425156-01

FMC CORPORATION
AGRICULTURAL CHEMICAL GROUP
Princeton, New Jersey

PC-0130 Revised
Page 1 of 42

REPORT TITLE: METHOD VALIDATION FOR THE DETERMINATION OF BIFENTHRIN IN/ON PECAN AND WALNUT

TEST SUBSTANCE: FMC 54800 (Bifenthrin)

DATA REQUIREMENT: Pesticide Assessment Guidelines, Subdivision O, 171-4, PR Notice 88-5

AUTHOR: David A. Winkler
(Study Director)

STUDY DATES:

Initiated:	April 19, 1989
Terminated:	October 11, 1989
Originally Reported:	February 26, 1990
Report Revised:	September 28, 1992

Note: This report supersedes the final report issued on February 26, 1990. All changes that were made are documented on the revision page which follows the GLP statement page of this report.

PERFORMING LABORATORY: EN-CAS Analytical Laboratories
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FMC STUDY NUMBER: 182MVL89R2

EN-CAS PROTOCOL NUMBER: 89-0021

Non Confidential
FMC Corporation Authorizes the Release or Use of This Method by Federal and State Agencies

FMC CORPORATION

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality 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: FMC CORPORATION, AG CHEM GROUP

Company Agent: Khalid H. Akkari, Ph.D.
Senior Research Chemist
Residue Chemistry Department

K. H. Akkari

Khalid H. Akkari, Ph.D.
Senior Research Chemist

Feb. 28, 1990

Date

GOOD LABORATORY PRACTICES STATEMENT

To the best of my knowledge the study reported herein, (Study ID: 182MVL89R2, Method Validation for the Determination of Bifenthrin in/on Pecan and Walnut, FMC Corporation, Ag Chem Group, PC-0130), was conducted and reported in accordance with the intent of the Good Laboratory Practice Standards set forth in Title 40, Part 160 of the Code of Federal Regulations of the United States of America.

STUDY DIRECTOR:

David A. Winkler — 9/28/92
David A. Winkler
Project Coordinator
Date

SPONSOR:

James E. Ridler — 10/2/92
James E. Ridler
Residue Data Chemist
Date

Based on the signature of the Study Director and Quality Assurance Officer, this study was, to the best of my knowledge, conducted in accordance with EPA FIFRA Good Laboratory Practice Standards.

SUBMITTER:

Eunice M. Cuirle — in October, 1992
Eunice M. Cuirle
Manager, Product Development and Registration
Date

QUALITY ASSURANCE STATEMENT

The analytical portion of this study was inspected by the EN-CAS Quality Assurance Unit for compliance with GLP standards, protocols and EN-CAS Standard Operating Procedures. The results were reported to Management and the Study Director as follows:

Inspection Dates	Dates Results Submitted to Study Director	Dates Results Submitted to Management
8/23/89	8/25/89	
9/29/89	9/29/89	
10/05/89	10/13/89	
10/25/89	10/27/89	
2/22/90	2/22/90	2/22/90

Kathleen H. Faltynski, M.S.
Quality Assurance Officer

2/26/90
Date

REVISION PAGE 1

This report was revised to satisfy a request from the EPA to provide a clean copy of the method validation for bifenthrin in/on pecan and walnut. The revised report was written following FMC report guidelines. The following changes were made:

Additions to revised report:

Page 9	The summary includes why a revised report was issued
Page 10	Table 1 summarizes the method recovery values for pecans
Page 11	Table 2 summarizes the method recovery values for walnuts
Page 13	This section provides additional information regarding the test substance, test commodity, study design, and analytical standard

Deletions from original report:

Page 4	All study personnel, except DA Winkler, are no longer employed by En-Cas
Pages 12, 13	The original method clean up 4 a i) and ii) was deleted to provide a clean copy of the validated method
Pages 32-36	Appendix B was deleted because it detailed the original method which was not validated by En-Cas

Sections of the original report moved or modified due to reformatting:

Page 1	Study Director, Rodney M.Bennett is no longer employed by En-Cas The termination date was changed based on a review of study records for this revised report
Page 2	Company Agent, Khalid H. Akkari is no longer employed by FMC
Page 3	Study Director, Rodney M.Bennett is no longer employed by En-Cas Sponsor, Khalid H. Akkari is no longer employed by FMC
Page 7	The Table of Contents was revised to follow FMC report format

REVISION PAGE 2

Sections of the original report moved or modified due to reformatting:

Page 12	The method flow scheme was moved from page 23
Page 19	Time required for analysis was modified to include time to complete the analytical procedure and not reviewing data and sample reinjection
Page 21	Limitations section modified since GPC was not used for method validation
Page 24	Table 4 includes information from Table 1 with USE column deleted
Page 25	Table 5 combines information from Tables 2 and 3
Page 26	Table 6 includes information from Table 4

We authorize the changes to this revised report.

David A. Winkler
Project Coordinator
for Study Director

9/28/92
Date

James E. Ridler
Residue Data Chemist
for Sponsor/Company Agent

10/2/92
Date

Kathleen H. Faltynski
Quality Assurance Officer

9/28/92
Date

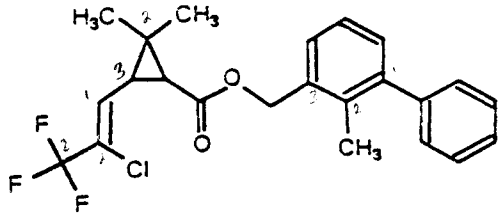
Eunice M. Cuirle
Manager, Product Development and
Registration

6 October, 1992
Date

TABLE OF CONTENTS

I. INTRODUCTION

Bifenthrin is the common name for the active ingredient in an insecticide/miticide produced by FMC Corporation. Its chemical name is [2-methyl-(1,1'-biphenyl)-3-yl]methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyclopropanecarboxylate and the code number is FMC 54800. The structure is as follows:



BIFENTHRIN

The analytical method contained in FMC reports P-1109 and RAN-0142 (Section IX, Reference 1 and 2) was provided to the contract laboratory for independent method validation. EN-CAS Laboratory conducted the independent method validation for FMC Corporation. This independent method validation was done to fulfill the EPA requirement contained in PR Notice 88-5. The method was successfully validated after modification in clean-up steps and reported as FMC report PC-0130. Although the original report contained a complete stepwise analytical procedure used in validation in Appendix C, rewriting of the report was requested by the EPA (Reference 3). The purpose of this report is to provide a complete clean copy of analytical method to the EPA. This was done by rewriting of the report PC-0130 with all required modifications included and with all old directions deleted. No laboratory work was conducted for this revised report.

II. SUMMARY

The analytical method for bifenthrin contained in FMC Reports P-1109 and RAN-0142 was provided to the contract laboratory for independent method validation. EN-CAS Laboratory validated the method for pecans and walnuts. The analytical method was validated successfully with some modifications in clean-up steps. The validated analytical method included an acetone extraction, concentration by vacuum rotary evaporation, acetonitrile partition, hexane partition, Florisil® column clean up, and analysis by a gas chromatograph equipped with an electron capture detector.

Pecans and walnuts known to have no bifenthrin residues were laboratory fortified at 0.05, 0.25, and 1.00 ppm, respectively. Fortified and control samples were analyzed for bifenthrin. Acceptable method recoveries were obtained from both pecan and walnut samples. The average method recovery for pecans was 90% with a standard deviation of ±22%. For walnuts, the average method recovery was 72% with a standard deviation of ±14%.

For both pecan and walnut samples, the limit of quantitation was established at 0.05 ppm and the limit of detection was set at 0.01 ppm. No particular interferences or limitations were experienced.

III. SUMMARY TABLES AND GRAPHICS

A. Summary of Method Recovery Data

TABLE 1

BIFENTHRIN METHOD RECOVERY VALUES FROM PECAN CONTROL SAMPLES

MATRIX	FORTIFICATION LEVEL (PPM)	NUMBER OF ANALYSES	CONTROL BACKGROUND (PPM)	RANGE OF RECOVERY (%)
PECAN NUTMEAT	0.05	3	ND	70 - 98
	0.25	3	ND	74 - 126
	1.00	3	ND	60 - 94
			Overall Average = 90 Standard Deviation = ±22 Number of Analyses = 9	

ND = Non-detectable (< 0.01 ppm)

TABLE 2

BIFENTHRIN METHOD RECOVERY VALUES
FROM WALNUT CONTROL SAMPLES

MATRIX	FORTIFICATION LEVEL (PPM)	NUMBER OF ANALYSES	CONTROL BACKGROUND (PPM)	RANGE OF RECOVERY (%)	
WALNUT NUTMEAT	0.05	3	ND	53 - 79	Ave 66%
	0.25	3	ND	70 - 95	
	1.00	3	ND	59 - 80	Ave - 67%

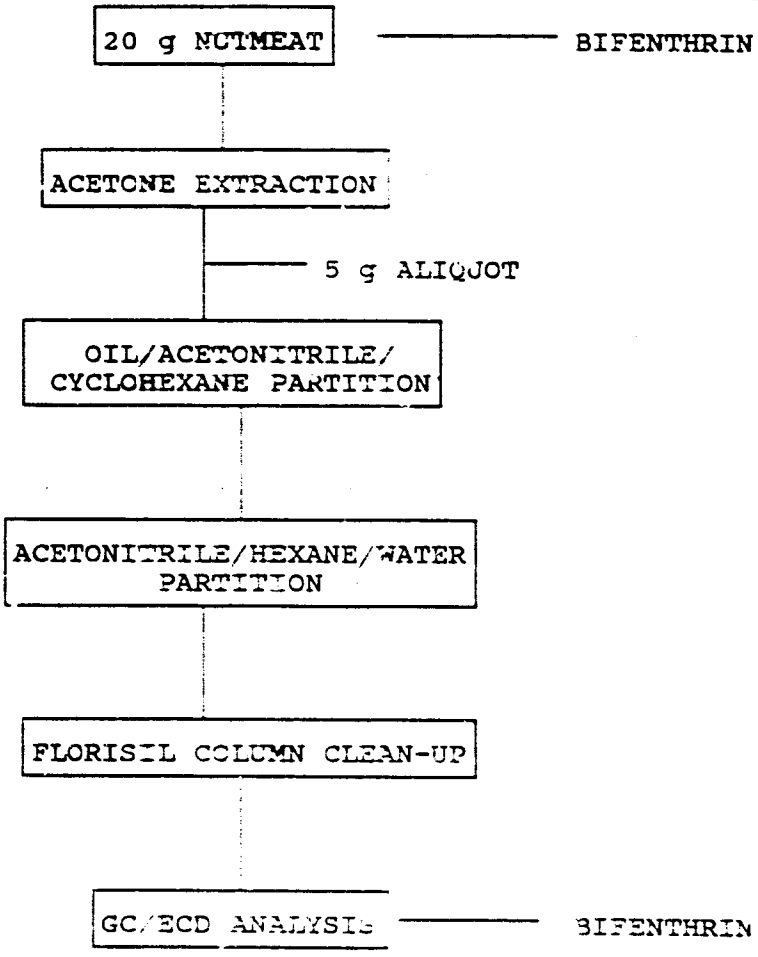
Overall Average = 72
Standard Deviation = ±14
Number of Analyses = 9

ND = Non-detectable (< 0.01 ppm)

B. Method Flow Scheme

FIGURE 1

FLOW SCHEME FOR BIFENTHRIN ON NUT MATRICES



IV. MATERIALS AND STUDY DESIGN

A. Test Substance

Bifenthrin has the chemical name [2-methyl(1,1'-biphenyl)-3-yl]methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate (see Section X, Table 3 for structure and other information). The code number for bifenthrin is FMC 54800 and the CAS number is 82657-04-3. The EPA registration number for bifenthrin technical is 279-3055.

B. Test Commodity

Pecans and walnuts are members of the tree nut crop group. Cherokee variety of mature trees were used for pecan. For walnut, English Franquette and Hartley varieties were used. Nutmeat is the only raw agricultural commodity of tree nuts required to be analyzed by the Pesticide Assessment Guidelines, Subdivision O, Table II. Mature, ripe pecans and walnuts were used to validate the bifenthrin analytical method.

C. Study Design and Procedures

Pecan and walnut samples which were known to contain no bifenthrin residues were used. Prior to analysis, the individual pecans and walnuts were hulled, ground with dry ice, and nutmeat obtained. Nutmeat samples were laboratory fortified at three different levels and analyzed for bifenthrin.

D. Analytical Standard

Information about the bifenthrin standard used in this study can be found Section X, Table 3.

E. Equipment

Similar or equivalent equipment, glassware, and instrument by other venders/manufacturers can be substituted for those listed.

Centrifuge
Chromatographic column (12 to 14 mm i.d.)
Filter paper (9 cm), Whatman #4
Filter paper, GF/C
Flasks, 1000 mL, flat bottom boiling
Flasks, 250 mL, 500 mL, Erlenmeyer
Flasks, 500 mL, sidearm filter
Funnels, 500 mL, separatory
Funnels (4" diameter), Metal powder
Glass jars, 32 oz, French square
Hobart food chopper
Homogenizer, Polytron
N-Evap evaporator, Organomation
Syringe, assorted size, Hamilton

F. Reagents

Acetone, Pesticide Grade, Fisher Scientific
Acetone, High Purity Grade, Burdick and Jackson
Acetonitrile, UV Grade, Burdick and Jackson
Analytical Standard, Bifenthrin (FMC 54800)
Inventory Number 198 and 97.9% purity, FMC Corporation, ACG, Princeton, NJ
Ethyl acetate, Pesticide Grade, Fisher Scientific
Florisil, 100-200 mesh, deactivated 3% with water, Fisher Scientific
Sodium chloride, Reagent Grade, Fisher Scientific
Sodium sulfate, anhydrous, Reagent Grade, oven dried
Water, Milli-Q deionized

V. ANALYTICAL PROCEDURE

A. Residue Method

The analytical method was validated on pecans and walnuts. The steps of this method include the following:

1. Sample Preparation

Prior to analysis, all matrices required processing in order to obtain a homogeneous sample. The pecan or walnut nutmeats were separated from the hulls and a homogeneous control sample was prepared by grinding the nutmeats using dry ice in a Hobart mixer.

2. Extraction

A schematic representation of the analytical procedure steps is shown in Section III, Figure 1. Twenty grams of subsample were weighed in a 32 oz French square bottle and blended (using a Polytron Tissue Extractor, Brinkman Inc.) for approximately 2 minutes with 200 mL of acetone. The mixture was vacuum filtered through a GF/C filter paper and transferred to a 500 mL graduated cylinder. The volume in the graduated cylinder was adjusted to 280 mL with acetone.

3. Partition

A 5 gram aliquot (70 mL) was transferred to a 250 mL Erlenmeyer flask. The sample was evaporated using a rotary evaporator at a water bath temperature of 30-35°C until only an oily residue remained (incipient dryness). The sample was transferred to 40 mL glass centrifuge tube using 25 mL of acetonitrile. One milliliter of cyclohexane was added, and the tube was shaken for about 30 seconds. The sample was centrifuged at low speed for about 10 minutes, and the upper acetonitrile phase was transferred to a 500 mL separatory funnel containing 100 mL sodium chloride solution (1 g/10 mL in deionized water). Twenty five milliliters of acetonitrile was added to the centrifuge tube and the partition was repeated. One hundred milliliters of hexane was added to the separatory funnel and shaken vigorously for approximately one to two minutes. The separatory funnel was allowed to stand until phase separation was completed. The lower aqueous phase was drained into a second 500 mL separatory funnel. The top organic phase was drained through a sodium sulfate pad (pre-rinsed using 50 mL of hexane) into a 1000 mL flat bottom boiling flask. The aqueous phase was extracted two additional times with 100 mL of hexane. The hexane phases were passed through the sodium sulfate pad and collected. The sample was reduced to about 5 mL under rotary evaporation at a water bath temperature of 30-35°C.

4. Florisil Column Clean-up

A glass chromatographic column (12 to 14 mm I.D.) with a 250 mL reservoir was plugged with a glass wool in bottom of the column, and 100 mL of hexane was added. Ten grams of 3% water deactivated Florisil was added into the column for slurry packing. The Florisil was allowed to settle and a few grams of anhydrous sodium sulfate were added on top of the packing. The solvent was drained to about 1/16 inch above the sodium sulfate. The hexane extract from the previous step was loaded onto the column. The boiling flask was rinsed with 2 X 2 mL of hexane and it was transferred onto the column. The column was eluted with 100 mL of hexane. The hexane eluate was discarded. The column was eluted for bifenthrin with 100 mL of 5% ethyl acetate in hexane (v/v). The solution was evaporated using rotary evaporation to about 5 mL. This solution was transferred quantitatively to a 13 mL centrifuge tube and evaporated using nitrogen to the appropriate volume for gas chromatographic analysis.

B. Instrumentation

The bifenthrin residue was quantitated by gas chromatography employing an electron capture detector. A Hewlett-Packard (HP) 5890 gas chromatograph equipped with an electron capture detector (Ni^{63}), HP 7673A autosampler, and HP 3396A integrator was used. Instrument and column parameters are listed in Section XII, Appendix A.

C. Quality Control

1. Preparation of Standards

The structure and purity of the bifenthrin analytical standard used in this study is shown in Section X, Table 3. A stock solution of 1000 ug/mL was prepared by dissolving the appropriate amount of the above analytical standard in hexane. Working solutions in concentrations from 0.00625 to 100 ng/uL were prepared by appropriate dilutions of the stock solution in hexane. Working solutions were

used for fortification, injection standard, and calibration of the instrument. Section X, Table 4 shows the list of standard solutions used in this study.

2. Fortification Procedure

Control samples were fortified prior to any analytical manipulation. The fortification standards (1.0 ug/mL, 10 ug/mL, and 100 ug/mL) were added with an appropriately sized microliter syringe or with a volumetric pipet directly onto the crop matrix. The hexane solvent was allowed to evaporate prior to initiating the analytical procedure.

D. Method of Calculation

The amount of bifenthrin in a 3 uL injection was quantitated from the detector's electrical response transmitted to the instrument integrator in the peak area mode. The responses were converted to area units which in turn were calculated as nanograms (ng) of bifenthrin based on a 0.125 ug/mL injection (run) standard calibration. A run standard was injected at the beginning of every set and subsequently after every two sample extracts. Each pair of sample injections was bracketed by a bifenthrin standard at a concentration of 0.125 ug/mL. The bifenthrin residue content of each sample was determined by the ratio of the peak area units found in the sample compared to the peak area units of the run standard immediately preceding the sample pair. The ratio was multiplied by the concentration of the run standard to determine the concentration of bifenthrin in the unknown sample. The final volume including any dilution as well as the microliters injected and the gram equivalents carried through the method were taken into account. The following formula was used:

$$\text{ng of bifenthrin in sample} = \frac{\text{area units (sample)}}{\text{area units (standard)}} \times \text{ng (standard)}$$

The ng of bifenthrin in the sample was used to calculate the uncorrected ppm (ug/g) by following formula:

$$\text{uncorrected ppm (ug/g)} = \frac{\text{ng of bifenthrin in sample}}{\text{mg of sample injected}}$$

The uncorrected ppm of the fortified control sample was divided by fortification level followed by multiplication by 100 to calculate the method recovery. The following formula was used:

$$\text{method recovery (\%)} = \frac{\text{uncorrected ppm}}{\text{fortification level (ppm)}} \times 100$$

An example of how to calculate the method recovery using Section XII, Figure 4 is given below:

ng (standard) = 3.0 ul x 0.125 ng/ul = 0.375 ng

area units of standard = 89736

area units of fortified sample = 132125

ng of bifenthrin in sample = $\frac{132125 \times 0.375 \text{ ng}}{89736}$ = 0.552 ng

uncorrected ppm (ug/g) = $\frac{0.552 \text{ ng}}{3.0 \text{ mg}}$ = 0.184 ppm

method recovery (%) = $\frac{0.184 \text{ ppm}}{0.250 \text{ ppm}} \times 100$ = 74 %

E. Interference

1. Sample Matrices - There was no detectable bifenthrin residue (ND, < 0.01 ppm) in any of the pecan or walnut control samples. There were no interferences in any of the checks or reagent blanks (acetone, hexane, water, Florisil, ethyl acetate, cyclohexane).

2. Other Pesticide - Nutmeat samples were not analyzed for any other pesticides.

3. Solvents - Analytical standard solutions were prepared in high purity hexane which produced a clean chromatogram from the sensitive electron capture detector. Reagent blank run through the procedure showed that the solvents (acetone, hexane, ethyl acetate, cyclohexane) and other reagents (sodium chloride, sodium sulfate, Florisil, glass wool) did not introduce impurities into the samples.

4. Labware - All glassware were thoroughly washed in a Better Built Turbomatic dishwasher using the following cycle: water rinse, non-phosphorous detergent wash, and water rinse. The cleaned glassware was then rinsed with acetone before use. Reagent blank analysis showed all glassware to be clean and free of interference.

F. Confirmatory Technique

Bifenthrin residues in this study were not characterized other by than GC/ECD analytical technique.

G. Time Required for Analysis

The analytical procedure from sample weighing to gas chromatography injection requires about 8 hours for a set of 8 samples.

H. Modifications or Potential Problems

Regular rubber septa cannot be used to cap vials since they introduce contamination via the injection needle. Only double faced teflon sealed silicon septum were used.

The sodium sulfate used for the method was baked in a muffle furnace to remove trace organic contaminants.

Also, bifenthrin has a tendency to adsorb to glass thus, any concentration step (rotary

evaporation) must be carefully watched to prevent the sample from going to dryness.

There may be build-up of material at the injection port liner and front end of the column. Symptoms of this are peak tailing and broadening and wide variability in area counts of standards. This can be corrected by changing the injection port liner (insert) and cutting off about 2 inches of the front end of the column, in addition to other routine maintenance of the GC system.

VI. RESULTS AND DISCUSSION

A. Accuracy

The method for the determination of bifenthrin in/on pecan and walnut nutmeat samples was validated by analyses of reagent blank, check (control), and fortified checks.

Statistical treatment of the data was limited to the determination of averages and standard deviation (SD). Fortification levels range standard deviation of recoveries were: pecans - 90% ±22% (n = 9); walnut - 72% ±14% (n = 9).

B. Precision

The standard deviation of ±22% for pecans and 14% for walnuts achieved for method recoveries showed that the method functioned properly. The reproducibility of this method depended on following the procedure closely and applying good analytical techniques.

C. Limit of Detection and Quantitation

Quantitatively reliable lower limit of determination of bifenthrin (limit of quantitation) was established by analysis of reagent blanks, checks (control), and fortified samples of control samples. The limit of quantitation was established and validated at 0.05 ppm. Typically, the optimum instrument

response attained to achieve method sensitivities was at least a 3 mm peak height for 0.075 ng bifenthrin injection. This level of response was equivalent to 20% of the method sensitivity. The screening level standard (0.05 ppm) was a 0.375 ng bifenthrin injection and typically yielded a ~10-15 mm peak height. Recognition of detector response at the threshold level (limit of detection) was equivalent to a peak height response of approximately 3 mm (20% of limit of quantitation). This translates to 0.01 ppm. Any apparent response below limit of detection was considered and reported as non-detectable (ND).

D. Ruggedness

It appears that matrix effects due to variable oil content in nut meats could affect the accuracy and precision of the method. However, the analytical method was practiced on pecans and walnuts with good results.

E. Limitations

No particular limitation is expected. Similar kind of nut matrices can be analyzed for bifenthrin by this method.

VII. CONCLUSION

An analytical method for determination of bifenthrin was validated successfully for pecans and walnuts by an independent laboratory. The validated analytical method for bifenthrin included an acetone extraction, concentration by vacuum rotary evaporation, acetonitrile partition, hexane partition, Florisil® column clean up, and analysis by a gas chromatograph equipped with an electron capture detector. Acceptable method recoveries were obtained from three different fortification levels.

The average method recovery for the analytical method for pecan nutmeat samples was 90% ±22%. The average method recovery for the analytical method for walnut nutmeat samples was 72% ±14%. The average background in the check samples was non-detectable (< 0.01 ppm).

All reagents, glassware, and equipment used in the procedure are readily obtainable or already available in the standard residue analysis laboratory. The results are reproducible if the usual precautions are taken. These include avoidance of contamination by using clean glassware, using accurate measurements (weighing, diluting, dispensing, etc.), and optimizing instrument conditions.

XIII. TABLES AND FIGURES

TABLE 3

TEST AND REFERENCE SUBSTANCE

COMPOUND	CHEMICAL NAME AND STRUCTURE	CODE NUMBER	RESIDUE INVENTORY NUMBER	PERCENT PURITY
BIFENTHRIN	(2-methyl[-1,1'-biphenyl]-3-yl) -methyl-3-(2-chloro-3,3,3-tri fluoro-1-propenyl)-2,2-dimethyl -cyclopropane-carboxylate	FMC 54800	198	97.9

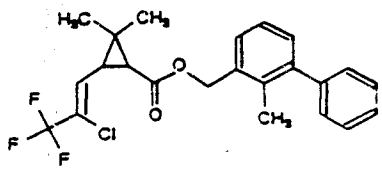


TABLE 4

REFERENCE SOLUTIONS

COMPOUND	SOLUTION SOLVENT	SOLUTION CONCENTRATION (NG/UL)	LAB. NOTEBOOK NUMBER/PAGE	DATE PREPARED
BIFENTHRIN	HEXANE	1000	121/130	05/24/89
BIFENTHRIN	HEXANE	100	121/130	05/25/89
BIFENTHRIN	HEXANE	10	121/130	05/25/89
BIFENTHRIN	HEXANE	5.0	121/130	05/25/89
BIFENTHRIN	HEXANE	2.5	121/130	05/25/89
BIFENTHRIN	HEXANE	1.0	121/130	05/15/89
BIFENTHRIN	HEXANE	1.0	133/138	09/11/89
BIFENTHRIN	HEXANE	0.5	121/130	05/25/89
BIFENTHRIN	HEXANE	0.25	121/130	05/25/89
BIFENTHRIN	HEXANE	0.125	121/130	05/25/89
BIFENTHRIN	HEXANE	0.125	133/80	06/07/89
BIFENTHRIN	HEXANE	0.125	149/24	07/03/89
BIFENTHRIN	HEXANE	0.125	131/177	10/12/89
BIFENTHRIN	HEXANE	0.10	121/130	05/25/89
BIFENTHRIN	HEXANE	0.05	121/130	05/25/89
BIFENTHRIN	HEXANE	0.025	121/130	05/25/89
BIFENTHRIN	HEXANE	0.0125	133/102	07/12/89
BIFENTHRIN	HEXANE	0.0625	133/102	07/12/89
BIFENTHRIN	HEXANE	0.00625	133/102	07/12/89

TABLE 5

METHOD RECOVERY OF BIFENTHRIN FROM LABORATORY FORTIFIED PECAN SAMPLES

MATRIX	SAMPLE IDENTIFICATION FMC CODE	SAMPLE IDENTIFICATION EN-CAS NO.	FORTIFICATION LEVEL (PPM)	CONTROL BACKGROUND	BIFENTHRIN RECOVERY (%)
NUTMEAT	EVG-87-7A	EG5078-101S	0.05	ND	98
		EG5078-102S	0.05	ND	83
		EG5078-103S	0.05	ND	70
	EVG-87-7A	EG5078-104S	0.25	ND	74
		EG5078-105S	0.25	ND	126
		EG5078-106S	0.25	ND	117
	EVG-87-7A	EG5078-107S	1.00	ND	94
		EG5078-108S	1.00	ND	60
		EG5078-109S	1.00	ND	92
				OVERALL AVERAGE	90
				STANDARD DEVIATION	±22
				NUMBER OF ANALYSIS	9

ND = Not-detectable (< 0.01 ppm)

TABLE 6

METHOD RECOVERY OF BIFENTHRIN FROM LABORATORY FORTIFIED WALNUT SAMPLES

MATRIX	SAMPLE IDENTIFICATION FMC CODE	SAMPLE IDENTIFICATION EN-CAS NO.	FORTIFICATION LEVEL (PPM)	CONTROL BACKGROUND	BIFENTHRIN RECOVERY (%)
WUTMEAT	88-JMT-28C	EGS080-110S	0.05	ND	66
		EGS080-111S	0.05	ND	53
		EGS080-112S	0.05	ND	79
	88-JMT-28C	EGS080-113S	0.25	ND	95
		EGS080-114S	0.25	ND	70
		EGS080-115S	0.25	ND	87
	88-JMT-28C	EGS080-116S	1.00	ND	63
		EGS080-117S	1.00	ND	59
		EGS080-118S	1.00	ND	80
				OVERALL AVERAGE	72
				STANDARD DEVIATION	±14
				NUMBER OF ANALYSIS	9

ND = Not-detectable (< 0.01 ppm)

IX. REFERENCES

1. Martin, F.D., "Determination of Bifenthrin Residues in/on Pecans," FMC Corporation, ACG. Princeton, NJ. P-1109, 6/06/85.

2. Rizzi, L.A., "Determination of FMC 54800 in/on Walnut Meats," FMC Corporation, ACG. Richmond, CA. Ran-0142, 11/29/84.

3. Chemistry Branch Tolerance Support review Conclusion 6a(2) of an FMC resubmission for EPA Pesticide Number 6F3454. Cover letter to EM Cuirle from George LaRocca dated 1/15/91.

X. APPENDICES

A. Chromatograms

Index to Chromatograms

Figure	Description	Amount Injected
2	0.125 ug/ml BIFENTHRIN Std	0.375 ng
3	Pecan Meat Control	7.5 mg
4	Pecan Meat Fortification (+ 0.25 ppm)	3.0 mg
5	Pecan Fortification (+ 1.0 ppm)	1.5 mg
6	0.125 ug/ml BIFENTHRIN Std	0.375 ng
7	Walnut Meat Control	7.5 mg
8	Walnut Meat Fortification (+ 0.05 ppm)	7.5 mg
9	Walnut Meat Fortification (+ 1.0 ppm)	1.5 mg

The chromatograms were reduced to 35% of the original size

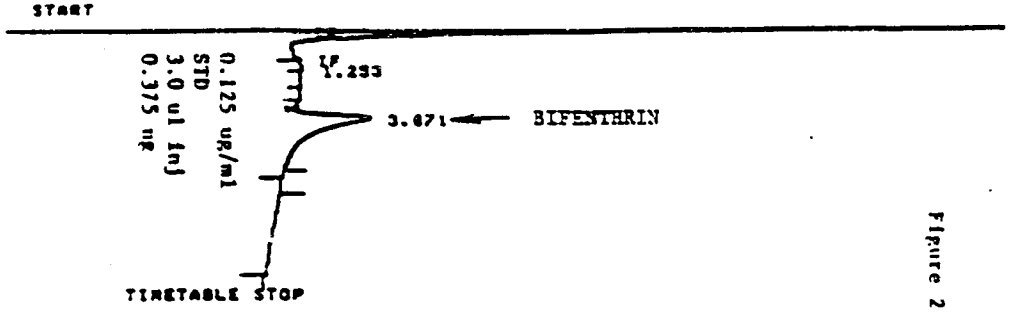


Figure 2

Closing signal file M:\G91E63CC.SMC

RUN# 11 OCT 4, 1989 16:16:11

SAMPLE NAME: 0.125 ng/µl Bifenthrin STD = 0.05 ppm SAMPLE# 11

GC Run #209C

RUN # 7 OCT 4, 1989 15:29:18
START

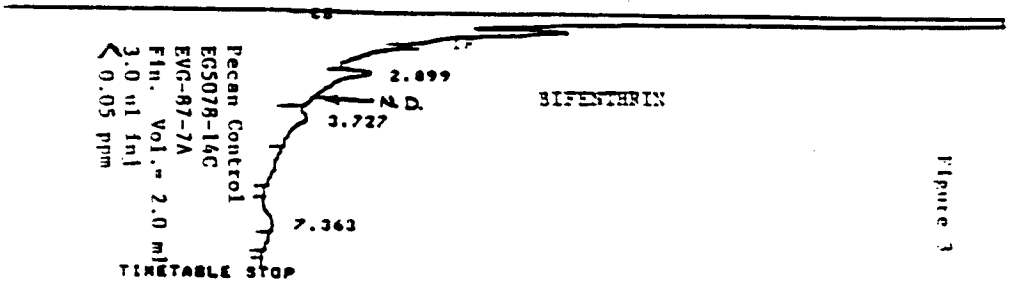


Figure 3

Sample Chromatograms of Bifenthrin Validation Analyses in Pecan Nut Meat

Closing signal file M:\G91E58CF.SMC

RUN# 7 OCT 4, 1989 15:29:18

SAMPLE NAME: EG5078-14C SAMPLE# 7

Sample Chromatograms of Bifenthrin Validation Analyses in Pecan Nut Meat

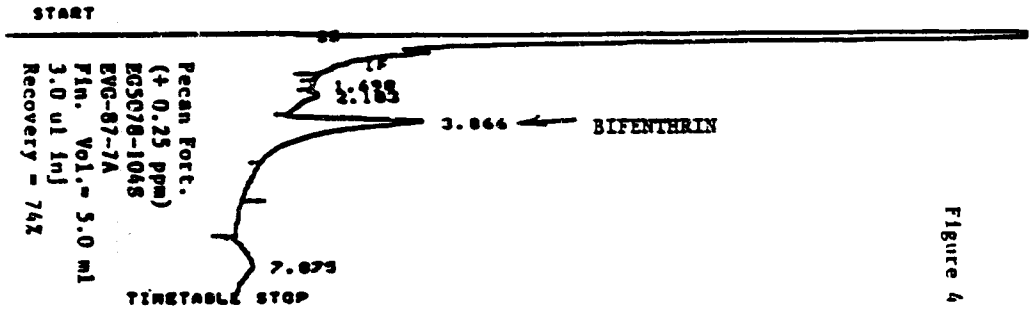


Figure 4

Closing signal file M:891E6948.BHC

RUN# 13 OCT 4, 1989 16:39:35

SAMPLE NAME: EGSC78-104S SAMPLE# 13

GC Run # 20904

RUN # 18 OCT 4, 1989 17:38:06
START

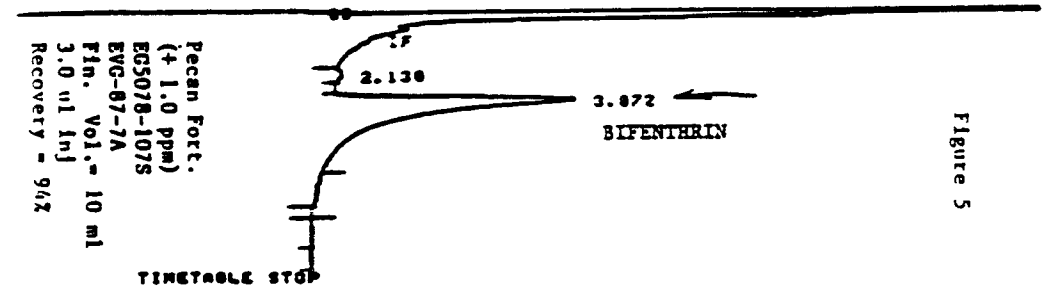


Figure 5

Closing signal file M:891E76FF.BHC

RUN# 18 OCT 4, 1989 17:38:06

SAMPLE NAME: EGSC78-107S SAMPLE# 18

RUN # 13 OCT 6, 1989 17:37:15
START

GC RUN 20960

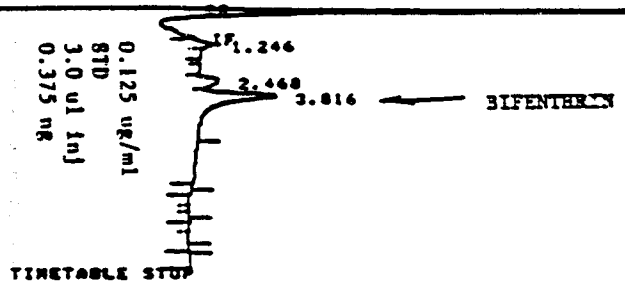


Figure 6

Closing signal file H:G92119CC.BNC

RUN# 13 OCT 6, 1989 17:37:15

SAMPLE NAME: 0.125 ng/mL DCAA in Hexane STD 20.05 ppm SAMPLE# 13

RUN # 9 OCT 6, 1989 16:58:19
START

GC RUN 20960

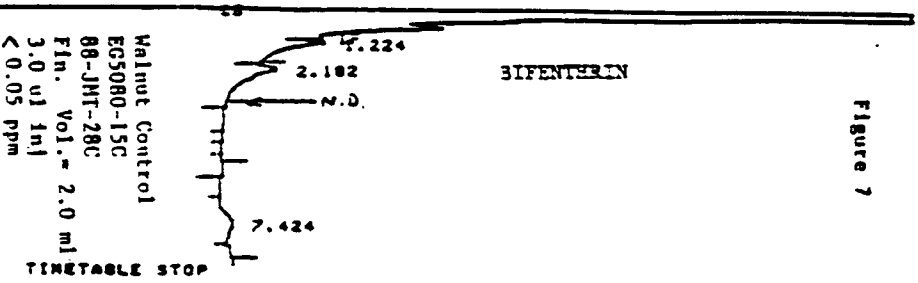


Figure 7

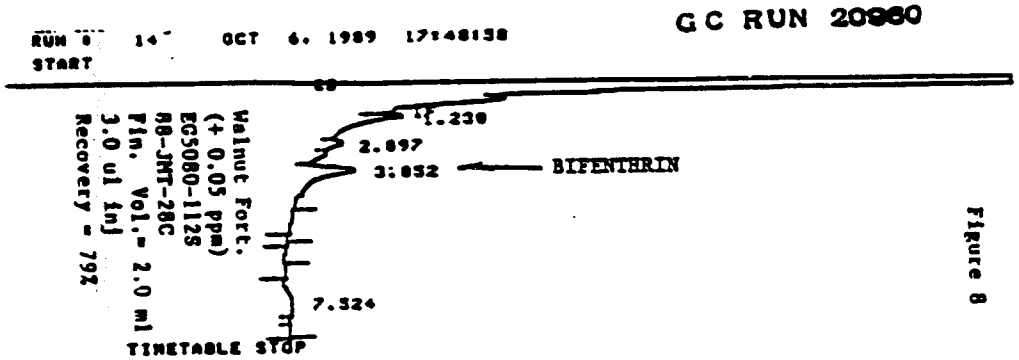
Sample Chromatograms of Bifenthrin Validation Analyses in on Walnut Nut Meat

Closing signal file H:G92118ECC.BNC

RUN# 9 OCT 6, 1989 16:58:19

SAMPLE NAME: EG5080 15C SAMPLE# 9

GC RUN 20980



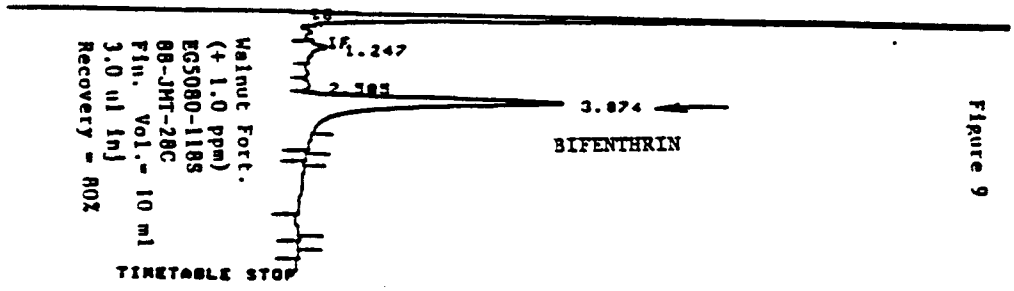
Closing signal file H:892110BA.BHC

RUN# 14 OCT 6, 1989 17:48:58

SAMPLE NAME: EG5080 112S SAMPLE# 14

Figure 8

GC RUN 20980



Closing signal file H:892135A9.BHC

RUN# 23 OCT 6, 1989 19:34:32

SAMPLE NAME: EG5080 118S SAMPLE# 23

Figure 9

Sample Chromatograms of Bifenthrin Validation Analyses in on Walnut Nut Meat

B. Method Validation Outline

Outline of General Method of Analysis for Bifenthrin in Nut Matrices

1. Weigh out 20.0 g of sample into a 32 oz. French square bottle.
2. Add 200 ml acetone to sample and allow to sit for about 10 minutes.
3. Polytron sample for about 2 minutes.
4. Vacuum filter sample into a 500 ml sidearm flask through GF/C filter paper. Rinse quart jar throughly with acetone and add to sample.
5. Rinse filter cake with 40-50 ml acetone.
6. Quantitatively transfer sample using acetone to a 500 ml graduated cylinder and bring up to 280 ml with acetone.
7. Take a 70 ml aliquot (5.0 g sample equivalent) and transfer to a 250 ml Erlenmeyer flask. Store remaining 210 ml of sample in a sealed glass container.
8. Reduce aliquoted sample, using rotary evaporation, down to just an oily residue remains, in a water bath temperature of 30 - 40° C.
9. Transfer oily residue to a 40 ml screw-top, glass centrifuge tube using a total of 25 ml acetonitrile.
10. Add 1.0 ml cyclohexane to the sample in the centrifuge tube.
11. Shake tube for about 30 seconds.
12. Centrifuge sample at low speed for about 10 minutes (a phase separation between the oils and acetonitrile should be evident).
13. Using a Pasteur pipet, draw off the acetonitrile phase (upper phase) and transfer to a 500 ml separatory funnel containing 100 ml of 1g NaCl:20 ml DI H_2O solution.
14. Add 25 ml acetonitrile to the centrifuge tube (still containing the oil) and repeat steps 11, 12, and 13.

15. Partition the NaCl:H_2O:acetonitrile solution (from Step 14) with 100 ml hexanes, shaking for 1-2 minutes.

16. Allow phases to separate.

17. Drain lower phase (aqueous) into another 500 ml separatory funnel and upper phase (organic) through a pre-wetted sodium sulfate pad into a 1000 ml flat-bottom boiling flask.

18. Repeat Steps 16-18 two more times, with 100 ml of hexanes each time, and discard the aqueous phase.

19. Rinse sulfate pad with 50 ml hexanes into the flat-bottom flask.

20. Reduce sample, using rotary evaporation, to less than 5 ml (do not allow the sample to go dry) in a water bath temperature of 35-45° C.

21. Proceed to Florisil column clean-up.

22. Prepare a 12 mm nominal diameter (I.D.) chromatography column (with a 250 ml integral reservoir) by inserting a glass wool plug into the bottom of the column and slurry packing the column with 10.0 g of 3% water deactivated florisil using hexanes. Cap column packing with about 1/2" of granular sodium sulfate.

23. Load sample (from Step 21) onto column with 5 ml hexanes. Allow the solvent to drain to top of the sodium sulfate cap. Discard eluent.

24. Rinse flask (from Step 21) with 100 ml hexanes and transfer to column. Allow the solvent to drain to the top of the sodium sulfate cap. Discard eluent.

25. Add 100 ml of 5% ethyl acetate in hexanes to the column. Allow the column to drain dry into a 250 ml Erlenmeyer flask. (This contains the Bifenthrin).

26. Reduce the sample, using rotary evaporation, to less than 2 ml (do not allow to go dry) in a water bath temperature of 35-45° C.

27. Quantitatively transfer to a 15 ml graduated centrifuge tube using hexanes.

28. Reduce sample, using a needle evaporator with a water bath temperature of 35-40°C, to below the desired final sample volume and reconstitute to the final volume with hexanes.

29. Proceed to gas chromatographic analysis.

GC Analysis

Instrument: HP5890 gas chromatograph equipped with an HP7673A auto-sampler, HP3396A integrator, and an electron capture detector (Ni^{+3}).

Conditions: Injector temperature: 290° C
Detector temperature: 300° C
Column temperature: 220° C (Isothermal)

Carrier Gas: Helium (carrier gas) 10 psi backpressure; 23 ml/min

Make-up Gas: 5% Methane:95% Argon; 60 ml/min.

Column: HP-1 10 m x 0.53 mm 2.65 um film thickness

Injection Volume: 3 ul

Injection Type: Solitless injection technique was used.

C. Study Protocol

EN-CAS ANALYTICAL PROTOCOL

[METHOD VALIDATION PROTOCOL]

TITLE: Method Validation for the Determination of BIFENTHRIN in Pecan and Walnut Substrates and in Alfalfa: Seed, Green Hay, Dry Hay, and Plant Debris

EN-CAS PROTOCOL NUMBER: 89-0021

FMC CORP. STUDY NUMBER: 182MVL89R2
DATA REQUIREMENT: Pesticide Assessment Guidelines, Subdivision O, 171-4: PR Notice 88-5

STUDY DIRECTOR: Rodney M. Bennett, EN-CAS Laboratories

SPONSOR REPRESENTATIVE: Khalid H. Akkari, FMC Corporation

SPONSOR/LOCATION: FMC Corporation
Agricultural Chemical Group
Box 8 US Highway 1
Princeton, New Jersey 08543
PH: (609) 452-2300

TEST FACILITY/LOCATION: EN-CAS Analytical Laboratories
2339 Farrington Point Drive
Winston-Salem, N.C. 27107
PH: (919) 785-3252

TEST/REFERENCE SUBSTANCE: Bifenthrin (FMC 54800)

Substance	Appearance	Source	Date Rec'd	EN-CAS #	Lot/ Batch #	% Purity	Exp Date	Store Cond.
Bifenthrin	White Granules	FMC Corp	3/22/89	EE3746	Invent# 198 E4468-83-105	97.9	10/90	Frzn

Chemical Name: [2-methyl[1,1'-biphenyl]3-yl]-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyclopropanecarboxylate.
Code Name: FMC 54800
CAS No.: 82657-04-3, cis; 83322-02-5, trans

PROPOSED INITIATION DATE: April 19, 1989
PROPOSED COMPLETION DATE: July 31, 1989

OBJECTIVE: The purpose of this study is to verify and validate a proposed method for the determination of bifenthrin (FMC 54800) in pecan and walnut meat and in alfalfa: seed, green hay, dry hay, and plant debris.

Total Number of Pages = 7

EXPERIMENTAL DESIGN:

A) Control Matrix

Pecans and Walnuts:

Control (untreated) samples (as whole nuts) shall be provided by FMC Corporation for the method trials. The matrices for analysis shall be pecan and walnut nut meat.

Alfalfa:

Control (untreated) samples shall be provided by FMC Corporation for the method trials. The matrices for analysis shall be alfalfa seed, green hay, dry hay, and plant debris.

B) Processing

Pecans and Walnuts:

The (pecan and walnut) nut meats shall be separated from the hull. A homogeneous control sample will be prepared by grinding the nut meats in dry ice using a Hobart mixer. These control samples shall be prepared for the pecan and walnut substrates individually.

Alfalfa:

Homogeneous control samples will be prepared by grinding each matrix in dry ice using a Hobart mixer or equivalent.

C) Method of Analysis

Pecans and Walnuts:

The method of analysis shall be the analytical Method I as extracted from FMC Corporation Reports P-1109 and RAN-0142, entitled Analytical Method for the Determination of Bifenthrin in Pecans and Walnuts, as provided by Dr. Khalid H. Akkari, FMC Corporation, Princeton, New Jersey.

Alfalfa:

The method of analysis shall be the analytical Method I as extracted from FMC Corporation reports P-1109 and RAN-0142 entitled Analytical Method for the Determination of Bifenthrin in Pecans and Walnuts, or Method II extracted from FMC Corporation reports P-1073, P-1089 and P-1090 as provided by Dr. Khalid H. Akkari, FMC Corporation, Princeton, New Jersey. Bifenthrin will be quantitated by gas chromatography utilizing electron capture detection.

D) Method Sensitivity/Detectability

Pecans, Walnuts, and Alfalfa:

A screening level (method sensitivity) of 0.05 ppm (ug/gram) bifenthrin shall be used. Method detectability will be set at 0.01 ppm.

E) Program Design

Pecans, Walnuts and Alfalfa:

For validation, one control sample and triplicate fortified samples at each of three levels (0.05 ppm, 0.25 ppm, and 1.00 ppm) shall be analyzed for each matrix. A reagent blank shall be analyzed along with each matrix set. These eleven validation samples per matrix shall be analyzed as listed below:

Sample Listing

Reagent Blank
Control Matrix
Control Matrix + 0.05 ppm bifenthrin
Control Matrix + 0.05 ppm bifenthrin
Control Matrix + 0.05 ppm bifenthrin
Control Matrix + 0.25 ppm bifenthrin
Control Matrix + 0.25 ppm bifenthrin
Control Matrix + 0.25 ppm bifenthrin
Control Matrix + 1.00 ppm bifenthrin
Control Matrix + 1.00 ppm bifenthrin
Control Matrix + 1.00 ppm bifenthrin

F) Quantitation

For all gas chromatographic runs, a standard curve ranging from above the highest quantifiable peak to approximately fifty percent of the screening level shall be run to show that a linear response is obtained from the detector over this standard range. The injection sequence shall be continued as: a (screening level) standard injection followed by two samples; then another (screening level) standard injection followed by two samples. [i.e. standard, sample, sample, standard, sample, sample, ...] Quantitation shall be performed by comparing the peak area of the residue found in each of the two samples with the peak area of the standard immediately preceeding those samples. The standard used for quantitation shall be the same for all samples. The screening level standard shall be that standard which is equivalent to a 0.05 ppm bifenthrin residue, when carried through the analytical method.

Any residue values below the screening level shall be reported in parentheses, provided that the residue is at least one fifth of the peak height of the screening level standard and is twice the peak height of any background (instrument baseline) noise.

Calculation shall be performed on a separate spreadsheet (either for manual or computer calculation). The ratio of the sample to the standard, as well as all other pertinent information required for calculation, will be shown in the spreadsheet. No calculation will be performed on the chromatogram.

REPORTING REQUIREMENTS:

EN-CAS Laboratories will issue one method validation report covering the work performed on all pecan, walnut and alfalfa substrates.

The reporting format shall be according to the Data Reporting Guidelines of Subdivision O, and PR Notice 86-5.

RECORDS TO BE MAINTAINED:

Photocopies of all raw data, transformations or calculations of the raw data, and the final report will be maintained in the Archives of EN-CAS Analytical Laboratories, 2339 Farrington Point Drive, Winston-Salem, North Carolina 27107. All original raw data will be returned to FMC Corporation, Princeton, New Jersey 08543 with the final report.

QUALITY ASSURANCE/GLP STATEMENT:

EN-CAS Analytical Laboratories has established a Quality Assurance Unit (QAU) as a commitment to performing laboratory studies in compliance with current Good Laboratory Practices (GLP) as established by the Environmental Protection Agency. The QAU shall conduct periodic inspections of this study as well as the EN-CAS facility to assure conformance to the study protocol, standard operating procedures, and GLP's. The final report shall be reviewed by the QAU, and a signed statement shall be included which specifies the dates inspections/audits were made and reported to management and the Study Director. The Quality Assurance unit of FMC Corporation will also conduct an inspection and audit the raw data and final report.

REFERENCES:

1) FMC Corporation Method I Actachment 1 letter from Dr. Khalid Akkari January 10, 1989 extracted from FMC reports P-1109 and RAN-0142 Analytical Method for the Determination of Bifenthrin in Pecan and Walnuts.

2) FMC Corporation Method II Attachment 2 letter from Dr. Khalid Akkari January 10, 1989 extracted from FMC reports P-1073, P-1089, and P-1090 Analytical Method for the Determination of Bifenthrin in Strawberries, Pears and Peaches.

3) "Pesticide Assessment Guidelines, Subdivision O Residue Chemistry", U.S. Environmental Protection Agency, Office of Pesticide and Toxic Substances, Washington, D.C. 204060: EPA 540/9-023, October, 1982. NTIS PB83-153981.

4) U.S. Environmental Protection Agency. 1983. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). Federal Register, Vol. 48, No. 230: 53946-53969.

5) U.S. Environmental Protection Agency: 1988. Tolerance Enforcement Methods - Independent Laboratory Confirmation by Petitioner PP Notice 88-5.

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SIGNATURES:

Contract Lab Study Director:

Mr. Rodney M. Bennett — 4/19/89
Mr. Rodney M. Bennett, B.S. — Date
Laboratory Manager

Quality Assurance:

Kathleen H. Faltynski — 4/10/89
Kathleen H. Faltynski, M.S. — Date
Quality Assurance Officer

Sponsor Representative:

K. H. Akkari — 4/20/89
Khalid H. Akkari, Ph.D. — Date
Senior Research Chemist
FMC Corporation