined. The combined extracts are evaporated to <1 mL. The final quantitative determination based on analyte peak area is performed by ion spray LC/MS/MS. The method validation was performed by Advanced BioAnalytical Services. Fortification levels for the validation were 0.010 ppm, 0.100 ppm, 0.350 ppm, and 1.00 ppm. The mean recovery of BAS 490 F, BF 490-1, and BF 490-5 from the test soil at all spiking levels was 99.5%, 83.6%, and 71.4%, respectively. The method validation also includes precision, accuracy and stability data. The accuracy and precision for individual BAS 490 F and BF 490-1 and BF 490-5 calibration curves were within 6%, 7% and 10%, respectively. BAS 490 F and BF 490-1 were determined to be stable for up to 21 days at ambient temperature in reconstituted extracts and BF 490-5 to be stable up to 7 days at ambient temperature. The lowest fortification level used for this study was 0.010 ppm and that is the working quantitation limit. A statistical limit of quantitation was calculated at 0.004 ppm for BAS 490 F and BF 490-1 and 0.006 ppm for BF 490-5.

96VIRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

STATEMENT OF THE QUALITY ASSURANCE UNIT

Report Number: 96VJRP01.BAS  
BASF Protocol Number: 95088  
ABS Study Number: BAS-490-F, Protocol 95088

Name/Number of the Test / Reference substances

Name: BAS 490 F  
Lot Number: 39-184-1  
Purity: 99.7%

Name: BF 490-1  
Lot Number: 00820-251  
Purity: 100%

Name: BF 490-5  
Lot Number: 00436-83  
Purity: 99.6%

Type of Study: Method Validation

The quality assurance unit of Advanced BioAnalytical Services, Inc. has inspected and/or audited this study and report, and reported its findings to the study director and to management.

Inspection Dates: October 6, 1995, November 8, 9, 14, 15, 17, 18 and 29, 1995; February 22, 1996; September 11, 12, 13 and 16, 1996

Reported to  
Study Director  
and Management: September 17, 1996

  
Signature of QAU

  
Date

96VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

STATEMENT OF THE BASF QUALITY ASSURANCE UNIT

Report Number: 96VJRP01.BAS  
BASF Protocol Number: 95088  
ABS Study Number: BAS-490-F, Protocol 95088

Name/Number of the Test / Reference substances

Name: BAS 490 F  
Lot Number: 39-184-1  
Purity: 99.7%

Name: BF 490-1  
Lot Number: 00820-251  
Purity: 100%


Name: BF 490-5  
Lot Number: 00436-83  
Purity: 99.6%

Type of Study: Method Validation

The quality assurance unit of BASF has inspected and/or audited this study and report, and reported its findings to the study director and to management.

Inspection Dates: 10/03/96

Reported to  
Study Director  
and Management: 10/03/96

  
Signature of QAU  
10/10/96  
Date

96VJRP01.BAS



## PAGES OF REPORT:

## TABLE OF CONTENTS

	Page
GOOD LABORATORY PRACTICES STATEMENT	3
ABSTRACT	4
STATEMENT OF THE QUALITY ASSURANCE UNIT	5
STATEMENT OF THE BASF QUALITY ASSURANCE UNIT	6
1.0 INTRODUCTION AND SUMMARY	14
1.1 Scope and Source of the Method	14
1.2 Test / Reference Substances	14
1.3 Principle of the Method	15
2.0 MATERIAL AND METHODS	16
2.1 Equipment	16
2.2 Reagents and Chemicals	18
2.3 Standard Substances and Solutions	19
3.0 ANALYTICAL PROCEDURE	22
3.1 Preparation of Samples for Extraction	22
3.2 Extraction of Residue	23
3.3 Dichloromethane Partition Clean-up	24
3.4 LC/MS/MS Analysis Sample Preparation	24
3.5 Instrumentation Common to LC/MS/MS Methods 1 and 2	25
3.6 Typical Operating Conditions for Lower Organic Content Soils (Method 1)	25
3.7 Typical Operating Conditions Higher Organic Content Soils (Method 2)	26
3.8 Calibration Procedures	28
3.9 Sample Analysis	29
3.10 Interferences	29
3.11 Confirmatory Techniques	30
3.12 Time Required for Analysis	30
3.13 Potential Problems	30
3.14 Moisture Content	30
4.0 METHODS OF CALCULATION	31
4.1 Calibration	31
4.2 Analyte in Sample	31
4.3 Calculation of Procedural Recoveries	31
5.0 VALIDATION	32
5.1 Recovery	32
5.2 Intra- and Inter-Assay Precision	32
5.3 Inter-Assay Accuracy	33
5.4 Linearity	34
5.5 Reconstituted Sample Stability	34
5.6 Sensitivity	34

96VIRP01.BAS



5.7	Use of Control Blanks	35
5.8	Limitations	35
6.0	CROSS-VALIDATION WITH VARIOUS SOILS	35
6.1	Cross-Validation of LC/MS/MS Method 1 to California Soil 94017	35
6.2	Cross-Validation of LC/MS/MS Method 1 to Oregon Soil 94016	36
6.3	Cross-Validation of LC/MS/MS Method 1 to New York Soil 94015	36
6.4	Cross-Validation of LC/MS/MS Method 2 to Oregon Soil 94016	36
6.5	Cross-Validation of LC/MS/MS Method 2 to New York Soil 94015	37
7.0	CONCLUSION	37
8.0	QUALITY ASSURANCE PROCEDURES	37
9.0	REFERENCES	37
10.0	SAFETY AND HEALTH CONSIDERATIONS	37
11.0	PROTOCOL CHANGES	38
	ADVANCED BIOANALYTICAL SERVICES SIGNATURE PAGE	39
12.0	TABLES	40
13.0	FIGURES	66
14.0	APPENDIX I: ANALYTICAL PROCEDURE	113
15.0	APPENDIX II: NOTES TO METHOD	121
TABLES:		
Table 1.	Analytical Standards Dilution Scheme	22
Table 2:	Summary of Validation Experiments.	40
Table 3:	Procedural Recoveries (%) for BAS 490 F from Canadian Soil 94041.	41
Table 4:	Procedural Recoveries (%) for BF 490-1 from Canadian Soil 94041.	42
Table 5:	Procedural Recoveries (%) for BF 490-5 from Canadian Soil 94041.	43
Table 6:	Inter-Assay Precision and Accuracy from BAS 490 F Analytical Standard Curve Results - Method 1.	44
Table 7:	Inter-Assay Precision and Accuracy from BF 490-1 Analytical Standard Curve Results - Method 1.	45
Table 8:	Inter-Assay Precision and Accuracy from BF 490-5 Analytical Standard Curve Results - Method 1.	46

96VJRP01.BAS

ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

Table 9:	Inter-Assay Precision and Accuracy from BAS 490 F Analytical Standard Curve Results - Method 2.	47
Table 10:	Inter-Assay Precision and Accuracy from BF 490-1 Analytical Standard Curve Results - Method 2.	48
Table 11:	Inter-Assay Precision and Accuracy from BF 490-5 Analytical Standard Curve Results - Method 2.	49
Table 12:	BAS 490 F Calibration Curve Summary Statistics - Method 1.	50
Table 13:	BF 490-1 Calibration Curve Summary Statistics - Method 1.	50
Table 14:	BF 490-5 Calibration Curve Summary Statistics - Method 1.	50
Table 15:	BAS 490 F Calibration Curve Summary Statistics - Method 2.	51
Table 16:	BF 490-1 Calibration Curve Summary Statistics - Method 2.	51
Table 17:	BF 490-5 Calibration Curve Summary Statistics - Method 2.	51
Table 18:	Summary of BAS 490 F Reconstituted Sample Stability at Ambient Temperature.	52
Table 19:	Summary of BF 490-1 Reconstituted Sample Stability at Ambient Temperature.	53
Table 20:	Summary of BF 490-5 Reconstituted Sample Stability at Ambient Temperature.	54
Table 21:	Summary of BAS 490 F Procedural Recovery (%) at Level of Quantitation in Soil.	55
Table 22:	Summary of BF 490-1 Procedural Recovery (%) at Level of Quantitation in Soil.	55
Table 23:	Summary of BF 490-5 Procedural Recovery (%) at Level of Quantitation in Soil.	56
Table 24:	Summary of Reconstitution Solvent and Control Soil Blank Extracts - Method 1.	57
Table 25:	Summary of Reconstitution Solvent and Control Soil Blank Extracts - Method 2.	58
Table 26:	Summary of Procedural Recoveries (%) from Cross Validation of LC/MS/MS Method 1 to California Soil 94017.	59
Table 27:	Summary of Procedural Recoveries (%) from Cross Validation of LC/MS/MS Method 1 to Oregon Soil 94016.	60
Table 28:	Summary of Procedural Recoveries (%) from Cross Validation of LC/MS/MS Method 1 to New York Soil 94015.	61

96V/RP01.BAS

ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

Table 29:	Summary of Procedural Recoveries (%) from Cross Validation of LC/MS/MS Method 2 to Oregon Soil 94016.	62
Table 30:	Summary of Procedural Recoveries (%) from Cross Validation of LC/MS/MS Method 2 to New York Soil 94015	63
Table 31:	Summary of Procedural Recoveries (%) from the Extraction Method using Reduced Extraction Times and Three Partition Steps.	64
Table 32:	Summary of Procedural Recoveries (%) from the Extraction Method using Reduced Extraction Times and Two Partition Steps.	65

#### FIGURES

Figure 1.	Full-Scan Mass Spectra of BAS 490 F, BF 490-1 and BF 490-5 - Method 1.	66
Figure 2:	Full-Scan MS/MS Spectra of BAS 490 F, BF 490-1 and BF 490-5 - Method 1.	67
Figure 3:	MS/MS Fragmentation For BAS 490 F, BF 490-1 and BF 490-5 - Method 1.	68
Figure 4:	Full-Scan Mass Spectra of BF 490-1 and BF 490-5 - Method 2.	69
Figure 5:	Full-Scan MS/MS Spectra of BF 490-1 and BF 490-5 - Method 2.	70
Figure 6:	Postulated Fragmentation Pathway to the Formation of the $m/z = 116$ Product Ion of BF 490-5.	71
Figure 7:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (2 ng/mL, 6 pg of each injected on column) - LC/MS/MS Method 1.	72
Figure 8:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (5 ng/mL, 15 pg of each injected on column) - LC/MS/MS Method 1.	73
Figure 9:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (10 ng/mL, 30 pg of each injected on column) - LC/MS/MS Method 1.	74
Figure 10:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (20 ng/mL, 60 pg of each injected on column) - LC/MS/MS Method 1.	75
Figure 11:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (50 ng/mL, 150 pg of each injected on column) - LC/MS/MS Method 1.	76
Figure 12:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (100 ng/mL,	

96VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

	300 pg of each injected on column) - LC/MS/MS Method 1.	77
Figure 13:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (200 ng/mL, 600 pg of each injected on column) - LC/MS/MS Method 1.	78
Figure 14:	Calibration Curve for BAS 490 F Analytical Standards - LC/MS/MS Method 1.	79
Figure 15:	Calibration Curve for BF 490-1 Analytical Standards - LC/MS/MS Method 1.	80
Figure 16:	Calibration Curve for BF 490-5 Analytical Standards - LC/MS/MS Method 1.	81
Figure 17:	Selected Reaction Monitoring Chromatograms from a Blank Soil Procedural Recovery Sample - LC/MS/MS Method 1.	82
Figure 18:	Selected Reaction Monitoring Chromatograms from 0.006 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (3 ng/mL in Final Extract Solution Assuming 100% Recovery) - LC/MS/MS Method 1.	83
Figure 19:	Selected Reaction Monitoring Chromatograms from 0.010 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (5 ng/mL in Final Extract Solution Assuming 100% Recovery) - LC/MS/MS Method 1.	84
Figure 20:	Selected Reaction Monitoring Chromatograms from 0.100 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (50 ng/mL in Final Extract Solution Assuming 100% Recovery) - LC/MS/MS Method 1.	85
Figure 21:	Selected Reaction Monitoring Chromatograms from 0.350 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (175 ng/mL in Final Extract Solution Assuming 100% Recovery) - LC/MS/MS Method 1.	86
Figure 22:	Selected Reaction Monitoring Chromatograms from 1.00 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (500 ng/mL in Final Extract Solution Assuming 100% Recovery) (Diluted x4 Prior to Injection) - LC/MS/MS Method 1.	87
Figure 23:	Selected Reaction Monitoring Chromatograms from a Blank Soil Procedural Recovery Sample (California Soil 94017) - LC/MS/MS Method 1.	88
Figure 24:	Selected Reaction Monitoring Chromatograms from 0.010 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (California Soil 94017) - LC/MS/MS Method 1.	89
Figure 25:	Selected Reaction Monitoring Chromatograms from 0.100 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (California Soil 94017) - LC/MS/MS Method 1.	90

96VTRP01.BAS

ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

Figure 26:	Selected Reaction Monitoring Chromatograms from 0.350 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (California Soil 94017) - LC/MS/MS Method 1.	91
Figure 27:	Selected Reaction Monitoring Chromatograms from 1.00 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (California Soil 94017) (Diluted x4 Prior to Injection) - LC/MS/MS Method 1.	92
Figure 28:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (2 ng/mL, 30 pg of each injected on column) - LC/MS/MS Method 2.	93
Figure 29:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (5 ng/mL, 75 pg of each injected on column) - LC/MS/MS Method 2.	94
Figure 30:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (10 ng/mL, 150 pg of each injected on column) - LC/MS/MS Method 2.	95
Figure 31:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (20 ng/mL, 300 pg of each injected on column) - LC/MS/MS Method 2.	96
Figure 32:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (50 ng/mL, 750 pg of each injected on column) - LC/MS/MS Method 2.	97
Figure 33:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (100 ng/mL, 1.5 ng of each injected on column) - LC/MS/MS Method 2.	98
Figure 34:	Selected Reaction Monitoring Chromatograms from an Analytical Standard Calibration Solution of BAS 490 F, BF 490-1 and BF 490-5 (200 ng/mL, 3.0 ng of each injected on column) - LC/MS/MS Method 2.	99
Figure 35:	Calibration Curve for BAS 490 F Analytical Standards - LC/MS/MS Method 2.	100
Figure 36:	Calibration Curve for BF 490-1 Analytical Standards - LC/MS/MS Method 2.	101
Figure 37:	Calibration Curve for BF 490-5 Analytical Standards - LC/MS/MS Method 2.	102
Figure 38:	Selected Reaction Monitoring Chromatograms from a Blank Soil Procedural Recovery Sample (Oregon Soil 94016) - LC/MS/MS Method 2.	103
Figure 39:	Selected Reaction Monitoring Chromatograms from 0.010 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (Oregon Soil 94016) - LC/MS/MS Method 2.	104

96VJRP01.BAS

ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

Figure 40:	Selected Reaction Monitoring Chromatograms from 0.100 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (Oregon Soil 94016) - LC/MS/MS Method 2.	105
Figure 41:	Selected Reaction Monitoring Chromatograms from 0.350 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (Oregon Soil 94016) - LC/MS/MS Method 2.	106
Figure 42:	Selected Reaction Monitoring Chromatograms from 1.00 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (Oregon Soil 94016) (Diluted x4 Prior to Injection) - LC/MS/MS Method 2.	107
Figure 43:	Selected Reaction Monitoring Chromatograms from a Blank Soil Procedural Recovery Sample (New York Soil 94015) - LC/MS/MS Method 2.	108
Figure 44:	Selected Reaction Monitoring Chromatograms from 0.010 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (New York Soil 94015) - LC/MS/MS Method 2.	109
Figure 45:	Selected Reaction Monitoring Chromatograms from 0.100 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (New York Soil 94015) - LC/MS/MS Method 2.	110
Figure 46:	Selected Reaction Monitoring Chromatograms from 0.350 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (New York Soil 94015) - LC/MS/MS Method 2.	111
Figure 47:	Selected Reaction Monitoring Chromatograms from 1.00 ppm Spiked Procedural Recovery Sample of BAS 490 F, BF 490-1 and BF 490-5 (New York Soil 94015) (Diluted x4 Prior to Injection) - LC/MS/MS Method 2.	112



## 1.0 INTRODUCTION AND SUMMARY

### 1.1 Scope and Source of the Method

#### 1.1.1 Scope

This method is to be used to determine the residues of BAS 490, BF 490-1 and BF 490-5 in soil. The BAS 490 F residues are first extracted from the soil with methanol; and then the soil is re-extracted with 0.1 N sodium hydroxide to release the acid moieties: BF 490-1 and BF 490-5. The alkaline extract is acidified and partitioned with dichloromethane/ethyl acetate. The partition extract and the methanol extract are concentrated under dry nitrogen separately then combined and further dried to < 1 mL. The residues are analyzed using LC/MS/MS.

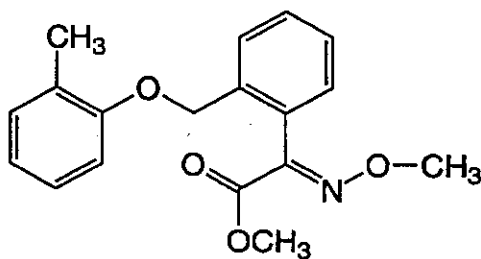
#### 1.1.2 Source

This method was developed at Advanced BioAnalytical Services, 15 Catherwood Road, Ithaca, NY 14850 and BASF Corporation, 26 Davis Drive, Research Triangle Park, North Carolina 27709.

### 1.2 Test / Reference Substances

Experimental name: BAS 490 F  
Chemical name: methyl-(E)-methoximino[ $\alpha$ -(o-tolyloxy)-o-tolyl] acetate  
Empirical formula:  $C_{18}H_{19}NO_4$   
Molecular weight: 313.36  
Melting point: ---  
Appearance: White crystalline  
Odor: Mildly aromatic  
Solubility - at 20°C  
Water:  $2 \times 10^{-4}$  g/100 g  
Dichloromethane: 93.9 g/100 mL  
methane: 1.49 g/100 mL

Structure:



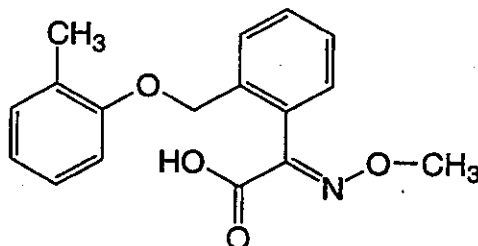
96VIRPO1.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

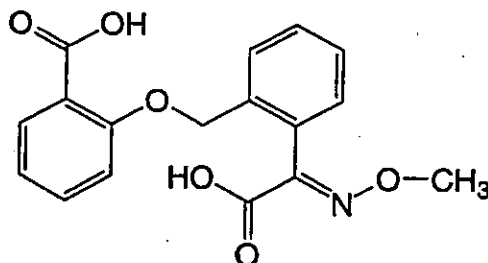
Experimental name: BF 490-1

Chemical name: (E)-methoximino[ $\alpha$ -(o-tolyloxy)-o-tolyl] acid  
Empirical formula:  $C_{17}H_{17}NO_4$   
Molecular weight: 299.33  
Structure:



Experimental name: BF 490-5

Chemical name: (E)-methoximino[ $\alpha$ -(o-tolyloxy)-o-tolyl] diacid  
Empirical formula:  $C_{17}H_{15}NO_6$   
Molecular weight: 329.31  
Structure:



### 1.3 Principle of the Method

Control soil samples were homogenized using a Fitzmill grinder at BASF and were sent to Advanced BioAnalytical Services. Samples were shipped to Advanced BioAnalytical Services from BASF and all samples were received in good condition and frozen on dry ice in insulated boxes. The soil samples were stored frozen at -30 °C. The assay uses 10 g aliquots of soil. ABS sample numbers were assigned in order of receipt by the software package DMLIMS+, Version 4.3.1i (PennComp Inc., Wayne, PA 19087).

96VIRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.



BAS 490 F, BF 490-1 and BF 490-5 were extracted from soil using a sequential solvent extraction procedure. Following extraction with 2 x 40 mL of methanol, samples were extracted with 30 mL sodium hydroxide (0.1 N). The alkaline extracts were acidified using 1000 µL of concentrated hydrochloric acid and back-extracted with 3 x 40 mL of 90:10 dichloromethane : ethyl acetate. The methanol extracts and partition extracts are concentrated under dry nitrogen separately to 5 mL and 30 mL, respectively. The extracts are combined and further concentrated to < 1 mL followed by sequential reconstitution in 10 mL of methanol with subsequent addition of 10 mL 8 mM ammonium formate, 0.2% formic acid. The concentrated extracts were then analyzed using ion spray liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) in the selected reaction monitoring (SRM) mode. The desired lower level of quantitation of the analytes in soil was 0.010 ppm.

## 2.0 MATERIALS AND METHODS

2.1 Equipment	<u>Suggested Sizes/Manufacturer</u>
PE-SCIEX API III <sup>plus</sup> Triple Quadrupole Mass Spectrometer	PE-SCIEX, Concord, Canada
LC Pump, Shimadzu LC 10-AD	Shimadzu Co., Kyoto, Japan
Autosampler, Waters 717 Plus	Waters, Milford, MA 01757
Alltech On Line Degassing System	Alltech Associates Inc., Deerfield, IL 60015, Cat# 590100
Asahipak ODP LC Column	5 µm particle size, 2 x 100 mm. Keystone Scientific, Bellefonte, PA 16823
Kromasil ODS LC Column	5 µm particle size, 2 x 50 mm Keystone Scientific, Bellefonte PA 16823
Column Heater, Model 7970	Jones Chromatography USA Inc., Lakewood, CO 80228
Harvard Syringe Pump 11	Harvard Apparatus Inc., South Natick, MA 01760
Multi-tube Vortexer	VWR Scientific, West Chester, PA 19380 Cat# 58816-115
Microbalance, Model MT-5	Mettler-Toledo Inc., Hightstown, NJ 08520
Balance, Model FX-300	AND Ltd., Tokyo, Japan
pH Meter, Model 340	Corning Inc., Corning, NY 14830
TurboVap LV Evaporator	Zymark, Hopkinton, MA 01748

96VIRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

	Cat# 43750
NANOpure-UV Water Purification System	Barnstead, Dubuque, IA 52001
Centrifuge:	Dupont RT-6000D Refrigerated or Equivalent
Mechanical shaker:	Eberbach Corp., Ann Arbor, MI 48106 Cat# 6000
Bench Top Shaker	Glas-Col, Terre Haute, IN Cat# 099A S60012
Whatman Autovial Processor, P20	Whatman Inc., Clifton, NJ 07014
Whatman Polypropylene membrane autovials	VWR Scientific, Rochester, NY 14603 Cat# 28297-106
Separatory funnels:	250 mL
Flat bottom flasks:	500 mL
Polypropylene Centrifuge tubes with screw caps	VWR Scientific, West Chester, PA 19380 Cat# 21008-146
Polypropylene Centrifuge tubes with screw caps	VWR Scientific, West Chester, PA 19380 Cat# 21008-725
Volumetric flasks:	10, 25, 50, 100, 250, 500, 1000, 2000 mL
Autosampler vial/caps:	Sun Brokers Inc., Wilmington, NC 28402 Cat# 500 765
Eppendorf Repeater Pipettes	Brinkman Instruments, Westbury, NY 11950, Cat# 22 26 000-6
Eppendorf Repeater Pipette Tips	5 mL, 12.5 mL, 25 mL, 50 mL Brinkman Instruments, Westbury, NY 11950
Pipettes	Cat# M-25, M-50, M-250, P-200, P-1000, P-5000, P-10ML Rainin Instrument Co., Woburn, MA 01888
Pipette Tips	Cat# CP-25, CP-50, CP-250, GPS-96, GPS-1000, C-5000, RC-10ML Rainin Instrument Co., Woburn, MA 01888
Glass Scintillation Vials	20 mL, Wheaton, Millville, NJ 08332

96VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

Polypropylene Scintillation Vials	20 mL, Wheaton, Millville, NJ 08332
Repeating Dispenser	Brinkman Dispensette 10-50 mL Brinkman Instruments, Westbury, NY 11950. Cat# 50 10 050-2
Repeating Dispenser	EM Optifix Basic 5, Cat# EM-108033 VWR Scientific, Rochester, NY 14603
Universal REPIPET Dispenser	10 mL, Cat# 53528-168 VWR Scientific, Rochester, NY 14603
Oxford Adjustable Dispenser	3.0 to 10 mL, Cat# 53519-520 VWR Scientific, Rochester, NY 14603
Solvent Filtration Apparatus	Krackeler Scientific, Albany, NY 12201 Cat# 32-00610
Nylon Titan Membrane Filters	0.45 mm, Cat# 74547-NN Scientific Resources Inc., Eatontown, NJ 07724
Volumetric pipettes:	25, 50, 100 mL

Other general laboratory glassware and supplies.

## 2.2 Reagents and Chemicals Suggested Source/Preparation

All chemicals may be substituted by that of an equivalent manufacturer and grade of chemical.

Dichloromethane Cat# 300-4	Baxter/Scientific Products, McGaw Park, IL 60085
Methanol, Cat# 230-4	Baxter/Scientific Products, McGaw Park, IL 60085
Water, Cat# 365-4	Baxter/Scientific Products, McGaw Park, IL 60085
Ethyl Acetate, Cat# EX0245-1	EM Science, Gibbstown, NJ 08027
Formic Acid Suprapur Grade, Cat# 11670-1	EM Science, Gibbstown, NJ 08027
Hydrochloric Acid, Cat# 9535-05	J.T. Baker, Danvers, MA 01923
Ammonium Formate, Cat# 09735	Fluka Chemical Corporation, Ronkonkoma, NY 11779
Sodium Hydroxide, Cat# 3722-11	J.T. Baker, Danvers, MA 01923
Acetonitrile, Cat# 015-4	J.T. Baker, Danvers, MA 01923

%VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

Ammonium Acetate, Cat# JT0596-11 J.T. Baker, Danvers, MA 01923

Polypropylene Glycol, Aldrich Chemical Co., Milwaukee, WI  
Average MW=425, Cat# 20230-4 53233

## 2.3 Standard Substances and Solutions

### 2.3.1 Preparation of Solutions

Solutions of 0.1 N sodium hydroxide, 2 mM ammonium acetate, 50:50 methanol : acetonitrile and 8 mM ammonium formate, 0.2% formic acid are assigned a three month expiration date. 90:10 dichloromethane : ethyl acetate was prepared fresh for each experimental run.

**1N Sodium Hydroxide:** Add 40 g of sodium hydroxide pellets to a 1000 mL volumetric flask and dissolve in HPLC grade water. Allow to cool to room temperature before diluting to the mark with HPLC grade water. Prepare a fresh solution every 12 months.

**0.1 N Sodium Hydroxide:** Place 200 mL of 1 N sodium hydroxide in a 2000 mL volumetric flask and dilute to the mark with HPLC grade water.

**1 M Ammonium Acetate:** Add 77 g of ammonium acetate to a 1000 mL volumetric flask and dissolve in HPLC grade water. Dilute to the mark with HPLC grade water. Filter the solution through a 0.45  $\mu$ m filter with a vacuum flask. Store refrigerated at approximately 4° C and bring to room temperature before use. Prepare a fresh solution every twelve months.

**2 mM Ammonium Acetate:** Add 1 mL of 1 M ammonium acetate to a 500 mL volumetric flask and dilute to the mark with HPLC grade water and mix thoroughly. Filter the solution through a 0.45  $\mu$ m filter with a vacuum flask. Store refrigerated at approximately 4° C and bring to room temperature before use.

**90:10 Dichloromethane : Ethyl Acetate:** Mix 1800 mL of dichloromethane with 200 mL ethyl acetate.

**50:50 Methanol : Acetonitrile:** Add 500 mL acetonitrile to a 500 mL graduated cylinder. Add 500 mL methanol to a 500 mL graduated cylinder. Combine in a 1000 mL Pyrex bottle and mix thoroughly. Degas by helium sparge.

**35:35:30 Methanol : Acetonitrile: 2 mM Ammonium Acetate, Eluent:** Add 700 mL of 50:50 methanol : acetonitrile to a 1000 mL graduated cylinder. Add 300 mL of 2 mM ammonium acetate to a 500 mL graduated cylinder. Combine the solutions in a 1000 mL Pyrex bottle and degas by helium sparge.

**1 M Ammonium Formate:** Add 31.5 g of ammonium formate to a 500 mL volumetric flask and dilute to the mark with HPLC grade water and mix thoroughly. Prepare a fresh solution every twelve months.

96VRF01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

8 mM Ammonium Formate, 0.2% Formic Acid: Add 8 mL of 1 M ammonium formate to a 1000 mL volumetric flask and dilute with approximately 800 mL of HPLC grade water. Add 2 mL formic acid to the flask and then dilute to the mark with HPLC grade water and mix thoroughly.

50:50 Methanol : 8 mM Ammonium Formate, 0.2% Formic Acid: Add 100 mL of 8 mM ammonium formate, 0.2% formic acid to a 100 mL graduate cylinder and 100 mL methanol to a separate 100 mL graduated cylinder. Combine solutions and mix thoroughly.

Aqueous Formic Acid (pH 2.5 - 2.75): Place 500 mL of NANOpure water in a 500 mL graduated cylinder. With constant monitoring using a calibrated pH meter add formic acid dropwise until the pH registers a constant pH 2.5 to 2.75. Filter through a 0.45  $\mu$ m Nylon filter and degas by helium sparge.

30:30:40 Methanol : Acetonitrile: Water: Mix 600 mL of 50:50 methanol : acetonitrile solution with 400 mL NANOpure water. Degas by helium sparge.

31.5:31.5:37 Methanol : Acetonitrile: Aqueous Formic Acid (pH 2.5-2.75): Mix 315 mL of 50:50 methanol : acetonitrile solution with 185 mL Aqueous Formic Acid (pH 2.5-2.75). Degas by helium sparge.

## Standards Used:

BASF Code	Lot Number	Purity
BAS 490	39-184-1	99.7%
BAS 490-1	00820-251	100%
BAS-490-5	00436-83	99.6%

## Standards supplied by:

BASF Corporation  
Agricultural Research Center  
P. O. Box 13528  
Research Triangle Park, NC 27709

Standards were maintained frozen (<-5 °C) until their use in this study. They were characterized as required by 40 CFR part 160, FIFRA Good Laboratory Practices. Information on the synthesis and subsequent characterization of this substance is available from BASF and is located at BASF Corporation, Agricultural Research Center, Research Triangle Park, North Carolina.

Solutions of standards were refrigerated (approximately +4 °C) during their use in this study. Stock solutions (1 mg/mL) were made fresh every three months. Dilutions of the stock solution were made every three months.

HPLC grade water or equivalent should be used wherever water is called for. Mix

96VIRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

all solutions well. The concentrations of all solutions are based on the free or unionized form of the analytes.

### 2.3.2 Standard Solutions for Fortifications

BAS 490 F, BF 490-1      1.0 mg/mL, 10.0, 2.0, 0.20 µg/mL in Acetonitrile  
BF 490-5                    1.0 mg/mL, 10.0, 2.0, 0.20 µg/mL in Methanol

NOTE: Storage containers for 1.0 mg/mL standard solutions and calibration standard solutions are bottles with Teflon-lined screw caps. Storage containers for other stock solutions are Pyrex bottles with polypropylene caps. All storage bottles are foil-wrapped to protect the solutions from light. These concentrations are suggested. Different preparation and concentration schemes may be used and additional standard concentrations may be prepared and used as needed.

#### Stock Solution Preparation

Separate 1.0 mg/mL solutions of BAS 490 F and BF 490-1 are prepared by weighing 5 mg of the analytical standard solid on a micro balance and transferring to a 5 mL class "A" volumetric flask. Dilution to the mark with acetonitrile yields a 1 mg/mL solution. A 1.0 mg/mL solution of BF 490-5 is prepared by weighing 5 mg of the analytical standard solid on a micro balance and transferring to a 5 mL class "A" volumetric flask. Dilution to the mark with methanol yields a 1 mg/mL solution. The solutions are transferred to scintillation vials, covered with aluminum foil and stored at approximately 4 °C. Fresh solutions are prepared every three months.

### 2.3.3 Preparation of BAS 490 F, BF 490-1 and BF 490-5 Analytical Standard Working Solutions

A 2 µg/mL Working Solution I of BAS 490 F and BF 490-1 is prepared by transferring appropriate volumes of each of the 1.0 mg/mL stock solutions to a 100 mL volumetric flask. Dilution to the mark with acetonitrile yields a 2 µg/mL solution. A 2 µg/mL Working Solution III of BF 490-5 is prepared by transferring an appropriate volume of the 1.0 mg/mL stock solution to a 100 mL volumetric flask. Dilution to the mark with methanol yields a 2 µg/mL solution. The solutions are transferred to Pyrex bottles, covered with aluminum foil and stored at approximately 4 °C. Fresh solutions are prepared every three months.

A 20 µg/mL Working Solution II of BAS 490 F and BF 490-1 is prepared by transferring appropriate volumes of each of the 1.0 mg/mL stock solutions to a 10 mL volumetric flask. Dilution to the mark with acetonitrile yields a 20 µg/mL solution. A 20 µg/mL Working Solution IV of BF 490-5 is prepared by transferring an appropriate volume of the 1.0 mg/mL stock solution to a 10 mL volumetric flask. Dilution to the mark with methanol yields a 20 µg/mL solution. The solutions are transferred to Pyrex bottles, covered with aluminum foil and stored at approximately 4 °C. Fresh solutions are prepared every three months.

### 2.3.4 Standard Solutions for LC/MS/MS Analysis

Calibration curves were prepared from analytical standard solutions (non-extracted)

96VIRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

for each analytical run according to the procedure outlined in Table 1. The concentration of the standards in the calibration curves for the assay were 2.0, 5.0, 10.0, 20.0, 50.0, 100, and 200 ng/mL for BAS 490 F, BF 490-1 and BF 490-5. The standards were prepared by dilution of Working Solutions I to IV with 50:50 methanol : 8 mM ammonium formate, 0.2% formic acid as outlined in Table 1. Analytical standards were placed at the beginning and end of each run and with regular distribution throughout the tray.

Table 1. Analytical Standards Dilution Scheme

Standard	BAS 490F Concentration (ng/mL)	BF 490-1 Concentration (ng/mL)	BF 490-5 Concentration (ng/mL)	Working Solution I (mL)	Working Solution II (mL)	Working Solution III (mL)	Working Solution IV (mL)	Total Volume (mL)*
1	2	2	2	0.025	---	0.025	---	25
2	5	5	5	0.025	---	0.025	---	10
3	10	10	10	0.05	---	0.05	---	10
4	20	20	20	0.1	---	0.1	---	10
5	50	50	50	0.25	---	0.25	---	10
6	100	100	100	---	0.05	---	0.05	10
7	200	200	200	---	0.1	---	0.1	10

\* Analytical Standards were diluted to the 10 mL or 25 mL mark with 50:50 methanol : 8 mM ammonium formate, 0.2% formic acid

### 3.0 ANALYTICAL PROCEDURES

#### 3.1 Preparation of Samples for Extraction

3.1.1 Weigh 10 g of soil sample using a top loading balance into a 50 mL polypropylene centrifuge tube.

3.1.2 As required, fortify control samples with appropriate volumes of fortification solutions.

#### 3.1.3 Fortifications for Procedural Recoveries

Stock solutions containing 1.0 mg/mL BAS 490 F and BF 490-1 in acetonitrile and 1.0 mg/mL BF 490-5 in methanol were prepared separately from those used to prepare the standard curves. These solutions were further diluted and used to prepare the procedural recovery samples.

The recoveries of BAS 490 F, BF 490-1 and BF 490-5 were determined using procedural recovery samples prepared following the procedure outlined below. Acceptable recoveries were defined as not less than 70% and not more than 120% for all samples.

It is recommended that at least two fortifications and one untreated sample (control) are run with each set of samples to monitor method efficiency. Typically, one fortification sample at the limit of quantitation is run along with one fortification sample at a higher level. For each fortified sample, an appropriate volume of standard solution is added to a control soil sample by volumetric pipette. For

96VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

example, for a 0.010 ppm fortification, pipette 500  $\mu\text{L}$  of the 0.2  $\mu\text{g}/\text{mL}$  standard solution into the control sample. Fortifications are made into the sample before the methanol is added to the sample for the initial extraction.

Soil Procedural Recovery Samples were prepared according to the following procedure:

Procedural Recovery Sample-1 (QC-1), 0.010 ppm: Place 10 g of control soil into a 50 mL polypropylene centrifuge tube. Using an Eppendorf repeater pipette add 500  $\mu\text{L}$  each of 0.2  $\mu\text{g}/\text{mL}$  BAS 490 F and BF 490-1 in acetonitrile and 0.2  $\mu\text{g}/\text{mL}$  BF 490-5 in methanol. Evaporate the organic solvent under a gentle stream of nitrogen (~2 psi) at 45  $^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for approximately 30 minutes.

Procedural Recovery Sample-2 (QC-2), 0.100 ppm: Place 10 g of control soil into a 50 mL polypropylene centrifuge tube. Using an Eppendorf repeater pipette add 500  $\mu\text{L}$  each of 2  $\mu\text{g}/\text{mL}$  BAS 490 F and BF 490-1 in acetonitrile and 2  $\mu\text{g}/\text{mL}$  BF 490-5 in methanol. Evaporate the organic solvent under a gentle stream of nitrogen (~2 psi) at 45  $^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for approximately 30 minutes.

Procedural Recovery Sample-3 (QC-3), 0.350 ppm: Place 10 g of control soil into a 50 mL polypropylene centrifuge tube. Using an Eppendorf repeater pipette add 1750  $\mu\text{L}$  each of 2  $\mu\text{g}/\text{mL}$  BAS 490 F and BF 490-1 in acetonitrile and 2  $\mu\text{g}/\text{mL}$  BF 490-5 in methanol. Evaporate the organic solvent under a gentle stream of nitrogen (~2 psi) at 45  $^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for approximately 30 minutes.

Procedural Recovery Sample-4 (QC-4), 1.00 ppm: Place 10 g of control soil into a 50 mL polypropylene centrifuge tube. Using an Eppendorf repeater pipette add 1000  $\mu\text{L}$  each of 10  $\mu\text{g}/\text{mL}$  BAS 490 F and BF 490-1 in acetonitrile and 10  $\mu\text{g}/\text{mL}$  BF 490-5 in methanol. Evaporate the organic solvent under a gentle stream of nitrogen (~2 psi) at 45  $^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for approximately 30 minutes.

### 3.2 Extraction of Residue

- 3.2.1 Add 40 mL of methanol to the sample and shake on a mechanical shaker for 15 minutes using the mechanical shaker. Shake on the high setting at the maximum speed throughout this procedure. Centrifuge the sample for 5 minutes at 3000 rpm, and decant the supernatant into a centrifuge tube or other collection vessel. Repeat the methanol extraction and decant the supernatant into a centrifuge tube or other collection vessel.
- 3.2.2 Resuspend the soil in the centrifuge tube with 30 mL of 0.1 N sodium hydroxide and shake for 15 minutes using the mechanical shaker. Centrifuge the extract for 5 minutes at 3000 rpm or until the soil is completely separated from the extract, then decant into a centrifuge tube or other collection vessel.
- 3.2.3 Resuspend the soil in the centrifuge bottle with 15 mL of NANOpure water and shake for 15 minutes using the mechanical shaker. Centrifuge the extract for 5 minutes at 3000 rpm, or until the soil is completely separated from the extract, then carefully decant into the centrifuge tube or other collection vessel containing the sodium hydroxide extract.

96VIRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.



- 3.2.4 Add 1000  $\mu$ L of concentrated hydrochloric acid to the centrifuge tube or other collection vessel containing the sodium hydroxide/water extract to lower the pH to  $\sim$ 1. Cap and vortex mix the solution.
- 3.2.5 Centrifuge the extract for 5 minutes at 3000 rpm to precipitate the humics from the extract.

### 3.3 Dichloromethane Partition Clean-up

- 3.3.1 Transfer the acidified extract to a 250 mL separatory funnel (If the supernatant from the centrifugation step 3.2.5 is transferred to a fresh centrifuge tube or other collection vessel, rinse the vessel with NANOpure water and add the aqueous rinse to the separatory funnel).
- 3.3.2 Extract the aqueous contents of the separatory funnel with 40 mL portions of dichloromethane: ethyl acetate (90:10). Shake the funnel on a mechanical shaker for 2 minutes, and allow the layers to separate. Remove the organic layer into a new centrifuge tube or other collection vessel. Repeat for a total of 3 partitions.

**NOTE:** The centrifuge step 3.2.5 should have removed all particulates that will cause emulsions. During shaking of the separatory funnels, soils with a high organic content may form emulsions which will not disperse on standing. When this occurs, remove the organic layer and the emulsified layer into a centrifuge tube and centrifuge at 3000 rpm for 3 minutes. Following centrifugation, transfer the aqueous layer back into the separatory funnel.

- 3.3.3 Concentrate the combined dichloromethane fraction to approximately 30 mL using a TurboVap or rotary evaporator with water bath at  $45^{\circ}\text{C} \pm 5^{\circ}\text{C}$ .
- 3.3.4 Concentrate the methanol fraction to 20 mL using a TurboVap or rotary evaporator with water bath at  $45^{\circ}\text{C} \pm 5^{\circ}\text{C}$ . Combine the fractions in one centrifuge tube. Concentrate the fraction to  $< 5$  mL but avoid concentrating to dryness.
- 3.3.5 Combine the methanol and dichloromethane fractions and concentrate to  $< 1$  mL but avoid going to dryness, using a TurboVap or rotary evaporator with water bath at  $45^{\circ}\text{C} \pm 5^{\circ}\text{C}$ . Add sufficient methanol to the 10 mL mark on the centrifuge tubes.

### 3.4 LC/MS/MS Analysis sample preparation

- 3.4.1 Add 10 mL 8 mM ammonium formate, 0.2% formic acid, vortex mix and filter the suspensions using polypropylene membrane autovials.

**NOTE:** The reconstitution process for procedural recovery and study samples is a two-step process. The samples are initially diluted to 10 mL with methanol and then a further 10 mL of 8 mM ammonium formate, 0.2% formic acid is added before filtration. Addition of 10 mL of 8 mM ammonium formate, 0.2% formic acid to methanol will yield a final volume of  $>19.2$  mL. All results from procedural recovery and study samples were multiplied by 0.96 to compensate for this volume compression.

96VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

3.4.2 Transfer 100  $\mu\text{L}$  of each sample to a labeled WISP autosampler vial for LC/MS/MS analysis. For the 1.00 ppm Procedural Recovery samples, pipette 225  $\mu\text{L}$  of the reconstitution solution into a WISP vial and add 75  $\mu\text{L}$  of the 1.00 ppm Procedural Recovery solution and vortex mix.

3.4.3 Prepare a 10 ng/mL post-extract spike solution by adding 25  $\mu\text{L}$  of the 2  $\mu\text{g}/\text{mL}$  Spiking solution of BAS 490 F and BF 490-1 and 25  $\mu\text{L}$  of the 2  $\mu\text{g}/\text{mL}$  Spiking solution of BF 490-5 to a 5 mL volumetric flask and diluting to the mark with control sample extract. Vortex mix and transfer 100  $\mu\text{L}$  to a labeled WISP autosampler vial for LC/MS/MS analysis.

### 3.5 Instrumentation Common to LC/MS/MS Methods 1 and 2

Instrument: PE SCIEX API III<sup>™</sup> Triple Quadrupole Mass Spectrometer  
 Degasser: Alltech On-Line Degassing System  
 HPLC pump: Shimadzu LC-10AD  
 Autosampler: Waters WISP 717<sup>™</sup>  
 Column Heater: Jones Chromatography, Model 7970  
 Data System: Macintosh Quadra 900 Data System

Other equivalent hardware may be used.

### 3.6 Typical Operating Conditions for Lower Organic Content Soils (Method 1)

LC Column	2 mm x 100 mm Asahipak ODP, 5 $\mu\text{m}$ particle size, 200 $\text{\AA}$ pore size
Mobile Phase	35:35:30 Methanol : Acetonitrile : 2 mM Ammonium Acetate (Helium sparged)
Column Temperature	45 $^{\circ}\text{C}$
Flow Rate	200 $\mu\text{L}/\text{min}$ .
Initial Pressure	60 bar
Injection Volume	3 $\mu\text{L}$
Autosampler Wash	Acetonitrile (Helium sparged)
Run Time	6.30 minutes
MS Acquisition Time	5 minutes

The time difference between run time and MS acquisition time is explained in Section 3.10.1

MS Mode	Negative Ion for 2.8 minutes then Positive Ion for 2.2 minutes.
Ion Spray Voltage	-3500 V for 2.8 minutes then +4600 V for 2.2 minutes.
Declustering Potential	-15 V for 2.8 minutes then 21 V for 2.2 minutes
Curtain Gas	U.H.P. Nitrogen @ 1.2 Liters/minute
Nebulizer Gas	Nitrogen @ 70 psi
Turbo Ion Spray Temperature	300 $^{\circ}\text{C}$
Turbo Ion Spray Auxiliary Gas	8 Liters/minute

96VJRP01.BAS



ADVANCED  
 BIOANALYTICAL  
 SERVICES, INC.

Dwell Time	100 ms for 2.8 minutes then 350 ms for 2.2 minutes.
Collision Gas	Argon at $1.9 \times 10^{15}$ atom/cm <sup>2</sup>
Collision Energy	-4 eV for 2.8 minutes then 11 eV for 2.2 minutes.

The infusion full-scan single MS positive ion spray mass spectrum for BAS 490 F demonstrated abundant ions for the protonated molecules ( $[M+H]^+$ ) at  $m/z = 314.1$  (Figure 1). The full-scan single MS negative ion spray mass spectra for BF 490-1 and BF 490-5 demonstrated abundant ions for the deprotonated molecules ( $[M-H]^-$ ) at  $m/z = 298.2$  and  $328.2$ , respectively (Figure 1). The full-scan MS/MS mass spectrum of BAS 490 F showed product ions at  $m/z = 116, 131, 132, 162, 206, 222, 223, 235, 238, 267$  and  $282$  with a relative ion abundance  $>10\%$  from the  $[M+H]^+$  precursor ion at  $m/z = 314.1$  (Figure 2). The full-scan MS/MS mass spectrum of BF 490-1 showed product ions at  $m/z = 102$  and  $222$  with a relative ion abundance  $>10\%$  from the  $[M-H]^-$  precursor ion at  $m/z = 298.2$  (Figure 2). The full-scan MS/MS mass spectrum of BF 490-5 showed product ions at  $m/z = 102$  and  $252$  with a relative ion abundance  $>8\%$  from the  $[M-H]^-$  precursor ion of  $m/z = 328.1$  (Figure 2). Plausible MS/MS bond cleavage pathways for each compound are shown in Figure 3. The following selected reaction monitoring (SRM) transitions of the deprotonated molecules BF 490-1 and BF 490-5 and the protonated molecule of BAS 490 F were used to quantify the analytes in soil (Figures 1 to 3):

Analyte	Transition monitored	Retention Time
BF 490-5	$m/z = 328.2 \rightarrow m/z = 252.0$	1.75 min.
BF 490-1	$m/z = 298.2 \rightarrow m/z = 222.0$	1.83 min.
BAS 490 F	$m/z = 314.2 \rightarrow m/z = 116.0$	3.22 min.

To achieve the desired lower level of quantitation the use of Turbo Ion Spray was determined to be vital. In contrast to many other test compounds, it was found that standard operation at  $500^\circ\text{C}$  would initially increase the response observed for BAS 490 F, BF 490-1 and BF 490-5, but then the response rapidly decreased with time. Optimization of the Turbo Ion Spray temperature showed that the response for BF 490-1 and BF 490-5 optimized at  $350^\circ\text{C}$  and then dropped off rapidly and BAS 490 F optimized around  $400^\circ\text{C}$ . To ensure sensitivity without subsequent reduction in signal for BF 490-1 and BF 490-5 from overheating, the Turbo Ion spray temperature was maintained at  $300^\circ\text{C}$  for all experiments.

During method development, the presence of formic acid in the reconstitution solvent was observed to be vital for maintenance of the retention and peak shape of BF 490-1 and BF 490-5. The separation of the three analytes was achieved within four minutes.

It was observed that the LC/MS/MS experiments required some system equilibration time because the response for BF 490-5 did not immediately optimize and the baseline in the chromatogram for BAS 490 F started relatively high. Therefore, it is recommended that several test injections of high standards be injected to determine that the observed peak heights are constant. The low analytical standard should also be injected to verify that it is readily seen above the baseline.

### 3.7 Typical Operating Conditions for Higher Organic Content Soils (Method 2)

96VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

A second LC/MS/MS approach, using an acidified mobile phase, where the fragmentation of the protonated molecules was used to provide the basis for the selected reaction monitoring transitions was developed for the determination of BAS 490 F, BF 490-1 and BF 490-5 residues for higher organic content soils.

LC Column	2 mm x 50 mm Kromasil ODS, 5 $\mu$ m particle size
Mobile Phase	31.5 : 31.5 : 37 Acetonitrile : Methanol : Aqueous Formic Acid (pH 2.5-2.75) (Helium sparged)
Column Temperature	45°C
Flow Rate	180 $\mu$ L/min.
Initial Pressure	35 bar
Injection Volume	15 $\mu$ L
Autosampler Wash	Acetonitrile : Methanol : Water (30:30:40) (Helium sparged)
MS Mode	Positive ion
Ion Spray Voltage	+4000 V
Declustering Potential	+14.3 V
Curtain Gas	U.H.P. Nitrogen @ 2.2 Liters/minute
Nebulizer Gas	Nitrogen @ 60 psi
Turbo Ion Spray Temperature	400°C
Turbo Ion Spray Auxiliary Gas	>8 Liters/minute
Dwell Time	200 ms
Collision Gas	Argon at $2.4 \times 10^{15}$ atom/cm <sup>2</sup>
Collision Energy	15.7 eV

The full-scan single MS positive ion spray mass spectra for BF 490-1 and BF 490-5 demonstrated weak ions for the protonated molecules ( $[M+H]^+$ ) of  $m/z = 300.2$  and  $330.2$ , respectively, with ions of much higher abundance corresponding to the ammoniated molecules, sodiated molecules and weaker potassiumated molecules (Figure 4). The full-scan MS/MS mass spectrum of the protonated molecule of BF 490-1 showed product ions at  $m/z = 116, 209, 222$  and  $235$  with a relative ion abundance >10% from the  $[M+H]^+$  precursor ion at  $m/z = 300.2$  (Figure 5). The full-scan MS/MS mass spectrum of the protonated molecule of BF 490-5 showed product ions at  $m/z = 116, 168, 192$  and  $236$  with a relative ion abundance >5% from the  $[M+H]^+$  precursor ion at  $m/z = 330.2$  (Figure 5). A plausible MS/MS fragmentation pathway for the most abundant product ion at  $m/z = 116$  seen for BF 490-5 and BF 490-1 is shown in Figure 6. The following selected reaction monitoring (SRM) transitions of the protonated molecules of BAS 490 F, BF 490-1 and BF 490-5 were used to quantify the analytes in soil (Figures 4 to 6):

Analyte	Transition monitored	Retention time
BF 490-5	$m/z = 330.2 \rightarrow m/z = 116.0$	1.63 min.
BF 490-1	$m/z = 300.2 \rightarrow m/z = 116.0$	3.60 min.
BAS 490 F	$m/z = 314.2 \rightarrow m/z = 116.0$	6.00 min.

A dummy transition from  $m/z = 600$  to  $m/z = 250$  is inserted between BF 490-5 and BF 490-1 to minimize crosstalk in the  $Q_2$  collision cell.

96VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

compounds readily form adduct ions, and in the presence of ammonium salts, the base peak in Q1 scans for each compound will be the ammonium adduct. During method development we attempted to use  $(M+NH_4)^+$   $\rightarrow$  product ion transitions, but encountered severe problems where the peak area increased throughout the analytical run. This directed us to focus on formation of the  $(M+H)^+$  ions. Conversion of the LC system and autosampler from running with ammonium formate to running with formic acid takes about three days. Additionally, removal of all ammonium salts from the system leads to extended run times. The presence of ammonium formate in the reconstitution solvent introduces sufficient ammonium salt into the system to maintain reasonable run times without compromising sensitivity. Once the LC/MS/MS system is equilibrated, system sensitivity is verified by injection of a 2 ng/mL standard solution.

LC/MS/MS Method 2 was originally developed using the  $m/z = 314.2$  to  $m/z = 222$  transition of BAS 490 F to minimize the potential for cross talk in the  $Q_2$  collision cell. This was the transition monitored during the cross-validation to Oregon Soil 94016 and New York Soil 94015. When the method was applied to the analysis of samples an interfering peak was observed for BAS 490 F. A subsequent tray was acquired where both the  $m/z = 314.2$  to  $m/z = 222$  and the  $m/z = 314.2$  to  $m/z = 116$  transitions were monitored. The  $m/z = 314.2$  to  $m/z = 116$  transition resulted in increased sensitivity together with minimal interference. This transition was used in all subsequent analyses using LC/MS/MS Method 2.

The preceding specifications are suggested and may be altered as needed. Actual use conditions and any changes must be documented.

### 3.8 Calibration Procedures

Calibrate the mass axis of the instrument by infusion of a  $10^{-4}$  M solution of PPG 425 in 50:50 methanol : water containing 0.1% formic acid, 0.1% acetonitrile and 2 mM ammonium acetate, at a flow rate of 20  $\mu$ L/min. Mass calibrate at  $m/z = 94, 251, 326, 384, 442, 558$  and 674. For LC/MS/MS Method 1 tune and optimize the instrument by infusion of a 1 mg/mL solution of BF 490-5 at 20  $\mu$ L/min into 180  $\mu$ L/min mobile phase via a zero dead volume T-junction by monitoring the change in relative ion abundance of the  $m/z = 252$  product ion. For LC/MS/MS Method 2 tune and optimize the instrument by infusion of a 1 mg/mL solution of BF 490-5 at 20  $\mu$ L/min into 180  $\mu$ L/min mobile phase via a zero dead volume T-junction by monitoring the change in relative ion abundance of the  $m/z = 116$  product ion. When the mobile phase flow rate is initiated the sensitivity of the  $m/z = 116$  product ion will optimize as the system temperature equilibrates (approximately 5 minutes). Be aware that tuning with the ester for either method will mean that a high background will occur. This will require a few hours to diminish to an acceptable level before sample analysis.

Peak widths should be approximately 0.6 amu at half-height in both single MS and MS/MS mode.

On each day of analysis, analytical standard solutions were employed as described. In each tray (run) one set of analytical standards was inserted at the beginning and one set was inserted at the end of the tray. Additionally, analytical standards were distributed every one to three samples so that at least three replicates of each calibration standard level are injected per analytical run. Quantitation of all samples was achieved using calibration curves generated from injection of the analytical standard solutions.

96VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

Standard curve correlation coefficients were  $>0.98$ .

### 3.9 Sample Analysis

Inject an appropriate amount of sample into the LC/MS/MS system depending on the LC/MS/MS method used.

Directly compare the peak area of unknown samples injected with the standard curve to obtain ng/mL values for BAS 490 F, BF 490-1 and BF 490-5 concentrations in the sample extract.

Bracket every 1-3 samples with standards to check for shifts in sensitivity. If the peak area of the unknown is larger than the highest standard, dilute the unknown appropriately and reinject. Reconstitution solvent blanks are injected at the beginning and end of each analytical tray to test for sample carryover. Standard solutions (10 ng/mL) prepared in control blank soil extract are injected to test for analyte signal suppression.

### 3.10 Interferences

#### 3.10.1 Sample Matrices

LC/MS/MS Method 1 was suitable for the determination of BAS 490 F, BF 490-1 and BF 490-5 in soils with a low amount of endogenous organic material. However, further application of the method revealed limitations when it was employed for the analysis of extracts from soils with a high organic acid content. The presence of co-extractants led to suppression of BF 490-1 and BAS 490 F which displayed low apparent recoveries. Additionally sample carryover was observed for BF 490-1 and BF 490-5.

Full-scan LC/MS acquisition using LC/MS/MS Method 1 revealed that endogenous soil components co-eluted with BAS 490 F, BF 490-1 and BF 490-5. However, using liquid chromatography in conjunction with tandem mass spectrometry, no co-eluting interferences were observed in the selected reaction monitoring chromatograms. When soil extracts were analyzed in the full-scan acquisition mode some late eluting soil components were observed. When the LC run time matched the MS acquisition time (four minutes) these components co-eluted with BF 490-1 and BF 490-5 from a subsequent injection causing a signal enhancement for those two analytes. A longer LC run time (six minutes) meant that the late eluting components had eluted from the column before injection of the next sample.

A second LC/MS/MS method was developed using an acidic mobile phase where all of the analytes were subsequently monitored as their protonated molecule ions. BF 490-1 was retained relative to co-extractants so suppression was minimized. In comparison to Method 1, the sensitivity for BF 490-1 and BF 490-5 was reduced but was sufficient for the analysis of samples. Sample carryover did not occur.

During the experimental procedure the amount of concentrated hydrochloric acid added was observed to be critical, particularly for soils from the 12-18" depth. Method D9503 stipulated that the post-addition pH of the solution should be less than 2. For California Soil 94017 the addition of 300  $\mu$ L of concentrated hydrochloric acid lowered the pH to  $\sim 1.6$ . However, for lower depth samples, the apparent recoveries of BAS 490 F were around 60% because of interfering components. Raising the volume of

96VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

hydrochloric acid to 500  $\mu\text{L}$  (pH ~ 1.4) solved this and no further difficulties were observed for California Soil 94017 or Oregon Soil 94016. For New York soil 94015 the problem was observed for BF 490-5 where the lower depth recoveries were around 60%. For New York soil procedural recovery samples from 6-12" and 12-18" depths varying amounts of hydrochloric acid were added. It was observed that a greater precipitate of humic acids was observed for additions of 300  $\mu\text{L}$  (pH ~2.6) and 500  $\mu\text{L}$  (pH ~ 1.6) hydrochloric acid but recoveries of BF 490-5 were <70%. Addition of 800  $\mu\text{L}$  (pH ~ 1.3) or 1000  $\mu\text{L}$  (pH ~ 1.1) of hydrochloric acid gave reduced precipitates but experimental recoveries of ~90%.

### 3.10.2 Other Sources

Other Pesticides:

None known to date

Solvents:

None known to date

### 3.11 Confirmatory Techniques

No problems with questionable peak identity have been encountered to date. Interference from centrifuge tubes from Lot Numbers  $\geq 6000$  yielded a peak of high abundance which eluted from the column before BF 490-5. The peak for BF 490-5 was observed as a shoulder on the tail of this much larger peak making quantitation difficult. Re-extraction of these samples for BF 490-5 only, using centrifuge tubes from appropriate Lot Numbers showed peaks for BF 490-5 which were free of any interfering peaks.

### 3.12 Time Required for Analysis

Using the Method as supplied by BASF, a run of 20 to 50 samples with additional duplicate blanks and eight procedural recovery samples would take four working days from soil weighing to LC/MS/MS analysis. Further investigation of the length and number of steps required for analysis revealed that reduction of shake times to 15 minutes, centrifuge times to 5 minutes and elimination of the fourth set of separatory funnels did not affect procedural recoveries. This meant that an equivalent number of samples could be processed in about two working days.

### 3.13 Potential Problems

Use of inappropriate centrifuge tubes can lead to interference of BF 490-5.

### 3.14 - Moisture Content

The procedure for the moisture determination utilized oven drying of the soil samples to constant weight. The soils were weighed as 5 g aliquots into glass scintillation vials in batches of 40. The batches of weighed soils were dried at 110  $^{\circ}\text{C}$  for 24 hours in a temperature-controlled oven. Some batches were re-weighed after a second 24-hour drying period to verify that they were dried to constant weight. Results indicated that 24 hours was a sufficient time period at this temperature to dry the soils. Percentage

96VTRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

water was calculated on a dry-mass basis. Details of the procedure are described in Appendix I.

#### 4.0 METHODS OF CALCULATION

##### 4.1 Calibration

All calculations of BAS 490 F, BF 490-1 and BF 490-5 are based on SRM LC/MS peak areas. For LC/MS/MS Method 1, concentrations (ng/mL) of BAS 490 F, BF 490-1 and BF 490-5 in the procedural recovery samples are determined using the slope and intercept values from a weighted quadratic regression (1/y) of the peak area versus concentration data from the calibration standards. For LC/MS/MS Method 2, concentrations (ng/mL) of BAS 490 F, BF 490-1 and BF 490-5 in the procedural recovery samples are determined using the slope and intercept values from a weighted linear regression (1/y) of the peak area versus concentration data from the calibration standards.

Results are reported as ng/mL of BAS 490 F, BF 490-1 and BF 490-5 which are extrapolated to provide % recovery for the procedural recovery samples following correction for change in volume with the two-step reconstitution. Sample residues are reported as ppm of analyte following correction for moisture content and reconstitution volume.

##### 4.2 Analyte in Sample

Convert concentration (ng/mL) interpolated from standard curve to total ng in sample i.e. For 20 mL reconstitution volume (ng/mL x 20)

Convert ng to µg (Divide by 1000)

This is µg obtained from ~10 g of soil:- Divide by measured weight of the soil sample to obtain ppm (µg/g) value for each analyte.

Multiply x 0.96 to compensate for the change in volume during reconstitution (See Section 3.4.1).

To correct for moisture;

Moisture corrected concentration = ppm x [(%Water/100) + 1]

##### 4.3 Calculation of Procedural Recoveries

% Recovery =  $\frac{\text{ppm concentration interpolated from standard curve} \times 0.96 \times 100}{\text{ppm BAS 490 F, BF 490-1 and BF 490-5 added}}$

Where 0.96 is the factor included to compensate for the change in volume during reconstitution.

Do not correct treated sample results for either control residues or procedural recoveries.

96VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.



## 5.0 VALIDATION

All response data and associated sample identification were directly transferred to an EXCEL™ (v4.0) spreadsheet from DMLIMS + for further statistical calculations. The data for the soil validation are stored in ABS Notebook 1003.

The schedule of the experiments acquired for inclusion in the validation study is summarized in Table 2.

The results for the calibration standards and procedural recovery samples of BAS 490 F, BF 490-1 and BF 490-5 obtained from the analysis of Tray 8 were inaccurate. It was thought that this resulted from poor chromatography. This tray was rejected.

The schedule for Tray 9 was compiled for a simultaneous study on another soil. The samples from this extraction were included in a subsequent tray and Tray 9 was not used. Tray 11 was acquired to provide 24-hour stability data. The reproducibility of the peak areas was poor, particularly for BF 490-5. This resulted from air in the LC and autosampler system providing irreproducible injections. The run was re-acquired as Tray 12 to provide 48-hour stability data.

The calculations in all of the Tables were performed on non-rounded numbers. The calculated numbers were then rounded to the appropriate number of significant figures as outlined in the BASF guidelines.

### 5.1 Recovery

The extraction recovery of each analyte was determined by comparing the response obtained from samples which were spiked with BAS 490 F, BF 490-1 and BF 490-5 prior to extraction (pre-extract) with the predicted response obtained from the calibration curve obtained from injection of analytical standard solutions. The percent recovery is obtained by the ratio of post-extract peak area to predicted peak area. Blank soil extracts were spiked at known levels of BAS 490 F, BF 490-1 and BF 490-5 to monitor whether suppression of the signal by co-eluting endogenous material occurred where a 100% match of experimental response to theoretical response indicated that suppression did not occur.

The procedural recovery results for BAS 490 F, BF 490-1 and BF 490-5 in a Canadian soil, BASF RCN number 94041 are summarized in Tables 3 to 5, respectively. The mean within-tray recovery ranged from 93.3% to 101% for BAS 490 F, except for the procedural recovery sample 4 in Tray 10 which showed a mean recovery of 113%. The mean within-tray recovery ranged from 76.4% to 85.1% for BF 490-1, except for the procedural recovery sample 4 in Tray 10 which showed a mean recovery of 105%. The mean within-tray recovery ranged from 66.2% to 81.3% for BF 490-5. For BAS 490 F, the overall recovery was 99.5% and the %RSD of the recovery experiments from soil at all levels were within 7%. For BF 490-1, the overall recovery was 83.6% and the %RSD of the recovery experiments from soil at all levels were within 11%. For BF 490-5, the overall recovery was 71.4% and the %RSD of the recovery experiments from soil at all levels were within 9%.

### 5.2 Intra- and Inter-Assay Precision

The inter-assay precision represents the precision observed between different trays (analytical runs). It was assessed from both the calibration standards and the

%VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

procedural recovery sample data.

For each day of analysis, the mean value and relative standard deviation (%RSD) were calculated for each calibration standard level. For LC/MS/MS Method 1 the concentration values were obtained by a weighted (1/y) quadratic regression and for LC/MS/MS Method 2 the concentration values were obtained by a weighted linear (1/y) regression. Acceptable inter-assay precision of the calibration standards was defined as a %RSD of  $\leq 20\%$  for each calibration level within individual trays and an overall mean %RSD of  $\leq 15\%$  for each calibration level.

Intra-assay and inter-assay precision was also assessed from the procedural recovery samples. Similar to the calibration standards above, the daily determinations for each procedural recovery concentration level were averaged and reported. The mean and %RSD for all replicates were calculated over all analyses for each soil. These data were then used to calculate the intra- and inter-assay precision (%RSD). The intra-assay precision is represented by the within-run variability of the procedural recovery samples. Acceptable precision of the procedural recovery samples was defined as a %RSD  $\leq 20\%$  for individual trays and an overall mean %RSD of  $\leq 15\%$  for each procedural recovery level.

Tables 6 to 8 present the BAS 490 F, BF 490-1 and BF 490-5 inter-assay precision results for the analytical calibration standards over nine analytical runs using LC/MS/MS Method 1, respectively. The inter-assay precision (%RSD) did not exceed 6% for BAS 490 F, 7% for BF 490-1 and 10% for BF 490-5 at any calibration level. This indicates acceptable inter-assay precision.

Tables 9 to 11 present the BAS 490 F, BF 490-1 and BF 490-5 inter-assay precision results for the analytical calibration standards over four analytical runs using LC/MS/MS Method 2, respectively. The inter-assay precision (%RSD) did not exceed 9% for BAS 490 F, 10% for BF 490-1 and 10% for BF 490-5 at any calibration level. This indicates acceptable inter-assay precision.

Tables 3 to 5 present the BAS 490 F, BF 490-1 and BF 490-5 inter and intra-assay precision results for the procedural recovery samples in Canadian soil 94041, respectively. The intra- and inter-assay precision for BAS 490 F was within 10%, the intra- and inter-assay precision for BF 490-1 was within 11% and the intra- and inter-assay precision for BF 490-5 was within 9% at each concentration level. This indicates acceptable inter- and intra-assay precision.

### 5.3 Inter-Assay Accuracy

The inter-assay accuracy of the method was assessed by determining the percent error (%DEV) observed in the analysis of analytical standards. For LC/MS/MS Method 1 the theoretical concentration for each analytical standard level was subtracted from the grand mean concentration determined from 9 analytical runs. For LC/MS/MS Method 2 the theoretical concentration for each analytical standard level was subtracted from the grand mean concentration determined from 4 analytical runs for BAS 490 F and BF 490-1 and BF 490-5. The residual was divided by the theoretical value and converted to percent. Therefore, %Error (%DEV) represents the average percent difference of the determined value of an analytical standard from its theoretical determination. Acceptable accuracy was defined as an overall mean %DEV of  $\leq 15\%$ .

96VIRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

Tables 6 to 8 present the inter-assay accuracy results for the BAS 490 F, BF 490-1 and BF 490-5 calibration curves using LC/MS/MS Method 1, respectively. The inter-assay accuracy (%DEV) did not exceed 2% for any calibration level. This indicates acceptable inter-assay accuracy.

Tables 9 to 11 present the inter-assay accuracy results for the BAS 490 F, BF 490-1 and BF 490-5 calibration curves using LC/MS/MS Method 2, respectively. The inter-assay accuracy (%DEV) did not exceed 4% for any calibration level. This indicates acceptable inter-assay accuracy.

#### 5.4 Linearity

The calibration curves for LC/MS/MS Method 1 were fit by a weighted (1/y) quadratic regression. Coefficients of determination ( $R^2$ ) ranged from 0.9976 to 0.9997 for BAS 490 F, from 0.9955 to 0.9999 for BF 490-1 and from 0.9971 to 0.9997 for BF 490-5. The summary of the calibration curve statistics is shown in Tables 12 to 14.

The calibration curves for LC/MS/MS Method 2 were fit by a weighted (1/y) linear regression. Coefficients of determination ( $R^2$ ) ranged from 0.9950 to 0.9988 for BAS 490 F, from 0.9925 to 0.9995 for BF 490-1 and from 0.9933 to 0.9992 for BF 490-5. The summary of the calibration curve statistics is shown in Tables 15 to 17.

#### 5.5 Reconstituted Sample Stability

The stability of BAS 490 F, BF 490-1 and BF 490-5 in reconstituted soil extracts was determined by injecting a full set of procedural recovery samples prepared as described in Section 3 and calibration standards. The samples were re-injected after 48 hours, 7 days and 21 days storage at ambient temperature. A reported mean concentration deviation of  $\geq 15\%$  from the T=0 results would indicate the possibility of sample degradation.

The stability of BF 490-5 in reconstituted soil extracts was demonstrated to be at least 7 days at ca. 25 °C, while the stability of BAS 490 F and BF 490-1 in reconstituted soil extracts was demonstrated to be at least 21 days at ca. 25 °C (Tables 18 to 20, respectively). At 7 days the mean %DEV for the BAS 490 F was -18.8%. After 21 days this improved to -4.44%. At 21 days the reconstituted samples were observed to have solid material in the tubes which were then vortexed prior to aliquotting samples which may explain the improved stability results observed for BAS 490 F. The %DEV of the 7 day samples from the 0 hour samples ranged from -28.4% to -3% for BAS 490 F, from -21.6% to 6.88% for BF 490-1 and from -11.2% to 5.07% for BF 490-5. The %DEV of the 21 day samples from the 0 hour samples ranged from -10.8% to 9.17% for BAS 490 F, from -25.5% to 6.37% for BF 490-1 and from -61.9% to -35.4% for BF 490-5.

For all concentrations of BAS 490 F, BF 490-1 and BF 490-5 stability procedural recovery samples, the %DEV of the 48 hour samples from the 0 hour samples did not exceed 7%, 15% and 12% respectively.

#### 5.6 Sensitivity

Using LC/MS/MS Method 1 the %RSD at the 0.010 ppm spiking level for BAS 490 F,

%VIRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

BF 490-1 and BF 490-5 was less than 7%, 6% and 9% respectively.

Additional lower level of quantitation (LLQ) experiments for soils spiked at 0.004 ppm (where 100% recovery would yield a solution concentration of 2 ng/mL) and 0.006 ppm (where 70% recovery would yield a solution concentration of 2.1 ng/mL) showed that 0.004 ppm was acceptable as the lower limit of quantitation for BAS 490 F and BF 490-1 where the %RSD was less than 11%. An LLQ of 0.006 ppm was acceptable for BF 490-5 where the %RSD was less than 10%. (Tables 21 to 23).

#### 5.7 Use of Control Blanks

The specificity of the assay was determined by the mass spectrometric technique used for analysis. To monitor for interference from blank soil or reconstitution solvent, samples containing neither BAS 490 F, BF 490-1 nor BF 490-5 (e.g. Control Blanks) were assayed with all validation experiments.

This assay has proven to be highly specific for BAS 490 F, BF 490-1 and BF 490-5. Control blanks were injected twice during each tray and some carryover associated with the autosampler was observed. The first control blank was injected after the highest analytical standard. BAS 490 F showed no evidence of carryover but those samples where a peak was observed suggest contamination of the sample. The carryover peaks for BF 490-1 were determined at less than 0.65 ng/mL. BF 490-5 showed higher carryover which in the worst case was determined to correspond to a solution concentration of 1.26 ng/mL. Blank reconstitution solvent was injected at the beginning and end of each tray where the sample injected at the beginning of the tray was blank but the sample at the end showed evidence of carryover from the 200 ng/mL standard injected immediately prior to the blank (Table 24). Observed peak areas for the lowest calibration (2 ng/mL) standards showed no correlation with the concentration of the previously injected sample.

For Method 2, no carryover was observed for BAS 490 F, BF 490-1 and BF 490-5 in the control blanks or the blank reconstitution solvents. These data are summarized in Table 25.

#### 5.8 Limitations

None known to date.

### 6.0 CROSS-VALIDATION WITH VARIOUS SOILS

#### 6.1 Cross-Validation of LC/MS/MS Method 1 to California Soil 94017

The procedural recovery results for BAS 490 F, BF 490-1 and BF 490-5 in Californian Soil 94017 are summarized in Table 26. The mean within-tray recovery ranged from 98.7% to 111% for BAS 490 F and the overall recovery was 103%. The mean within-tray recovery ranged from 86.9% to 90.9% for BF 490-1, except for the procedural recovery sample 4 which showed a mean recovery of 103% and the overall recovery was 92.7%. The mean within-tray recovery ranged from 78.9% to 81.5% for BF 490-5, except for the procedural recovery sample 4 which showed a mean recovery of 101% and the overall recovery was 85.2%.

The intra-assay precision for the procedural recovery samples of each analyte was calculated as described in Section 5.2. The intra-assay precision for BAS 490 F was

96V/RP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

within 6%, the intra-assay precision for BF 490-1 was within 8% and the intra-assay precision for BF 490-5 was within 13% at all concentration levels. This indicates acceptable intra-assay precision.

#### 6.2 Cross-Validation of LC/MS/MS Method 1 to Oregon Soil 94016

The procedural recovery results for BAS 490 F, BF 490-1 and BF 490-5 in Oregon Soil 94016 are summarized in Table 27. The mean within-tray recovery ranged from 84.2% to 91.6% for BAS 490 F and the overall recovery was 86.8%. The mean within-tray recovery ranged from 64.3% to 70.7% for BF 490-1 and the overall recovery was 66.5%. The mean within-tray recovery ranged from 72.6% to 79.8% for BF 490-5 and the overall recovery was 76.0%.

The intra-assay precision for the procedural recovery samples of each analyte was calculated as described in Section 5.2. The intra-assay precision for BAS 490 F was within 5%, the intra-assay precision for BF 490-1 was within 5% and the intra-assay precision for BF 490-5 was within 9% at all concentration levels. This indicates acceptable intra-assay precision.

The low apparent recoveries of BF 490-1 observed for the procedural recovery samples meant that LC/MS/MS Method 1 was not applicable to the analysis of samples from Oregon Soil 94016.

#### 6.3 Cross-Validation of LC/MS/MS Method 1 to New York Soil 94015

The procedural recovery results for BAS 490 F, BF 490-1 and BF 490-5 in New York Soil 94015 are summarized in Table 28. The mean within-tray recovery ranged from 89.1% to 91.6% for BAS 490 F and the overall recovery was 90.3%. The mean within-tray recovery ranged from 62.0% to 70.2% for BF 490-1 and the overall recovery was 64.3%. The mean within-tray recovery ranged from 63.0% to 76.2% for BF 490-5 and the overall recovery was 70.1%.

The intra-assay precision for the procedural recovery samples of each analyte was calculated as described in Section 5.2. The intra-assay precision for BAS 490 F was within 4%, the intra-assay precision for BF 490-1 was within 7% and the intra-assay precision for BF 490-5 was within 9% at all concentration levels. This indicates acceptable intra-assay precision.

The low apparent recoveries of BF 490-1 and BF 490-5 observed for the procedural recovery samples meant that LC/MS/MS Method 1 was not applicable to the analysis of samples from New York Soil 94015.

#### 6.4 Cross-Validation of LC/MS/MS Method 2 to Oregon Soil 94016

The procedural recovery results for BAS 490 F, BF 490-1 and BF 490-5 in Oregon Soil 94016 using LC/MS/MS Method 2 are summarized in Table 29. The mean within-tray recovery ranged from 89.5% to 93.8% for BAS 490 F and the overall recovery was 92.1%. The mean within-tray recovery ranged from 76.4% to 88.6% for BF 490-1 and the overall recovery was 81.2%. The mean within-tray recovery ranged from 83.0% to 90.8% for BF 490-5 and the overall recovery was 87.5%.

The intra-assay precision for the procedural recovery samples of each analyte was

96VIRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

calculated as described in Section 5.2. The intra-assay precision for BAS 490 F was within 8%, the intra-assay precision for BF 490-1 was within 12% and the intra-assay precision for BF 490-5 was within 10% at all concentration levels. This indicates acceptable intra-assay precision.

#### 6.5 Cross-Validation of LC/MS/MS Method 2 to New York Soil 94015

The procedural recovery results for BAS 490 F, BF 490-1 and BF 490-5 in New York Soil 94015 are summarized in Table 30. The mean within-tray recovery ranged from 82.7% to 86.1% for BAS 490 F and the overall recovery was 84.8%. The mean within-tray recovery ranged from 70.9% to 79.4% for BF 490-1 and the overall recovery was 74.8%. The mean within-tray recovery ranged from 83.0% to 84.3% for BF 490-5 and the overall recovery was 83.7%.

The intra-assay precision for the procedural recovery samples of each analyte was calculated as described in Section 5.2. The intra-assay precision for BAS 490 F was within 4%, the intra-assay precision for BF 490-1 was within 6% and the intra-assay precision for BF 490-5 was within 6% at each concentration level. This indicates acceptable intra-assay precision.

#### 7.0 CONCLUSION

The analytical procedure is applicable for measuring the residues of BAS 490, BF 490-1 and BF 490-5 in soil at levels down to 0.01 ppm. This method has been used successfully for the analysis of BAS 490, BF 490-1 and BF 490-5 in soil in a contract lab at levels down to 0.01 ppm.

#### 8.0 QUALITY ASSURANCE PROCEDURES

The raw data, final report and analytical standards of the studies referenced in this method will be stored in the BASF archives at:

BASF Corporation  
Agricultural Products  
Agricultural Research Center  
26 Davis Drive  
Research Triangle Park, NC 27709-3528

Instrumental log books and associated notebooks are stored in the ABS archives. Copies of pertinent information from these logbooks and notebooks will be archived at BASF.

#### 9.0 REFERENCES

1. Methods of Soil Analysis, Part 1. Physical and Mineralogical Properties, Including Statistics of Measurement and Sampling. C.A. Black, Editor, American Society of Agronomy and American Society for Testing and Materials. American Society of Agronomy, Madison, Wisconsin, 1965, pages 92-93.

#### 10.0 SAFETY AND HEALTH CONSIDERATIONS

1. Dichloromethane is a suspected cancer agent-use with adequate ventilation, avoid

%VIRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

breathing vapors and use it in the hood. Avoid contact with skin and eyes.

2. Methanol is extremely flammable, moderately toxic. Keep away from heat, spark, and open flame. Keep container closed. Use with adequate ventilation, avoid breathing vapors and use it in the hood. Avoid contact with skin and eyes.

#### 11.0 PROTOCOL CHANGES

Amendment 1 alters the names of the metabolites in the title of the protocol to BF 490-1 and BF 490-5 and corrects the test / reference substance section of the protocol for the lot number of BAS 490 F to 39/184-1 and the lot number of BF 490-1 to 00820-251. Amendment 1 also specifies the soils to be used in the method validation as California soil RCN 94017, New York RCN 94015, Oregon RCN 94016 and Canada RCN 94041.



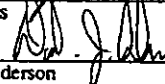
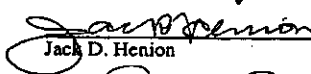
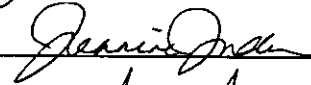
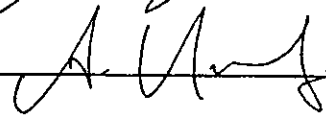
06VJRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.

ADVANCED BIOANALYTICAL SERVICES  
SIGNATURE PAGE

We, the undersigned, hereby declare that this report provides a true and accurate record of the results obtained.

Author:	 _____ John R. Perkins	<u>10/8/96</u> Date
	 _____ Steve Lowes	<u>10/8/96</u> Date
	 _____ David J. Anderson	<u>10/8/96</u> Date
	 _____ Jack D. Henion	<u>10/8/96</u> Date
Approved By:	 _____ Jackie Jones	<u>10-10-96</u> Date
Issued By:	 _____ A. Henry	<u>10/10/96</u> Date

96VZRP01.BAS



ADVANCED  
BIOANALYTICAL  
SERVICES, INC.



## 12.0 TABLES

Table 2: Summary of Validation Experiments

Experiment	Tray #	Soil RCN Number	LC/MS/MS Method
Recovery	7, 10, 13	94041	1
Inter and Intra Assay Precision	7, 10, 13	94041	1
48 Hour Recon. Stability	12	94041	1
7 Day Recon. Stability	14	94041	1
21 Day Recon. Stability	15	94041	1
Lower Level of Quantitation	7, 10, 13	94041	1
Cross Validation	17	94017	1
Cross Validation	21	94016	1
Cross Validation	20	94015	1
Cross Validation	29	94016	2
Cross Validation	30	94015	2
Reduced Shakes and Partition Steps	35	94015	2
Two Partition Steps	36	94015	2

96VJRP01.BAS

ADVANCED  
BIOANALYTICAL  
SERVICES, INC.