Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Cymoxanil

MRID #: 441807-53

Matrix: Soil

Analysis: LC/MS

441807-53

TRADE SECRET

Study Title

A CONFIRMATORY ANALYTICAL METHOD FOR THE DETERMINATION OF CYMOXANIL RESIDUES IN SOIL, WATER, AND POTATOES USING LC/MS

Data Requirement

EU Council Directive 91/414 Annex II, Section 4.2.1, Residues in Plants, Plant Products, Foodstuffs, Feedingstuffs

U.S. Environmental Protection Agency Residue Chemistry Test Guidelines, OPPTS 860.1340 (Residue Analytical Method) (formerly Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry: Section 171-4(b))

Authors

Richard K. Trubey
Peter T. J. DeLuke
Robert W. Sund
Joseph P. McClory

Date Study Completed

November 5, 1996

Performing Laboratories

E. I. du Pont de Nemours and Company
DuPont Agricultural Products
Global Technology Division
Experimental Station
Wilmington, Delaware 19880-0402

Laboratory Project ID

AMR 4171-96

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 Section 10(d)(1)(A), (B), or (C).

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Company Agent: Mary Lou Hawkins
(Typed Name)

U. S. Product Registration Manager
(Title)

Mary Lou Hawkins
(Signature)

Nov. 8, 1996
(Date)

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GOOD LABORATORY PRACTICE STATEMENT

The EPA Good Laboratory Practice (GLP) requirements specified in 40 CFR Part 160 do not require analytical methods to be developed under Good Laboratory Practices (GLP). However, the methods development presented in this report was done under GLP except that no protocol was written, no conduct audit was performed, and no QA audit of the study records was done. Analytical procedures, documentation, and archiving of the validation data were done by Standard Operating Procedures.

Sponsor

E. I. du Pont de Nemours and Company

Submitter

E. I. du Pont de Nemours and Company

Study Director

Richard K. Trubey 05-Nov-96

Richard K. Trubey
Senior Research Chemist

Nov. 8, 1996
Date

Company Representative

Mary Lou Hawks

DuPont Registration Representative

Nov. 8, 1996
Date

CERTIFICATION

A CONFIRMATORY ANALYTICAL METHOD FOR THE DETERMINATION OF CYMOXANIL RESIDUES IN SOIL, WATER, AND POTATOES USING LC/MS

We, the undersigned, declare that the work described in this report was performed under our supervision, and that this report, provides an accurate record of the procedures and results.

Report by:

Richard K. Trubey 05-Nov-1996
Richard K. Trubey Date
Study Director

Approved by:

Nov. 5, 1996
Thomas P. Fuesler Date
Research Supervisor

Date Study Completed:

November 5, 1996

Storage Location of Records and Final Report:

E. I. du Pont de Nemours and Company
DuPont Agricultural Products
Global Technology Division
Experimental Station
Wilmington, Delaware 19880-0402

Sponsor:

E. I. du Pont de Nemours and Company
DuPont Agricultural Products
Global Technology Division
Experimental Station
Wilmington, Delaware 19880-0402

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ABBREVIATIONS

Abbreviation	Explanation
Cat.	catalog
Coeff.	Coefficient
HPLC	High Performance Liquid Chromatography
LC/MS	Liquid Chromatography with Mass Spectrometric Detection
LC/UV	Liquid Chromatography with Ultraviolet Absorbance Detection
LOQ	Limit of Quantitation
min	minutes
m/z	mass-to-charge ratio
n	sample population
no.	number
ppm	parts per million
Q1	Quadrupole 1
quad.	quadrupole
R^2	Correlation Coefficient
RIC	Reconstructed Ion Chromatogram
S.D.	Standard deviation, $\sqrt{\frac{n\sum x^2 - (\sum x)^2}{n(n-1)}}$
sec	seconds
ser.	serial
SIM	Selected Ion Monitoring
SPE	Solid Phase Extraction

A CONFIRMATORY ANALYTICAL METHOD FOR THE DETERMINATION OF CYMOXANIL RESIDUES IN SOIL, WATER, AND POTATOES USING LC/MS

Richard K. Trubey, Peter T.J. DeLuke, Robert W. Sund, and Joseph P. McClory

1.0 SUMMARY

A thermospray LC/MS based residue method is described for confirmation of cymoxanil residues in soil, water, and potato tubers. This document describes LC/MS conditions and results. Matrix extraction and clean up procedures are described in the residue method reports intended for enforcement purposes (References 1-3).

Recoveries ranged from 84-101% in soil fortified at 0.050-0.25 ppm. Recoveries ranged from 78-111% in water fortified at 0.0020-0.010 ppm. Recoveries ranged from 76-88% in white potato tubers fortified at 0.020-0.10 ppm. Average recoveries were 94%, 98%, and 81% in soil, water, and potatoes, respectively.

2.0 INTRODUCTION

Cymoxanil (DuPont No. DPX-T3217) is the active ingredient in Curzate® fungicide, registered for the control of many important plant diseases. Analyte structure, chemical name, DuPont code number, and *Chemical Abstracts* registry number are provided in Figure 1.

This method was developed as a confirmatory method for the determination of cymoxanil residues in soil, water, and potato tubers. Applicability to other matrix types has not been determined, although this method should be applicable to similar matrices (e.g., other watery crops such as tomatoes). Residue methods intended for primary support of regulatory needs, including enforcement, are described in References 1-3. Extracts processed by the "primary" methods can be directly analyzed (without additional work up) via this LC/MS method.

This LC/MS analysis provides confirmatory information in the form of very selective detection: our MS conditions select only for ions corresponding to the molecular weight of cymoxanil. (Also, the use of HPLC conditions that differ from the related enforcement methods offers an additional, but less important, mode of selectivity.)

Soil, water, and potato tubers were fortified and extracted using the "primary" residue methods (References 1-3). Extracts were cleaned up and concentrated according to these methods, as well.

Aliquots of cleaned up samples were filtered and then analyzed using positive ion, thermospray LC/MS. The mass analyzer was set to detect ions having mass-to-charge ratios (m/z) of 199 and 216, which correspond to the protonated molecular ion of cymoxanil (MW = 198) and an ammonium adduct (i.e., cymoxanil + NH_4^+), respectively. The MS was operated in the Selected Ion Monitoring (SIM) mode. Quantitation was based on the Reconstructed Ion Chromatogram (RIC).

Recoveries ranged from 84-101% in soil fortified at 0.050-0.25 ppm. Recoveries ranged from 78-111% in water fortified at 0.0020-0.010 ppm. Recoveries ranged from 76-88% in white potato tubers fortified at 0.020-0.10 ppm. Average recoveries were 94%, 98%, and 81% in soil, water, and potatoes, respectively.

3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified; note any specifications in the following descriptions before making substitutions. The equivalency/suitability of any substitution should be verified with acceptable control and fortification recovery data.

3.1 EQUIPMENT

Standards Preparation: Mettler AE 160 series, 4-place analytical balance (Mettler Instrument Corp., Hightstown, N.J.); Pyrex® Class A glass 10-, 50-, and 100-mL volumetric flasks (VWR, Bridgeport, N.J.); Syringes, glass, high-performance, Luer-Lok® tip, 250-µL and 1000-µL capacities (Model Nos. 1725 and 1001, respectively [Hamilton Company, Reno, Nev.]); Syringe needles, #22 x 2", blunt (perpendicular) tip with Luer® hub, Cat. No. 72-15 (Alltech Associates, Deerfield, Ill.).

Sample Extraction: Covered in References 1-3.

LC/MS/MS Analysis (supplies): Millipore "HV" Durapore® polyvinylidene fluoride membrane filters, 47-mm diameter, 0.45-µm pores, Cat. No. HVLP 047 00 (Millipore Corp., Bedford, Mass.); Filter apparatus, all-glass, Cat. No. XX15 04700 (Millipore Corp.); Filter units (disposable), Millipore Millex®-HV13, 13-mm diameter, 0.45-µm pores, Durapore® polyvinylidene fluoride membrane, Cat No. SJHV 013 NS, (Millipore Corp.); Syringes, Fortuna® disposable, sterilized, 2.5-mL capacity, all-polypropylene/polyethylene, Cat. No. Z11685-8 (Aldrich Chemical Company, Milwaukee, Wisc.); Vials, Waters autosampler, 1-mL capacity, clear glass with caps, Cat. No. WAT025054 (Waters Corporation, Milford, Mass.).

LC/MS/MS Analysis (instrumentation):

Liquid Chromatograph (Waters Corporation, Milford, Mass.).
Waters 616 HPLC System, consisting of Model 616 Pump (Ser. No. MXSKM3052M) and Model 600S Controller (Ser. No. SXSKM0105M)
Waters Temperature Control Module (Ser. No. 1837)
Waters Column Oven Heater Module (Ser. No. CEM002354)
Waters Model 717 plus Autosampler (Ser. No. MXSKM2944M)

HPLC Columns (Mac-Mod Analytical, Inc., Chadds Ford, Pa.)
DO NOT SUBSTITUTE ANALYTICAL COLUMNS WITHOUT EVALUATING EQUIVALENCY
Zorbax® C8 Guard Cartridge, Part No. 820674-906
followed by
Zorbax® Rx-C8 Analytical Column, 4.6 mm x 25 cm, Part No. 880967-901

Mass Spectrometer (Finnigan MAT Corporation, San Jose, Calif.)
Finnigan Model TSQ7000 Mass Spectrometer (Ser. No. TS 010025)
Finnigan MAT Thermospray LC Interface Module, Model TSP-2 (Ser. No. 01011001)
Digital DEC3000 Model 300LX Computer (Ser. No. NI51000X10)
ICIS Software (Version 8.2.1)

Other Instrumentation
Mettler PB602, 2-place, toploading analytical balance (Mettler Instrument Corp., Hightstown, N.J.); Fisher Accumet® 15 pH meter (Fisher Scientific, Pittsburgh, Pa.)

3.2 *REAGENTS AND STANDARDS*

Reagents: *Substitutions of equivalent reagents should only be made if equivalency/suitability has been verified.* Water, Milli-Q™ Type I deionized, distilled water system (Millipore Corporation, Bedford, Mass.); methanol, OmniSolv® distilled, Product #MX0488-1 (EM Science, Gibbstown, N.J.); acetic acid (glacial), 'Baker Analyzed'®, HPLC Reagent grade, Cat. #9515-03 (J. T. Baker); ammonium acetate, 'Baker Analyzed'®, HPLC Reagent grade, Cat. #0599-08 (J. T. Baker).

Standards: Cymoxanil, DPX-T3217-101, 99.9% pure (DuPont Agricultural Products, Wilmington, Del.).

3.3 *SAFETY AND HEALTH*

No unusually hazardous materials are used in this method. All appropriate material safety data sheets should be read and followed, and proper personal protective equipment should be used.

4.0 METHODS

4.1 PRINCIPLES OF ANALYTICAL METHODS

4.1.1 Sample Extraction

Sample extractions are performed according to procedures detailed in **References 1-3.**

4.1.2 Extract Purification

SPE clean ups of sample extracts are described in **References 1-3**. The only additional purification required is preanalysis filtration (0.45 µm) of an aliquot of the final analysis solution, to prevent introduction of particulates into the HPLC system.

4.1.3 LC/MS Analysis

An HPLC separation coupled to a mass spectrometer (via a thermospray interface) is the basis for detection and quantitation of cymoxanil residues. The MS is operated in the positive, selected ion monitoring (SIM) mode, set to monitor ions having m/z of 199 and 216, which correspond to the protonated molecular ion of cymoxanil (MW = 198) and an ammonium adduct (i.e., cymoxanil + NH_4^+), respectively. Quantitation is based on the Reconstructed Ion Chromatogram (RIC).

4.2 ANALYTICAL PROCEDURE

4.2.1 Glassware and Equipment Cleaning Procedures

Disposable labware are generally used in this method. Reusable labware, including volumetric flasks for standard solutions, are cleaned by washing with a laboratory-grade detergent followed by tap-water rinses (3) and distilled water rinses (3). A final acetone rinse may be used to remove the residual water and promote drying.

4.2.2 Preparation and Stability of Reagent Solutions

Prepare an aqueous 0.10 M ammonium acetate solution by weighing 7.71±0.02 g of the reagent (CH_3COONH_4, FW = 77.08 g) in a plastic weighboat, transferring to 1 L of Milli-Q® water (already contained in a 1-L beaker), and thoroughly mixing (magnetic stirring). Adjust the pH

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to 4.5 ± 0.1 with glacial acetic acid (magnetic stirring). Transfer this solution to a suitable container for storage at ambient temperature. Replace solution monthly (≤30 days), or sooner if turbidity is observed. Prior to use as an HPLC mobile phase component, this solution must be filtered through 47-mm diam. filters (0.45-µm pore size).

4.2.3 Stock Solutions Preparation

If possible, use standards with a purity greater than 95%. Prepare a 100±2-µg/mL stock standard solution by diluting 10.0±0.20 mg of cymoxanil to volume with methanol, using a 100-mL volumetric flask.

Determine sample weights to 3 significant figures. The analytical balance must provide a weight precision to at least 3 significant figures.

Clearly label as a stock solution with date prepared, analyte, and concentration. Store this stock solution under refrigeration (4±2°C). Replace stock solutions at 6 months, or sooner if chromatography indicates significant degradation.

4.2.4 Fortification Solutions Preparation

Prepare fortification solutions according to procedures given in **References 1-3.**

4.2.5 Calibration Solutions Preparation

Prepare a 1.00-µg/mL intermediate standard (cymoxanil) by transferring 1.00 mL of the 100-µg/mL stock solution (use a syringe or equivalent) to a 100-mL volumetric flask and diluting to volume with methanol.

Prepare calibration solutions at concentrations over a range of 0.00500-0.100 µg/mL from dilutions of the 1.00-µg/mL intermediate standard. The dilution solvent is 20% methanol / 80% 0.10 M ammonium acetate (pH 4.5) (v/v). A minimum of 4 standard concentrations should be prepared over this range including the lower and upper limits. Use the following table as a guide for standards preparation.

1.0 µg/mL Standard Aliquot (mL)	Volumetric Size (mL)	Final Concentration (µg/mL)*
0.0500	10.0	0.00500
0.100	10.0	0.0100
0.200	10.0	0.0200
0.500	10.0	0.0500
1.00	10.0	0.100

Clearly label calibration solutions with date prepared, analytes, and concentration. Store calibration solutions in a refrigerator (4±2°C) and replace weekly (≤7 days).

CAUTION: Glassware is a source of possible contamination if not cleaned properly. Rinse flasks 2-3 times with 50% methanol / 50% water prior to preparing standard solutions.

4.2.6 Source of Samples

Soil samples (see Table 1 for characterization information) were obtained from two sources:

- Madera, Calif. (Sample Barcode No. S00089759, DuPont Study No. AMR 3401-95)
- Environmental Studies Soil Bank, DuPont Agricultural Products, Experimental Station, Wilmington, Del. ("Drummer" Soil)

The "Drummer" soil has a high clay and % organic matter content, making it an especially good soil for residue-method testing.

Water was obtained (28-Nov-95) from the Brandywine Creek (near the DuPont Experimental Station, Wilmington, Del.).

Untreated potatoes (tubers) were purchased locally (supermarket), homogenized (ground with dry ice), and stored frozen 19-Nov-94.

4.2.7 Storage and Preprocessing of Samples

This information is available in References 1-3.

4.2.8 Sample Fortification Procedure

This information is available in References 1-3.

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[*] equivalent to ppm

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4.2.9 *Analyte Extraction Procedures*

This information is available in **References 1-3**.

4.2.10 *Analyte Purification Procedure*

SPE clean-up procedures are available in **References 1-3**. Prior to instrumental analysis, an approximately 1-mL aliquot from each final sample solution is filtered into an autosampler vial using a 2.5-mL plastic syringe equipped with a 0.45-µm filter (Millex®-HV13).

4.3 ***Instrumentation***

4.3.1 *Description*

This method uses an LC/MS system for determination of cymoxanil residues in a wide variety of sample matrix types. Mass spectrometry is the most widely accepted technique for confirmation of residue concentrations.

The LC system components are listed in ***Equipment***. An isocratic, reversed-phase separation using a Zorbax® Rx-C8 analytical column is performed.

HPLC requirements for an LC/MS system are more stringent than for LC/UV; the chromatograph should have minimal pressure fluctuations, as this has a major effect on mass spectrometer baseline noise.

A low dead-volume, 0.5-µm pore filter is critical in the tubing leading to the MS interface to prevent exposure of the MS interface to particulates. (Finnigan MAT supplies an appropriate in-line filter for their MS system.)

In addition to being a useful mobile phase buffer, ammonium acetate is necessary to provide a proton source as part of the ionization process occurring in the MS interface (in a thermospray interface, droplet desolvation and analyte ionization occur fairly simultaneously following introduction of LC effluent — in the form of a hot aerosol — into the MS source region (References 4 and 5)).

The MS system is equipped with a high-pressure switching valve, between the LC and the MS source, to allow convenient diversion of LC effluent to waste during time periods irrelevant to analyte quantitation. **This feature is essential** in residue methods such that early eluting sample materials are prevented from fouling the MS source (especially over the course of numerous analyses).

The mass spectrometer is a triple-quadrupole design equipped with a thermospray source (see Section *4.1.3*). However, a single-quadrupole instrument would be equivalent for this method (only one quadrupole — Quad 1 — was involved in mass selectivity).

A typical full-scan spectrum is shown in Figure 2. The base peak (greatest abundance) at m/z 216 corresponds to the ammonium adduct (cymoxanil + NH_4^+). The other relevant peak (m/z 199) corresponds to the protonated molecular ion.

For quantitation of cymoxanil residues, the mass spectrometer was operated in the Selected Ion Monitoring (SIM) mode, such that Quad 1* selected both relevant ions (m/z 199,216). Quantitation was based on the RIC.

A low-level chromatographic standard (cymoxanil) should be analyzed prior to the start of analyses to establish suitability of selected MS parameters. In our work, the electron multiplier voltage was adjusted such that the peak area for a 0.100-µg/mL standard (100 µL injection) yielded at least 10^6 counts for cymoxanil peak area (sum of both ions – same as RIC).

The typical linear range for calibration standards was 0.00500–0.100 µg/mL (see Figure 3).

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* Quads 2 and 3 were not involved in mass selectivity (they were operated to pass all masses through).

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4.3.2 LC/MS Operating Conditions

Operating Conditions (HPLC):

Column Temp: 40°C
Injection Vol: 100 µL

Mobile phase: 40% methanol /
60% 0.10 M ammonium acetate, pH 4.5
(pH adjusted with glacial acetic acid)

Flow Rate: 1.00 mL/min

Operating Conditions (MS):

(Reference: Conditions were recorded 28-Aug-1996, in instrument records; these would be considered typical.)

Vaporizer Temp: 90°C
Aerosol Temp: 103°C
Source Block Temp.: 200°C
Repeller: 100 V
Electron multiplier voltage: 1600 V

SIM Mode (Q1MS), m/z 199.0, 216.0 (0.50 sec/mass [1 scan/sec])

4.3.3 Calibration Procedures

Instrumentation calibration is based on average response factors (defined in Section *4.4.1*) calculated as the ratio of detector response (chromatographic peak area) to concentration of standard injected (all injections should be same volume [100 µL suggested]).

If the relative standard deviation for an individual group of 3 standards is less than 20%, then the method is considered to be operating correctly (otherwise, appropriate corrective action, such as instrument maintenance or trouble-shooting, should be taken). Standards at 3 concentration levels (minimum) should be used to verify this criterion at the beginning of a particular day's analysis set as well as throughout the day's run.

The successful detection (S/N > 5:1) of the lowest calibration standard is an additional criterion to be used to verify proper tuning and calibration of the instrument. Again, this criterion should be evaluated (and appropriate action taken, if needed) prior to running samples.

4.3.4 Sample Analysis

A sample analysis run should start out with 3-4 (minimum) injections of standards (covering at least 3 calibration concentrations), for assessment of system suitability and performance (see previous section). Subsequently, no more than 4 samples should be injected between standards. The analysis run should conclude with at least one standard injection.

4.4 CALCULATIONS

4.4.1 Methods

The Finnigan data system was used to integrate, report, and print the relevant area counts for each chromatogram. The analyst examined each chromatogram (using the Finnigan program "CHRO" -- see Table 2 for typical integration parameters), manually reintegrated the peak(s) if necessary, then printed each chromatogram.

Area count responses (RIC) were manually entered into an EXCEL Spreadsheet table (Microsoft Corp., Version 5.0a). Spreadsheet formulas were used to complete the data analysis, using the Response Factor Method described below.

Response Factor Method

$$\textbf{Response Factor (RF)} = \frac{\textbf{Peak Area (counts)}}{\textbf{Standard Concentration (ppm)}}$$

Quantitation of analyte concentration in fortified or treated samples is performed based on the average response for standards preceding and following the samples, as follows:

$RF_{AVG} = [(RF_1) + (RF_2) + (RF_3)] / 3$, where

RF_1 = response factor of first standard,

RF_2 = response factor of second standard, and

RF_3 = response factor of third standard.

Normally, RF_1 and RF_2 will precede the sample and RF_3 will follow (other, similar bracketing schemes are also legitimate). These response factors (and also RF_{AVG}) were calculated by appropriate formulas contained in the EXCEL spreadsheet.

Analyte residue concentrations are calculated as follows:

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$$\text{ppm Found} = \frac{(\text{Peak Area})(\text{AF})(\text{FV})(\text{DF})}{(\text{RF}_{\text{AVG}})(\text{SW})}$$

where

AF = aliquot factor,
FV = final volume of sample in mL,
DF = dilution factor (if additional sample dilution by the LC/MS analyst is required), and
SW = weight of sample in grams.

The percent recovery is calculated using the "ppm Found" and the fortification concentration (ppm) in the following formula:

$$\text{Percent Recovery} = \frac{\text{ppm Found} \times 100}{\text{Fortification Level}}$$

4.4.2 Examples

The concentration of cymoxanil found in white potato sample 1B fortified at 0.020 ppm is calculated as follows (see Data Sheet Number 093096 in Appendix 1):

Peak Area = 157,974 counts
Aliquot Factor = 99 mL/7.5 mL = 13.2 (a 7.5-mL aliquot was removed out of a 99-mL acetonitrile layer, during sample work up)
Final Volume = 3.00 mL
Dilution Factor = 1
RF_{AVG} = 17,743,397 counts/ppm
Sample Weight = 20 g

$$\text{ppm Found} = \frac{(157{,}974)(13.2)(3.00)(1)}{(17{,}743{,}397)(20)} = 0.0176$$

This value was reported as 0.018 ppm in Table 4.

The concentration of cymoxanil found in Brandywine water sample 2C fortified at 0.004 ppm is calculated as follows (see Data Sheet Number 091396 in Appendix 1):

Peak Area = 1,629,778 counts
Aliquot Factor = 1
Final Volume = 3.00 mL
Dilution Factor = 1
RF_{AVG} = 12,593,338 counts/ppm
Sample Weight = 100 g

$$\text{ppm Found} = \frac{(1{,}629{,}778)(1)(3.00)(1)}{(12{,}593{,}338)(100)} = 0.00388$$

This value was reported as 0.0039 ppm in Table 3.

5.0 RESULTS AND DISCUSSION

5.1 *METHOD VALIDATION RESULTS*

5.1.1. Detector Response

Figure 2 shows a full scan LC/MS (Quad 1) spectrum of a 10-µg/mL cymoxanil standard (a total Reconstructed Ion Chromatogram is also shown, for reference purposes). The base peak (greatest abundance) at m/z 216 corresponds to the ammonium adduct (cymoxanil + NH_4^+). The other relevant peak (m/z 199) corresponds to the protonated molecular ion.

For routine residue analysis, the mass spectrometer parameters were established as described in Section *4.3.2*. Selected Ion Monitoring (SIM) was performed, with Quad 1 selecting for m/z 199 and 216. Quantitation was based on the sum of both signals (RIC).

Figure 3 shows selected ion chromatograms for typical calibration standards. Standards typically yielded a linear response (Correlation Coeff. > 0.99) over a range somewhat greater than one order of magnitude (but less than 2 orders of magnitude), such as the range 0.005-0.100 µg/mL (Figure 4 depicts such a standard curve).

As would be expected for the thermospray interface, system response tended to drift over long periods of time (> 6-8 hr). Therefore, the response factor method described in Section *4.4.1* was utilized (this method uses the response of 3 standards injected closely in time to the sample of interest, to quantify the sample).

Representative chromatograms for each analyte in each sample matrix are presented in Figures 5-7.

5.1.2 Controls

Control samples were analyzed with each fortification set. No interferences were observed.

5.1.3 Recoveries (Accuracy & Precision)

This subject is thoroughly addressed in the original method reports (References 1-3). However, to assess performance of this confirmatory method, fortifications were made at various levels to each matrix type.

Recovery results for the LC/MS analysis of soil, water, and potato tubers are presented in Tables 3-5. The following summary table gives the percent recovery range, average, and standard deviation for each matrix:

Matrix	Recovery Range	Average	S.D. (n)
soil	84-101	94	6 (9)
water	78-111	98	9 (9)
potato	77-88	81	5 (9)

S.D. = standard deviation; n = sample population

5.1.4 Extraction Efficiency

This subject is addressed in the original method reports (References 1-3).

5.1.5 Limit of Quantitation (LOQ)

This subject is thoroughly addressed in the original method reports (References 1-3). However, based on S/N of the lowest fortifications evaluated for LC/MS, LOQs for this confirmatory method appear to be somewhat below the level of the lowest fortification analyzed (also, control samples showed no significant chemical interference for either mass "channel", in the vicinity of the retention time for cymoxanil). This LC/MS method is clearly suitable for confirmation, from both sensitivity and selectivity standpoints.

5.2 Timing

This subject is addressed in the original method reports (References 1-3), relative to the time required for sample preparation/extractions. The LC/MS instrumentation is highly automated so that analyses are typically performed unattended (e.g., overnight). Each chromatogram requires 25 min to acquire.

Therefore, extraction, purification, and analysis of test sample sets should routinely be completed within 24 hours.

5.3 Modifications or Special Precautions

This subject is addressed in the original method reports (References 1-3).

5.4 *Method Ruggedness*

The method has been tested on soil, water, and potato tubers.

Only the instrumentation specified in this report has been used for this method to date. Due to excellent sensitivity and selectivity afforded by the LC/MS technique, method ruggedness (from an instrumental analysis standpoint) should be excellent.

5.4.1 Stability

The solid standards should be stored at room temperature, preferably in a dessicator. Other stability issues are addressed in the original method reports (References 1-3).

5.4.2 Specificity/Potential Interference

Due to the nature of LC/MS, this method has excellent specificity and low likelihood of chemical interference.

6.0 CONCLUSIONS

This analytical method is clearly suitable for the confirmation (including quantitation) of cymoxanil residues in soil, water, and potatoes. Limits of Quantitation/Detection are dictated primarily by aspects of the original methods (References 1-3).

The average recoveries for cymoxanil were 94%, 98%, and 81% for soil, water, and potato tubers, respectively. All recoveries were acceptable relative to current regulatory standards.

7.0 REFERENCES

1. McClory, J. P.; Jones, W.; DeLuke, P. T. J., *Analytical Method for the Determination of Residues of Cymoxanil in Soil Using Liquid Chromatography*, DuPont Report No. AMR 3595-95, E. I. du Pont de Nemours and Company, Wilmington, Del.
2. Nathan, E. C.; Hill, S. J., *Analytical Method for the Determination of Cymoxanil in Water Using Liquid Chromatography*, DuPont Report No. AMR 3430-95, E. I. du Pont de Nemours and Company, Wilmington, Del.
3. DeMario, D.; Westberg, G.L.; Hill, S. J.; Nathan, E. C., *Analytical Method for the Determination of DPX-JE874 and Cymoxanil Residues in Various Matrices*, DuPont Report No. AMR 3705-95, E. I. du Pont de Nemours and Company, Wilmington, Del.

4. Garteiz, D. A.; Vestal, M. L. *LC Magazine* **1986**, *4*, 334-46.
5. Vestal, M. L. *Science* **1984**, *226*, 275-281.
6. McClory, J. P.; Jones, W., *Field Soil Dissipation of Cymoxanil Following Application of Curzate® M-8 Fungicide,* DuPont Report No. AMR 3401-95, E. I. du Pont de Nemours and Company, Wilmington, Del.

TABLE 1: SOIL CHARACTERIZATION DATA

	Madera, Calif.*	"Drummer"†
Texture	loam	silty clay loam
% Sand	39.2	17.2
% Silt	44.0	52.0
% Clay	16.8	30.8
% Organic Matter	1.5	5.2
pH	7.8	5.7
DuPont Sample ID	89745	1602-120

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* from a soil dissipation study with cymoxanil (pre-application, 0- to 15-cm depth [see Reference 6])

† from South Dakota

TABLE 2: TYPICAL INTEGRATION PARAMETERS

(Finnigan MAT "CHRO" software application)

Minimum peak width	9 or 10
Label noise factor	20 or 21
Baseline window	50
Multiplet resolution	10
Area tail extension	10
Area noise factor	20
Baseline subtraction	none
Smoothing	3 points

TABLE 3: SOIL RECOVERY RESULTS

Sample ID	Soil	Fort. Level (ppm)	Found (ppm)	% Rec.
819B	Madera	0.050	0.043	86
819C	Madera	0.10	0.084	84
819D	Madera	0.25	0.241	96
820B	Madera	0.050	0.048	96
820C	Madera	0.10	0.099	99
820D	Madera	0.25	0.246	99
821B	Drummer	0.050	0.044	89
821C	Drummer	0.10	0.100	100
821D	Drummer	0.25	0.253	101
			Average =	94
			S.D. =	6

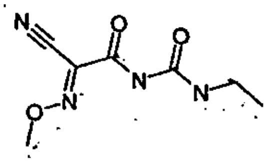
TABLE 4: WATER RECOVERY RESULTS

Sample ID	Water	Fort. Level (ppm)	Found (ppm)	% Rec.
1B	Brandywine	0.0020	0.0016	78
1C	Brandywine	0.0040	0.0042	105
1D	Brandywine	0.010	0.0111	111
2B	Brandywine	0.0020	0.0020	98
2C	Brandywine	0.0040	0.0039	97
2D	Brandywine	0.010	0.0097	97
3B	Brandywine	0.0020	0.0020	102
3C	Brandywine	0.0040	0.0040	100
3D	Brandywine	0.010	0.0092	92
			Average =	98
			S.D. =	9

TABLE 5: WHITE POTATO RECOVERY RESULTS

Sample ID	Matrix	Fort. Level (ppm)	Found (ppm)	% Rec.
1B	tubers	0.020	0.018	88
1C	tubers	0.040	0.035	88
1D	tubers	0. 10	0.082	82
2B	tubers	0.020	0.016	78
2C	tubers	0.040	0.031	77
2D	tubers	0. 10	0.077	77
3B	tubers	0.020	0.016	80
3C	tubers	0.040	0.034	86
3D	tubers	0. 10	0.076	76
			Average =	81
			S.D. =	5

FIGURE 1: CHEMICAL STRUCTURES AND NAMES



Exact MW = 198.075

DuPont Code Number: DPX-T3217

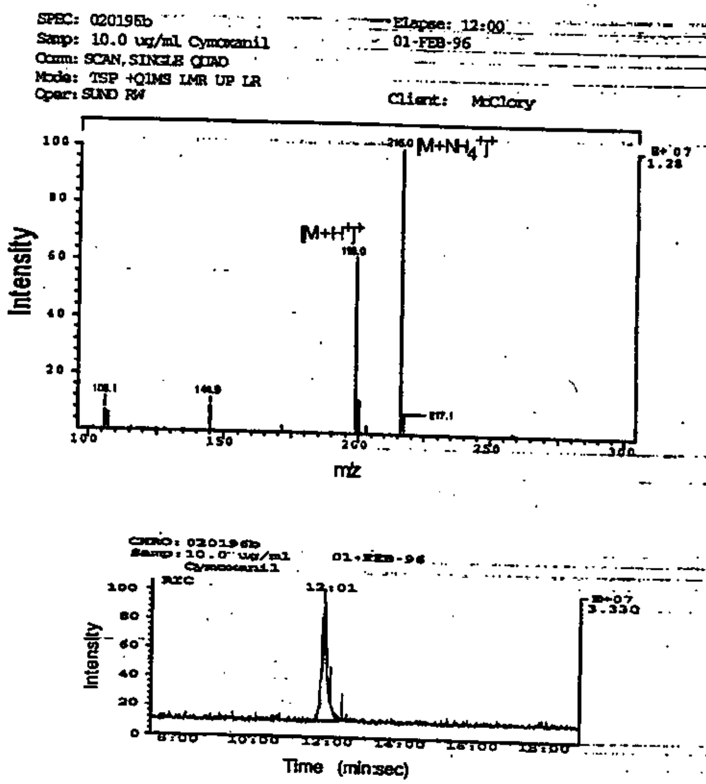
Trivial Name: cymoxanil

CAS Chemical Name:
2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide

CAS Registry No.: 57966-95-7

IUPAC Chemical Name: 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea

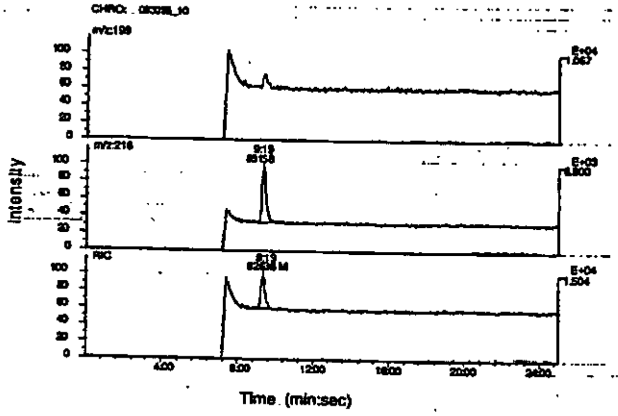
FIGURE 2: FULL SCAN THERMOSPRAY LC/MS SPECTRUM OF CYMOXANIL STANDARD (10.0 µG/ML)



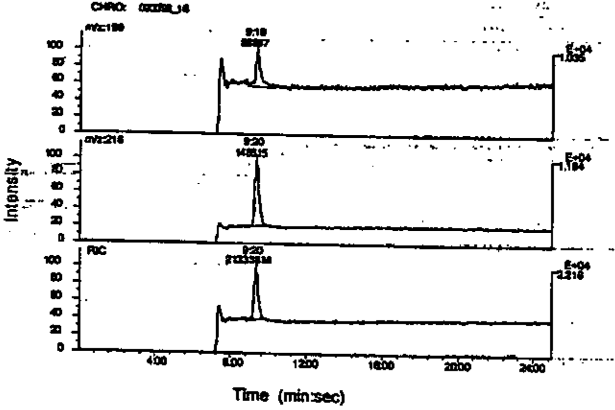
SPEC: 020196b
Samp: 10.0 ug/ml Cymoxanil
Comm: SCAN, SINGLE QUAD
Mode: TSP +Q1MS LMR UP LR
Oper: SUND RW
Elapse: 12:00
01-FEB-96
Client: McClory

CHRO: 020196b
Samp: 10.0 ug/ml Cymoxanil
01-FEB-96

FIGURE 3: REPRESENTATIVE CHROMATOGRAMS: STANDARDS

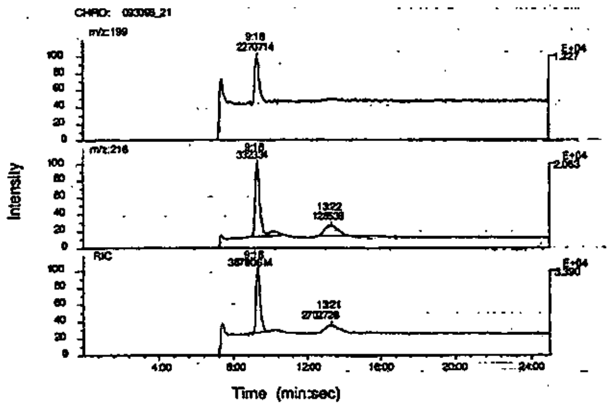


A. 0.005 µg/mL standard

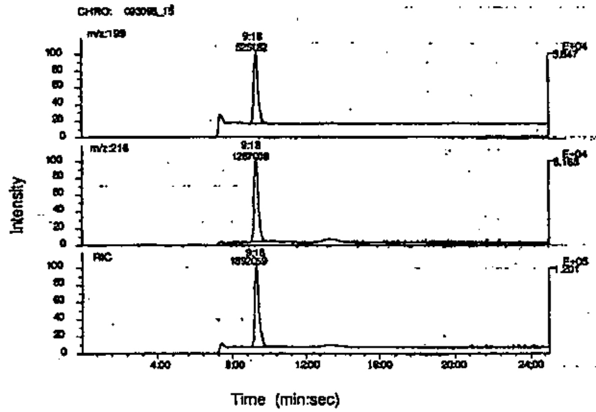


B. 0.010 µg/mL standard

FIGURE 3 (CONTINUED): REPRESENTATIVE CHROMATOGRAMS: STANDARDS



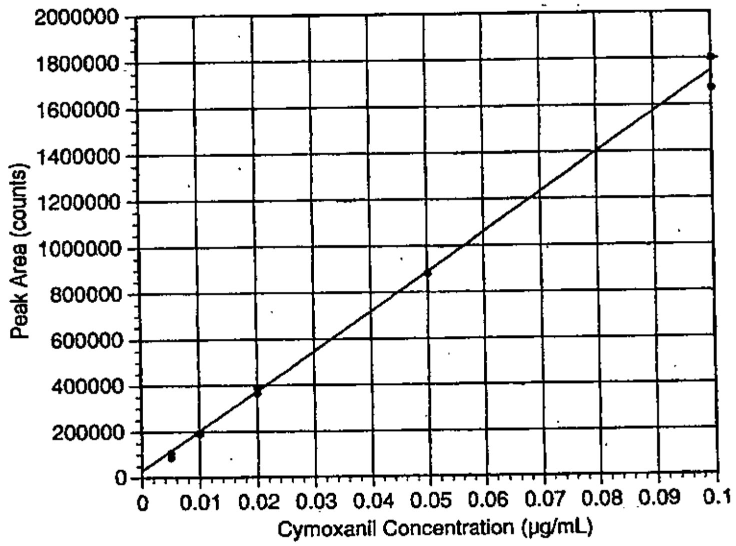
A. 0.020 µg/mL standard



B. 0.100 µg/mL standard

FIGURE 4: REPRESENTATIVE STANDARD CURVE

(Reference: Data Sheet No. 093096, Analysis Date 30-Sep-96)

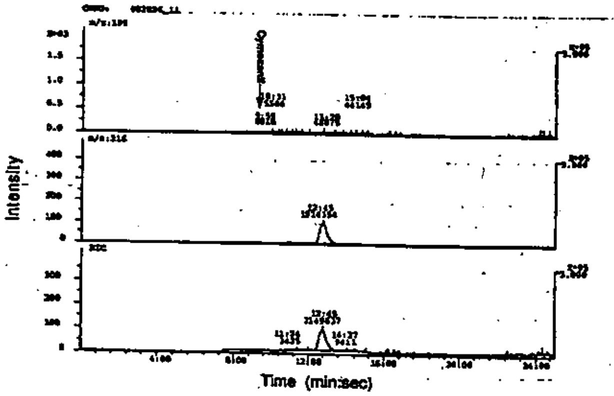


Linear Regression Results

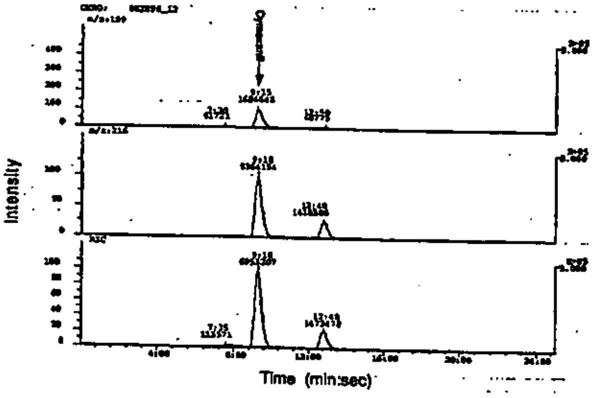
f(x) = 1.72E+7*x + 3.00E+4

R^2 = 0.9974

FIGURE 5: REPRESENTATIVE CHROMATOGRAMS: SOIL

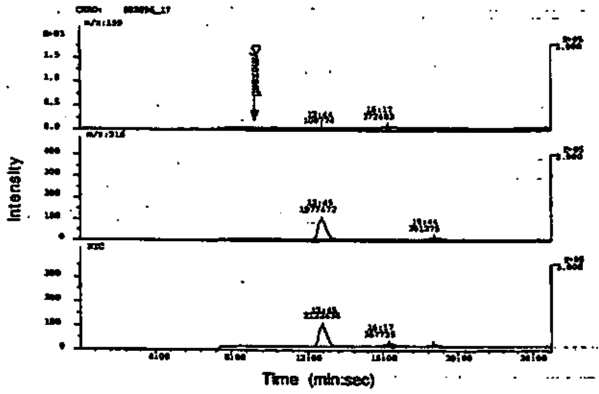


A. Unfortified control (Sample No. 820A)

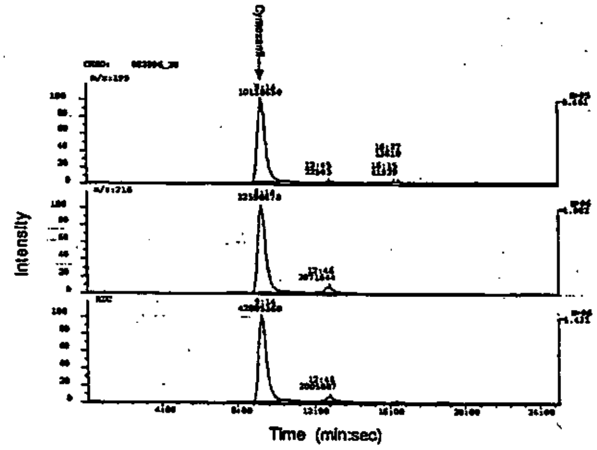


B. Fortified at 0.050 ppm (Sample No. 820B, 96% recovery)

FIGURE 5 (CONTINUED): REPRESENTATIVE CHROMATOGRAMS: SOIL

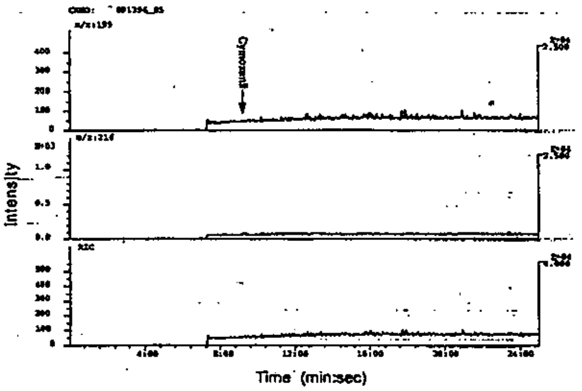


C. Unfortified control (Sample No. 821A)

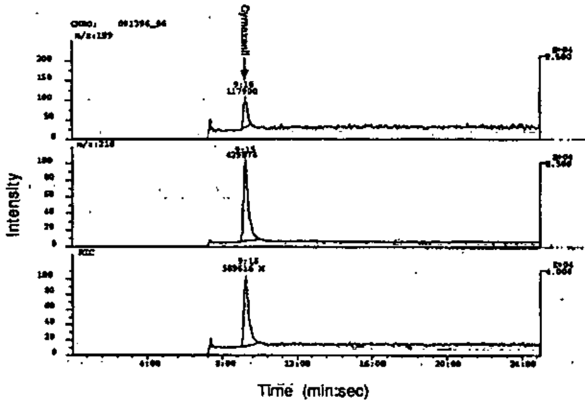


D. Fortified at 0.25 ppm (Sample No. 821D, 101% recovery)

FIGURE 6: REPRESENTATIVE CHROMATOGRAMS: WATER

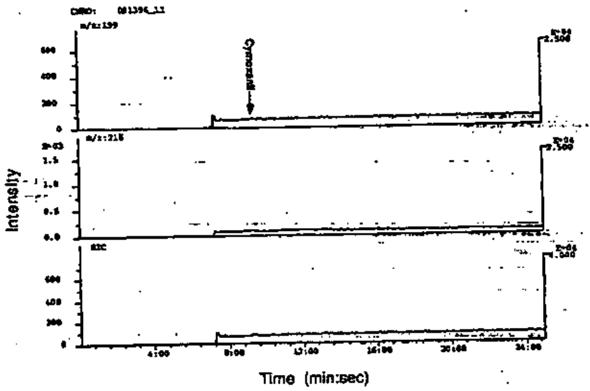


A. Unfortified control (Sample No. Brandywine 1A)

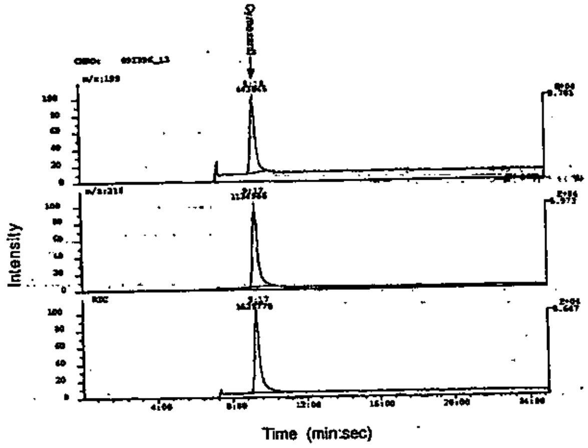


B. Fortified at 0.0020 ppm (Sample No. Brandywine 1B, 78% recovery)

FIGURE 6 (CONTINUED): REPRESENTATIVE CHROMATOGRAMS: WATER

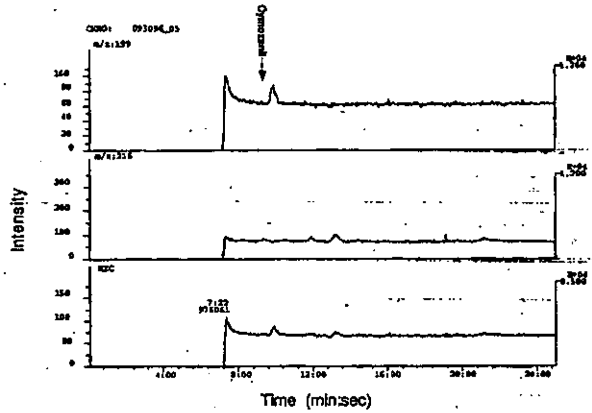


C. Unfortified control (Sample No. Brandywine 2A)

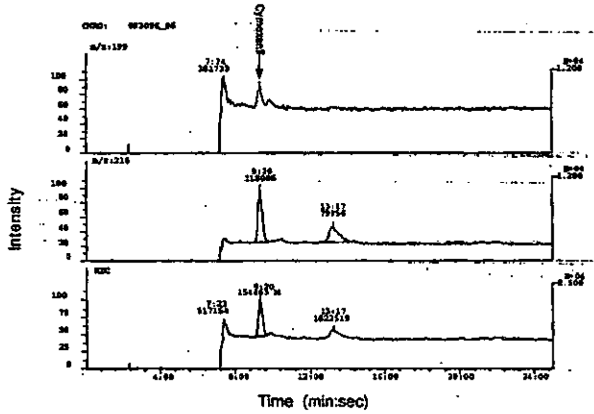


D. Fortified at 0.0040 ppm (Sample No. Brandywine 2C, 97% recovery)

FIGURE 7: REPRESENTATIVE CHROMATOGRAMS: WHITE POTATOES

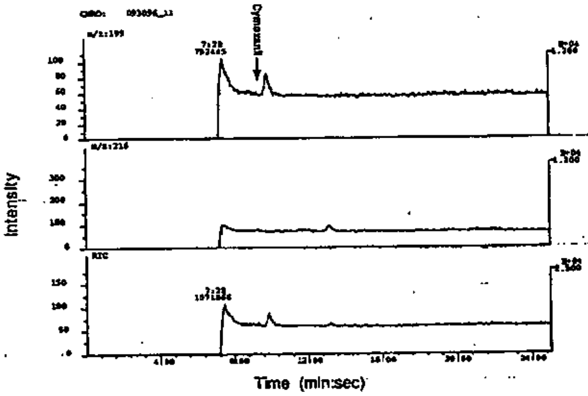


A. Unfortified control (Sample No. 1B)

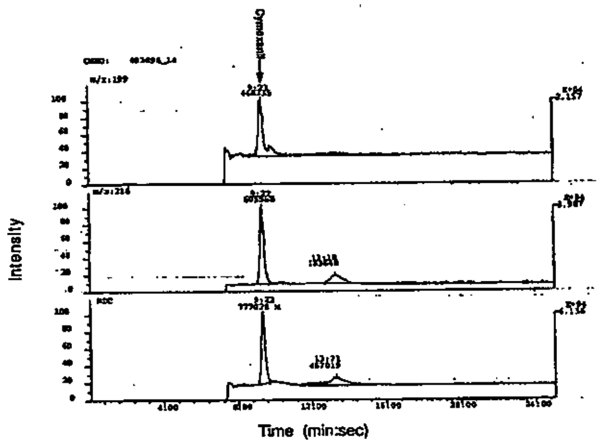


B. Fortified at 0.020 ppm (Sample No. 1B, 88% recovery)

FIGURE 7 (CONTINUED): REPRESENTATIVE CHROMATOGRAMS: WHITE POTATOES



C. Unfortified control (Sample No. 2A)



D. Fortified at 0.100 ppm (Sample No. 2D, 77% recovery)

APPENDIX 1: METHOD VALIDATION RAW DATA SHEETS

Study Number AMR 4171-96
Sample prep PETER DELUKE
Analyte CYMOXANIL

Extraction Date	8/19,20,21/1996
Analysis Date	28-Aug-96
Matrix/Crop	SOIL

Data Sheet Number	082896

LC/MS Method	MTH 3401-95.r1	Injection Volume
Instrument Logbooks	#L0651, #L0589	250 µL

LC/MS Method Deviations
INJECTION VOLUME SHOULD HAVE BEEN 100 ULS, AND VAPORIZER TEMPERATURE SHOULD HAVE BEEN 90 DEGREES
LC STANDARDS WERE MADE IN 20 % METHANOL, 80% .1M AMMONIUM ACETATE PH 4.5

Vial	Sample	Vial	Sample
1	Std-1 (0.2 ppm)	14	820D
2	Std-2 (1.0 ppm)	15	Std-7 (0.4 ppm)
3	Std-3 (0.4ppm)	16	Std-8 (0.2 ppm)
4	Std-4 (.15 ppm)	17	821A
5	819A	18	821B
6	819B	19	821C
7	819C	20	821D
8	819D	21	Std-9 (0.15 ppm)
9	Std-5 (0.2 ppm)	22	Std-10 (1.0 ppm)
10	Std-6 (1.5 ppm)	23	Std-11 (1.5 ppm)
11	820A	24	
12	820B	25	
13	820C	26	

Standard	Conc. (ppm)	Area (counts)	Response Factor (counts/ppm)	Ave. Response Factor (counts/ppm)	RSD (%)
1	0.200	8.00E+06	4.00E+07		
2	1.000	4.11E+07	4.11E+07	4.10E+07	2.38
3	0.400	1.68E+07	4.19E+07	4.22E+07	2.71
4	0.150	OUT OF RANGE			
5	0.200	8.68E+06	4.34E+07	4.17E+07	4.21
6	1.500	5.99E+07	3.99E+07	4.10E+07	4.98
7	0.400	1.58E+07	3.96E+07	4.05E+07	2.64
8	0.200	8.34E+06	4.17E+07	4.22E+07	8.22
9	0.150	6.76E+06	4.51E+07	4.38E+07	3.99
10	1.000	4.41E+07	4.41E+07	4.43E+07	1.63
11	1.500	6.56E+07	4.37E+07		

FORTIFIED SAMPLES	Sample Wt (g)	Fortified Level (ppm)	Aliquot Factor	Final Vol. (mL)	Dilution Factor	MW Factor	Area (counts)	ppm Found	% Recovery
1 819B	20.00	0.05	1	5.00	1	1	7.22E+06	0.043	86
2 819C	20.00	0.100	1	5.00	1	1	1.42E+07	0.084	84
3 819D	20.00	0.25	1	5.00	1	1	4.06E+07	0.241	96
4 820B	20.00	0.05	1	5.00	1	1	7.91E+06	0.049	98
5 820C	20.00	0.100	1	5.00	1	1	1.62E+07	0.099	99
6 820D	20.00	0.25	1	5.00	1	1	4.04E+07	0.248	99
7 821B	20.00	0.05	1	5.00	1	1	7.47E+06	0.044	88
8 821C	20.00	0.100	1	5.00	1	1	1.69E+07	0.100	100
9 821D	20.00	0.25	1	5.00	1	1	4.27E+07	0.253	101

TREATED SAMPLES	Sample Wt (g)	Area (counts)	ppm Found*

[#AF=Aliquot Factor, FV=Final Volume, DF=Dilution Factor]

CONTROL MATRIX	Sample Wt (g)	Area (counts)	ppm Found
1 819A	20.00	0.00E+00	0.000
2 820A	20.00	0.00E+00	0.000
3 821A	20.00	0.00E+00	0.000
4			
5			

Response Factor (RF) = Peak Area/ Standard Concentration
Fort. Level = (Standard Volume Used*[Standard])/Sample weight
ppm Found = [(Peak Area)(Aliquot Factor)(Final Volume)(Dilution Factor)]/[(RF)(Sample Weight)(MW Factor)]
%Recovery = [(ppm Found)/(Fort. Level)] * 100

Analyst _______ Date 10/2/96
Analytical Project Director _______ Date 10/9/96

E.I. du Pont de Nemours and Co.
Du Pont Agricultural Products
Experimental Station
Wilmington, DE 19880-0402

Study Number AMR 4171-96
Sample prep PETER DELUKE
Analyte CYMOXANIL

Extraction Date	9/5,6,11/1996
Analysis Date	13-Sep-96
Matrix/Crop	WATER

Data Sheet Number	091396

LC/MS Method	MTH 3401-95.r1	Injection Volume
Instrument Logbooks	#L0651, #L0569	100

LC/MS Method Deviations
LC STANDARDS WERE MADE IN 20 % METHANOL, 80% .1M AMMONIUM ACETATE PH 4.5

Standard	Conc. (ppm)	Area (counts)	Response Factor (counts/ppm)	Ave. Response Factor (counts/ppm)	RSD (%)
1	0.200	2.32E+06	1.16E+07		
2	0.400	4.37E+06	1.09E+07	1.12E+07	3.04
3	0.050	5.68E+05	1.12E+07	1.09E+07	2.76
4	0.075	7.92E+05	1.06E+07	1.11E+07	4.42
5	0.050	5.77E+05	1.15E+07	1.15E+07	8.51
6	0.400	5.01E+06	1.25E+07	1.26E+07	6.64
7	0.100	1.37E+06	1.37E+07	1.34E+07	5.48
8	0.050	6.94E+05	1.39E+07	1.38E+07	0.84
9	0.200	2.75E+06	1.37E+07	1.40E+07	2.98
10	0.075	1.09E+06	1.45E+07		

Vial	Sample	Vial	Sample
1	Std-1 (0.2 ppm)	14	BRANDYWINE2,D
2	Std-2 (.4 ppm)	15	Std-7 (0.1 ppm)
3	Std-3 (0.05ppm)	16	Std-8 (0.05 ppm)
4	Std-4 (.075 ppm)	17	BRANDYWINE3,A
5	BRANDYWINE1,A	18	BRANDYWINE3,B
6	BRANDYWINE1,B	19	BRANDYWINE3,C
7	BRANDYWINE1,C	20	BRANDYWINE3,D
8	BRANDYWINE1,D	21	Std-9 (0.2 ppm)
9	Std-5 (0.05 ppm)	22	Std-10 (.075 ppm)
10	Std-6 (.4 ppm)		
11	BRANDYWINE2,A		
12	BRANDYWINE2,B		
13	BRANDYWINE2,C		

FORTIFIED SAMPLES	Sample Wt (g)	Fortified Level (ppm)	Aliquot Factor	Final Vol. (mL)	Dilution Factor	MW Factor	Area (counts)	ppm Found	% Recovery
1 BRANDYWINE1,B	100.00	0.002	1	3.00	1	1	6.73E+05	0.002	78
2 BRANDYWINE1,C	100.00	0.004	1	3.00	1	1	1.55E+06	0.004	105
3 BRANDYWINE1,D	100.00	0.010	1	3.00	1	1	4.12E+06	0.011	111
4 BRANDYWINE2,B	100.00	0.002	1	3.00	1	1	8.21E+05	0.002	98
5 BRANDYWINE2,C	100.00	0.004	1	3.00	1	1	1.63E+06	0.004	97
6 BRANDYWINE2,D	100.00	0.010	1	3.00	1	1	4.07E+06	0.010	97
7 BRANDYWINE3,B	100.00	0.002	1	3.00	1	1	9.33E+05	0.002	102
8 BRANDYWINE3,C	100.00	0.004	1	3.00	1	1	1.84E+06	0.004	100
9 BRANDYWINE3,D	100.00	0.010	1	3.00	1	1	4.21E+06	0.009	92

TREATED SAMPLES	Sample Wt (g)	Area (counts)	ppm Found*

[#AF=Aliquot Factor, FV=Final Volume, DF=Dilution Factor]

CONTROL MATRIX	Sample Wt (g)	Area (counts)	ppm Found
1 BRANDYWINE1,A	100.00	0.00E+00	0.000
2 BRANDYWINE2,A	100.00	0.00E+00	0.000
3 BRANDYWINE3,A	100.00	0.00E+00	0.000
4			
5			

Response Factor (RF) = Peak Area/ Standard Concentration
Fort. Level = (Standard Volume Used*[Standard])/Sample weight
ppm Found = [(Peak Area)(Aliquot Factor)(Final Volume)(Dilution Factor)/[(RF)(Sample Weight)(MW Factor)]
%Recovery = [(ppm Found)/(Fort. Level)] * 100

Analyst ______ Date 10/2/96
Analytical Project Director Richard L. Arubey Date 10/9/96

E.I. du Pont de Nemours and Co.
Du Pont Agricultural Products
Experimental Station
Wilmington, DE 19880-0402

21

Same as previous, but add'l. sig. figs. for "ppm Found" RLSruby 10/10/96

Study Number AMR 4171-96
Sample prep PETER DELUKE
Analyte CYMOXANIL

Extraction Date	9/5,9,11/1996
Analysis Date	13-Sep-96
Matrix/Crop	WATER

Data Sheet Number	091396

LC/MS Method	MTH 3401-95#1	Injection Volume 100
Instrument Logbooks	#L0651, #L0889	

LC/MS Method Deviations
LC STANDARDS WERE MADE IN 20 % METHANOL,
80% .1M AMMONIUM ACETATE PH 4.5

Standard	Conc. (ppm)	Area (counts)	Response Factor (counts/ppm)	Ave. Response Factor (counts/ppm)	RSD (%)
1	0.200	2.32E+06	1.16E+07		
2	0.400	4.37E+06	1.09E+07	1.12E+07	3.04
3	0.050	5.59E+05	1.12E+07	1.09E+07	2.76
4	0.075	7.92E+05	1.06E+07	1.11E+07	4.42
5	0.050	5.77E+05	1.15E+07	1.16E+07	5.61
6	0.400	5.01E+06	1.25E+07	1.20E+07	5.64
7	0.100	1.37E+06	1.37E+07	1.34E+07	6.48
8	0.050	6.94E+05	1.39E+07	1.38E+07	0.84
9	0.200	2.75E+06	1.37E+07	1.40E+07	2.98
10	0.075	1.09E+06	1.45E+07		

Vial	Sample	Vial	Sample
1	Std-1 (0.2 ppm)	14	BRANDYWINE2.D
2	Std-2 (.4 ppm)	15	Std-7 (0.1 ppm)
3	Std-3 (0.05ppm)	16	Std-8 (0.05 ppm)
4	Std-4 (.075 ppm)	17	BRANDYWINE3.A
5	BRANDYWINE1.A	18	BRANDYWINE3.B
6	BRANDYWINE1.B	19	BRANDYWINE3.C
7	BRANDYWINE1.C	20	BRANDYWINE3.D
8	BRANDYWINE1.D	21	Std-9 (0.2 ppm)
9	Std-5 (0.05 ppm)	22	Std-10 (.075 ppm)
10	Std-6 (.4 ppm)		
11	BRANDYWINE2.A		
12	BRANDYWINE2.B		
13	BRANDYWINE2.C		

FORTIFIED SAMPLES	Sample Wt (g)	Fortified Level (ppm)	Aliquot Factor	Final Vol. (mL)	Dilution Factor	MW Factor	Area (counts)	ppm Found	% Recovery
1 BRANDYWINE1.B	100.00	0.002	1	3.00	1	1	5.73E+05	0.0016	78
2 BRANDYWINE1.C	100.00	0.004	1	3.00	1	1	1.65E+06	0.0042	105
3 BRANDYWINE1.D	100.00	0.010	1	3.00	1	1	4.12E+06	0.0111	111
4 BRANDYWINE2.B	100.00	0.002	1	3.00	1	1	8.21E+05	0.0020	99
5 BRANDYWINE2.C	100.00	0.004	1	3.00	1	1	1.63E+06	0.0039	97
6 BRANDYWINE2.D	100.00	0.010	1	3.00	1	1	4.07E+06	0.0097	97
7 BRANDYWINE3.B	100.00	0.002	1	3.00	1	1	8.33E+05	0.0020	102
8 BRANDYWINE3.C	100.00	0.004	1	3.00	1	1	1.64E+06	0.0040	100
9 BRANDYWINE3.D	100.00	0.010	1	3.00	1	1	4.21E+06	0.0092	92

TREATED SAMPLES	Sample Wt (g)	Area (counts)	ppm Found*

[#AF=Aliquot Factor, FV=Final Volume, DF=Dilution Factor]

CONTROL MATRIX	Sample Wt (g)	Area (counts)	ppm Found
1 BRANDYWINE1.A	100.00	0.00E+00	0.000
2 BRANDYWINE2.A	100.00	0.00E+00	0.000
3 BRANDYWINE3.A	100.00	0.00E+00	0.000
4			
5			

Response Factor (RF) = Peak Area/ Standard Concentration
Fort. Level = (Standard Volume Used*[Standard])/Sample weight
ppm Found = [(Peak Area)(Aliquot Factor)(Final Volume)(Dilution Factor)/[(RF)(Sample Weight)(MW Factor)]
%Recovery = [(ppm Found)/(Fort. Level)] * 100

Analyst _______ Date 10/21/96
Analytical Project Director R. L. Sruby Date 21-Oct-96

E.I. du Pont de Nemours and Co.
Du Pont Agricultural Products
Experimental Station
Wilmington, DE 19880-0402

Study Number AMR 4171-96
Sample prep PETER DELUKE
Analyte CYMOXANIL

Extraction Date	27-Sep-96
Analysis Date	30-Sep-96
Matrix/Crop	WHITE POTATOES

Data Sheet Number	093096

LC/MS Method	MTH 3401-95.r1	Injection Volume
Instrument Logbooks	#L0851, #L0859	100

LC/MS Method Deviations
LC STANDARDS WERE MADE IN 20 % METHANOL, 80% .1M AMMONIUM ACETATE PH 4.5

Standard	Conc. (ppm)	Area (counts)	Response Factor (counts/ppm)	Ave. Response Factor (counts/ppm)	RSD (%)
1	0.005	1.17E+05	2.34E+07		
2	0.010	2.00E+05	2.00E+07	2.07E+07	11.79
3	0.020	3.73E+05	1.87E+07	1.88E+07	5.86
4	0.050	8.88E+05	1.78E+07	1.77E+07	5.18
5	0.100	1.68E+06	1.68E+07	1.79E+07	6.49
6	0.005	9.56E+04	1.91E+07	1.80E+07	6.43
7	0.100	1.81E+06	1.81E+07	1.92E+07	5.73
8	0.010	2.03E+05	2.03E+07	1.94E+07	5.98
9	0.020	3.96E+05	1.98E+07	1.93E+07	6.71
10	0.050	8.91E+05	1.78E+07		

Vial	Sample	Vial	Sample
1	Std-1 (0.005 ppm)	14	WHITE POTATOES2,D
2	Std-2 (.01 ppm)	15	Std-7 (0.1 ppm)
3	Std-3 (0.02ppm)	16	Std-8 (0.01 ppm)
4	Std-4 (.05 ppm)	17	WHITE POTATOES3,A
5	WHITE POTATOES1,A	18	WHITE POTATOES3,B
6	WHITE POTATOES1,B	19	WHITE POTATOES3,C
7	WHITE POTATOES1,C	20	WHITE POTATOES3,D
8	WHITE POTATOES1,D	21	Std-9 (0.02 ppm)
9	Std-5 (0.1 ppm)	22	Std-10 (.05 ppm)
10	Std-6 (.005 ppm)		
11	WHITE POTATOES2,A		
12	WHITE POTATOES2,B		
13	WHITE POTATOES2,C		

FORTIFIED SAMPLES	Sample Wt (g)	Fortified Level (ppm)	Aliquot Factor	Final Vol. (mL)	Dilution Factor	MW Factor	Area (counts)	ppm Found	% Recovery
1 WHITE POTATOES 1,B	20.00	0.020	13.2	3.00	1	1	1.66E+05	0.018	88
2 WHITE POTATOES 1,C	20.00	0.040	13.2	3.00	1	1	3.14E+05	0.036	89
3 WHITE POTATOES 1,D	20.00	0.100	13.1	3.00	1	1	7.37E+05	0.082	82
4 WHITE POTATOES 2,B	20.00	0.020	12.3	3.00	1	1	1.53E+05	0.016	78
5 WHITE POTATOES 2,C	20.00	0.040	12.3	3.00	1	1	2.99E+05	0.031	77
6 WHITE POTATOES 2,D	20.00	0.100	12.3	3.00	1	1	7.52E+05	0.077	77
7 WHITE POTATOES 3,B	20.00	0.020	11.9	3.00	1	1	1.73E+05	0.016	80
8 WHITE POTATOES 3,C	20.00	0.040	12	3.00	1	1	3.71E+05	0.034	85
9 WHITE POTATOES 3,D	20.00	0.100	12.3	3.00	1	1	7.95E+05	0.076	76

TREATED SAMPLES	Sample Wt (g)	Area (counts)	ppm Found*

(#AF=Aliquot Factor, FV=Final Volume, DF=Dilution Factor)

CONTROL MATRIX	Sample Wt (g)	Area (counts)	ppm Found
1 WHITE POTATOES 1 A	20.00	0.00E+00	0.000
2 WHITE POTATOES 2 A	20.00	0.00E+00	0.000
3 WHITE POTATOES 3 A	20.00	0.00E+00	0.000
4			
5			

Response Factor (RF) = Peak Area/ Standard Concentration
Fort. Level = (Standard Volume Used*[Standard])/Sample weight
ppm Found = ((Peak Area)(Aliquot Factor)(Final Volume)(Dilution Factor)/((RF)(Sample Weight)(MW Factor))
%Recovery = ((ppm Found)/(Fort. Level)) * 100

Analyst R.S. Date 10/2/96
Analytical Project Director Richard E. Lubry Date 10/9/96

E.I. du Pont de Nemours and Co.
Du Pont Agricultural Products
Experimental Station
Wilmington, DE 19880-0402