Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Clomazone

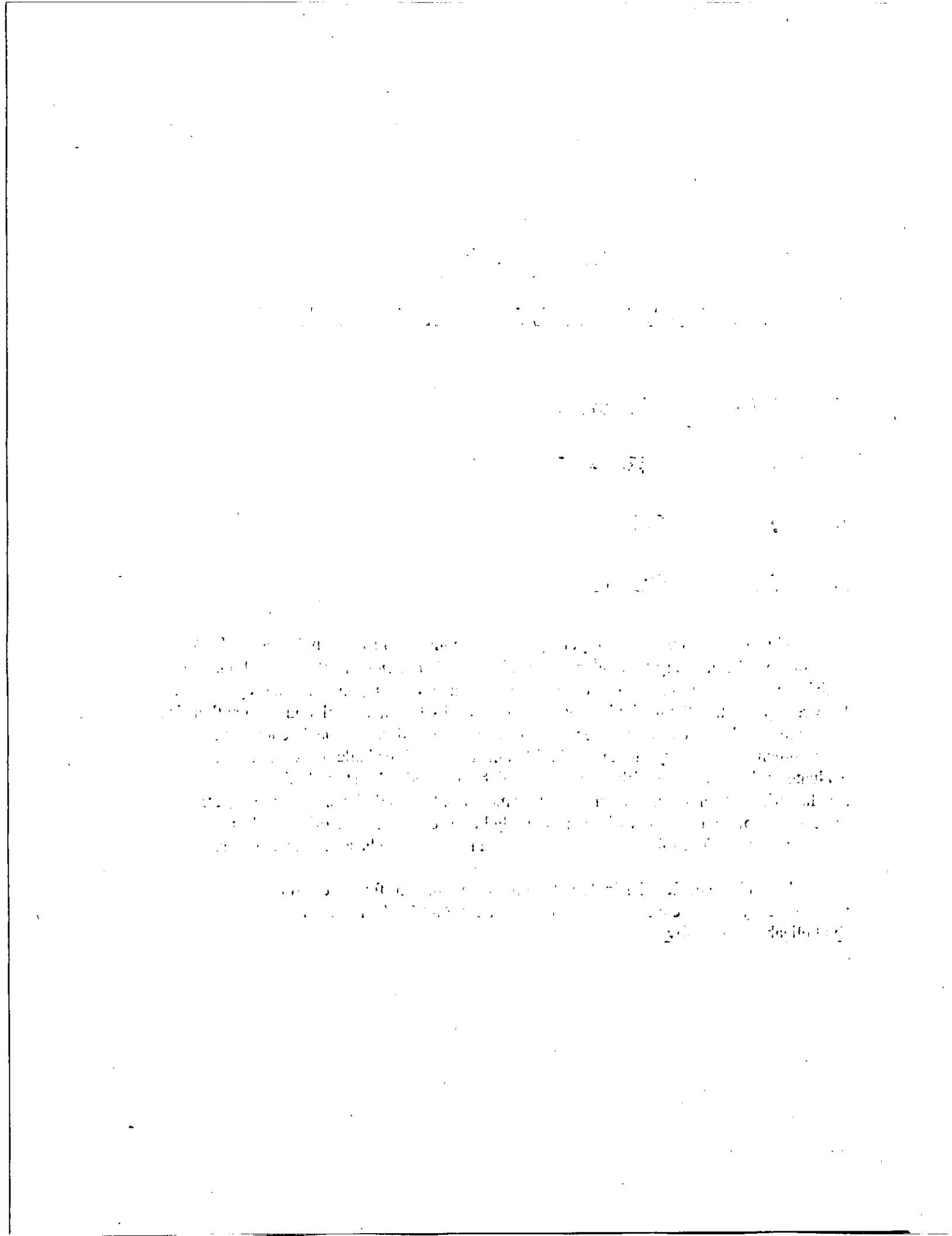
MRID #: 443484-07

Matrix: Soil

Analysis: GC/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.

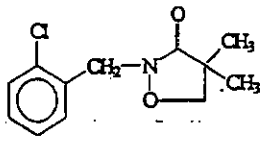
If you have difficulties in downloading the method, or further questions concerning the methods, you may contact Elizabeth Flynt at 228-688-2410 or via e-mail at flynt.elizabeth@epa.gov.



FMC CORPORATION
FMC Study Number: 164E4295E1
Battelle Study Number: N001417D

1. INTRODUCTION

Clomazone, the active ingredient in Command 3ME Herbicide, is being developed for registration on rice. Command 3ME contains 3 pounds of active ingredient per gallon formulated as a micro-encapsulation. The chemical name of clomazone is 2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone. The chemical structure of clomazone is as follows:



Clomazone

An aquatic field dissipation study was conducted at two sites (Proctor, AR and Pattison, TX) in the southern United States (Reference 1). Two plots per trial site were planted with rice, with one plot being treated once with Command 3ME at the rate of 0.6 lbs ai/acre. At the AR site, treatment was made as an early post-emergent broadcast application. At the TX site, treatment was made as a pre-emergent broadcast application. The second plot at each site was untreated and served as the control plot. The plots were on agriculturally viable lands at sites that are representative of key rice growing areas in the southern United States. Soil and water samples from the sites were analyzed to determine the dissipation characteristics of the test material. Based on the method in FMC report P-2640 (Reference 2), a residue analytical method was modified for clomazone and two of its metabolites, FMC 55657 and FMC 65317 in soil and water. This report describes the modified residue analytical method for clomazone, FMC 55657, and FMC 65317 in soil and water.

II. SUMMARY

A method was developed and validated for the measurement of clomazone, FMC 55657, and FMC 65317 in soil and water by gas chromatography - mass selective detection, (GC-MSD) in the selected ion monitoring (SIM) mode. The method was based on the method in FMC Report P-2640. Modifications were made to accommodate the metabolites.

Clomazone, FMC 55657, and FMC 65317 residues were extracted from a five-gram sample of soil by sonicating with acidic methanol. Water samples were filtered through glass fiber filters and then acidified. The soil extract and acidified water samples were cleaned with a C18 solid phase extraction (SPE) cartridge followed by a florisil cartridge. The eluate was concentrated, solvent exchanged into acetonitrile, and then derivatized using N-methyl-bis-trifluoroacetamide (MBTFA). The derivatized eluate was analyzed by GC-MSD in the SIM mode. The concentrations of clomazone, FMC 55657, and FMC 65317 were calculated from a least squares linear regression curve of analytical standards using the peak area response.

The limits of detection and quantitation are 10 ppb and 50 ppb for soil and 0.20 ppb and 1.0 ppb for water, respectively. The standard curve was linear over the concentration range of 0.05 to 1.5 µg/mL and showed an acceptable degree of variation in the slope of the regression line. Accuracy and precision of the method were assessed by measuring the recovery and the standard deviation found for control soil and water samples fortified with clomazone, FMC 55657, and FMC 65317. The degree of accuracy and precision was acceptable at all concentration levels and the method was consistent between the two matrices. Recoveries obtained for clomazone from soil and water averaged 76% and 79% with relative standard deviations of 8% and 7%, respectively. The average recovery obtained for FMC 55657 in both soil and water was 113% with relative standard deviations of 10% and 16%, respectively. FMC 65317 gave average recoveries of 98% from soil and 95% from water, with relative standard deviations of 11% and 12%, respectively.

The method was radio-validated by analyzing a soil sample which had been prepared as part of an aerobic aquatic metabolism study. Radioactivity measurements indicated that the method was able to extract an average of 95% of the total residue. The nature of, and concentration of, the residues detected by the method were comparable to the results obtained during the metabolism study.

FMC CORPORATION
FMC Study Number: 164E4295E1
Battelle Study Number: N001417D

III. SUMMARY TABLES AND GRAPHICS

A. Method Recoveries

TABLE 1A

METHOD RECOVERY VALUES FOR CLOMAZONE FROM LABORATORY FORTIFIED SOIL AND WATER

Matrix	Analyte	Fortification Level (ppb)	Number of Analyses	Recovery Range (%)	Average Recovery (%)	Standard Deviation (%)	Relative Standard Deviation
Soil	clomazone	50	3	68 - 81	76	7	10
		250	2*	74 - 79	76	3	4
		500	3	68 - 85	76	9	12
		Summary:	8	68 - 85	76	6	8
Water	clomazone	0.63	3	73 - 87	79	7	9
		3.13	3	79 - 86	83	3	4
		6.25	3	72 - 78	75	3	4
		Summary:	9	72 - 87	79	5	7

Number of observations outside 70-120% range = 2

* One sample lost during preparation.

FMC CORPORATION
FMC Study Number: 164E4295E1
Battelle Study Number: N001417D

PC-0271M
Page 12

TABLE 1B

METHOD RECOVERY VALUES FOR FMC 55657
FROM LABORATORY FORTIFIED SOIL AND WATER

Matrix	Analyte	Nominal Fortification Level (ppb)	Number of Analyses	Recovery Range (%)	Average Recovery (%)	Standard Deviation (%)	Relative Standard Deviation
Soil	FMC 55657	50	3	97 - 125	115	16	14
		250	2*	110 - 113	111	2	2
		500	3	100 - 124	113	12	11
		Summary:	8	97 - 125	113	11	10
Water	FMC 55657	0.63	3	93 - 154	115	34	29
		3.13	3	113 - 117	115	2	2
		6.25	3	100 - 116	108	8	8
		Summary:	9	93 - 154	113	18	16

Number of observations outside 70-120% range = 3

* One sample lost during preparation.

TABLE 1C

METHOD RECOVERY VALUES FOR FMC 65317
FROM LABORATORY FORTIFIED SOIL AND WATER

Matrix	Analyte	Nominal Fortification Level (ppb)	Number of Analyses	Recovery Range (%)	Average Recovery (%)	Standard Deviation (%)	Relative Standard Deviation
Soil	FMC 65317	50	3	83 - 109	92	15	16
		250	2*	104 - 105	104	1	1
		500	3	89 - 108	99	10	10
		Summary:	8	83 - 108	98	11	11
Water	FMC 65317	0.63	3	91 - 120	103	16	15
		3.13	3	93 - 100	96	4	4
		6.25	3	84 - 89	86	3	3
		Summary:	9	84 - 120	95	11	12

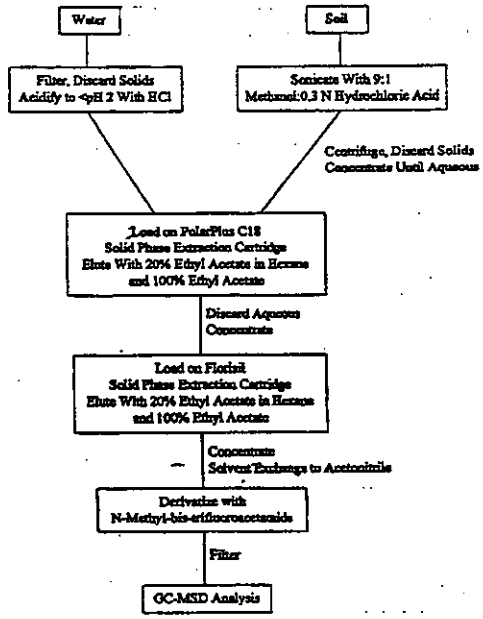
Number of observations outside 70-120% range = 0

* One sample lost during preparation.

B. Method Flow Scheme

FIGURE 1

FLOW SCHEME FOR ANALYSIS OF CLOMAZONE FROM SOIL AND WATER



IV. MATERIALS

A. Analytical Standards

The chemical names, CAS numbers, structures, and purities of the analytical standards are listed in Section XI, Table 2. Individual stock solutions of approximately 1000 µg/mL of clomazone, FMC 55657, and FMC 65317 were prepared by dissolving 10 mg of the analytical standard in 10 mL of acetonitrile using a 10 mL volumetric flask. Mixed fortification solutions containing approximately 10 µg/mL of each standard were prepared by mixing 100 µL aliquots of each stock solution and diluting to 10 mL with acetonitrile. Dilutions of the 10 µg/mL fortification solutions were prepared in acetonitrile to produce individual calibration solutions ranging from 0.05 to 1.50 µg/mL. All standard solutions were stored at approximately -20°C when not in use. A summary of the analytical standard solutions prepared is shown in Section XI, Table 3.

B. Equipment

Balance, Model AE200, Mettler.
Balance, Model PM2000, Mettler.
Centrifuge, Centra-7, IEC.
Centrifuge Tubes, 50 mL, Glass.
Centrifuge Tubes, 50 mL, Polypropylene.
Flasks, Erhlenmeyer, Normal and Side-arm, 2 L
Filters, Gelman, A/E Glass Fiber
Funnels, Buchner.
Graduated Cylinders, various
Pasteur Pipets, disposable
Pipettors -100-1000 µL, EDP, Rainin (electronic air displacement).
250 µL, Microman, Gilson (manual positive displacement).
25 µL, Microman, Gilson (manual positive displacement).
Solid Phase Extraction Cartridges (C18), 2 g, 6 mL, PolarPlus™, Baker.
Solid Phase Extraction Cartridges (Florisil), 1 g, 6 mL, Baker Analyzed.
Solid Phase Extraction Reducing Adapter, Supelco.
Solid Phase Extraction Vacuum Manifold, Supelco.
Solid Phase Extraction Visidry Drying Attachment, Supelco.
Turbovap, Model II, Zymark
Ultrasonic Bath, Model 2200, Branson.
Vacuum Pump, Model 1400, Sargent Welch.
Vortexer, Genie, Vortexer II, VWR.
Volumetric Flasks, Class A (various sizes).

C. Reagents

Anhydrous Sodium Sulfate, Mallenkrodt
Deionized, distilled water (DDW), Milli-Q Plus water system, Millipore.
Hydrochloric Acid, Baker Analyzed, Reagent Grade, 37.4%.
Acetonitrile, High Purity, Burdick and Jackson.
Ethyl Acetate, Baker Analyzed.
Hexane, High Purity, Burdick and Jackson.
Methanol, High Purity, Burdick and Jackson.
N-Methyl-bis-trifluoroacetamide, Derivatization Grade, Aldrich.

D. Reagent Solutions

0.3 N Hydrochloric Acid Solution: Add 75 mL of concentrated hydrochloric acid to 2925 mL of deionized distilled water.

9:1 Methanol: 0.3 N Hydrochloric Acid: Dilute 200 mL 0.3 N hydrochloric acid to 2 L with methanol.

20% ethyl acetate in hexane: dilute 200 mL ethyl acetate to 1 L with hexane in a 1 L volumetric flask.

V. ANALYTICAL PROCEDURE

A. Initial Soil Extraction and Water Preparation.

For Soil:

1. Air dry the soil and homogenize using a suitable device.

2. Weigh five grams of soil and place it into 50-mL polypropylene centrifuge tubes. Fortify the required control samples with various volumes of the fortification solution using pipettors. Allow the tubes to remain uncovered for approximately 20 minutes to evaporate the fortification solvent. Add 40 mL 9:1 methanol:0.3 N HCl to each sample. Vortex the tubes until the soil is suspended and then sonicate for 20 minutes. Centrifuge for five minutes at approximately 3000 rpm and then decant the supernatants into 250-mL 1-mL endpoint Turbovap tubes. Repeat the extraction three additional times (collecting the supernatant for each extraction in the same Turbovap tube).

3. Bring each extract up to a total volume of 200 mL by adding ca. 40 mL of 0.3 N HCl. Concentrate each sample to 50 mL on a Turbovap. Bring each sample up to a final volume of 100 mL with 0.3 N HCl.

For Water:

1. Measure 250 mL aliquots of water using a 500-mL graduated cylinder. Fortify the required control water aliquots with various volumes of the fortification solution using pipettors. Filter each water aliquot through Gelman glass fiber filters using a Buchner funnel to remove any soil sediment. Add 6 mL of concentrated HCl to adjust the water aliquot to ~0.3 N. Check the pH of each aliquot with pH paper and adjust to less than 2 with concentrated HCl, if necessary.

B. Solid Phase Extraction

1. Assemble the SPE vacuum manifold with 2 gram, 6 mL PolarPlus™ C18 cartridges. Condition each cartridge with 24 mL methanol, followed by 24 mL 0.3 N HCl, discarding the waste. Do not allow the cartridges to dry before loading samples.

2 Using a Pasteur pipet, transfer portions of each sample to its corresponding cartridge. Connect each cartridge to a sample using a SPE reducing adapter and a length of Teflon® tubing. Raise the sample slightly above the manifold to create a siphon effect.

3. Apply the samples to the columns at a flow rate of approximately 5 mL/minute using vacuum. The vacuum should be set at <10" Hg. Once all of the sample has been applied, rinse each Turbovap tube with 10 mL 0.3 N HCl and apply the rinsate to the cartridges. Rinse each cartridge with 10 mL deionized distilled water to remove some of the acid. All of the eluate may be discarded.

4. When the solution has completely passed through the SPE cartridge, remove the tubing and add the nitrogen drying attachment. Dry the columns under a strong nitrogen flow for at least 30 minutes at ambient temperature. Remove any remaining droplets adhering to the sides of the cartridge with a cotton swab or clean tissue. It is crucial that the water is removed or problems will occur during subsequent steps.

5. Remove the drying attachment. Rinse the columns with 12 mL hexane, discarding the rinse.

6. Elute each column under vacuum at a flow rate of approximately 1 mL/minute into a 50 mL graduated centrifuge tube with 24 mL 20% ethyl acetate in hexane followed by 24 mL 100% ethyl acetate.

7. Concentrate the samples to 0.5-1 mL on a Turbovap; do not allow the sample volume to drop below 0.5 mL. Remove and discard any aqueous layer. Bring the sample volume up to 10 mL with hexane and mix. Reconcentrate to 2-3 mL. Bring the final volume up to 5 mL with hexane.

8. Assemble the SPE vacuum manifold with 1 gram florisil SPE columns. Add anhydrous sodium sulfate to a height of approximately 0.5 cm to each cartridge. Condition each cartridge with 12 mL of 20% ethyl acetate in hexane and then with 12 mL of hexane, discarding the waste.

9. Load the hexane samples from step 2-7 to the columns at a flow rate of 1-2 mL/minute with vacuum. Rinse each Turbovap tube with 2 mL hexane and pass the rinsate through the column.

10. Elute the columns under vacuum at a flow rate of approximately 1 mL/minute with 24 mL of 20% ethyl acetate in hexane followed by 24 mL 100% ethyl acetate.

11. Concentrate the sample to 0.5 - 1 mL using a Turbovap and tubes with 1 mL tips; do not allow the sample volume to drop below 0.5 mL. Add 5 mL of acetonitrile to each tube. Reconcentrate to 0.5 - 1 mL. Bring the final volume up to 1.0 mL with acetonitrile.

C. Derivatization

1. Add 100 µL MBTFA (N-methyl-bis-trifluoroacetamide) to each Turbovap tube. Using a disposable pipette, transfer each solution from B-11 to a 2-mL screw-capped glass vial.

2. To derivatize calibration solutions, aliquot 1 mL of each calibration solution into an empty 2 mL screw-cap glass vial. Add 100 µL MBTFA to each calibration solution. (Note: Calculate the concentration of the calibration solutions based on a 1.1 mL volume)

3. Incubate the vials for 30 minutes in a 70°C oven (a GC oven works fine for this). Allow the vials to cool to room temperature before opening.

4. Filter each derivatized solution through an Anopore® 0.2 µm, 10 mm syringe filter into GC vials for analysis.

D. Dilution

1. If after analysis any of the sample concentrations are outside the range of the calibration curve (or if a fortified sample is calculated to have an extract concentration outside the calibration range), dilute with ACN until the concentration falls within the range.

E. Analysis

1. Instrumentation:
   Gas Chromatograph, Hewlett-Packard, Model 5890 Series II.
   Mass Selective Detector, Hewlett-Packard, Model 5971A.
   Personal Computer, Hewlett-Packard ChemStation (DOS series)
   Software operating on a Hewlett-Packard Vectra Personal Computer Model QS/120(386/25)
   Column, capillary gas chromatography, DB-5, 15 m x 0.25 mm i.d., 0.25 µm film thickness, J&W, Supelco.

2. Analyze the extracts using the instrument operating conditions listed in Section XIII, Appendix A. Typically the autosampler is loaded with vials containing a solvent blank (acetonitrile), a complete set of calibration standards, and the sample solutions. Program the data system to inject calibration standards at the beginning of the analysis sequence and interspersed throughout the run between sample solutions. Another complete set of calibration solutions can also be analyzed at the end of the analysis sequence if desired.

F. Calculations

Calculate the equations for the least squares linear regression curves from the peak area response versus known concentrations of the calibration standards. The actual concentration of each analyte in the samples is determined from the linear regression curve.

The concentration of each analyte in a given sample is calculated as follows:

$$\textit{Analyte concentration (ppb)} = \left(\frac{A-b}{m}\right) \times \frac{V_f \times D}{W} \times 1000$$

where:

A = sample peak area for the analyte
m = slope of the calibration curve for the analyte
b = intercept of the calibration curve for analyte
V_f = final extract volume (1.1 mL)
D = dilution factor (if applicable)
W = sample weight (soil) or volume (water)

Analyte concentration in water sample fortified with clomazone at 6.25 ppb.

$$4.88\ ppb = \frac{(17931 - 176)}{20003} \times \frac{(1.1 \times 2)}{400} \times 1000$$

$$\textit{Method Recovery \%} = \frac{\textit{Concentration obtained}}{\textit{Nominal Concentration}} \times 1000$$

$$78.1\% = \frac{4.88\ ppb}{6.25\ ppb} \times 1000$$

G. Time Required for Analysis

One person can extract and prepare twelve water samples for analysis in one 8-hour work day and twelve soil samples in two 8-hour work days.

H. Modification or Potential Problems

1. Loading the 2-gram SPE cartridges can be difficult due to the cartridge's smaller solvent reservoirs. It is important that the C18 bed not go dry. The flow rate can be controlled both with vacuum pressure and siphon rate (raising or lowering the sample above the cartridge).

2. After loading the C18 SPE cartridge, it must be thoroughly dried to remove all traces of water before elution. No water can be present when the hexane eluate from the C18 cartridge is loaded onto the florisil SPE cartridge or the analytes will not be adequately retained on the cartridge. If the concentrated C18 eluant contains two layers or phases, this indicates that the

cartridges were not sufficiently dried before elution. This solution can be successfully dried by pipetting off most of the water and then adding anhydrous sodium sulphate.

3. Lower recoveries may result during the nitrogen concentration steps if the extracts are allowed to evaporate to less than 0.5 mL.

4. Other gas chromatographic columns also provide acceptable chromatographic performance. If the column performance deteriorates rapidly, adding a 1 m pre-column with a polar stationary phase (stabilwax) should provide more reproducible chromatography.

5. The purpose of step A-3 is to remove the MeOH before SPE. Sample preparation time might be reduced if the acidic methanol extracts are concentrated as they are produced instead of waiting for all four extracts to be combined. If this procedure is used, the 40 mL of 0.3 N HCl should be added to the final extract volume before MeOH is evaporated.

VI. METHOD VALIDATION

A. Experiment Design

Control samples of soil and water were fortified by adding known amounts of clomazone, FMC 55657, and FMC 65317 to each matrix. The analytical method was practiced at approximately 50, 250, and 500 ppb in soil and at 0.63, 3.13, and 6.25 ppb in water. A fortification solution containing clomazone, FMC 55657, and FMC 65317 was prepared and the solution was added using calibrated positive displacement pipettors onto an accurately weighed or measured aliquot of matrix. For each matrix an analysis set consisted of one reagent blank, one control sample, and three laboratory fortified control samples for each fortification level.

B. Test System

The test systems were comprised of soil (0-6" depth) and water from the control plot of trial number 03 located in Proctor, Arkansas. The trial site was representative of key rice growing regions in the southern United States. Soil cores from the control plot were shipped via Federal Express from Mid-South Ag Research, Inc. and received on May 20, 1995. The soil samples were dried, homogenized and stored frozen. The test systems chosen for these experiments is representative of the systems on which this method will be used.

1. Sample Preparation

Control soil was air dried and then homogenized using a mill. Because the water samples to which this method is applicable were taken from the standing irrigation water in flooded rice fields, a simulated standing water sample was prepared by mixing ~100 g of control soil and 2 L of water in an Erhlenmyer flask. The flask was shaken by hand for several minutes and then centrifuged to settle the soil. Aliquots of the "dirty" water were used for method validation.

C. Reference Substances

The reference substances for this study were clomazone, FMC 55657, and FMC 65317. Reference substances were received from FMC Agricultural Products Group (FMC APG), Princeton, NJ on April 25, 1995. The reference substances were stored at approximately -10°C. The chemical names, CAS numbers, structures, purities and reference numbers are listed in Section XI, Table 2. Documentation of the stability, solubility, as well as chemical and physical characterization of the reference substances are maintained by FMC APG.

1. Preparation of Standards

Stock solutions of approximately 1000 µg/mL of clomazone, FMC 55657, and FMC 65317 were prepared individually by dissolving 10 mg of the analytical standard in 10 mL acetonitrile using a 10 mL volumetric flask. A fortification solution of approximately 10 µg/mL was prepared by mixing 100 µL aliquots of each stock solution and diluting to 10 mL with acetonitrile. Dilutions of the 10 µg/mL fortification solution were prepared in acetonitrile to produce individual calibration solutions of 0.05, 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 µg/mL. The stock and standard solution were stored at ≤-10°C when not in use. Information on the reference solutions is in Section XI, Table 3.

D. Calculations

1. Evaluation of Accuracy and Precision

The method accuracy was assessed by measuring the recovery obtained for the fortified control soil and water samples. Recovery was calculated by dividing the obtained value of the fortified sample by the fortified expected value and multiplying that ratio by 100. The method was considered valid if the average recoveries were between 70% and 120%.

The method precision was assessed by measuring the standard deviation (SD) and the relative standard deviation (RSD) of the values for the fortified samples in each analytical run. The method was considered valid if the RSDs were ≤20% at each fortification level for each matrix, and ≤15% RSD for all fortifications for each matrix.

2. Evaluation of Standard Curve Linearity

A least squares linear regression analysis was performed comparing the concentration of the analytes in the calibration standards to the chromatographic response (peak area). The y-intercept, slope, and correlation coefficient for the regression analysis were determined. The standard curve linearity was evaluated by monitoring the slopes and the correlation coefficients for each calibration. The analyses were considered valid if the correlation coefficients were ≥0.95 for the curves. For sample sets with more than one curve, the slopes should be similar.

E. Interferences

Blank control soil or water were analyzed for the presence of interfering peaks at the retention time of clomazone, FMC 55657, and FMC 65317. Small interference peaks, representing approximately 10% of LOQ, were observed in the target ion chromatograms for each compound. These interference peaks did not contain the qualifier peaks in the correct ratios, if at all, indicating that they were not the analytes of interest. To determine accurate fortified sample recoveries, the peak areas of the interference peaks were subtracted from the analyte peak areas in fortified sample extract chromatograms.

F. Confirmatory Techniques

Mass spectrometry in the selected ion mode served as the confirmatory technique. Five ions were monitored. Three of the ions are fragments characteristic of an individual analyte (m/z 204 for clomazone, m/z 176 for FMC 55657, and m/z 302 for derivatized FMC 65317). The other two ions (m/z 125 and 127) are present in all three analytes and if these ions co-maximize with the characteristic ion and have the correct relative intensities, they can confirm the presence of a particular analyte.

G. Radio-Validation

The method was further evaluated by using the method to analyze an aged sediment sample which had been dosed with ^{14}C-labeled clomazone as part of an aerobic aquatic metabolism study. The data obtained were compared to the data obtained in the metabolism study (Section XIII, Appendix C).

FMC CORPORATION
FMC Study Number: 164E4295E1
Battelle Study Number: N001417D

VII. STORAGE STABILITY

Standards of the analytes are stable under frozen conditions. For the validation study, standard stock solutions were prepared once and stored at ≤ -10°C for this study.

VIII. RESULTS AND DISCUSSION

A. Accuracy

The method met all the acceptable criteria for accuracy, as the average recoveries for all fortified soil or water samples were between 70% and 120%. Acceptable accuracy criteria were met for both matrix types (Section XI, Tables 4 and 5). The overall average recoveries from soil for clomazone, FMC 55657 and FMC 65317 were 76, 113, and 98%, respectively (Table 4). The overall average recoveries from water for clomazone, FMC 55657 and FMC 65317 were 79, 113, and 95%, respectively (Table 5).

For soil, the average method recovery of clomazone at nominal fortification levels of 50, 250, and 500 ppb, was 76% at each level. The average method recovery of FMC 55657 at nominal fortification levels of 50, 250, and 500 ppb, was 115%, 111%, and 113%, respectively. The average method recovery of FMC 65317 at nominal fortification levels of 50, 250, and 500 ppb, was 92%, 104%, and 99%, respectively.

For water, the average method recovery of clomazone at nominal fortification levels of 0.63, 3.13, and 6.25 ppb, was 79%, 83%, and 75%, respectively. The average method recovery of FMC 55657 at nominal fortification levels of 0.63, 3.13, and 6.25 ppb, was 115%, 115%, and 108%, respectively. The average method recovery of FMC 65317 at nominal fortification levels of 0.63, 3.13, and 6.25 ppb, was 103%, 96%, and 86%, respectively.

B. Precision

The method met all the acceptable criteria for precision, as the SD's and RSD's for the fortified samples were ≤20% for each individual concentration level and ≤15% for all fortifications of each matrix with one exception. The LOQ fortification level in water matrix had a single high (154%) recovery value which raised the RSD's for this analyte above the target levels. With this one exception, acceptable precision criteria were obtained for both matrices (Section XI, Tables 4 and 5, and Tables 1A, 1B and 1C).

For soil, the RSD for clomazone at fortification levels of 50, 250, and 500 ppb was 10%, 4% and 12%, respectively (Table 1A). The RSD for FMC 55657 at

fortification levels of 50, 250, and 500 ppb was 14%, 2%, and 11%, respectively (Table 1B). The RSD for FMC 65317 at nominal fortification levels of 50, 250, and 500 ppb was 16%, 1%, and 10%, respectively (Table 1C).

For water, the RSD for clomazone at fortification levels of 0.63, 3.13, and 6.25 ppb was 9%, 4%, and 4%, respectively (Table 1A). The RSD for FMC 55657 at fortification levels of 0.63, 3.13, and 6.25 ppb was 29%, 2%, and 8%, respectively (Table 1B). The RSD for FMC 65317 at nominal fortification levels of 0.63, 3.13, and 6.25 ppb was 15%, 4%, and 3%, respectively (Table 1C).

C. Limit of Quantitation (LOQ) Limit of Detection (LOD)

The LOQ of clomazone, FMC 55657, and FMC 65317 was established at 50 ppb for soil since this was the lowest fortified concentration level of each standard that was tested. The LOQ for water has been set at 1.0 ppb by using a 250 mL sample size. Chromatograms for fortified and unfortified control soil and water extracts are presented in Section XIII Appendix B, Figures 6-11.

The LOD of clomazone, FMC 55657, and FMC 65317 was established at 10 ppb for soil and set to 0.20 ppb for water. These values correspond to the lowest calibration solution analyzed.

D. Ruggedness

The average recoveries and the standard deviations for the analytical method indicate that the analytical methods are reliable and accurate. Each step in the sample preparation procedure and instrumental analysis utilize routine residue techniques. Careful attention to the detailed method will ensure reliable performance of the method.

All the acceptance criteria for validating this method were met. The method has been proven to be accurate and precise for the analysis of clomazone, FMC 55657, and FMC 65317 in soil at concentrations ranging from 50 to 500 ppb and in water at concentrations ranging from 0.63 to 6.25 ppb.

The required sensitivity level for clomazone, FMC 55657, and FMC 65317 in soil of 50 ppb (227 ng/mL nominal extract concentration) and in water of 0.63 ppb (229 ng/mL nominal extract concentration) was easily met by this method. The lowest level calibration standard injected is equivalent to an analyte concentration of 10 ppb in soil and 0.13 ppb in water.

The method was determined to be linear over the range of 50 - 1500 ng/mL as shown by a correlation coefficient value ≥0.95. Representative chromatograms of the calibration standards are presented in Section XIII Appendix B, Figures 3-5.

The retention times for clomazone, FMC 55657, and FMC 65317 were consistent during the analysis of each set. The relative retention times for FMC 55657, clomazone, and FMC 65317 were approximately 5.7, 7.0, and 7.8 minutes on the J&W DB-5 column and 6.5, 8.3, and 8.8 minutes on the Supelco SPB-5 column.

E. Limitations

No limitations were experienced with this method. Any possible difficulties are described in both sections above on "Interferences" and "Modifications or Potential Problems".

F. Radio-Validation

A description of the radio-validation experiments and the results are included in Section XIII Appendix C.

IX. CONCLUSIONS

A residue analytical method was successfully developed and validated for the extraction and detection of clomazone, FMC 55657, and FMC 65317 in soil and water. The overall average method recoveries of clomazone, FMC 55657, and FMC 65317 from soil were 96% and from water, 96%. The method limit of quantitation was 50 ppb for each analyte in soil and 1.0 ppb for each analyte in water. The nature of, and concentration of, the residues detected by the method were comparable to the results obtained during previous ^{14}C-metabolism studies.

All equipment needed to perform these analyses is readily available in most analytical laboratories. An experienced residue analyst, following the procedure exactly as written and being aware of the potential problems, can obtain adequate recoveries of clomazone, FMC 55657, and FMC 65317 from soil and water.

FMC CORPORATION
FMC Study Number: 164E4295E1
Battelle Study Number: N001417D

X. 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.

Mark R. Bauer, Ph.D.
STUDY DIRECTOR/CO-AUTHOR
Battelle

12/19/96
Date

Timothy A. Cristy.
CO-AUTHOR
Battelle

12/19/96
Date

Additional Personnel:
José R. Marengo

SPONSOR CERTIFICATION

FMC Corporation certifies that Battelle Study No. N001417D is a complete and unaltered copy of the report as provided by the testing facility.

Joseph H. Finley, Ph.D.
Contract Coordinator
SPONSOR MONITOR

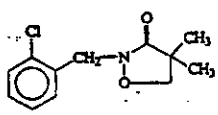
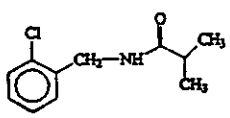
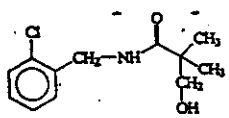
12/23/96
Date

FMC CORPORATION
FMC Study Number: 164E429SE1
Battelle Study Number: N001417D

XI. TABLES

TABLE 2

TEST AND REFERENCE SUBSTANCES

Common Name	Chemical Name/Structure	CAS Number	FMC Reference Number	Purity
Clomazone	2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone	81777-89-1	E6788:76	99.7%
				
FMC 55657	N-[(2-chlorophenyl)methyl]-2-methyl-propanamide	NA	C9698:133	95.0%
				
FMC 65317	N-[(2-chlorophenyl)methyl]-3-hydroxy-2,2-dimethyl propanamide	NA	E2028:104	99.0%
				

FMC CORPORATION
FMC Study Number: 164E4295E1
Battelle Study Number: N001417D

PC-0271M
Page 28

TABLE 3

REFERENCE SOLUTIONS

Name	Analyte(s)	Solution Solvent	Nominal Concentration (ng/µL)	Validation Solution ID #*
Stock	Clomazone	Acetonitrile	1000	9508-43-01
Stock	FMC 55657	Acetonitrile	1000	9508-43-03
Stock	FMC 65317	Acetonitrile	1000	9508-43-02
Fortification Solution	Clomazone, FMC 55657, and FMC 65317	Acetonitrile	10	9508-43-04
Calibration Solutions	Clomazone, FMC 55657, and FMC 65317	Acetonitrile	0.05	9508-44-07
			0.25	9508-44-06
			0.50	9508-44-05
			0.75	9508-44-04
			1.00	9508-44-03
			1.25	9508-44-02
			1.50	9508-44-01

* Prepared on 1/17/96 and stored at approximately -20°C when not in use.

FMC CORPORATION
FMC Study Number: 164E4295E1
Battelle Study Number: N001417D

TABLE 4

METHOD RECOVERIES OF CLOMAZONE, FMC 55657, AND FMC 65317 FROM LABORATORY FORTIFIED CONTROL SOIL

Matrix	Sample ID	Nominal Fortification Level (ppb)	Method Recovery %		
			Clomazone	FMC 55657	FMC 65317
Soil	9508-45-01	50	81	125	109
	9508-45-02	50	79	123	84
	9508-4503	50	68	97	83
	9508-45-04	250	74	110	105
	9508-45-05	250	79	113	104
	9508-45-06	250	*	*	*
	9508-45-07	500	68	100	89
	9508-45-08	500	85	124	101
	9508-45-09	500	76	115	108
		Average/n=8	76	113	98
		Standard Deviation	6	11	11
		Relative Standard Deviation	8	10	11

* Sample lost during preparation.

FMC CORPORATION
FMC Study Number: 164E4295E1
Battelle Study Number: N001417D

PC-0271M
Page 30

TABLE 5

METHOD RECOVERIES OF CLOMAZONE, FMC 55657, AND FMC 65317 FROM LABORATORY FORTIFIED WATER

Matrix	Sample ID	Nominal Fortification Level (ppb)	Method Recovery %		
			Clomazone	FMC 55657	FMC 65317
Water	9508-50-01	0.63	77	93	91
	9508-50-02	0.63	73	97	97
	9508-50-03	0.63	87	154	120
	9508-50-04	3.13	79	113	93
	9508-50-05	3.13	86	117	95
	9508-50-06	3.13	85	115	100
	9508-50-07	6.25	78	116	89
	9508-50-08	6.25	72	108	84
	9508-50-09	6.25	76	100	84
		Average/n=9	79	113	95
		Standard Deviation	5	18	11
		Relative Standard Deviation	7	16	12

XIII. APPENDICES

INDEX TO APPENDICES

XII. REFERENCES

1. Bauer, Mark R., "Command® 3ME Herbicide - Aquatic Field Dissipation", Battelle, Columbus, OH, FMC Report PC-0271, November, 1996.

2. Chen, Audrey W., "Residue Analytical Method for the Determination of Clomazone in/on Crop and Processed Part Matrices of Corn, Cottonseed, Soybean, and Tobacco", FMC Corporation, Agricultural Chemical Group, Princeton, New Jersey, FMC Report P-2640, June 1992.