STUDY TITLE: Residue Analytical Method for the Determination of FMC 97285 and FMC 106091 in/on Soybeans Treated with F6285 4F

TEST SUBSTANCE: FMC 97285 and FMC 106091

DATA REQUIREMENT: Pesticide Assessment Guidelines Subdivision O, 171-4: Residue Analytical Method

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429321-09

STUDY DATES:

Study Initiated:	March 1992
Experiment Terminated:	April 1993
Study Completed:	June 1993

PERFORMING LABORATORY: FMC Corporation
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STUDY NUMBER: 162SOY92R1

Non-Proprietary Information
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

John M. Becker 06/29/93

John M. Becker
Manager, Residue Chemistry
SPONSOR

Date

GOOD LABORATORY PRACTICES STATEMENT

The study reported herein (Study number: 162SOY92R1, "Residue Analytical Method for the Determination of FMC 97285 and FMC 106091 in/on Soybeans Treated with F6285 4F", FMC Corporation, Agricultural Chemical Group, P-2811M) was conducted and reported in compliance with the Good Laboratory Practice Standards set forth in Title 40, Part 160 of the Code of Federal Regulations of the United States of America with the following exception.

1. The weather and soil analysis data were not recorded in compliance with GLPs.

audrey w chen — 6/29/93

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Date

QUALITY ASSURANCE STATEMENT

It is the intent of FMC Corporation that all studies sponsored by or conducted by our facility shall be of the highest quality and meet or exceed the criteria promulgated by the EPA to assure the quality and integrity of the data generated. Study 162SOY92R1, "Residue Analytical Method for the Determination of FMC 97285 and FMC 106091 in/on Soybeans Treated with F6285 4F", reported herein, was inspected and the findings signed by the Study Director and management of FMC Corporation on the following dates:

Inspection Date	Signed by Study Director	Signed by Management	Signed by Director
6/09/92	4/07/93	4/08/93	4/09/93
10/01,02/92	10/15/92	10/16/92	10/19/92
10/30/92	4/07/93	4/08/93	4/09/93
11/17/92	4/07/93	4/08/93	4/09/93
12/08/92	4/07/93	4/08/93	4/09/93
2/24/93	2/24/93	2/24/93	2/25/93

This report and all records and raw data were audited and the report was found to be an accurate reflection of the study. All raw data will be maintained by FMC Corporation, PO Box 8, Princeton, NJ 08543 in the Quality Assurance Unit Archives.

Jane W. Brown
Quality Assurance Specialist

6/29/93
Date

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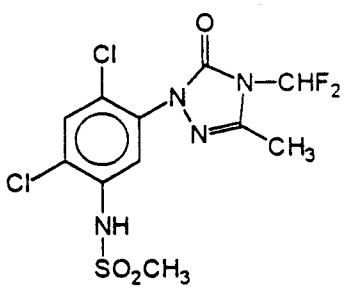
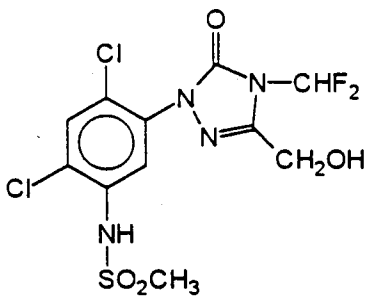
I. INTRODUCTION

F6285 4F herbicide is currently being developed by FMC Corporation for the control of grass and broadleaf weed species encountered in the growing of soybeans. The chemical name of the active ingredient is N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-phenyl]-methanesulfonamide. The compound is code numbered FMC 97285 and has no common name at this time. The major soybean metabolite of F6285 has been identified as 3-hydroxymethyl F6285. The chemical name of the metabolite is N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-hydroxymethyl-5-oxo-1H-1,2,4-triazol-1-yl]-phenyl]-methanesulfonamide, and is code numbered FMC 106091. The chemical structures are as follows:



FMC 97285



FMC 106091

This report describes an analytical method to determine FMC 97285 and FMC 106091 residues in/on soybeans. The report was generated as part of the study 162SOY92R1 to support the determination of FMC 97285 and its hydroxymethyl metabolite in/on soybeans. However, the use of this method report is not limited to a particular study.

The method was developed based on a solvent extraction, two solid phase extraction cartridges, a silylation reaction, and gas chromatographic quantitation.

II. SUMMARY

The analytical method for determination of FMC 97285 and FMC 106091 residues in/on soybeans consisted of an initial reflux with acetone/0.25N HCl (75/25, v/v), filtrations, a C_8 (octyl) solid phase extraction (SPE) cartridge, and a SI (silica gel) cartridge for the final clean-up. Prior to instrumental analysis, a silylation reagent (N,O-bis-(trimethylsilyl)-trifluoroacetamide , BSTFA) was used to convert FMC 106091 to its trimethylsilyl (TMS) derivative to increase its volatility and instrument response. The parent compound was not affected by the silylation reagent, so that both compounds could be analyzed simultaneously.

The final sample solution was quantitated using a gas chromatograph equipped with a HP-50+ Megabore® capillary column (50/50 phenyl methyl silicon) and an electron capture detector. The quantitation of analytes from the matrix was based on an external standard calibration.

Acceptable method recoveries were obtained at a limit of quantitation (LOQ) of 0.025 ppm, and a limit of detection (LOD) was set at 0.005 ppm. The average method recoveries for FMC 97285 and FMC 106091 were 113% (n=7) and 92% (n=10), with standard deviations of ±6 and ±19%, respectively. The higher standard deviation of FMC 106091 is based on combined results from GC/ECD and GC/MSD quantitations. The average recovery and the corresponding standard deviation for FMC 106091 were 84% and ±12%, if recovery data from GC/ECD only were considered.

III. SUMMARY TABLES AND GRAPHICS

A. Summary of Method Recovery Data

TABLE 1

FMC 97285 AND FMC 106091 METHOD RECOVERY VALUES FROM SOYBEAN CONTROL SAMPLES

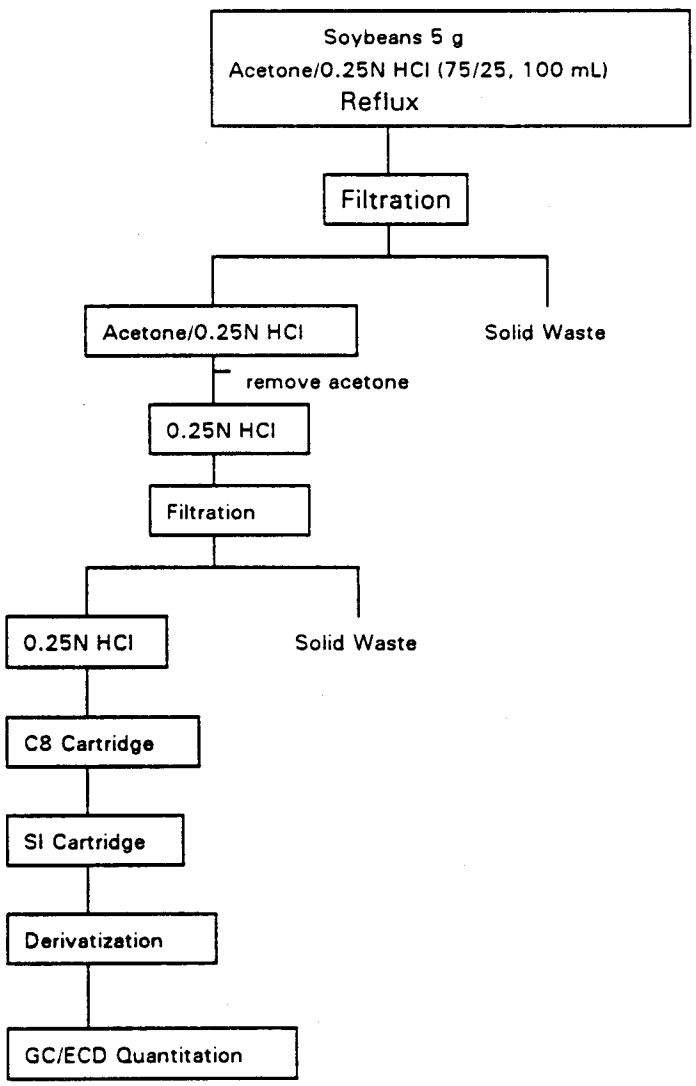
COMPOUND	FORTIFICATION LEVEL (ppm)	NUMBER OF ANALYSES	RECOVERY RANGE (%)	AVERAGE RECOVERY (%)	RECOVERY STD. DEV. (%)
FMC 97285 (ECD)	0.025	7	104 - 120	113	±6
FMC 106091 (ECD)	0.025	7	67 - 101	84	±12
FMC 106091 (MSD)	0.025	3	99 - 132	111	±18

ECD: Electron Capture Detector, MSD: Mass Spectrometry Detector

B. Method Flow Scheme

FIGURE 1

FLOW SCHEME FOR FMC 97285 AND FMC 106091 IN/ON SOYBEAN SAMPLES



IV. MATERIALS AND STUDY DESIGN

A. Test Substance

The chemical names of FMC 97285 and FMC 106091 are N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-phenyl]-methanesulfonamide and N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-hydroxymethyl-5-oxo-1H-1,2,4-triazol-1-yl]-phenyl]-methanesulfonamide, respectively. CAS registration number for FMC 97285 and FMC 106091 are 122836-35-5 and 134390-99-1, respectively. FMC 97285 has no common name at this time. EPA registration is in progress.

B. Test Commodities

The soybeans (seed) used as control samples were harvested at the mature stage, collected and shipped frozen to the FMC Residue Chemistry laboratory at Princeton, NJ. All the samples were maintained frozen (ca. -18°C) during shipping and storage to insure integrity of the samples. The beans were finely ground in a Wiley® mill in liquid nitrogen before analysis.

C. Study Design and Procedures

The residue method was validated with acceptable and reproducible recoveries. A method validation set consisted of one control sample and one fortified control sample. Fortification was accomplished by adding known amounts of FMC 97285 and FMC 106091 standards in acetonitrile directly onto the control sample matrix by a pipette. After fortification the sample was carried through as part of an assay set with the control sample to determine the method recovery.

D. Analytical Standards

The structures and purities of the two analytical standards are shown in Section X, Table 2. A stock solution of 1000 ng/µL was prepared by dissolving appropriate amounts of the above analytical standards in acetonitrile. Working standard solutions containing both analytes, were prepared in acetonitrile by appropriate dilutions from the individual stock solutions on a monthly basis. The working standard solutions ranging from 0.0625 to 1.0 ng/µL, were used for fortification, injection standards, and instrument linearity calibrations. Due to the low instrument response of FMC 106091, the standards were also derivatized with BSTFA at the time of analysis. Stock and working solutions were stored in volumetric containers in a refrigerator/freezer unit to insure maintenance of proper concentrations. Table 4 in Section X lists the index number, concentration, and date of preparation of some of the standard solutions.

E. Equipment

Balance, top-loading (Mettler PM600)
Capillary column, HP-50+ (Hewlett Packard)
Cartridge, C8, 1 g (Varian)
Cartridge, SI, 1 g (J.T. Baker)
Centrifuge tube, Pyrex®, 13 mL, 0.1 mL, graduation
Condenser, Pyrex, Graham coil, 41 mm X 500 mm with ⅋ 24/40 joint
Cylinder, graduated, 100 mL, 250 mL
Filtering flask, 500 mL, Pyrex
Flask, Erlenmeyer, 250 mL, 500 mL, 1000 mL
Flask, boiling, 500 mL with ⅋ 24/40 joint
Flask, volumetric, 100 mL
Gas chromatograph (Hewlett-Packard 5890A with ECD or MSD)
Glass microfibre filters (934-AH, 11.0 cm, Whatman)
Glass tube, 200 mL (ZA7516, Zymark)
Glass tube support rack (ZA7020, Zymark)
Heating mantles (Glas-Col®)
Magnetic stirrer (Model 200, VWR Scientific)
N-Evap® evaporator (Organomation Associates 111)
Pipette (E2-1000, Rainin Instrument Co.)
Porcelain Buchner filter funnel, 10.5 cm i.d., (Coors)
Reservoir (75 mL, Varian)
Solvent dispenser (501, VWR Scientific)
Teflon® stirring bars (VWR Scientific)
Test tube mixer (Thermolyne M16715)
Thomas-Wiley Mill, ED-5 (Thomas Co.)
TurboVap® evaporator (ZW640-3, Zymark)
TurboVap LV evaporator (ZW700, Zymark)
Visidry® vacuum manifold drying attachment (Supelco)
Visiprep® manifold (Supelco)

F. Reagents

Acetone, acetonitrile, ethyl acetate, hexane, methanol, all Resi-analyzed grade solvents (J.T. Baker)
Hydrochloric acid, reagent grade (J.T. Baker)
N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, Pierce)
0.25N HCl solution, prepared from reagent grade HCl
Distilled deionized water (house distilled)

V. ANALYTICAL PROCEDURE

A. Residue Method

1. Acetone/0.25N HCl Reflux

A 5 g macerated soybean subsample was weighed into a 500 mL boiling flask. Fortification was applied at this point by adding 250 uL of 0.5 ng/µL standards (0.125 µg each analyte) in acetonitrile directly onto the control sample by a pipette or a syringe. One hundred mL of acetone/0.25N HCl (75/25, v/v) and a teflon stirring bar were added to the flask, and the flask was attached to a condenser. The heating mantle Variac power supply was set at ~55 and the sample solution was boiled under gentle reflux for one hour.

The sample solution was allowed to cool to room temperature and filtered under vacuum through a Whatman glass microfibre filter (No. 934-AH) with a Buchner funnel. The flask and the sample matrix were rinsed and washed with 50 mL of acetone. The sample solution was transferred to a 200 mL Zymark glass tube. The solution was concentrated to a volume of less than 20 mL to remove the acetone by a TurboVap evaporator (ZW640-3, water bath temperature at ~45°C). The concentrated solution was again filtered under vacuum through another Whatman glass microfibre filter. This time the flask and the sample matrix were rinsed and washed with 50 mL of 0.25N HCl.

2. C_8 Cartridge

A C_8 SPE cartridge (1 g, 6 cc) was conditioned first with 12 mL of methanol, and then with 12 mL of 0.25N HCl on a vacuum manifold. The vacuum was turned off and a 75 mL reservoir was attached to the cartridge with an adaptor. The cartridge was kept wet and the acid filtrate (~70 mL) was passed through the cartridge by regulating the vacuum. The solution flow rate was maintained at ~5 mL/min. After loading, the cartridge was completely blown dry with nitrogen on a manifold drying attachment (~30 min). The residues were eluted with 12 mL of ethyl acetate/hexane (20/80, v/v) and collected in a 13 mL graduated test tube at a flow rate of ~2 mL/min. The solution was further concentrated to ~1 mL with an N-Evap or a TurboVap LV evaporator (ZW700). The temperature of the water bath was kept between 40 and 45°C.

3. SI (Silica gel) cleanup cartridge

The SI SPE cartridge was conditioned first with 12 mL of ethyl acetate/hexane (60/40, v/v) and then with 12 mL of hexane. The sample solution was loaded directly to the cartridge barrel by a pipette. One mL of ethyl acetate/hexane (20/80, v/v) was used to rinse the test tube and then was transferred to the cartridge. The cartridge was washed with 12 mL of ethyl acetate/hexane (20/80, v/v). Next, the residues were eluted with 12 mL of ethyl acetate/hexane (60/40, v/v) and collected in a graduated test tube. The sample solution was concentrated to dryness by an N-Evap and acetonitrile was added to ~1 mL.

4. Derivatization

One hundred μL of N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA)was added to the sample solution. The test tube was capped and the solution was mixed well. The test tube was then heated in a water bath at ~70°C for one hour. After reaction, the sample solution was concentrated to dryness by an N-Evap and acetonitrile was added to exactly 1.0 mL. The final solution was quantitated by a gas chromatograph equipped with an electron capture detector.

B. Instrumentation

The FMC 97285 and FMC 106091 residues were quantitated by a Hewlett-Packard 5890A gas chromatograph equipped with an electron capture detector, a HP 7673B autosampler, and a HP 3392A integrator. An equivalent gas chromatograph, integrator, data acquisition system, and autosampler can be used. A HP-50+ megabore capillary column was used to separate and analyze the residues. Section XII, Appendix A1 shows one example of the operating conditions. The chromatographic conditions can be modified or optimized for best resolution and detection sensitivity.

C. Method Validation and Quality Control

A method validation set consisted of one control sample and one fortified sample for each of seven field trials. The analytical method recovery was determined by the results from the fortified control samples. All of the control samples were determined to be free of FMC 97285 and FMC 106091 residues.

D. Method of Calculation

The magnitude of FMC 97285 and FMC 106091 residues in each sample were determined by an external standard calibration method based on the average of all run standards in an assay set. The run standard solution was injected at the beginning of every set and subsequently after every two sample solutions. The amount of analytes was quantitated from the detector response transmitted to the data acquisition system. The responses as peak areas were calculated as nanograms (ngs) of FMC 97285 and FMC 106091 based on the injection of run standards. The nanogram value reported was calculated by comparing the area units of unknown sample to the average run standard using the following formula:

$$\text{ng of analyte in sample} = \frac{\text{area unit (sample)}}{\text{average area (standard)}} \times \text{ng (standard)}$$

Amount of sample injected was calculated based on the initial 5 g sample weight, 1 mL of the final sample solution volume, and the 2 µL of injection volume. Results of each analysis were reported on a ppm (µg/g) basis by using the following formula:

$$\text{ppm (µg/g)} = \frac{\text{ng of analyte in sample}}{\text{mg of sample injected}}$$

Method recovery was then obtained by comparing the analyte amount recovered from the sample to the initial fortification level.

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

Since all of the standards were derivatized the same as the sample solutions, the calculations of FMC 106091 residue level were based on the amount of FMC 106091, without correcting for the molecular ratio between FMC 106091 and its TMS derivative. The calculations, therefore, were exactly the same for both analytes. An example of how to calculate the method recovery of FMC 106091 in a fortified soybean sample (Figure 6) is given below:

ng (Standard) = 2.0 µL x 0.125 ng/µL = 0.25 ng

Average area units of standards = 584857

Area units of fortified soybean sample = 580780

ng of FMC 97285 in sample $= \frac{580780 \times 0.25 \text{ ng}}{584857} = 0.248$ ng

mg of sample injected $= \frac{5000 \text{ mg}}{1000 \text{ µL}} \times 2\text{µL} = 10$ mg

FMC 97285 content (ppm) $= \frac{0.248 \text{ ng}}{10 \text{ mg}} = 0.0248$ ppm

Method recovery (%) $= \frac{0.0248 \text{ ppm}}{0.025 \text{ ppm}} \times 100\% = 99\%$

E. Confirmatory Techniques

A mass spectrometry detector could be used to confirm FMC 97285 and FMC 106091 residues in the final sample solution, if needed. An example of operating conditions for a Hewlett-Packard 5890A gas chromatograph equipped with a mass selective detector 5970 and a HP 7673A autosampler to confirm the FMC 106091 residues, are listed in Section XII, Appendix A2. The monitored ion m/e 459, the M-15 peak, resulted from loss of methyl from the TMS derivative molecular ion . The prominent M-15 peak determined by MSD for the TMS derivative compounds was in agreement with the literature results (Section XI, Reference 1).

F. Interferences

1. **Sample Matrices** - No interference was noted in the control soybean samples near the limit of detection level.

2. **Other Pesticides** - No interference due to other pesticides was expected.

3. **Solvents and Labware** - No interference was observed from solvents and labware.

G. Time Required for Analysis

The analytical procedures required approximately 10 hours for each sample set. During this time, one person can complete a set of eight samples from initial weighing of samples to gas chromatographic measurement.

H. Modifications or Potential Problems

1. Removing acetone completely from the sample solution before loading to the C_8 cartridge was critical to achieve adequate method recovery. Traces of acetone in the aqueous acid solution seemed to allow some of the analyte to pass through the cartridge rather than being retained on the C_8 cartridge.

2. C_{18} SPE cartridges were also tested. Acceptable method recoveries could be obtained for FMC 97285. However, recoveries for FMC 106091 were not satisfactory.

3. The second filtration step after acetone removal was necessary to eliminate some of the matrix background elements, which would otherwise show in the chromatogram.

4. Before adding 1.0 ml of acetonitrile in the final solution, the eluants from the SI cartridge had to be evaporated to dryness to assure the removal of any excess silylation reagent.

5. Conditioning the GC detection system with control or fortified samples before the actual run of the set is recommended. Programing the GC oven temperature to a higher final (post-run) temperature is also recommended after initial run conditions to bake out any possible matrix interferences.

6. Interference peaks sometimes could be observed when the soybean samples were from different trial locations. Switching the derivatization and the silica gel clean-up steps was found to be an effective means to eliminate such interferences. However, the sample solutions eluted from C_8 should be reconstituted into acetonitrile before the derivatization, and after reaction the solutions should be reconstituted into an appropriate combination of ethyl acetate and hexane before silica gel clean-up. Detailed stepwise procedures of the modified method can be found in Appendix B, if needed.

VI. STORAGE STABILITY

FMC 97285 and FMC 106091 analytical standards were assayed by the FMC Corporation Analytical Chemistry Department on a regular basis for percent purity. The standards had a proven pattern of stability. Stock solutions (1000 µg/mL) were prepared annually in acetonitrile. Fresh dilute solutions were prepared at suitable concentrations on a monthly basis. All solutions were stored in volumetric containers in a refrigerator/freezer unit and had proven stability for their respective storage periods.

VII. RESULTS AND DISCUSSION

A. Accuracy and Precision

The accuracy and the precision of the analytical method were determined by the average recovery and standard deviation of the results from the fortified control samples. Table 1 in Section III presents the average method recoveries and standard deviations for all the measurements. The average method recoveries for FMC 97285 and FMC 106091 for 7 analyses based on GC/ECD results were 113 and 84%, with standard deviations of $\pm$6 and $\pm$12%, respectively. The individual method recovery data can be found in Section X, Table 5.

B. Limits of Detection and Quantitation

The limit of quantitation (LOQ) has been validated at 0.025 ppm based on acceptable fortified recovery values. The limit of detection (LOD) has been set at 0.005 ppm. Any response below the limit of detection is considered non-detectable (ND).

C. Ruggedness Testing

The method was developed on soybean seed samples. The results of the average method recovery and the standard deviation indicated that this method is reliable and accurate.

D. Limitations

No potential limitations have been experienced during the assay analyses.

VIII. CONCLUSION

The analytical method utilizing an acetone/acid reflux, a C_8 SPE cartridge, a silica gel clean-up cartridge, and a silylation reagent was developed to simultaneously determine FMC 97285 and FMC 106091 residues in/on soybeans. This method was successfully performed at a limit of quantitation of 0.025 ppm. Also, the method was fast, easy to perform, and consumed only small volumes of solvents per sample.

All of the equipment needed to perform the analysis, e.g., gas chromatograph with an electron capture detector, is readily available in most residue analytical laboratories. An experienced residue analyst following the procedure exactly as written, and being aware of the possible potential problems, should not experience interference problems and should obtain adequate recoveries.

IX. CERTIFICATION

We, the undersigned, hereby declare that this study was performed under our supervision according to the procedures herein described, and that this report provides a true and accurate record of the results obtained.

Audrey Chen

Audrey W. Chen
Senior Research Chemist
AUTHOR/STUDY DIRECTOR

6/29/93
Date

John M. Becker

John M. Becker
Manager, Residue Chemistry
SUPERVISOR

06/29/93
Date

ADDITIONAL STUDY PERSONNEL:

Harvey R. Wendt, Research Technician
Dave Baffuto, Research Technician

X. TABLES AND FIGURES

TABLE 2

REFERENCE SUBSTANCE

COMPOUND	CHEMICAL NAME/STRUCTURE	FMC NUMBER	REFERENCE NUMBER	PERCENT PURITY
FMC 97285	N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-phenyl]-methanesulfonamide	97285	RIN 224	99.8
FMC 106091	N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-hydroxymethyl-5-oxo-1H-1,2,4-triazol-1-yl]-phenyl]-methanesulfonamide	106091	E6806:80A	97.2

TABLE 3

RECORD OF SOYBEAN TRIAL DATES

TRIAL LOCATION	RECEIVING NUMBER	SAMPLING DATE	SHIPMENT DATE	RECEIVING DATE	PREPARATION DATE	EXTRACTION DATE	ANALYSIS DATE	STORAGE LENGTH (months)
GA	PRI-173	11/17/92	12/01/92	12/14/92	01/08/93	02/04/93	02/11/93	~3
LA	PRI-175	11/17/92	12/04/92	12/14/92	01/08/93	02/19/93	02/23/93	~3
NE	PRI-135	09/24/92	09/26/92	10/09/92	01/08/93	02/04/93	02/11/93	~4
TN	PRI-181	11/09/92	12/02/92	12/14/92	01/08/93	02/04/93	02/11/93	~3
IL	PRI-150	10/01/92	10/19/92	10/28/92	01/08/93	02/10/93	02/17/93	~4
MN	PRI-168	10/30/92	11/14/92	11/19/92	01/08/93	02/10/93	02/17/93	~3
OH	PRI-139	10/18/92	10/20/92	10/21/92	01/08/93	03/01/93	03/03/93	~4

TABLE 4

REFERENCE SOLUTIONS OF FMC 97285 AND FMC 106091

STANDARD SOLUTION INDEX NUMBER	COMPOUND	SOLUTION SOLVENT	CONCENTRATION (ng/µL)	DATE PREPARED
509	FMC 106091	acetonitrile	1000	04/06/92
514	FMC 97285	acetonitrile	1000	05/06/92
579-1	FMC 97285 + FMC 106091	acetonitrile	10 (each)	02/02/93
590	FMC 97285	acetonitrile	1000	03/23/93
592	FMC 106091	acetonitrile	1000	03/23/93
593-7	FMC 97285 + FMC 106091	acetonitrile	10 (each)	04/12/93

Dilute reference solutions were prepared from the above reference solutions.

TABLE 5

METHOD RECOVERY OF FMC 97285 AND FMC 106091 FROM LABORATORY FORTIFIED CONTROL SOYBEAN SAMPLES

SAMPLE IDENTIFICATION	ASSAY NUMBER	FORTIFICATION LEVEL (ppm)	CONTROL BACKGROUND (ppm)	FMC 97285 RECOVERY (%)	FMC 106091 RECOVERY (%)
92-HGH-01C	1-2	0.025	ND[1]	120	80
92-SJS-01C	5-2	0.025	ND	111	76
92-GJZ-1C	2-2	0.025	ND	113	101
92-HRM-100C	2-6	0.025	ND	104	79
92-RSP-011C	3-2	0.025	ND	110	83
92-LKF-170C	3-6	0.025	ND	112	67
92-HLG-17C	4-2	0.025	ND	120	99
92-GJZ-1C	6-2	0.025	ND	NA[2]	103[3]
92-HRM-100C	6-6	0.025	ND	NA	132[3]
92-HLG-17C	6-10	0.025	ND	NA	99[3]
		OVERALL AVG		113 (n=7)	92 (n=10)
		OVERALL STD. DEV.		±6	±19

1. ND = Non-Detectable (< 0.005 ppm)
2. NA = Not-Analyzed
3. Determined by GC/MSD, others by GC/ECD

XI. REFERENCES

1. Pierce, A.E., "Silylation of Organic Compounds," Pierce Chemical Co., 1968

XII. APPENDICES

A. Instrument Parameters

1. **GC/ECD**

COLUMN:	HP-50+, 50% phenyl methyl silicone, 30 m x 0.53 mm x 1.0 µm
INLET:	Direct injection (260°C)
Inlet Sleeve:	Single gooseneck
OVEN TEMP:	
Initial Temp:	200°C
Initial Time:	4 min
Rate:	20°C/min
Final Temp:	260°C
Final Time:	12 min
Rate A:	20°C
Final Temp A:	280°C
Final Time A:	6 min
DETECTOR TEMP:	300°C
GAS FLOW RATE:	He, carrier, ~7.5 mL/min Ar/CH_4, make-up, ~42 mL/min
RETENTION TIME:	~16 min (FMC 97285) ~21 min (FMC 106091 TMS derivative)

Equivalent GC and GC columns can be used to determine the FMC 97285 and FMC 106091 residues. Chromatographic conditions can be modified or optimized for best detection sensitivity.

2. **GC/MSD**

COLUMN:	DB-5, 5% phenyl methyl silicone, 15 m x 0.25 mm x 0.25 µm
INLET:	Splitless mode (270°C)
OVEN TEMP:	
Initial Temp:	120°C
Initial Time:	2 min
Rate:	27°C/min
Final Temp:	280°C
Final Time:	5 min
DETECTOR TEMP:	280°C
GAS FLOW RATE:	He, carrier, ~1 mL/min
RETENTION TIME:	~7.9min (FMC 106091 TMS derivative)
ION MONITORED:	459 (FMC 106091 TMS derivative)

Equivalent GC/MSD and GC columns can be used to determine the FMC 106091 residues. Chromatographic conditions can be modified or optimized for best detection sensitivity.

B. Modified Method (when interferences were observed)

1. Weigh 5 g soybean sample into a 500 mL boiling flask. Apply fortification at this step. Add 100 ml of acetone/0.25N HCl (70/30, v/v) and a teflon stirring bar (or boiling chips). Set heating mantle Variac at ~55 and gently boil the sample under reflux for an hour.

2. Cool the sample solution to room temperature and filter the solution through a Whatman glass microfibre filter (No. 934-AH) with a Buchner funnel under vacuum. Rinse the flask and post-reflux solid with 100 mL of acetone.

3. Remove the organic solvent (acetone) from the sample solution and concentrate the solution to a volume of less than 20 mL by a TurboVap.

4. Filter the solution with another Whatman glass microfibre filter and rinse the flask and solid with 50 mL of 0.25N HCl.

5. Install a C_8 cartridge (1 g/6 cc, Varian) on a vacuum manifold. Condition the cartridge first with 12 mL of methanol, and then with 12 mL of 0.25N HCl.

6. While the cartridge remains wet, attach a 75 mL reservoir to the cartridge with an adaptor and pass the sample solution through the C_8 cartridge. Maintain the solution flow rate at ~5 mL/min by regulating a vacuum. Rinse the flask with ~10 mL of 0.25N HCl and transfer the rinse to the reservoir.

7. Blow the cartridge completely dry by nitrogen with a manifold drying attachment (~30 min).

8. Elute and collect the analytes with 12 mL of ethyl acetate/hexane (20/80, v/v) in a 13 mL test tube. Concentrate the solution to near dryness by a N_2-Evap or a TurboVap evaporator. Add 1 mL of acetonitrile.

9. Add 100 ul of concentrated BSTFA. Mix the solution well and cap the test tube. Place the test tube (with rack) in water bath for an hour with temperature at ~70°C.

10. Remove the test tubes from the water bath. N_2-Evap the sample solution to dryness (avoid overdry) and add 1 mL of ethyl acetate/hexane (20/80, v/v).

11. Condition a SI cartridge (1 g/6 cc, Baker) first with 12 mL of ethyl acetate/hexane (60/40, v/v), and then with 12 mL of hexane. Load the sample solution in the cartridge barrel. Rinse the test tube with 1 mL of ethyl acetate/hexane (20/80, v/v), and transfer the rinse to the cartridge. Wash the cartridge with 12 mL of ethyl acetate/hexane (20/80, v/v). Elute and collect the analytes with 12 mL of ethyl acetate/hexane (60/40, v/v).

12. N_2-Evap (or TurboVap) the sample solution to dryness (avoid overdry) and make the final volume exactly 1.0 mL with acetonitrile.

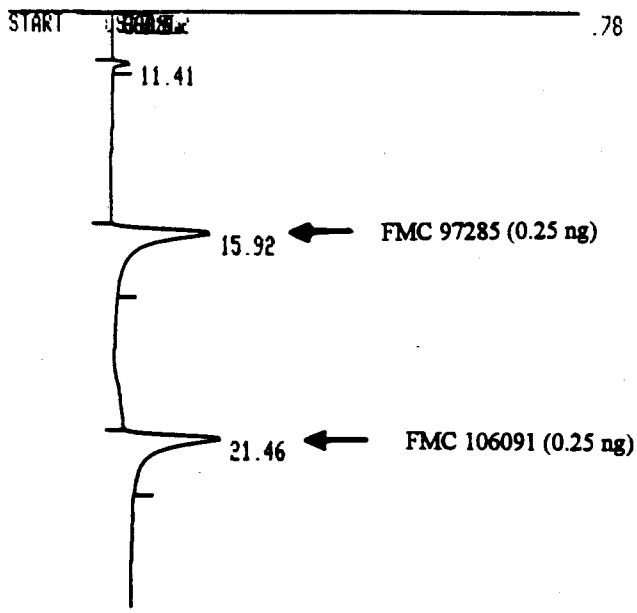
13. GC-ECD analysis.

C. Chromatograms

FIGURE NUMBER	DESCRIPTION
2	Standard, 0.25 ng (each analyte)
3	Soybeans, Control Sample (#3-1)
4	Soybeans, Fortified Sample (#3-2)
5	Soybeans, Control Sample (#4-1)
6	Soybeans, Fortified Sample (#4-2)

FIGURE 2

STANDARD (FMC 97285 + FMC 106091, #579-5)
2 µL X 0.125 ng/µL



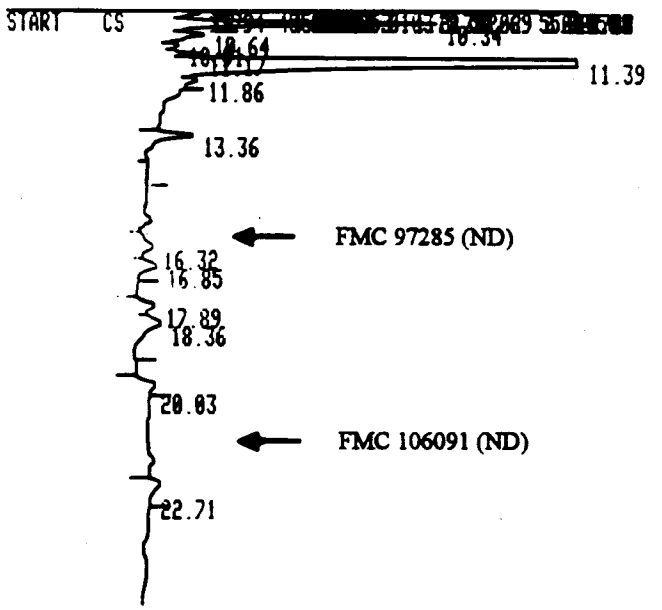
RUN # 560 MAR/03/93 15:30:35
WORKFILE ID: C
WORKFILE NAME:
SAMPLE # 8

ESTD

RT	AREA	TYPE	CAL #	AMOUNT
15.92	629680	PB	1R	0.193
21.46	578650	BB	2	0.212

FIGURE 3

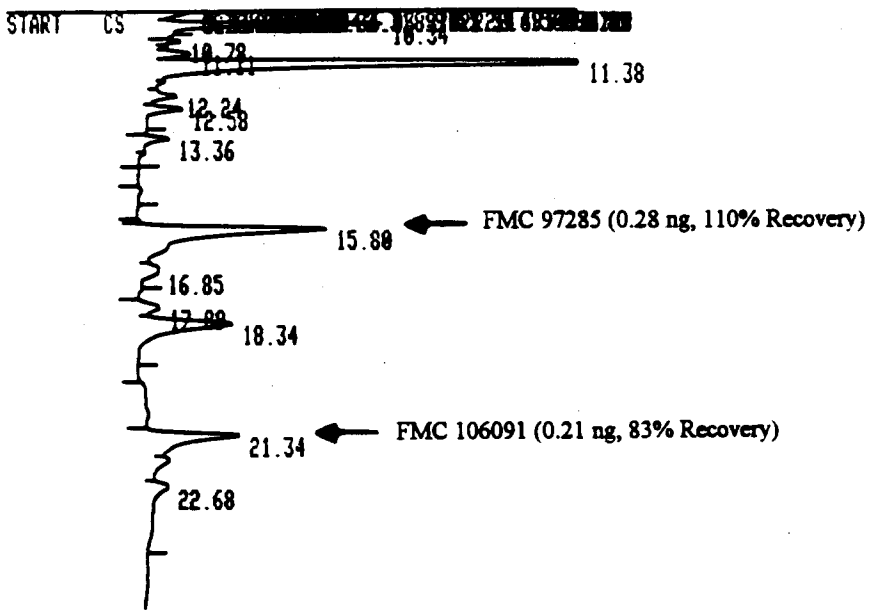
SOYBEAN, CONTROL
(PRI-150, 92-RSP-011C, #3-1)
10 mg injected



RUN: : 423 FEB/17/93 17:18:38
WORKFILE ID: C
WORKFILE NAME:
SAMPLE # 4
NO CALIB PEAKS FOUND

FIGURE 4

SOYBEAN, FORTIFIED @ 0.025 ppm
(PRI-150, 92-RSP-011C, #3-2)
10 mg injected

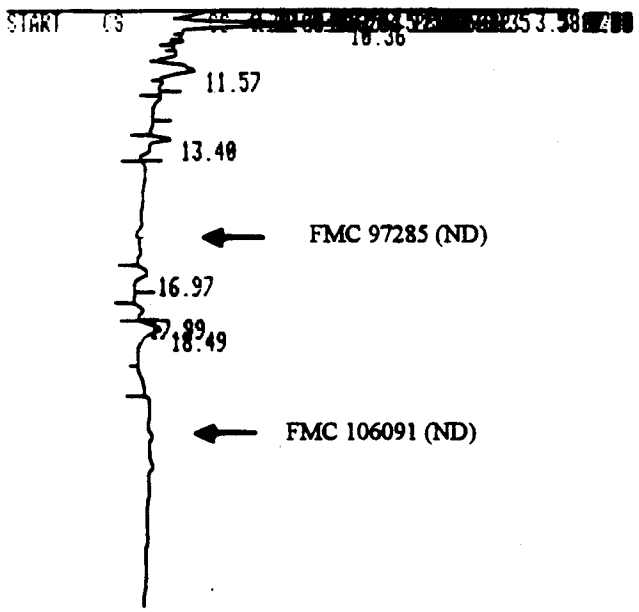


RUN # 424 FEB/17/93 17:49:07
WORKFILE ID: C
WORKFILE NAME:
SAMPLE # 5

ESTD RT	AREA	TYPE	CAL #	AMOUNT
15.80	788730	BV	1R	0.242
21.34	417960	PV	2	0.153

FIGURE 5

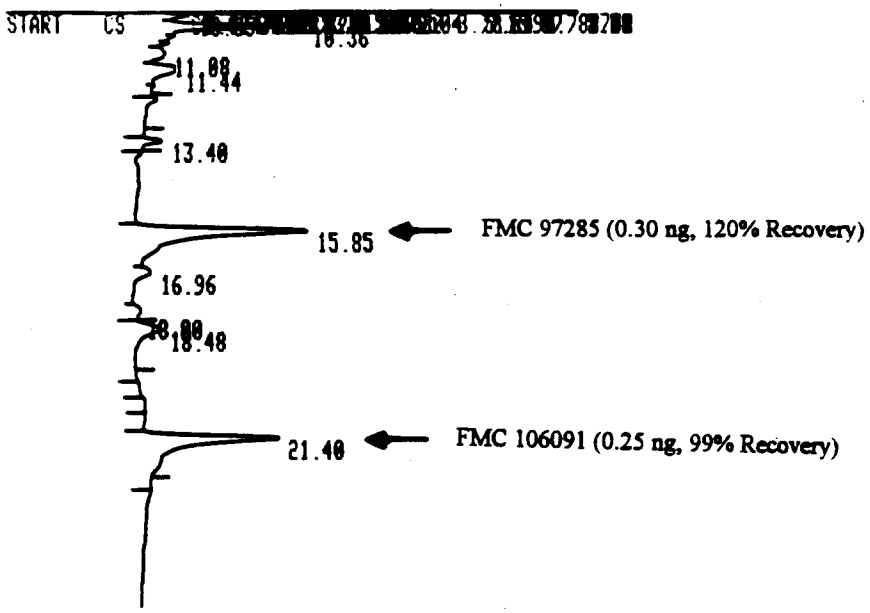
SOYBEAN, CONTROL
(PRI-139, 92-HLG-17C, #4-1)
10 mg injected



RUN # 555
WORKFILE ID: C
WORKFILE NAME:
SAMPLE # 3
NO CALIB PEAKS FOUND

FIGURE 6

SOYBEAN, FORTIFIED @ 0.025 ppm
(PRI-139, 92-HLG-17C, #4-2)
10 mg injected



RUN # 556 MAR/03/93 13:28:39
WORKFILE ID: C
WORKFILE NAME:
SAMPLE # 4

ESTD

RT	AREA	TYPE	CAL #	AMOUNT
15.85	754130	BV	1R	0.232
21.40	580780	PB	2	0.212