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VOLUME 2 OF 2 OF SUBMISSION

CGA-169374

TITLE

ANALYTICAL METHOD FOR THE CONFIRMATION OF RESIDUES OF CGA-169374 IN BARLEY BY GC/MSD

DATA REQUIREMENT

GUIDELINE NO. 171-4 (c)
(NEW GUIDELINE NO. 860-1300)

STUDY DIRECTOR

T. L. OAKES, Chemist IV

DATE COMPLETED

JULY 17, 1997

PERFORMING LABORATORY

Human Safety Department
Novartis Crop Protection, Inc. (Formerly Ciba Crop Protection)
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LABORATORY PROJECT IDENTIFICATION

ANALYTICAL METHOD NO. AG-676
STUDY NUMBER 313-97

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VOLUME 1 OF 1 OF REPORT

PAGE 1 OF 100

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Richard Pence 7/21/97

Signature Date

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STATEMENT CONCERNING GOOD LABORATORY PRACTICES

The Good Laboratory Practice Compliance Statement regarding the U. S. Environmental Protection Agency's Good Laboratory Practice Standards (40 CFR Part 160, October 16, 1989) provided on page 25 of this submittal volume for AG-676, and signed by the Study Director, is truthful and accurate.

Robert S. Hackett for R.K. Williams

Robert K. Williams
Manager of Residue Chemistry and
Authorized Representative of Submitter/Sponsor

7/18/97
Date

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Novartis Crop Protection, Inc.
Human Safety Department
Greensboro, North Carolina

ANALYTICAL METHOD FOR THE CONFIRMATION OF RESIDUES OF CGA-169374 IN BARLEY BY GC/MSD

ANALYTICAL METHOD NO. AG-676

Protocol No.: 313-97 Project No.: 420011

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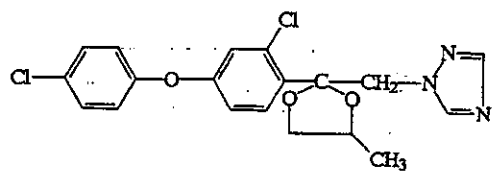
I. SUMMARY AND INTRODUCTION

A. SCOPE

Ciba Analytical Method AG-575B[1] is used for the determination of parent residues of CGA-169374 (1-[[2-[2-chloro-4-(4-chloro-phenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole) in wheat raw agricultural commodities. The chemical structure of CGA-169374 is below.



Method AG-575B is a re-issue of Method AG-575A[2] as a result of an EPA data review and method trial[3] conducted at the EPA Laboratory in Beltsville, Maryland. Method AG-575A and AG-575B were validated for wheat raw agricultural commodities. The outcome of the method trial was a request to add a confirmatory procedure for wheat generated by EPA as part of the methodology. This procedure included the use of a megabore gas chromatography column providing for improved separation of CGA-169374 from background matrix in wheat forage and straw. AG-575B also added reference to a method specificity study requested as part of the data review. In this specificity study, sixty-eight compounds with tolerances in wheat, barley and rye grain were examined for potential interference with the analysis of CGA-169374 by the procedures of the method.

Method AG-575B was also reviewed as part of petition PP# 6F04748 dated June 14, 1996 for difenconazole (Dividend) in or on Barley, Oats, and Triticale. In this review of petition PP# 6F04748 (received by Dr. Gregory T. Peters on September 26, 1996[4]), EPA stated that Novartis had two alternatives: 1)demonstrate the specificity of the analytical method by performing an interference study with all

pesticides with established tolerances on barley and oats, or 2) develop a selective confirmatory procedure (GC/MS).

Novartis Analytical Method AG-676 is applicable as a confirmatory method for detection of CGA-169374 in barley forage, grain, hay, and straw. Novartis Analytical Method AG-676 is identical to Analytical Method AG-575B except for the deletion of a charcoal purification, and the addition of a partition step placed at the end of the method for further purification of grain samples only. Further purification is not necessary for barley forage, straw, and hay. Method AG-676 includes a confirmatory procedure (GC/MSD) as well as the tolerance enforcement procedure outlined in Method AG-575B. See Figure 1 for the Method AG-676 flow diagram.

The limit of reliable quantitation is 0.01 ppm and the limit of detection is 0.02 ng of CGA-169374.

B. PRINCIPLE

A representative crop sample is extracted by refluxing in 8:2 (v/v) methanol:concentrated ammonium hydroxide. After filtering, an aliquot of the extract is diluted with water and saturated sodium chloride, followed by a hexane partition. The hexane fraction is partitioned with acetonitrile (ACN) and the ACN fraction is further purified with a silica gel Sep-Pak followed by a phenyl Bond-elute solid phase extraction. Forage, straw, and hay samples are evaporated and brought up to volume in toluene for GC analysis. Grain samples are purified by silica and phenyl bond elute cartridges (as forage, straw, and hay), the eluates are evaporated to dryness and samples are again reconstituted with ACN followed by an additional hexane partition. The resulting ACN fractions are evaporated to dryness and reconstituted in an appropriate volume of toluene for GC analysis.

Determination of CGA-169374 in barley by AG-676 utilizes gas chromatography and nitrogen/phosphorous (GC/NPD) with a DB-17 megabore column (15 meter x 0.53 mm ID, d_f = 1.0 µm). The confirmatory method described herein uses gas chromatography with a mass selective detector (GC/MSD) and a DB-1701 capillary column (30 meter x 0.25 mm ID, d_f = 0.1 µm).

II. MATERIALS AND METHODS

The materials in Analytical Method AG-676 are presented below. The apparatus and reagents in Method AG-676 are identical to those described in Analytical Method AG-575B with the following exceptions: 1) the deletion of all reagents and materials used in the original charcoal column cleanup procedure and 2) the addition of ethylene glycol (reagents 15 and 16, below).

A. APPARATUS

1.0. Condenser, Allihn, 60-cm.

2.0. Bond-elut, phenyl (Analytichem, #608303-1210-2032).

3.0. Bottle, Boston round, 8-oz.

4.0. Filter paper, Reeve Angel 802 (coarse porosity), and Whatman 2V (medium porosity), 24-cm.

5.0. Flask, Erlenmeyer, 250-mL.

6.0. Flask, round bottom, 50-mL, 100-mL, 250-mL and 500-mL.

7.0. Funnel, filter, 10-cm.

8.0. Funnel, separatory, 250-mL.

9.0. Heating mantle, 500-mL.

10.0. Rotary evaporator, Buchi, or equivalent.

11.0. Scintillation vial, 20-mL.

12.0. Sep-Pak, silica gel (Waters Assoc. #51900).

13.0. Syringe, 20-mL, Luer-Lok.

14.0. Visiprep solid phase extraction manifold (Supelco #57030 or equivalent).

B. REAGENTS

1.0. Acetone, reagent grade (Fisher cat. #A18SK-4).

2.0. Acetonitrile (ACN), HPLC grade (Fisher cat. #A998-4).

3.0. Ammonium hydroxide, concentrated (28-30%), reagent grade (Fisher cat. #A669-212).

4.0. Ethyl ether, anhydrous, Reagent A.C.S. (Fisher Cat. #E138-1).

5.0. Hexane, HPLC grade (Fisher Cat. #H302-4).

6.0. Methanol, HPLC grade (Fisher Cat. #A452-4).

7.0. 8:2 (v/v) Methanol:conc. ammonium hydroxide.

8.0. Sodium chloride, reagent grade (Fisher cat. #S271-500).

9.0. Sodium chloride, saturated solution in distilled water.

10.0. Toluene, HPLC grade (Fisher cat. #T290-4).

11.0. 85:15 (v/v) Toluene:acetone.

12.0. 9:1 (v/v) Hexane:ethyl ether.

13.0. Water, distilled.

14.0. CGA-169374 Analytical Standard, Novartis, P.O. Box 18300, Greensboro, NC, 27419.

15.0 Polyethylene glycol (Sigma Catalog) CAS #25322-68-3.

16.0 1:1 Polyethylene glycol:toluene

C. ANALYTICAL PROCEDURE

All analytical procedures described in this section are identical to those described in AG-575B except for the omission of the charcoal column cleanup and the addition of an extra partition step in Section 6.0 (for grain only). Polyethylene glycol has been added to the final fractions to correct injector enhancement problems. In addition, a confirmatory procedure is included for the identification of CGA-169374 residues by GC/MSD.

1.0. Sample Preparation

Samples are prepared under the general guidelines of the U.S. Food and Drug Administration Pesticide Analytical Manual Volume I, Section 141. Frozen forage and straw are chopped in a Hobart food chopper. Frozen grain is ground in a Wiley Mill.

2.0. Extraction

2.1. Weigh a 20-g subsample from a well-homogenized chopped or ground crop sample into a 500-mL round bottom flask. Add 200 mL of 8:2 methanol:concentrated ammonium hydroxide solution to grain or forage, and 300 mL to straw samples (include several boiling chips).

2.2. Fit the round bottom flask onto an Allihn condenser, place the flask in a heating mantle and reflux for two hours.

2.3. Cool the sample to room temperature and filter the extract through a Reeve Angel 802 filter paper placed inside a Whatman 2V filter paper into an 8-oz. Boston round bottle.

3.0. Partition Cleanup

3.1. Transfer a 40-mL aliquot of the filtered extract solution for grain, hay, or forage, (or a 60-mL aliquot for straw) into a 250-mL separatory funnel. Add 100 mL of distilled water and 4 mL of saturated sodium chloride to the separatory funnel.

3.2. Partition the aqueous methanol with 50 mL of hexane by shaking the separatory funnel for 30 seconds. Allow the layers to separate, and draw the lower aqueous layer into a 250-mL Erlenmeyer flask, leaving any emulsion in the separatory funnel. Add 4 mL of saturated sodium chloride to the separatory funnel to break any remaining emulsion. Draw the aqueous layer and any remaining emulsion and combine it with the aqueous layer in the 250-mL Erlenmeyer flask.

3.3. Pour the hexane layer from the top of the separatory funnel into a second 250-mL separatory funnel (minimize the transfer of water).

3.4. Transfer the aqueous fraction back into the first 250-mL separatory funnel and again partition with hexane as described (Section II.C.3.2).

3.5. Combine the hexane fraction from Section II.C.3.4 with that from Section II.C.3.3 in the second 250-mL separatory funnel. Partition the hexane with two 50-mL portions of ACN. Combine the ACN fractions in a 250-mL round bottom flask and evaporate to dryness on a rotary evaporator (bath temperature of approximately 40°C).

4.0. Silica Sep-Pak Cleanup

4.1. Dissolve the residue in the round bottom flask (Section II.C.3.5) with 5 mL of toluene.

4.2. **Note that the Silica Sep-Pak procedure is performed using gravity flow. Do not let the column bed go to dryness between elutions.** Connect a silica Sep-Pak to the Luer fitting on a 20-mL Luer Lok syringe barrel. Prewash the Sep-Pak with 5 mL of toluene. Load the toluene solution from Section II.C.4.1 onto the Sep-Pak. Discard the eluate.

4.3. Rinse the 250 mL round bottom flask with 5 mL of toluene and load the toluene onto the silica Sep-Pak. Discard the eluate.

4.4. Elute CGA-169374 from the Sep-Pak with 15-mL of 85:15 toluene:acetone and collect the eluate in a 50-mL round bottom flask.

4.5. Evaporate the contents of the flask to dryness on a rotary evaporator (bath temperature of approximately 40°C).

5.0. Phenyl Bond-Elut Solid Phase Extraction Cleanup

5.1. Dissolve the residue in the 50 mL round bottom flask (Section II.C.4.5) with 3 mL of hexane.

5.2. **Note that all Phenyl Bond-elut elutions are performed under low vacuum. Do not let the column bed go to dryness between elutions.** Connect the Phenyl Bond-elut column to a solid phase extraction manifold and prewash the column with 3 mL of hexane (under low vacuum). Discard the eluate.

5.3. Load the hexane solution from Section II.C.5.1 onto the Bond-elut column. Rinse the 50-mL round bottom flask with 3 mL of hexane and load onto the Bond-elut column. Repeat the rinse with an additional 3 mL of hexane and load onto the column. Discard the eluates.

5.4. Wash the Bond-elut column with 3 mL of 9:1 hexane:ethyl ether and discard the eluate. Repeat this wash three more times, discarding the eluate.

5.5. Elute CGA-169374 from the Bond-elut with 2 mL of methanol, collecting into a scintillation vial. Repeat this step three more times, collecting 8 mL of methanol in the scintillation vial.

5.6. Transfer the contents of the scintillation vial to a 50-mL round bottom flask. Rinse the scintillation vial with 3 mL of methanol and add to the 50-mL round bottom flask.

5.7. Evaporate the contents of the flask to dryness on a rotary evaporator (bath temperature of approximately 40°C).

5.8 For barley forage, hay, and straw, the residue is brought to final volume for GC analysis. For grain samples, an additional partition purification is performed (below).

6.0. <u>Partition Cleanup (grain only)</u>

6.1 Reconstitute the residue from Section 5.7 (above) in 25 mL of ACN.

6.2 Partition the ACN with 25 mL of hexane by shaking the separatory funnel for 30 seconds. Allow the layers to separate and draw the lower layer into a 250 round bottom flask. Discard the hexane portion.

6.3 Pour the ACN back into the separatory funnel and partition with an additional 25 mL of hexane by shaking the separatory funnel for 30 seconds. Allow the layers to separate and draw the lower layer (ACN) into a 250 round bottom flask. Discard the hexane portion

6.4 Evaporate the ACN using rotary evaporation (bath temperature of approximately 40°C).

6.5 Dissolve the samples in an appropriate volume of toluene to yield the final fraction for GC analysis.

7.0. Confirmatory Method GC/MSD Analysis - Final Fraction

7.1 Place 1 mL of sample solutions (diluted in appropriate final volume (Section 6.5)) and standard solutions into sample injection vials. Add 10 µl of 50% polyethylene glycol: toluene to both samples and standards. Mix thoroughly (vortex) and inject on GC/MSD system.

D. INSTRUMENTATION

1.0 Description and Operating Conditions

For tolerance enforcement purposes, residues are determined using the instrumentation described in Analytical Method AG-575B with modifications described herein. Method AG-575B was modified to employ a DB-17 (15 meter x 0.53 mm ID, d_f = 1.0 µm) megabore analytical column equipped with NPD (Table I).

For confirmation of residues, the same final fractions are analyzed using a GC/MSD system with a DB-1701 analytical column (30 m x 0.25 mm, d_f = 0.10 µm), using the conditions outlined in Table II.

2.0. Calibration and Standardization

2.1. The GC and GC/MSD systems are calibrated with each analytical set by checking the retention time and detector response relative to previous runs. Retention times should not vary by more than 5% and detector response should not vary more than 10% between runs.

2.2. The GC and GC/MSD systems are standardized by injecting aliquots of standard solutions of CGA-169374 in a working range of 0.02 - 1.0 ng/injection. A linear regression function is generated by comparing detector response and ng injected. Typical standard chromatograms and corresponding calibration curves for AG-575B (GC/NPD) and AG-676 (GC/MSD) are shown in Figures 3 through 6, respectively.

E. INTERFERENCES

1.0. Analysis of control samples of barley forage, hay, and straw show no significant interferences at a CGA-169374 screening level of 0.05 ppm. Analysis of control samples of barley grain show no significant interferences at a screening level of 0.01 ppm. No interferences have been observed in reagent blanks. See Figure 7 for example chromatograms of reagent blanks for both GC/NPD (tolerance enforcement) and GC/MSD (confirmatory) methods.

2.0. As part of a data review for an import tolerance petition[5], EPA requested that the specificity of the method (AG-575B) be demonstrated. A method specificity study[6] was conducted where 68 compounds having tolerances in wheat, barley and rye were examined for potential interferences with CGA-169374 using these procedures (AG-575B). Upon examination, eight compounds were

eliminated from further testing based on chemical or physical properties. The remaining sixty compounds were fortified at tolerance levels on wheat grain and the samples were analyzed by the method procedures. None of the compounds tested were found to interfere with the determination of CGA-169374 residues at the proposed tolerance level.

F. CONFIRMATORY TECHNIQUES

Presented in this method

G. TIME REQUIRED

A skilled analyst can carry out the extraction, purification, and analysis of a set (4-6 samples) in a 24-hour period including GC/MSD analysis.

H. MODIFICATIONS

None.

I. PREPARATION OF STANDARD CGA-169374 SOLUTIONS

1.0. Weigh 100 mg of the CGA-169374 analytical standard into a 100-mL volumetric flask and dilute to the mark with acetone.

1.1. Make serial dilutions of the 1 mg/mL standard solution with toluene to give a series of injection standards in a range of 0.01 to 1.0 ng/µl of CGA-169374.

J. DETERMINATION OF SAMPLE RESIDUES

1.0. Inject the sample extract aliquots into the GC/MSD system under the same conditions as for standards. Make appropriate dilutions of the samples (if necessary) with toluene to bring the sample peak heights within the range of the standard curve. Compare the peak heights of the unknown samples to the standard curve or enter the peak height into a least squares program to determine the

nanograms of CGA-169374 in the injected aliquot. (See Table IV for example sample calculation.) Typical chromatograms for control and fortified barley samples are shown in Figures 8 through 15.

2.0. Calculate CGA-169374 residues by the following equation:

$$(1)\quad \text{PPM CGA-169374} = \frac{\text{CGA-169374 found (ng)}}{\text{mg sample injected}} \times \frac{100}{R\%}$$

where mg sample injected is calculated as follows:

$$(2)\quad \frac{G}{V} \times V_a \times \frac{V_i}{V_f} \times \frac{V}{V + (W \times M/100)} = \text{mg inj.}$$

G = milligrams of sample extracted
V = the volume of the extraction solvent (mL)
V_f = total volume of final injection solution (µl)
R% = recovery ratio given by equation (3)
V_i = injection volume (µl)
V_a = aliquot volume (mL)
W = weight of sample extracted (g)
M = moisture content of sample (%)

3.0. Fortification Experiments

The method is validated for each set of samples analyzed by including an untreated control sample and one or more control samples fortified prior to extraction. Barley forage, hay, and straw samples are fortified at 0.05 ppm CGA-169374 (LOQ) Barley grain samples are fortified at 0.01 ppm (LOQ) or 0.1 ppm (tolerance level). Additional fortifications are also made at higher concentrations.

3.1. Add up to 2.0 mL of the appropriate standard solution of CGA-169374 to 20 g of the substrate prior to the addition of the extraction solvent (Section II.C.2.1). Allow the sample

to sit for a few minutes before adding the extraction solvent. Adjust the concentration of the fortification solution so that no more than 2 mL of solution is added to 20-g of substrate.

3.2. Extract, purify and analyze the fortified samples with the procedures outlined in this method.

3.3. Determine the recovery factor by first subtracting the detector response for CGA-169374, if any, in the control sample (if any) from the CGA-169374 response in the recovery sample. Then calculate the recovery factor expressed as a percentage (R%) by the following equation:

$$(3)\ R\% = \frac{\text{CGA - 169374 found (ng)}}{\text{ppm CGA - 169374 added}} \times 100\%$$

Note: Recovery corrections are not used for tolerance enforcement calculations.

4.0. Calculation Data for All Analyses

All chromatograms for all commodities analyzed are included in this report. All validation data needed to reproduce the calculations are included with the chromatogram figures. The data includes the slope and Y-intercept calibration values, spiking level, mg injected, resulting peak height, ng recovered, and percent recovery for each sample.

To calculate the ng found for each analysis, the equation Y=mX+b is utilized. For example, in Figure 10 (sample chromatogram 2), the slope (m) = 66068 millivolts per ng (mV/ng), the Y-intercept (b) = 0 mV, and the peak height = 7386 µV.

X = (7386 µV - 0)/66068 = 0.112 ng found.

To convert to ppm, divide the ng determined in the sample by the mg injected.

0.112 ng/1.98 mg = 0.057 ng/mg = 0.057 ppm

To determine percent recovery, divide the calculated ppm by the spiked level and multiply by 100.

(0.057 ppm/0.05 ppm) x 100 = 113% recovered.

III. RESULTS AND DISCUSSION

This method provides mass spectral confirmation of CGA-169374 in barley using established tolerance enforcement methodology (AG-575B). Confirmation is accomplished by analyzing ions unique to CGA-169374 using GC/MSD with selected ion monitoring. Figure 2 shows an example mass spectrum for CGA-169374 using GC/MSD in the full scanning mode under electron impact conditions. Note the absence of a molecular ion (m/z 406). However, fragmentation of the parent molecule affords ions at m/z = 265 and 323 with associated chlorine clusters which serve as marker ions in high abundance. Therefore, confirmation of CGA-169374 residues can be achieved using selected ion monitoring (SIM) to detect the following ions: (m/z = 265, 323, and 325). The chlorine ion (m/z = 267 associated with m/z = 265 was not used for quantitation due to inadequate sensitivity at low concentrations. The GC/MSD should be calibrated by injecting 2-µl aliquots of standard solutions containing CGA-169374 in a working range of 0.02 - 1.0 ng/injection. Quantitation is obtained by taking the ratio of the qualifying ions at m/z = 265 and 325 to the target ion at m/z = 323. Structural confirmation can be made if the sample ion ratios match the ratios obtained from the standard (+/- 25%).

This method validation was performed under Protocol 313-97[7]. Note that the tolerance enforcement method, AG-575B, has been previously validated on weathered samples for accuracy, precision, and extractability. Ruggedness has also been previously demonstrated during ruggedness trials for methods AG-537A and AG-575[8].

All procedures for sample extractions, purifications, and original GC/NPD conditions are contained in this method (AG-676). In addition, confirmatory GC/MSD conditions and associated validation are provided in this method. Typical standard chromatograms and the corresponding calibration plots are shown in Figures 3-4 and 5-6 for GC/NPD (tolerance enforcement) and GC/MSD (confirmatory) quantitation, respectively. A summary of the residue results, including copies of the raw data summary sheets are reported in Novartis Residue Test Report RI-MV-011-97[9].

Validations of Method AG-676 were performed by analyzing untreated controls, and fortified controls of barley hay, straw, forage, and grain.

Recovery results from control and fortified control samples were used to calculate accuracy as a function of mean and standard deviation (SD). The results are shown in Table III and are compared to results from the initial analysis by Method AG-575B. Recovery and control values from the confirmatory analyses compare well with the initial analyses of these samples (below).

Substrate	GC/NPD MEAN (SD)	GC/MSD MEAN (SD)
Hay	107 (6.1)	98.9 (4.1)
Forage	104 (6.7)	105 (10.2)
Straw	95.3 (7.3)	102 (1.9)
Grain	95.0 (3.7)	119 (27)

Figure 7 shows representative reagent blank chromatograms obtained from AG-575B (GC/NPD) and AG-676 (GC/MSD). Figures 8, 10, 12, and 14 show representative sample chromatograms (control and recoveries from analyses of barley straw, hay, forage, and grain, respectively), using the tolerance enforcement method (AG-575B). Figures 9, 11, 13, and 15 show representative chromatograms (control and recoveries) from the corresponding confirmatory analysis of barley straw, hay, forage, and grain, respectively, using AG-676.

Results show that using GC/MSD is adequate in determining residues of CGA-169374 at the proposed tolerance levels (0.05 ppm for hay, straw, and forage; 0.1 ppm for grain).

Grain recoveries were also run at the Limit of Quantitation (LOQ) of Method AG-575B (0.01 ppm). Recoveries using GC/MSD were somewhat enhanced at this low level due to matrix. Grain recoveries using AG-676 (confirmatory procedure) at 0.01 ppm (LOQ) were in the range of 150%-170%. However, overall grain recoveries ranged from 89.4% to 171% with a mean value of 119%.

The protocol, raw data, Residue Test Report (RTR) and final report (AG-Method) are archived at the Agricultural Group Archives, Novartis Corporation, Greensboro, North Carolina. Residue Samples will be retained in the Cold Storage Building, Greensboro, North Carolina until authorization for disposal by the Study Director and verification by the Quality Assurance Unit.

See Residue Test Report RI-MV-011-97" for test substance ID, protocol amendments, protocol deviations, and any circumstances affecting the quality and integrity of the study.

IV. CONCLUSION

Analytical Method AG-676 is a valid and accurate confirmatory method for the determination of parent residues of CGA-169374 in barley. This confirmatory technique offers a highly specific method for the verification of potential CGA-169374 residues in barley and other cereal grains at or above the tolerance level.

V. CERTIFICATION

The reports and experimental results included in this study, Laboratory Project ID. AG-676, are certified to be authentic accounts of the experiments.

Timothy L. Oakes 7/17/97

T. L. Oakes, Date
Chemist IV
Residue Chemistry
Human Safety Department

CERTIFICATION OF GOOD LABORATORY PRACTICES

The analytical work reported in AG-676 was performed in accordance with Good Laboratory Practice Standards, 40 CFR Part 160.

Timothy L. Oakes 7/17/97

T. L. Oakes, Date
Chemist IV
Residue Chemistry
Human Safety Department

Dennis S. Hazlett for R.K. Williams 7/17/97

Robert K. Williams Date
Manager, Residue Chemistry
Agent of Sponsor

Sponsor/submitter: Novartis Crop Protection, Inc.
Human Safety Department
Residue Chemistry Laboratory
Post Office Box 18300
Greensboro, NC 27419

Novartis Crop Protection, Inc.
Quality Assurance Unit
Greensboro, NC

QUALITY ASSURANCE STATEMENT

Study Title: Validation of Draft Method AG-676 for the Confirmation of CGA-169374 in or on Barley

Study Director: Tim L. Oakes

Study Number: 313-97

Report Number: Method AG-676

Pursuant to Good Laboratory Practice Regulations, this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

AUDIT TYPE	INSPECTION/AUDIT DATES	REPORTING DATE
Protocol	5/12/97	5/12/97
In-Progress	6/19/97	6/19/97
Final Report	7/10-11/97	7/11/97

Prepared by: Barbara S. North
Barbara S. North

Date: 7/11/97

VII. TABLES AND FIGURES

TABLE I. GC/NPD OPERATING PARAMETERS FOR CGA-169374 RESIDUES BY AG-575B

Instrumentation:	Hewlett-Packard 5890 Series II Gas Chromatograph Hewlett-Packard Nitrogen Phosphorous Detector
Column:	DB-17, J&W Scientific, Inc., 15 m x 0.53 mm, d_f = 1.0µm
Gases:	Carrier: Helium 4 psi head pressure at 80°C, constant flow mode.
Injection:	2 µl, Splitless
Temperatures:	Injector: 290°C Detector Temperature: 300°C Column Temperature Program: Oven initial temp: 80°C Oven initial time: 2 min. Ramp 1 rate: 25°C Oven final temp: 270°C Oven final time: 20 minutes Run time: 27 minutes
Timetable Events:	Purge A = Off

CGA-169374 retention time: ~16.5 minutes

TABLE II. GC/MSD OPERATING PARAMETERS FOR THE CONFIRMATION OF CGA-169374 RESIDUES BY AG-676

Instrumentation:	Hewlett-Packard 5890 Series II Gas Chromatograph Hewlett-Packard 5973 Mass Selective Detector (EI Mode)
System Software:	Microsoft DOS 5.0 Microsoft Windows 95
Application Software:	Hewlett Packard HPG1034C MS ChemStation, Version C.00.07
Column:	DB-1701, J&W Scientific, Inc., 30 m x 0.25 mm, d_f = 0.15µm Mode: Constant Flow Initial Flow: 1.0 mL/min Nominal Initial Pressure: 10 psi Outlet: MSD Outlet pressure: vacuum
Injection:	2 µl, Splitless
Oven:	Oven initial temp: 100°C Oven initial time: 1 min. Ramp 1 rate: 20°C/min Oven final temp: 290°C Final time: 20 min Run time: 30.5 min MSD Transfer Line temp: 280°C
Front Inlet (Split/Splitless)	Mode: Pulsed Splitless Initial temp: 240°C Pressure: 10 psi Pulsed Pressure: 50 psi Pulse time: 0.3 min Purge Flow: 50 mL/min Purge Time: 0.75 min Total Flow: 53.3 mL/min Gas: Helium
GC/MS Run Parameters:	Mode: SIM Dwell Time: 100 msec. EMV offset: 400
Timetable Events:	Time 0.0 Purge Off Time 0.75 min Purge On
CGA-169374 retention time:	14-15 min
Mode:	Selective Ion Monitoring
Target Ions:	m/z 265 m/z 323 m/z 325

TABLE III. RECOVERY RESULTS FOR CONTROL AND CGA-169374 FORTIFIED CONTROL SUBSTRATES USING METHODS AG-575B AND AG-676

Barley Substrate	Numeric ID	Fortification Level (ppm)	GC/MSD Recovery (%)	GC/NPD Recovery (%)
Hay	200043	control	--	--
Hay	201644	0.05	100	113
Hay	201645	0.1	102	101
Hay	201646	0.5	94.3	108
		Average =	98.9	107
		Standard Deviation =	4.1	6.1
Forage	203792	control	--	--
Forage	203849	0.05	96.1	110
Forage	203850	0.1	116	106
Forage	203851	0.5	103	96.5
		Average =	105	104
		Standard Deviation =	10.2	6.7
Straw	200044	control	--	--
Straw	201647	0.05	104	95.5
Straw	201648	0.1	100	87.9
Straw	201649	0.5	102	102
		Average =	102	95.3
		Standard Deviation =	1.9	7.3
Grain	200045	control	--	--
Grain	201463 (Set 1)	0.01	148	93.4
Grain	201463 (Set 2)	0.01	171	96.0
Grain	201463 (Set 2)	0.01	148	87.5
Grain	201464 (Set 2)	0.04	118	100
Grain	201464 (Set 1)	0.04	106	93.7
Grain	201465 (Set 1)	0.2	108	95.0
Grain	201465 (Set 2)	0.2	97.0	94.2
Grain	201463a	0.1	107	95.5
Grain	201464a	0.2	89.4	101
Grain	201465a	1.0	96.7	93.8
		Average =	119	95.0
		Standard Deviation =	27	3.7

FIGURE 1. FLOW DIAGRAM FOR AG-676

All Substrates

20-g Crop Sample

Reflux grain or forage for 2 hr. with 200 mL 8:2 methanol:ammonium hydroxide
(Reflux straw with 300 mL)

Cool and filter

Dilute a 40-mL aliquot of grain or forage extract with 100 mL of water and 4 mL sat. NaCl
(Use 60-mL aliquot with straw)

Partition with 2x50 mL hexane

Hexane — Aqueous-Methanol-Discard

Partition with 2x50 mL ACN

Hexane-discard — ACN

Evaporate to dryness

Dissolve residue in 5 mL toluene

Load onto a silica gel Sep-Pak

1) Load - discard

2) Wash with 5 mL of toluene - discard

3) Elute with 15 mL of 85:15 toluene:acetone - collect

Evaporate to dryness

Dissolve the residue in 3.0 mL of hexane
(cont.)

FIGURE 1. FLOW DIAGRAM FOR AG-676 (Continued)

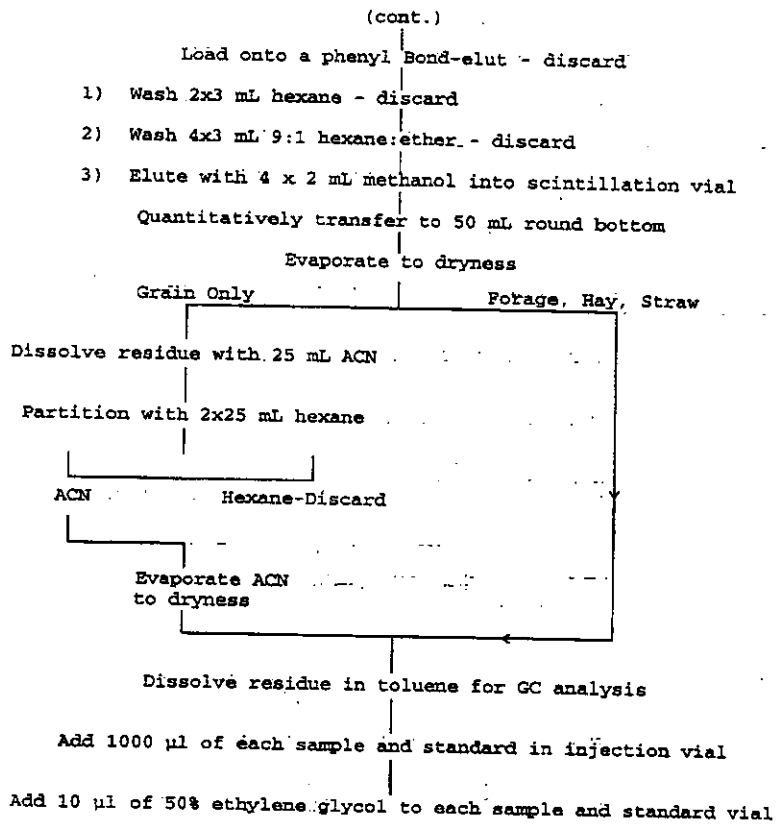


FIGURE 2. REPRESENTATIVE MASS SPECTRUM FOR CGA-169374 USING GC/MSD IN FULL SCAN MODE

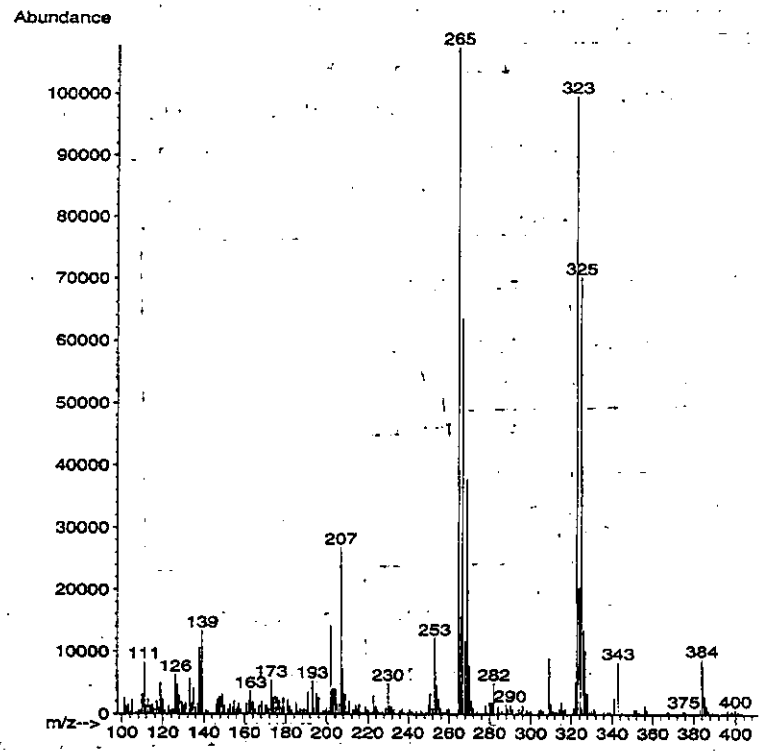
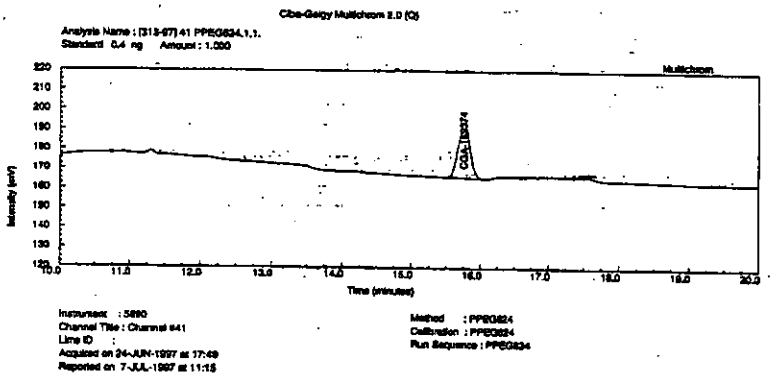
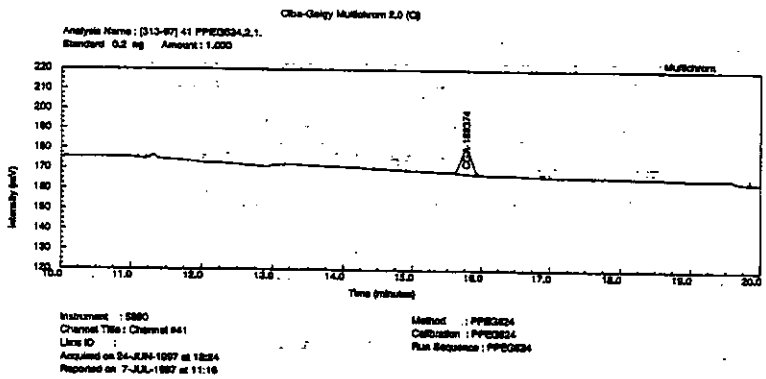


FIGURE 3. TYPICAL STANDARD CHROMATOGRAMS OF CGA-169374 USING GC/NPD (AG-575B)
(Multichrom Worksheet: 313-97-PEGG)

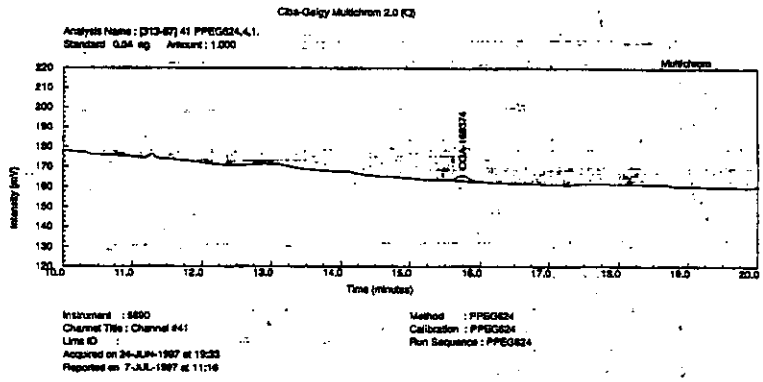


1. 0.4 ng standard CGA-169374 (Response Factor - 28485 µV)

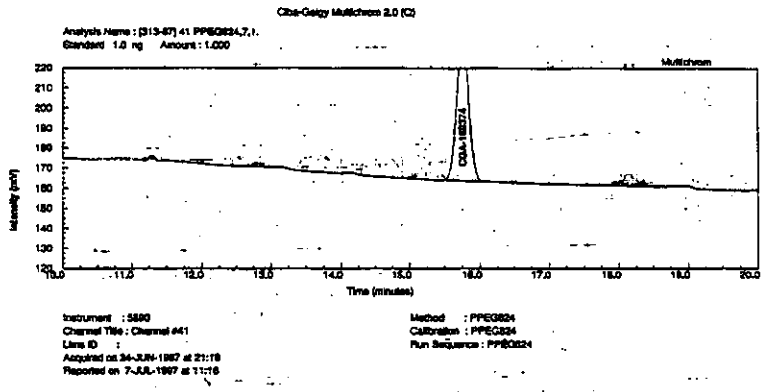


2. 0.2 ng standard CGA-169374 (Response Factor - 12495 µV)

FIGURE 3. TYPICAL STANDARD CHROMATOGRAMS OF CGA-169374 USING GC/NPD (AG-575B) (Continued)
(Multichrom Worksheet: 313-97-PEGG)

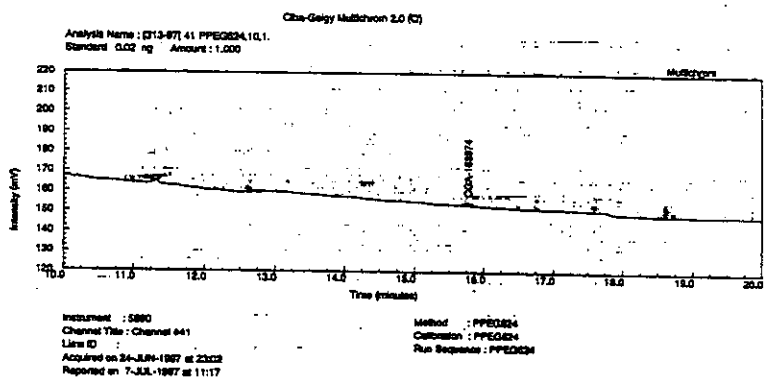


3. 0.04 ng standard CGA-169374 (Response Factor = 2739 µV)

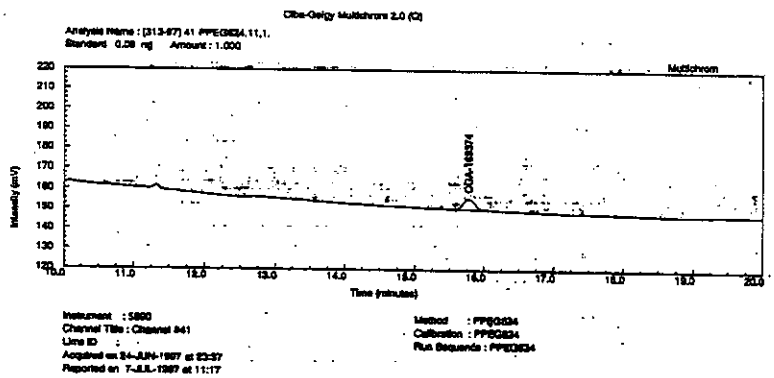


4. 1.0 ng standard CGA-169374 (Response Factor = 72856 µV)

FIGURE 3. TYPICAL STANDARD CHROMATOGRAMS OF CGA-169374 USING GC/NPD (AG-575B) (Continued)
(Multichrom Worksheet: 313-97-PEGG)



5. 0.02 ng standard CGA-169374 (Response Factor - 1313 µV)



6. 0.08 ng standard CGA-169374 (Response Factor - 5481 µV)

FIGURE 4. TYPICAL CALIBRATION PLOT OF CGA-169374 CORRESPONDING TO FIGURE 3 OBTAINED USING AG-575B (Multichrom Worksheet: 313-97-PEGG)

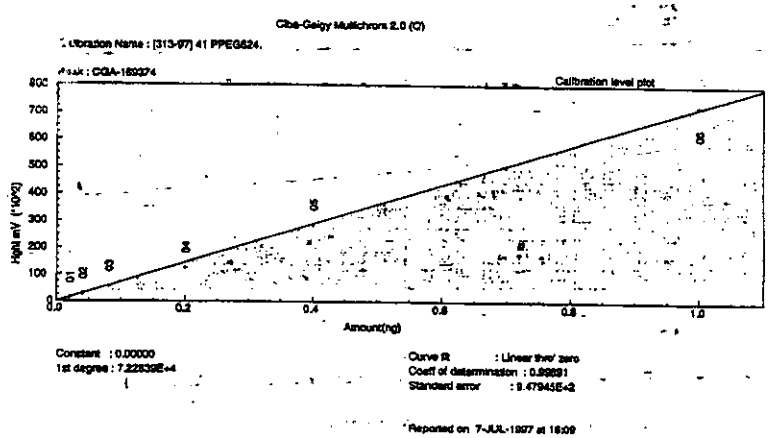
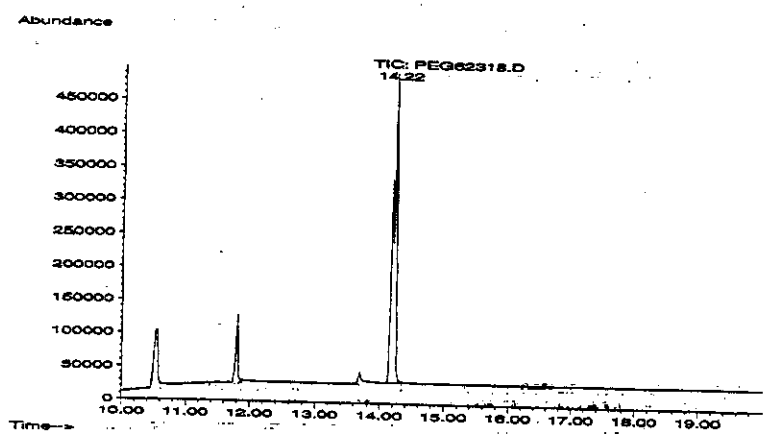
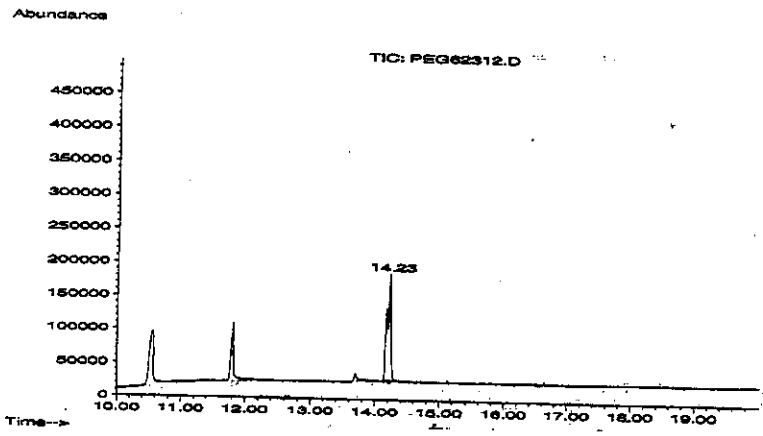


FIGURE 5. TYPICAL STANDARD CHROMATOGRAMS OF CGA-169374 USING GC/MSD (AG-676)
(Multichrom Worksheet: PRESSTRAW)

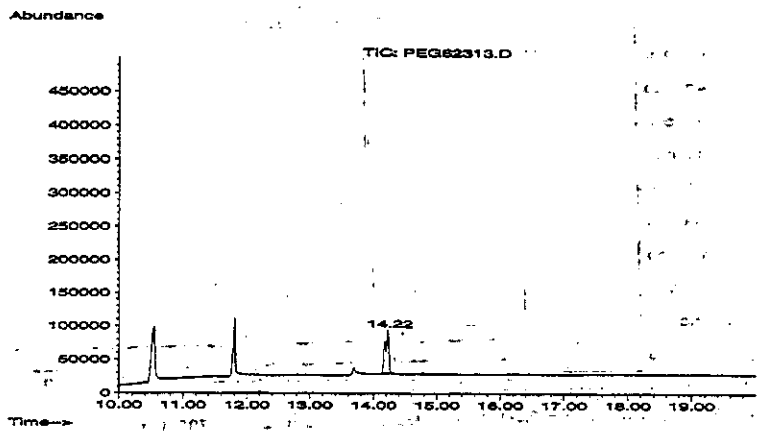


1. 1.0 ng standard CGA-169374 (Response Factor = 6770810 µV)

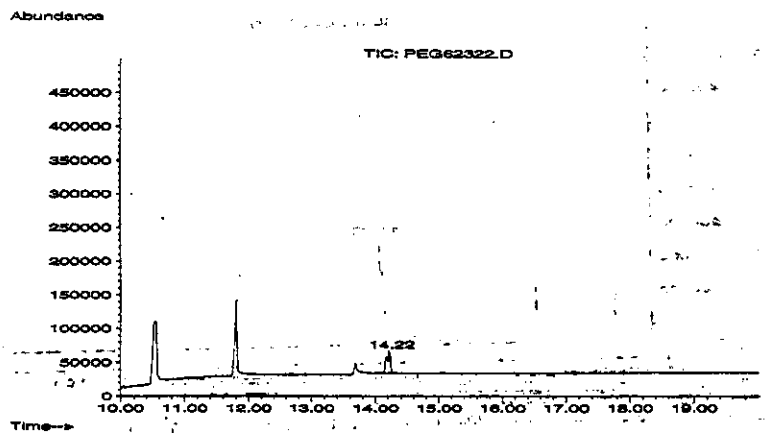


2. 0.4 ng standard CGA-169374 (Response Factor = 2482777 µV)

FIGURE 5. TYPICAL STANDARD CHROMATOGRAMS OF CGA-169374 USING GC/MSD (AG-676) (Continued)
(Multichrom Worksheet: PRESSTRAW)

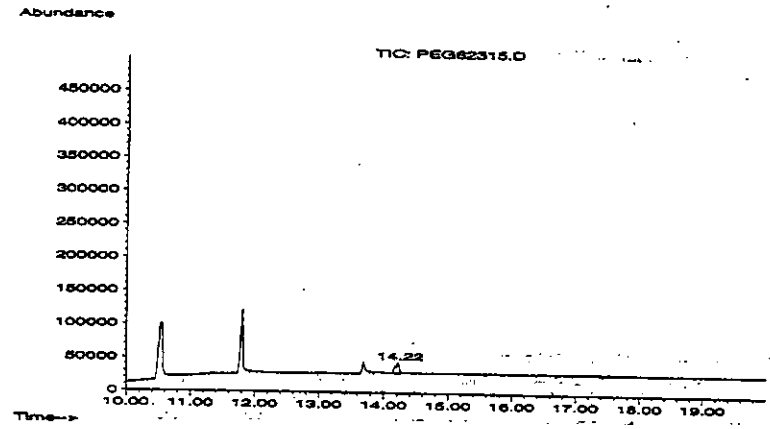


3. 0.2 ng standard CGA-169374 (Response Factor = 1069574 µV)

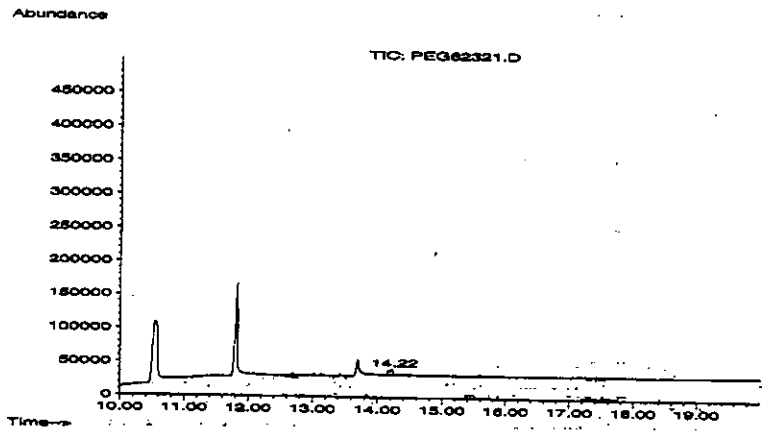


4. 0.08 ng standard CGA-169374 (Response Factor = 547157 µV)

FIGURE 5. TYPICAL STANDARD CHROMATOGRAMS OF CGA-169374 USING GC/MSD (AG-676) (Continued)
(Multichrom Worksheet: PRESSTRAW)



5. 0.04 ng standard CGA-169374 (Response Factor = 272824 µV)



6. 0.02 ng standard CGA-169374 (Response Factor = 153981 µV)

FIGURE 6. TYPICAL CALIBRATION PLOT OF CGA-169374 CORRESPONDING TO FIGURE 5 OBTAINED USING AG-676
(Multichrom Worksheet: PRESSTRAW)

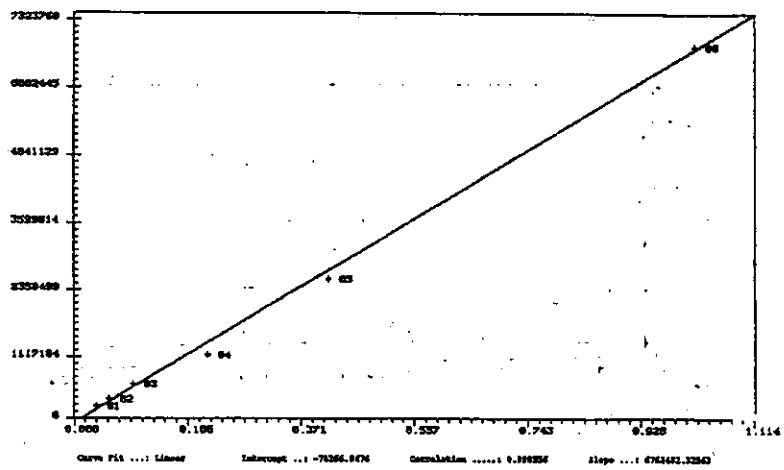
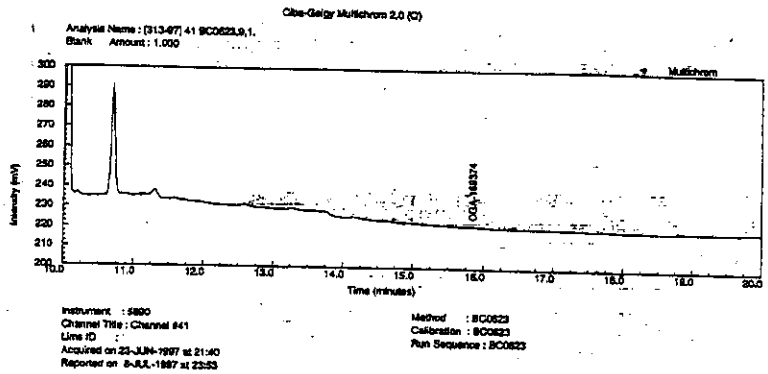
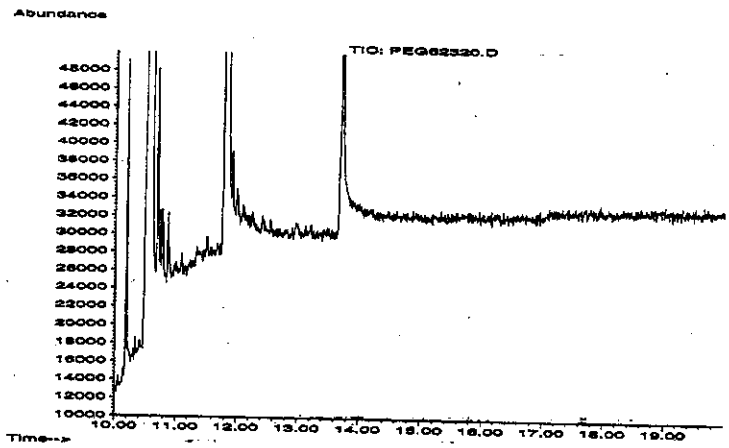


FIGURE 7. CHROMATOGRAMS OF THE REAGENT BLANKS FROM ANALYSIS OF CGA-169374 USING AG-575B AND AG-676



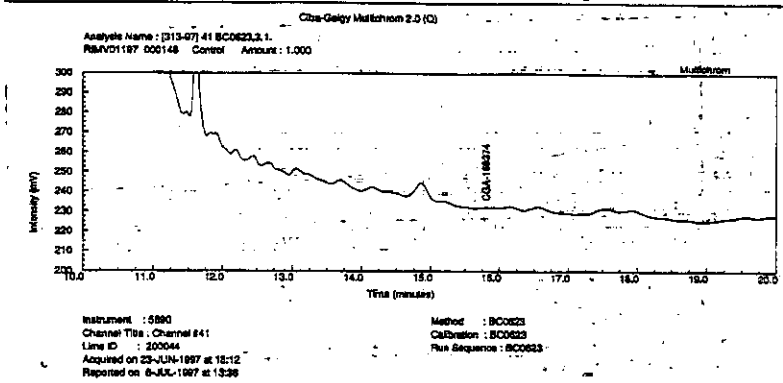
1. Reagent Blank, 3.97 mg injected, 0.003 ng found, <0.01 ppm (using GC/NPD - AG-575A).



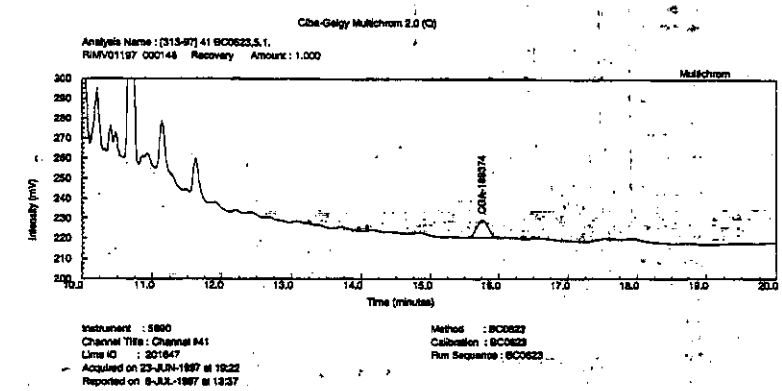
2. Reagent Blank, 3.97 mg injected, 0.00 ng found, <0.01 ppm (using GC/MSD - AG-676).

FIGURE 8. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY STRAW USING AG-575B
(Multichrom Worksheet: 313-97-BC4R)

CGA-169374: Slope = 89550 µV/ng Y-intercept = 0

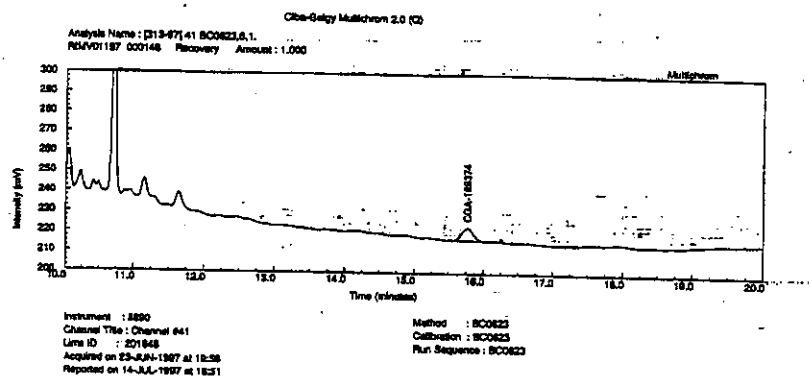


1. 200044, control, 3.97 mg injected, 0.004 ng found, <0.01 ppm (0.001 ppm). (Response Factor - 382 µV)

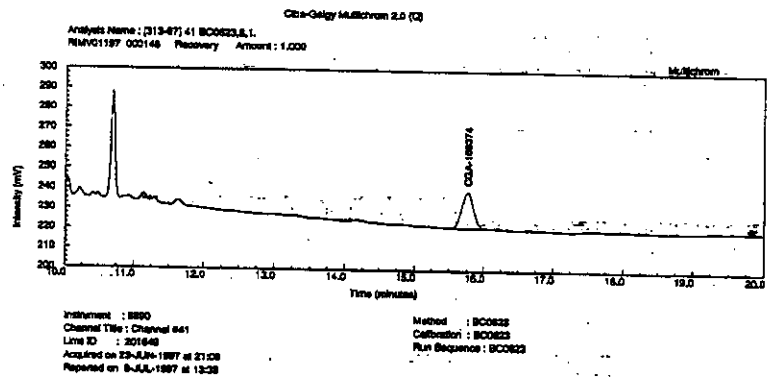


2. 201647, control + 0.05 ppm CGA-169374, 1.99 mg injected, 0.097 ng found, 0.049 ppm found, 0.048 ppm (corrected for control), 95.5% recovery. (Response Factor - 8677 µV)

FIGURE 8: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY STRAW USING AG-575B (Continued) (Multichrom Worksheet: 313-97-BC4R)



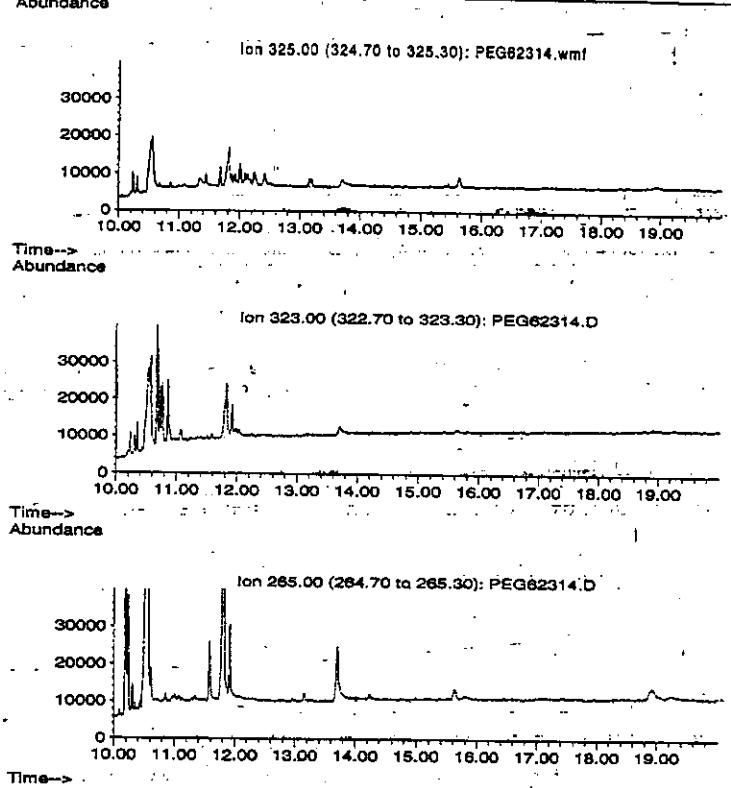
3. 201648, control + 0.10 ppm CGA-169374, 0.794 mg injected, 0.071 ng found, 0.089 ppm, 0.088 ppm (corrected for control) 87.9% recovery. (Response Factor - 6330 µV)



4. 201649, control + 0.5 ppm CGA-169374, 0.398 mg injected, 0.204 ng found, 0.513 ppm, 0.512 ppm (corrected for control), 102% recovery. (Response Factor - 18276 µV)

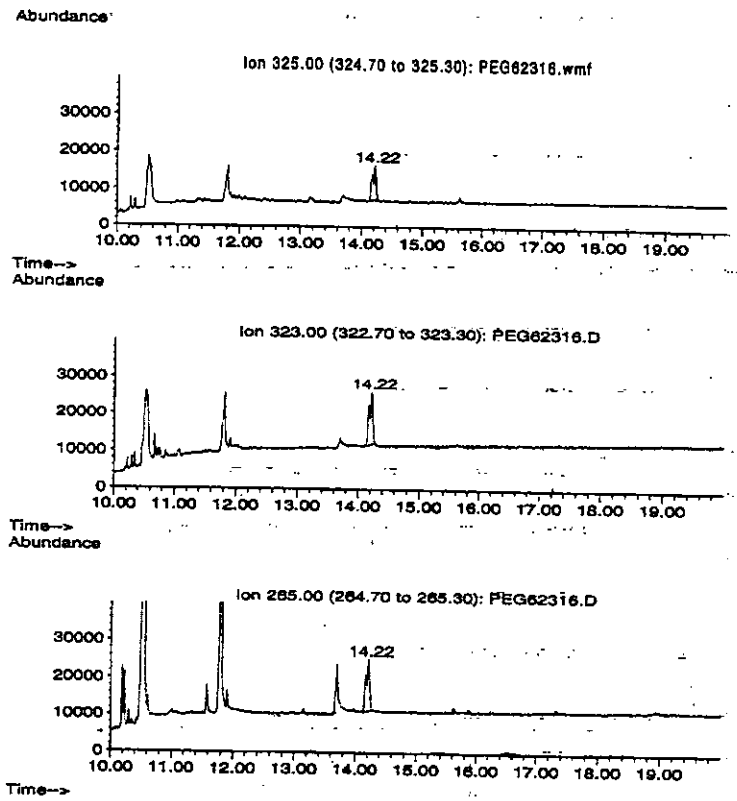
FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY STRAW USING AG-676
(Multichrom Worksheet: PRESSTRAW)

CGA-169374: Slope = 6762482 µV/ng Y-intercept = -78266 µV



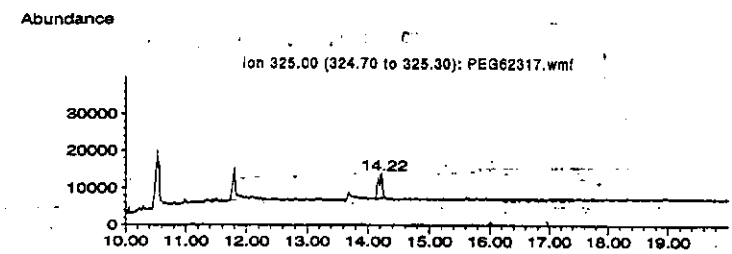
1. 200044, control, 3.97 mg injected, 0.00 ng found, <0.01 ppm.
(Response Factor - 0 µV)

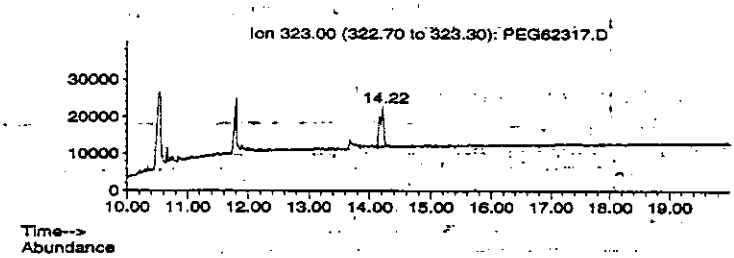
FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY STRAW USING AG-676
(Continued)
(Multichrom Worksheet: PRESSTRAW)

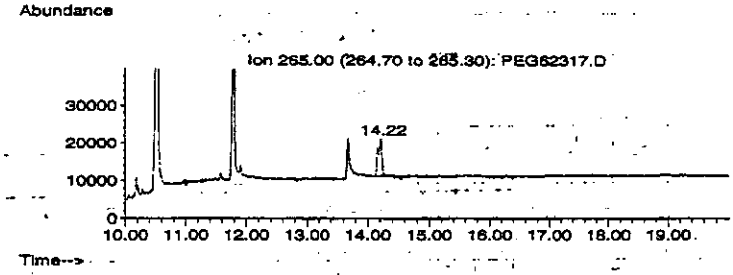


2. 201647, control + 0.05 ppm CGA-169374, 1.99 mg injected, 0.103 ng found, 0.052 ppm, 0.052 ppm (corrected for control), 104% recovery. (Response Factor - 620917 µV)

FIGURE 9.7 REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY STRAW USING AG-676
(Continued)
(Multichrom Worksheet: PRESSTRAW)

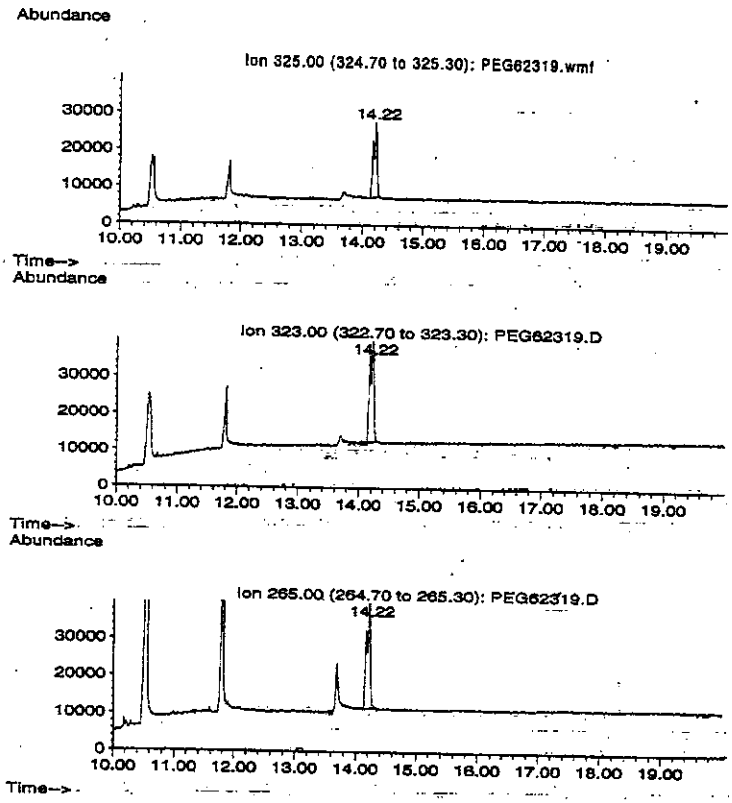






3. 201648, control + 0.10 ppm CGA-169374, 0.794 mg injected, 0.080 ng found, 0.100 ppm, 0.100 ppm (corrected for control), 100% recovery. (Response Factor - .461097 µV)

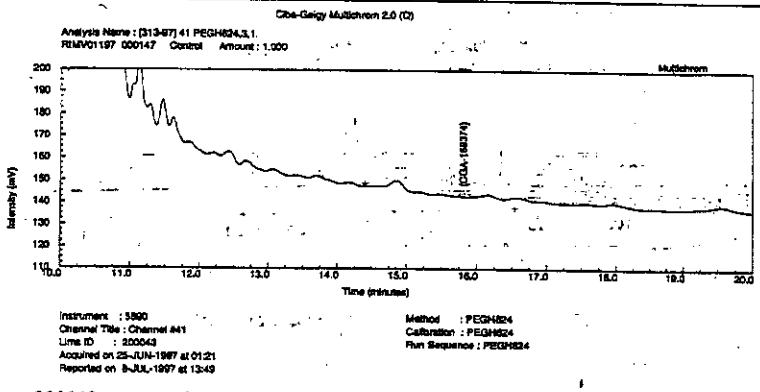
FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY STRAW USING AG-676
(Continued)
(Multichrom Worksheet: PRESSTRAW)



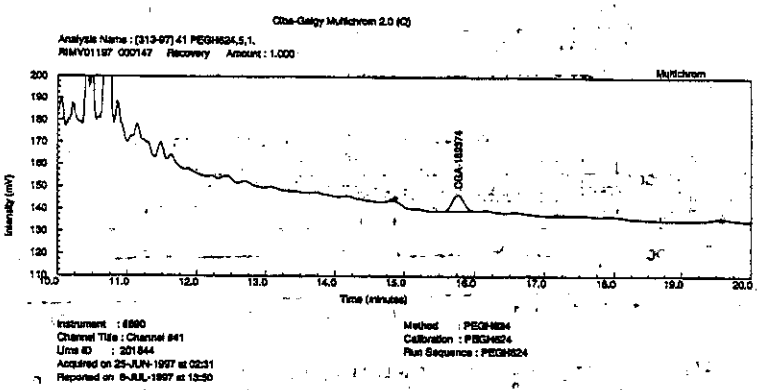
4. 201649, control + 0.5 ppm CGA-169374, 0.398 mg injected, 0.204 ng found, 0.512 ppm, 0.512 ppm (corrected for control), 102% recovery. (Response Factor - 1299378 µV)

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY HAY USING AG-575B
(Multichrom Worksheet: 313-97-PEGH)

CGA-169374: Slope = 66068 µV/ng Y-intercept = 0

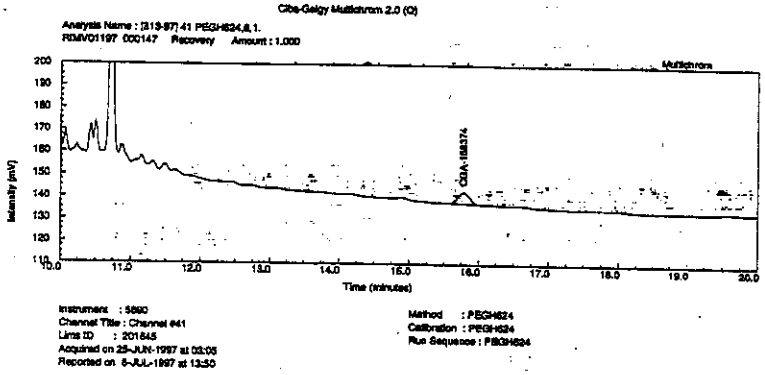


1. 200043, control, 3.96 mg injected, 0.000 ng found, <0.01 ppm (0.000 ppm). (Response Factor - 0 µV)

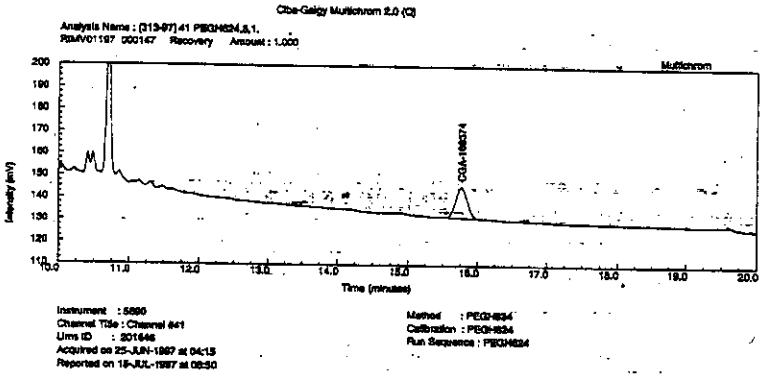


2. 201644, control + 0.05 ppm CGA-169374, 1.98 mg injected, 0.112 ng found, 0.057 ppm, 0.057 ppm (corrected for control), 113% recovery. (Response Factor - 7386 µV)

FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY HAY USING AG-575B (Continued)
(Multichrom Worksheet: 313-97-PEGH)



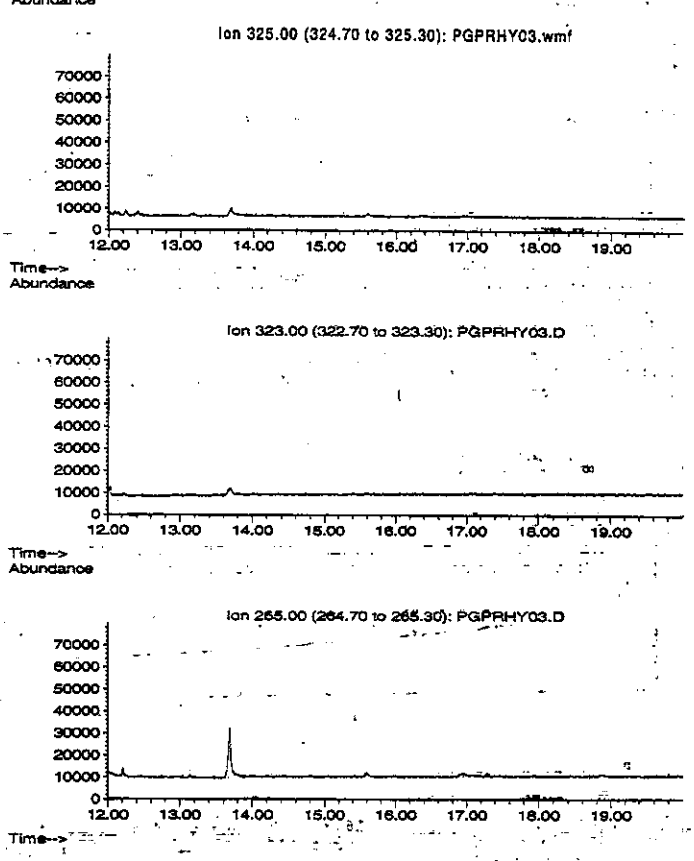
3. 201645, control + 0.1 ppm CGA-169374, 0.791 mg injected, 0.08 ng found, 0.101 ppm, 0.101 ppm (corrected for control), 101% recovery. (Response Factor - 5278 µV)



4. 201646, control + 0.5 ppm CGA-169374, 0.396 mg injected, 0.215 ng found, 0.542 ppm, 0.542 ppm (corrected for control), 108% recovery. (Response Factor - 14188 µV)

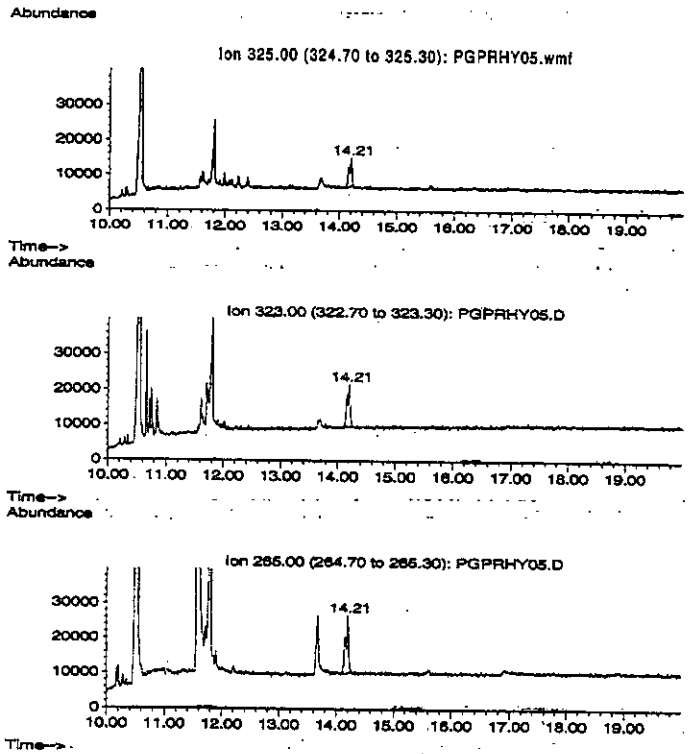
FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY HAY USING AG-676
(Multichrom Worksheet: PRESSHAY)

CGA-169374: Slope = 5248423 µV/ng Y-intercept = -12582 µV



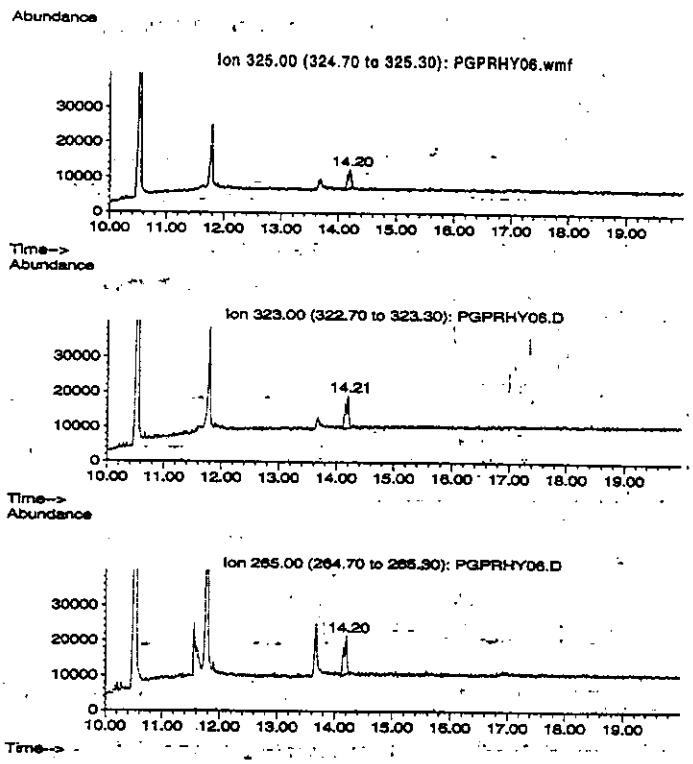
1. 200043, control, 3.96 mg injected, 0.00 ng found, <0.01 ppm (0.000 ppm).
(Response Factor = .0 µV)

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY HAY USING AG-676 (Continued)
(Multichrom Worksheet: PRESSHAY)



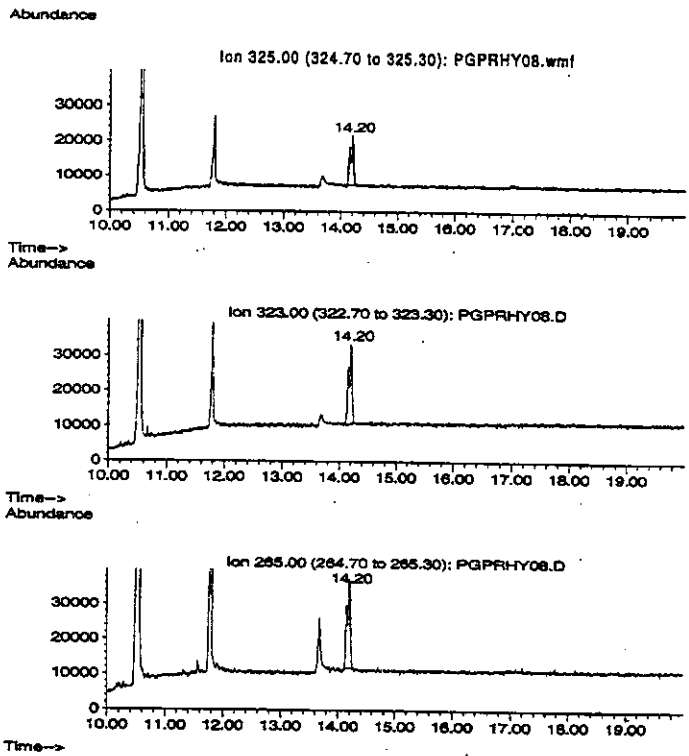
2. 201644, control + 0.05 ppm CGA-169374, 1.98 mg injected, 0.099 ng found, 0.050 ppm, 0.050 ppm (corrected for control), 100% recovery. (Response Factor - 508165 µV)

FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY HAY USING AG-676 (Continued)
(Multichrom Worksheet: PRESSHAY)



3. 201645; control + 0.1 ppm CGA-169374, 0.791 mg injected, 0.081 ng found, 0.102 ppm, 0.102 ppm (corrected for control), 102% recovery. (Response Factor - 411723 µV)

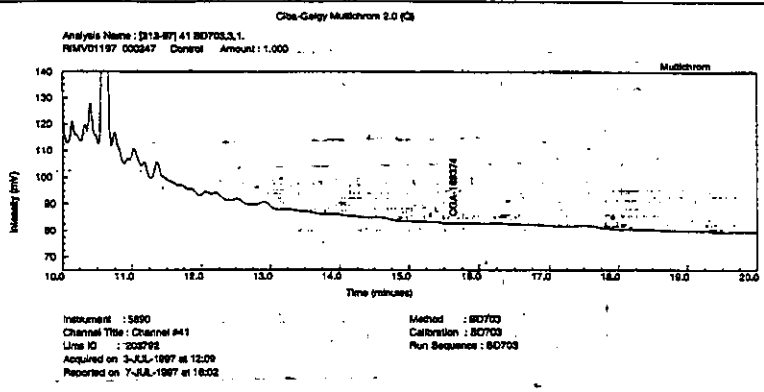
FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY HAY USING AG-676 (Continued)
(Multichrom Worksheet: PRESSHAY)



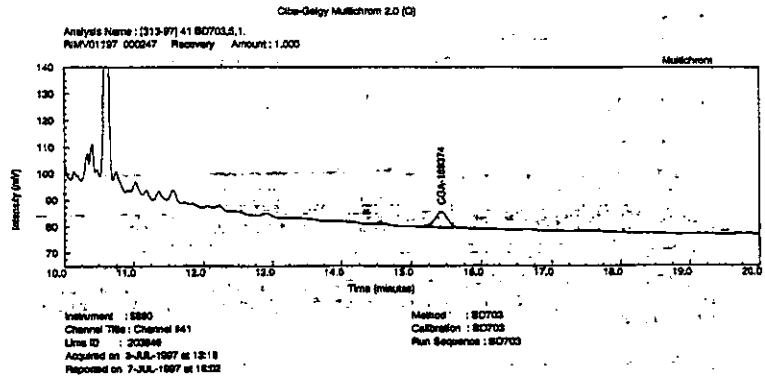
4. 201646, control + 0.5 ppm CGA-169374, 0.396 mg injected, 0.187 ng found, 0.472 ppm, 0.472 ppm (corrected for control), 94.3% recovery. (Response Factor - .968127 µV)

FIGURE-12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY FORAGE USING AG-575B
(Multichrom Worksheet: 313-97-BD2)

CGA-169374: Slope = 57349 µV/ng Y-intercept = 0

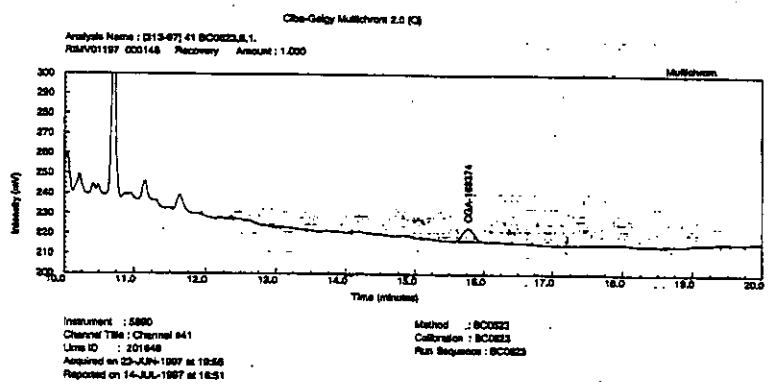


1. 203792, control, 3.74 mg injected, 0.003 ng found, <0.01 ppm (0.001 ppm). (Response Factor - 180 µV)

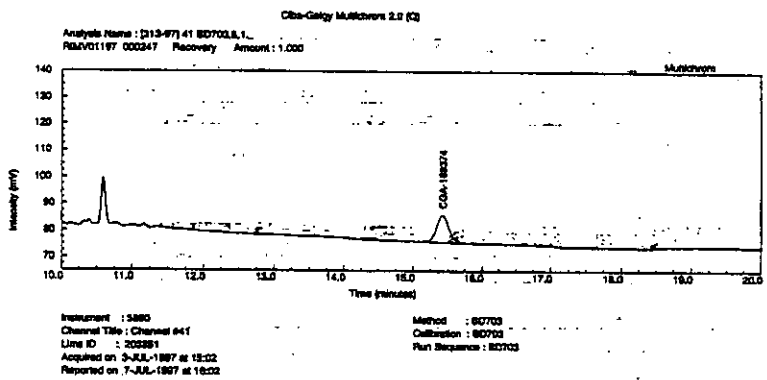


2. 203849, control + 0.05 ppm CGA-169374, 1.87 mg injected, 0.104 ng found, 0.056 ppm, 0.055 ppm (corrected for control), 110% recovery. (Response Factor - 5961 µV)

FIGURE 12. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY FORAGE USING AG-575B
(Continued)
(Multichrom Worksheet: 313-97-BD2)



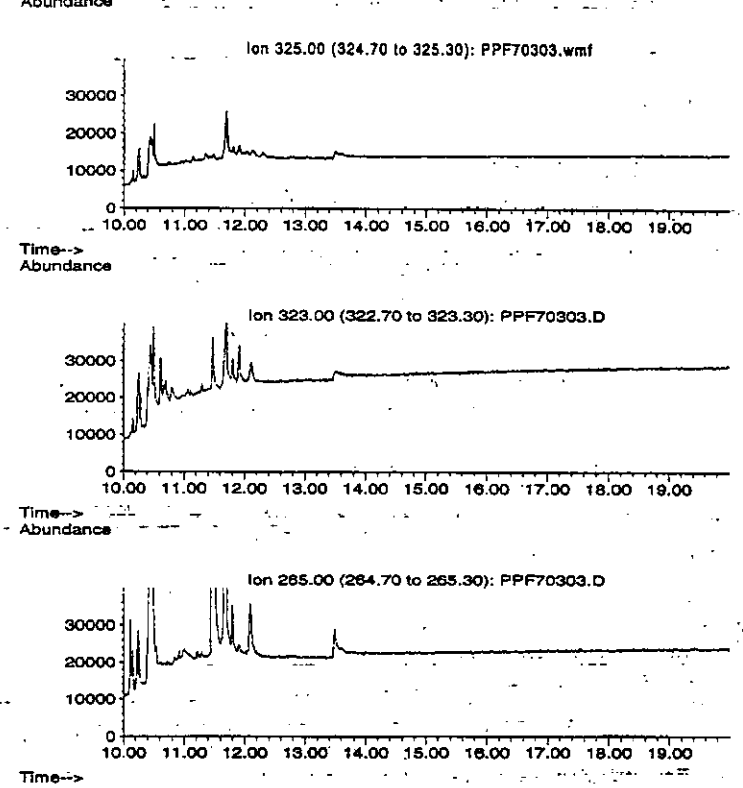
3. 203850, control + 0.1 ppm CGA-169374, 0.748 mg injected, 0.080 ng found, 0.107 ppm, 0.106 ppm (corrected for control), 106% recovery. (Response Factor - 4572 µV)



4. 203851, control + 0.50 ppm CGA-169374, 0.374 mg injected, 0.181 ng found, 0.484 ppm, 0.483 ppm (corrected for control), 96.5% recovery. (Response Factor - 10367 µV)

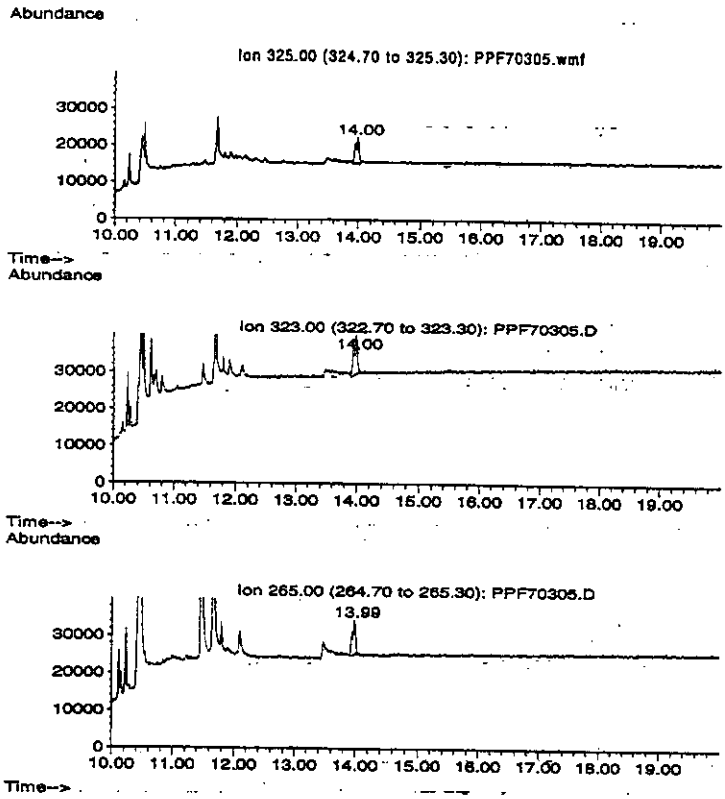
FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY FORAGE USING AG-676
(Multichrom Worksheet: TARGETFORAGE4C)

CGA-169374: Slope = 4121214 µV/ng Y-intercept = -20189 µV



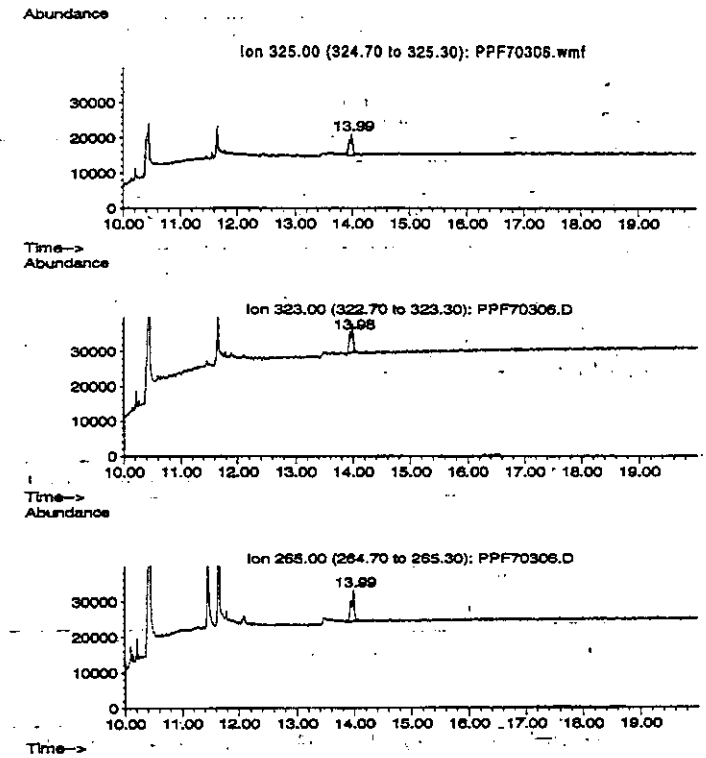
1. 203792, control, 3.74 mg injected, 0.00 ng found, <0.01 ppm.
(Response Factor - 0 µV)

FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY FORAGE USING AG-676
(Continued)
(Multichrom Worksheet: TARGETFORAGE4C)



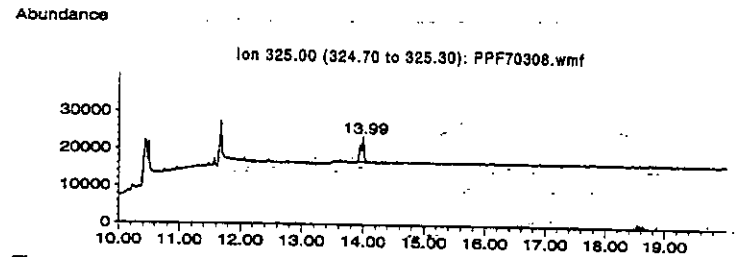
2. 203849, control + 0.05 ppm CGA-169374, 1.87 mg injected, 0.090 ng found, 0.048 ppm, 0.048 ppm (corrected for control), 96.1% recovery. (Response Factor - 390332 µV)

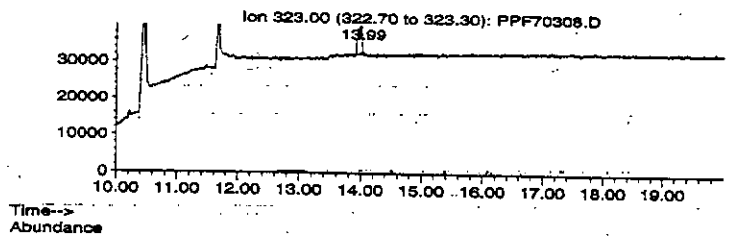
FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY FORAGE USING AG-676
(Continued)
(Multichrom Worksheet: TARGETFORAGE4C)

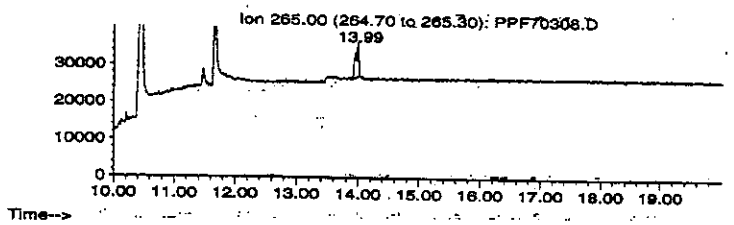


3. 203850, control + 0.1 ppm CGA-169374, 0.748 mg injected, 0.087 ng found, 0.116 ppm, 0.116 ppm (corrected for control), 116% recovery. (Response Factor - 378405 µV)

FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY FORAGE USING AG-676
(Continued)
(Multichrom Worksheet: TARGETFORAGE4C)



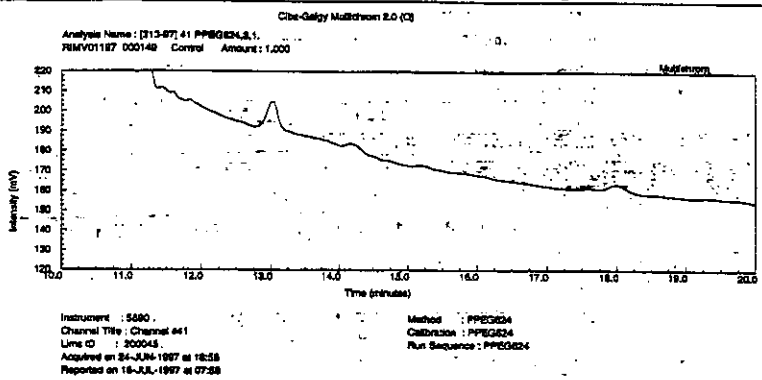




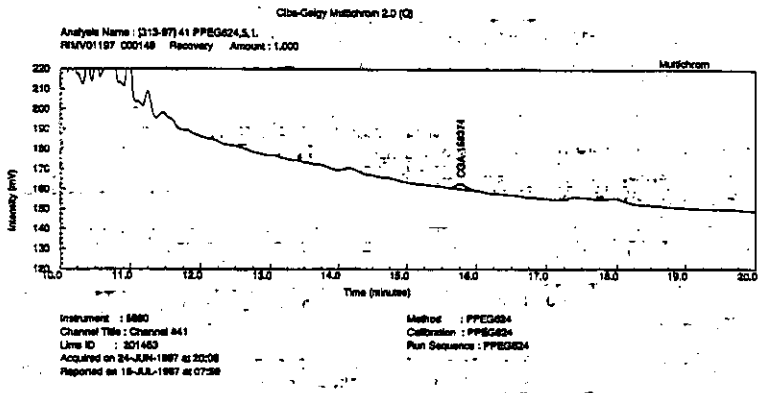
4. 203851, control + 0.50 ppm CGA-169374, 0.187 mg injected, 0.097 ng found, 0.517 ppm, 0.517 ppm (corrected for control), 103% recovery. (Response Factor - 418623 pV)

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-575B (SET 1)
(Multichrom Worksheet: 313-97-PEGG)

CGA-169374: Slope = 72264 µV/ng Y-intercept = 0

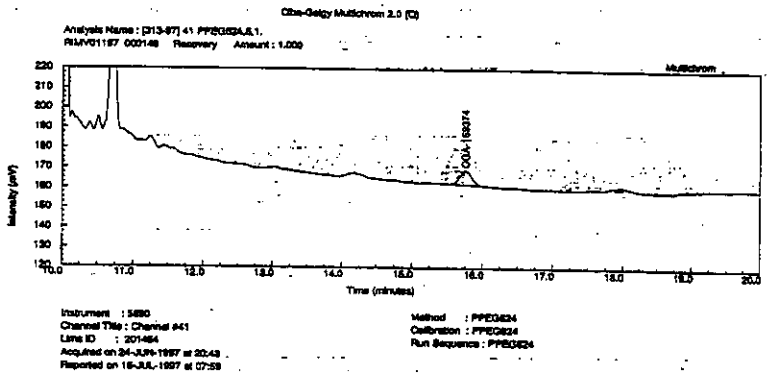


1. 200045, control, 3.95 mg injected, 0.00 ng found, <0.01 ppm. (Response Factor - 0 µV)

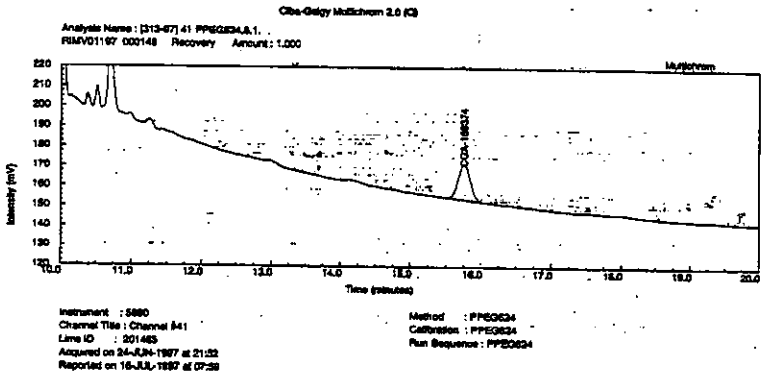


2. 201463, control + 0.01 ppm CGA-169374, 3.95 mg injected, 0.037 ng found, 0.009 ppm, 0.009 ppm (corrected for control), 93.4% recovery. (Response Factor - 2668 µV)

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-575B (SET 1) (Continued)
(Multichrom Worksheet: 313-97-PEGG)



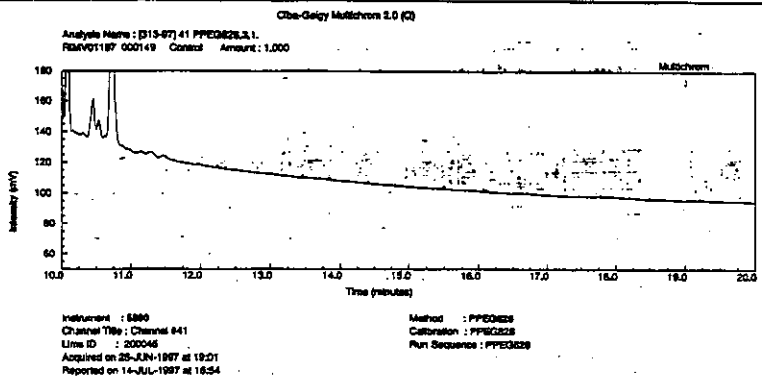
3. 201464, control + 0.04 ppm CGA-169374, 2.64 mg injected, 0.099 ng found, 0.037 ppm, 0.037 ppm (corrected for control), 93.7% recovery. (Response Factor - 7138 µV)



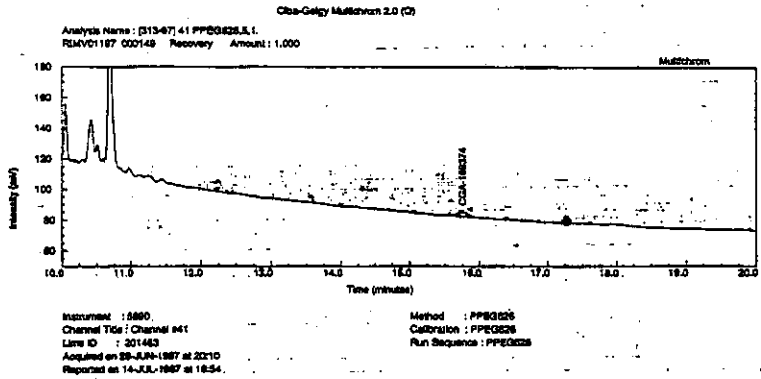
4. 201465, control + 0.2 ppm CGA-169374, 1.32 mg injected, 0.250 ng found, 0.190 ppm, 0.190 ppm (corrected for control), 95% recovery. (Response Factor - 18086 µV)

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-575B (SET 2) (Continued)
(Multichrom Worksheet: 313-97-PEGG2)

CGA-169374: Slope = .71569 µV/ng Y-intercept = 0

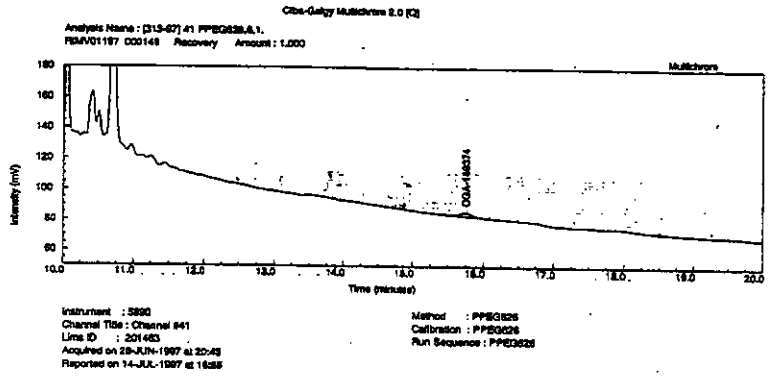


1. 200045, control, 3.96 mg injected, 0.00 ng found, 0.00 ppm (ND). (Response Factor - 0 µV)

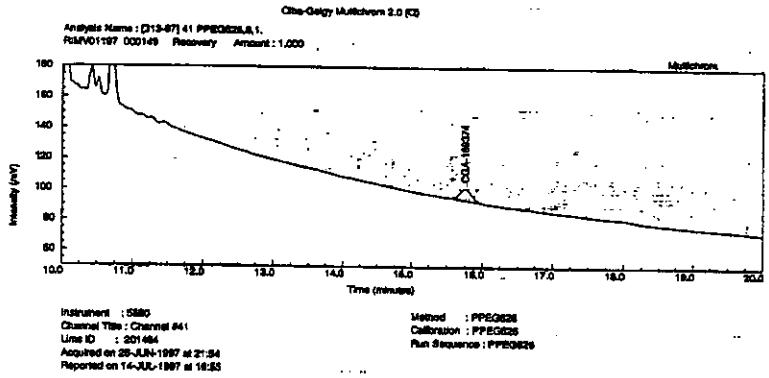


2. 201463, control + 0.01 ppm CGA-169374, 3.95 mg injected, 0.038 ng found, 0.010 ppm, 0.010 ppm (corrected for control), 96.0% recovery. (Response Factor - 2716 µV)

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-575B (SET 2) (Continued)
(Multichrom Worksheet: 313-97-PEGG2)

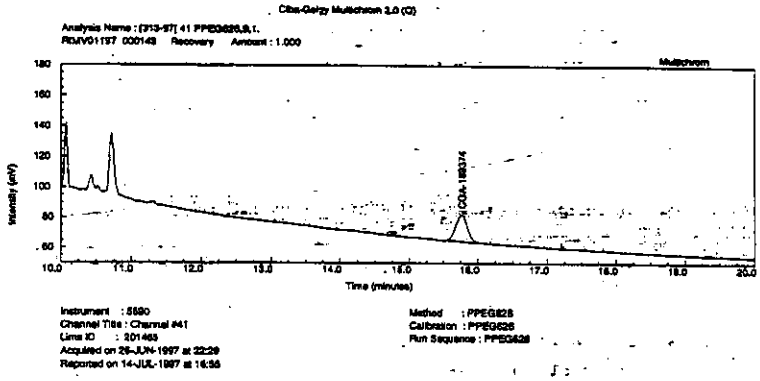


3. 201463, control + 0.01 ppm CGA-169374, 3.95 mg injected, 0.035 ng found, 0.009 ppm, 0.009 ppm (corrected for control), 87.5% recovery. (Response Factor - 2477 µV)



4. 201464, control + 0.04 ppm CGA-169374, 2.64 mg injected, 0.106 ng found, 0.040 ppm, 0.04 ppm (corrected for control), 100% recovery. (Response Factor - 7584 µV)

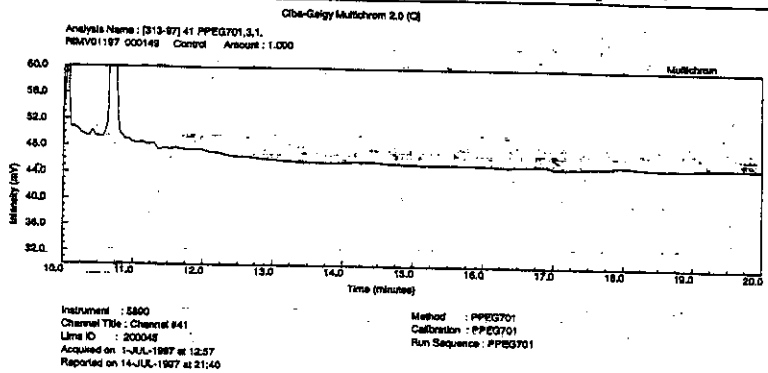
FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-575B (SET 2) (Continued)
(Multichrom Worksheet: 313-97-PEGG2)



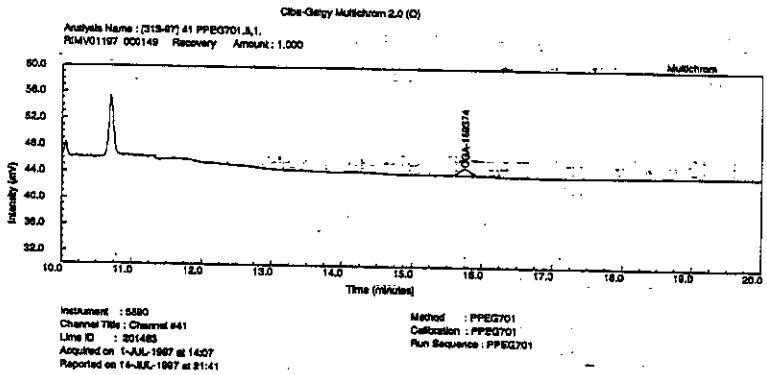
5. 201465, control + 0.20 ppm CGA-169374, 1.32 mg injected, 0.249 ng found, 0.188 ppm, 0.188 ppm (corrected for control), 94.2% recovery. (Response Factor ~ 17786 µV)

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-575B (SET 3) (Continued)
(Multichrom Worksheet: 313-97-PEGG3)

CGA-169374: Slope = 12854.5 µV/ng Y-intercept = 0

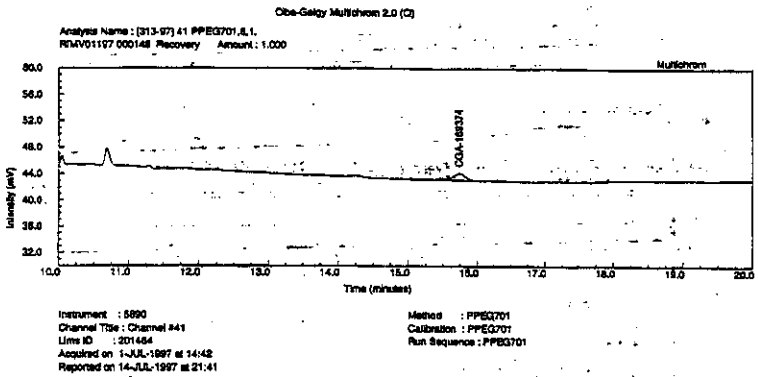


1. 200045, control, 3.96 mg injected, 0.00 ng found, <0.01 ppm (ND). (Response Factor - 0 µV)

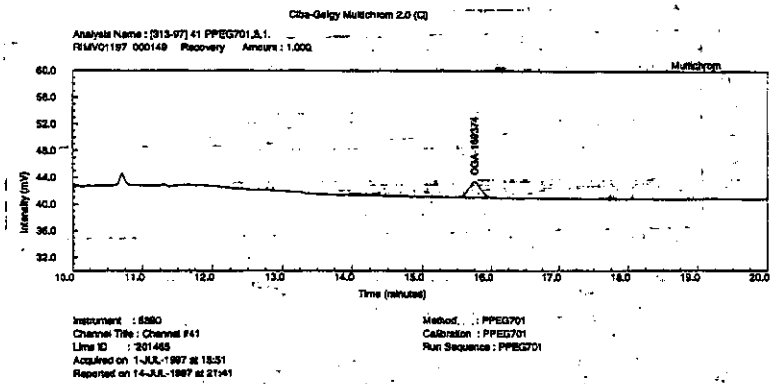


2. 201463, control + 0.10 ppm CGA-169374, 0.791 mg injected, 0.076 ng found, 0.096 ppm, 0.096 ppm (corrected for control), 95.5% recovery. (Response Factor - 971 µV)

FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-575B (SET 3) (Continued)
(Multichrom Worksheet: 313-97-PEGG3)



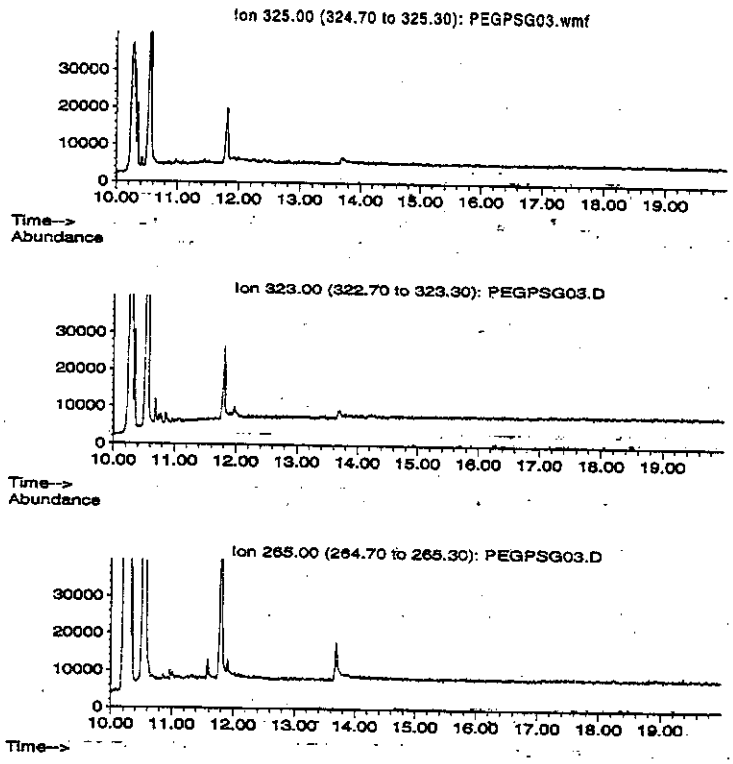
3. 201464, control + 0.2 ppm CGA-169374, 0.395 mg injected, 0.08 ng found, 0.201 ppm, 0.201 ppm (corrected for control), 101% recovery. (Response Factor - 1024 µV)



4. 201465, control + 1.0 ppm CGA-169374, 0.198 mg injected, 0.185 ng found, 0.938 ppm, 0.938 ppm (corrected for control), 93.8% recovery. (Response Factor - 2384 µV)

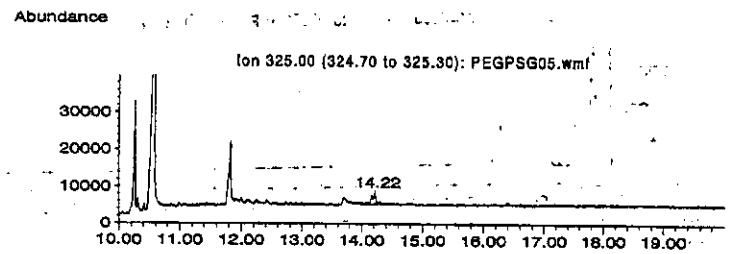
FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 1)
(Multichrom Worksheet: PRESSGRAIN)

CGA-169374: Slope = 5403530 µV/ng Y-intercept = -124813 µV



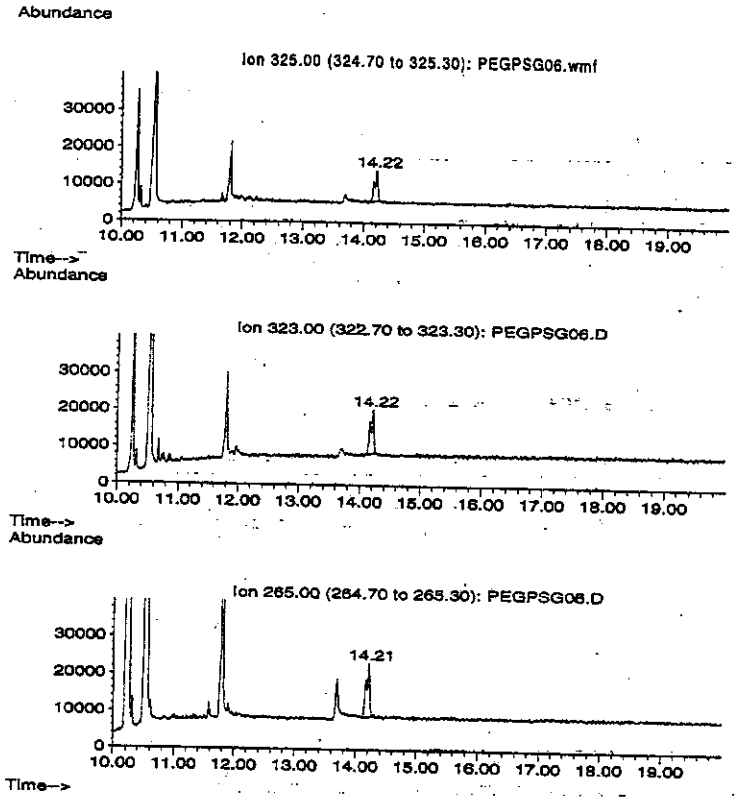
1. 200045, control, 3.96 mg injected, 0.00 ng found, <0.01 ppm.
(Response Factor = 0 µV)

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 1) (Continued)
(Multichrom Worksheet: PRESSGRAIN)



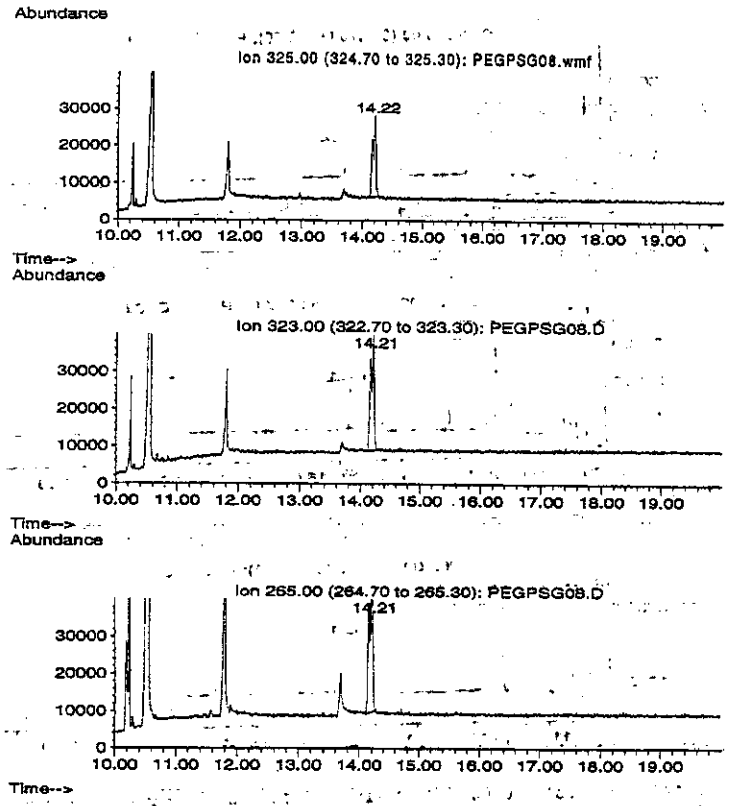
2. 201463, control + 0.01 ppm CGA-169374, 3.95 mg injected, 0.058 ng found, 0.015 ppm, 0.015 ppm (corrected for control), 148% recovery. (Response Factor - 191189 µV)

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 1) (Continued)
(Multichrom Worksheet: PRESSGRAIN)



3. 201464, control + 0.04 ppm CGA-169374, 2.64 mg injected, 0.112 ng found, 0.042 ppm, 0.042 ppm (corrected for control), 106% recovery. (Response Factor = 479213 µV)

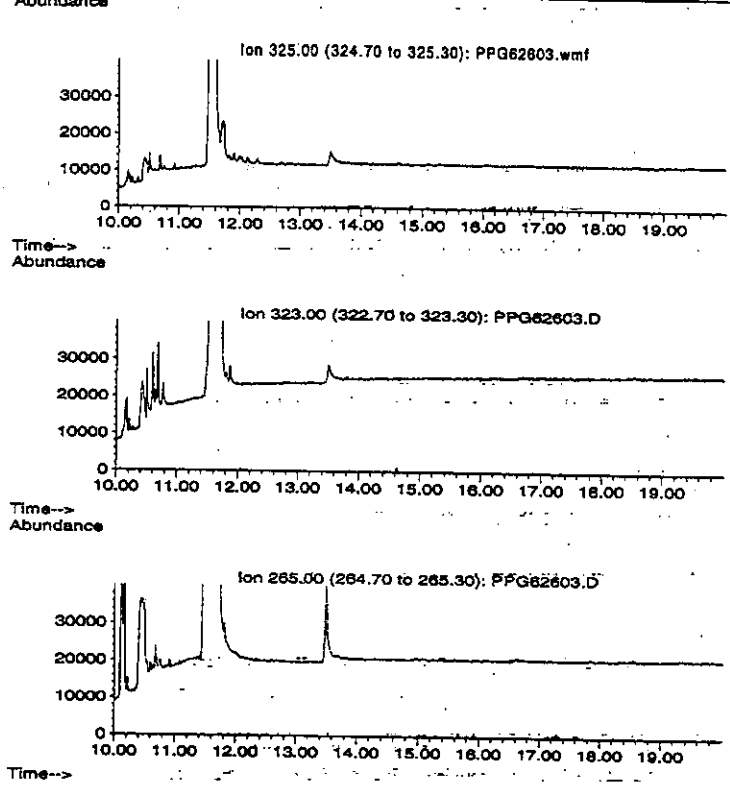
FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 1)
(Continued)
(Multichrom Worksheet: PRESSGRAIN)



4. 201465, control + 0.2 ppm CGA-169374, 1.32 mg injected, 0.288 ng found, 0.216 ppm, 0.216 ppm (corrected for control), 108% recovery. (Response Factor = 1409492 µV)

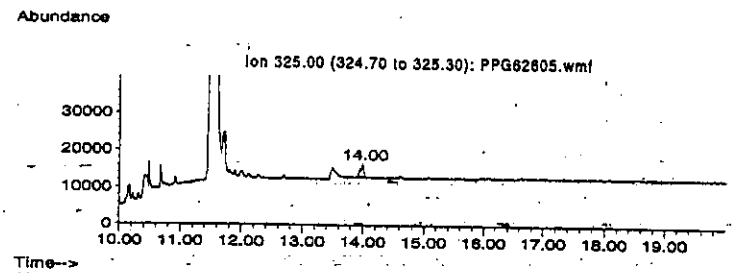
FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 2)
(Continued)
(Multichrom Worksheet: PREPEGGRAIN2)

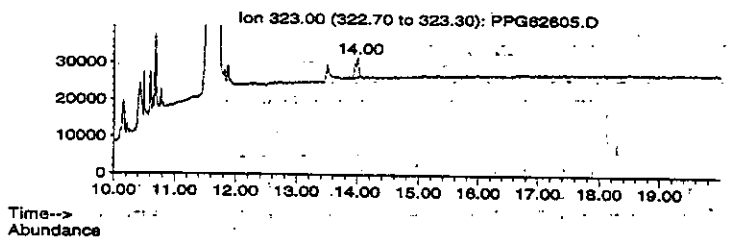
CGA-169374: Slope = 6174976 µV/ng Y-intercept = -174935 µV

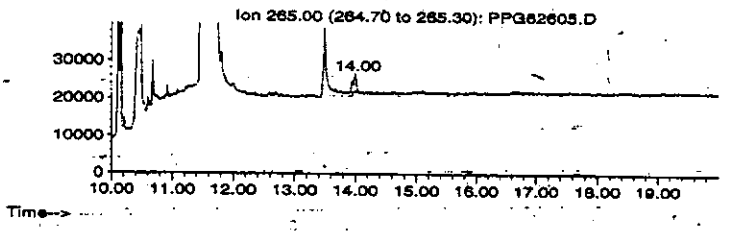


1. 200045, control, 3.96 mg injected, 0.00 ng found, 0.00 ppm (ND).
(Response Factor - 0 µV)

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 2)
(Continued)
(Multichrom Worksheet: PREPEGGRAIN2)

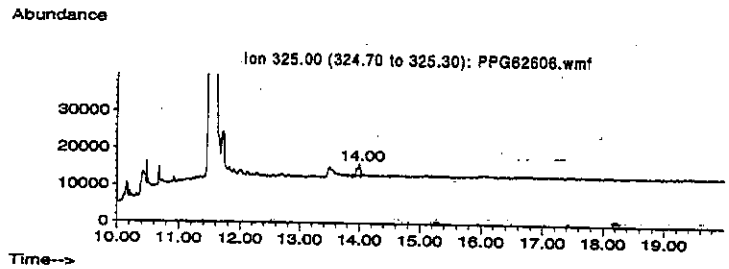


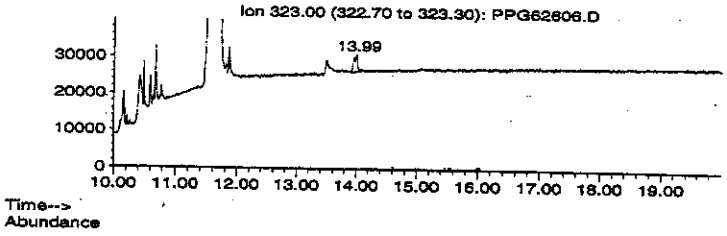


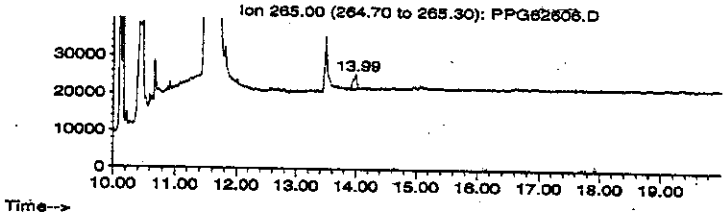


2. 201463, control + 0.01 ppm CGA-169374, 3.95 mg injected, 0.068 ng found, 0.017 ppm, 0.017 ppm (corrected for control), 171% recovery. (Response Factor = .242257 µV)

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 2) (Continued)
(Multichrom Worksheet: PREPEGGRAIN2)

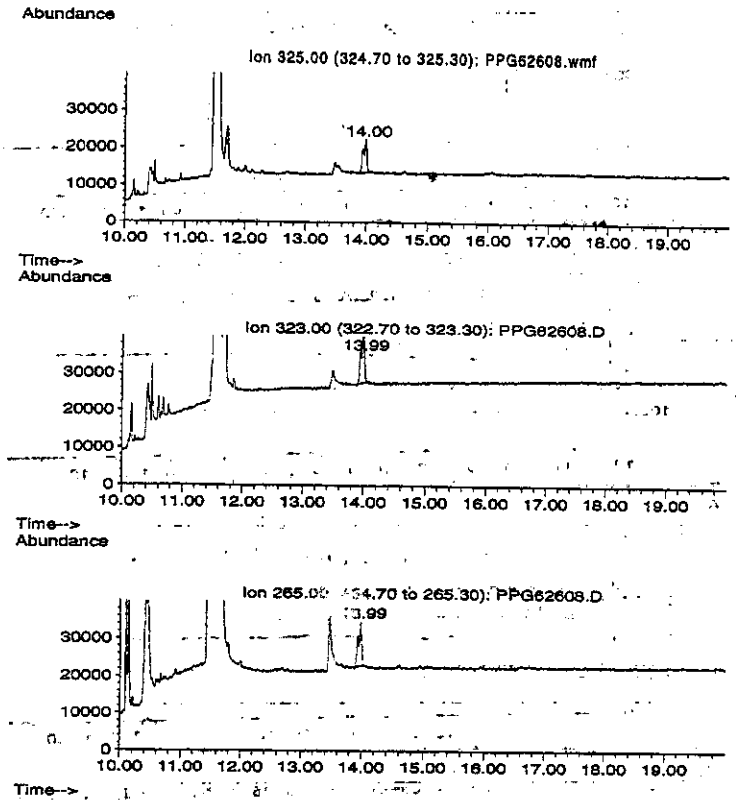






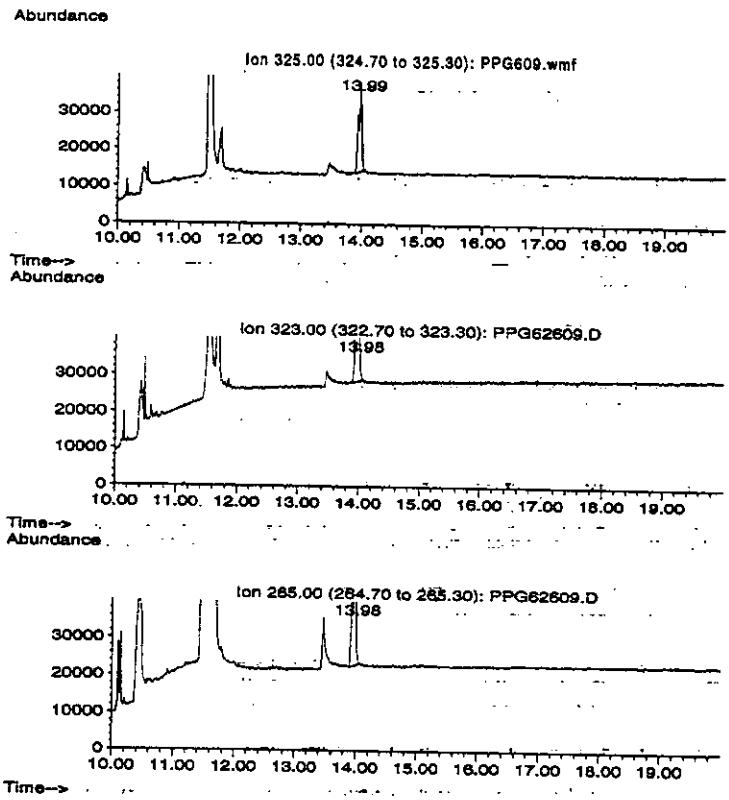
3. 201463, control + 0.01 ppm CGA-169374, 3.96 mg injected, 0.058 ng found, 0.015 ppm, 0.015 ppm (corrected for control), 148% recovery. (Response Factor - 186185 µV)

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 2) (Continued)
(Multichrom Worksheet: PREPEGGRAIN2)



4. 201464, control + 0.04 ppm CGA-169374, 2.64 mg injected, 0.124 ng found, 0.047 ppm, 0.047 ppm (corrected for control), 118% recovery. (Response Factor = 593837 µV)

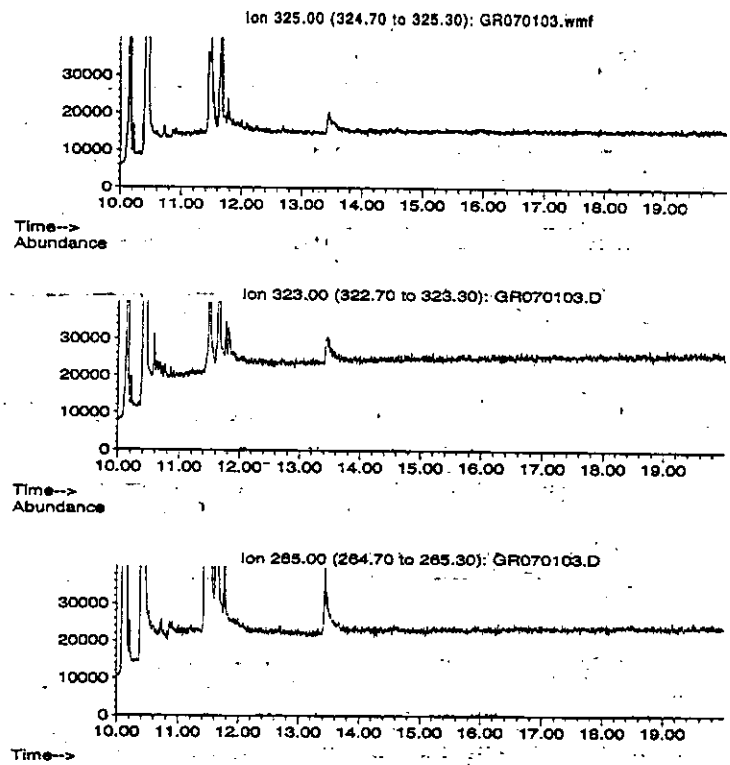
FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 2)
(Continued)
(Multichrom Worksheet: 169GR4)



5. 201465, control + 0.2 ppm CGA-169374, 1.32 mg injected, 0.256 ng found, 0.194 ppm, 0.194 ppm (corrected for control), 97.0% recovery. (Response Factor - 1406385 µV)

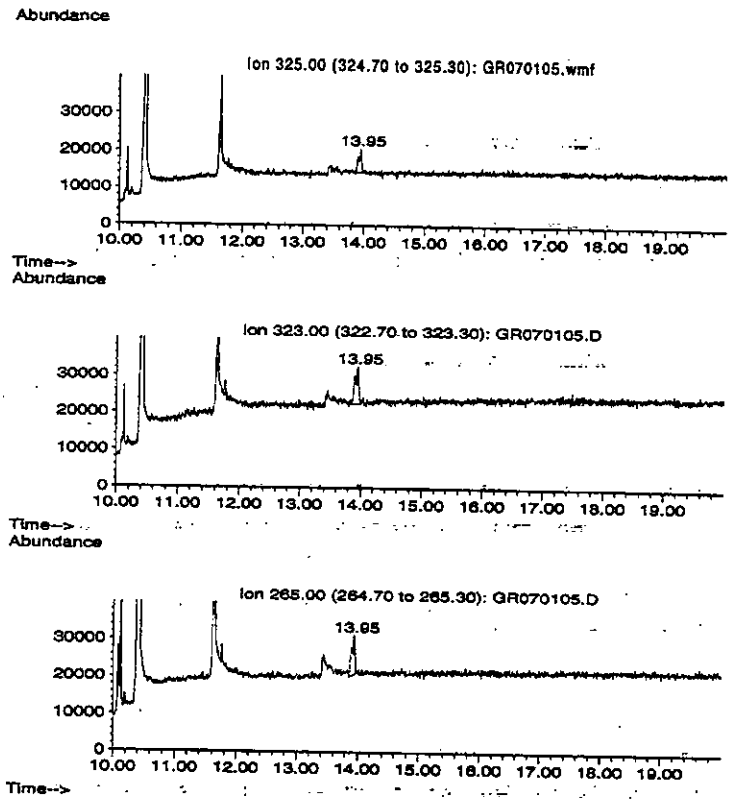
FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 3) (Continued)
(Multichrom Worksheet: 169GR4)

CGA-169374: Slope = 5086355 µV/ng Y-intercept = -53035 µV



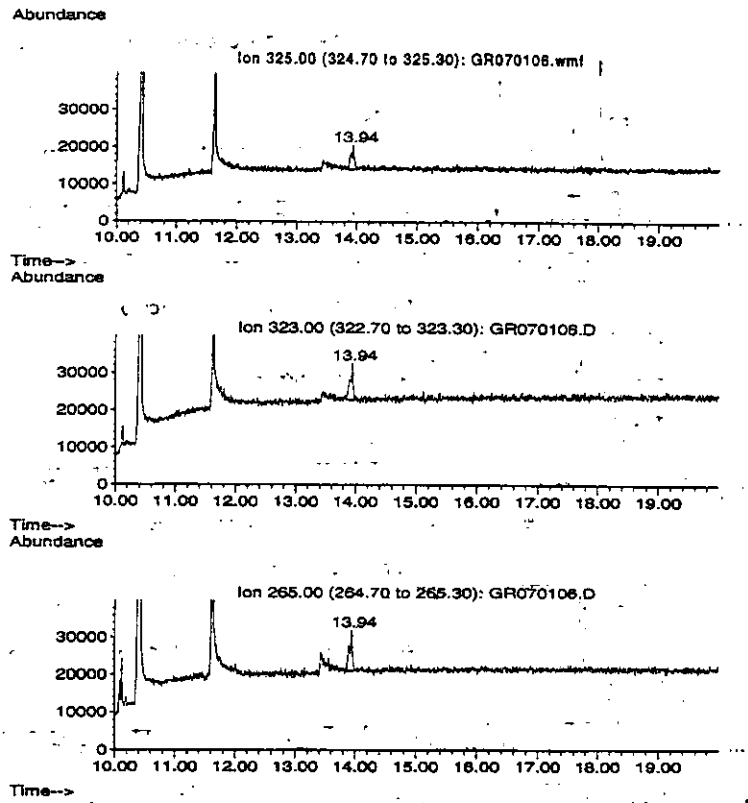
1. 200045, control, 3.96 mg injected, 0.00 ng found, <0.01 ppm. (Response Factor = 0 µV)

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 3) (Continued)
(Multichrom Worksheet: 169GR4)



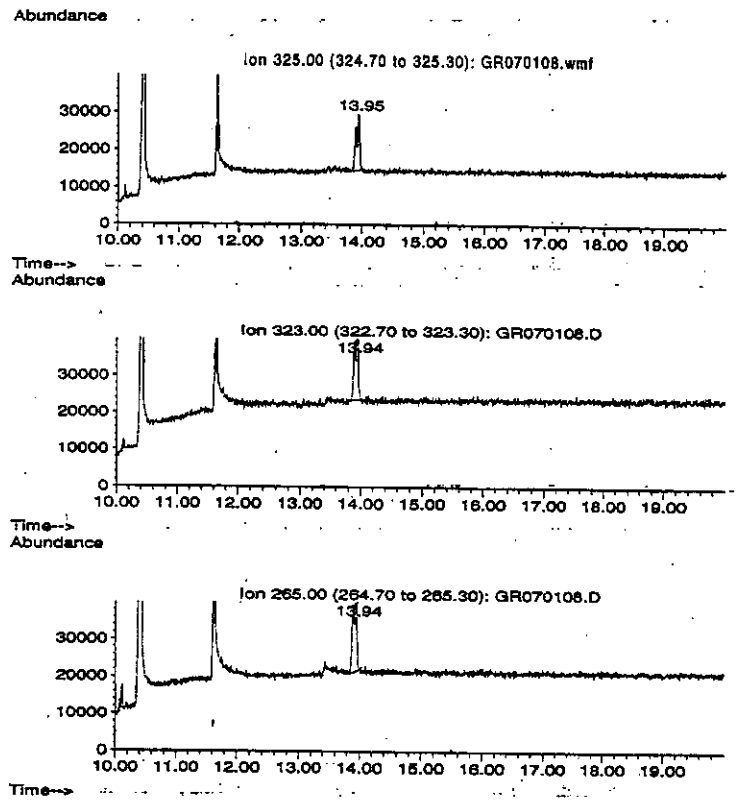
2. 201463, control + 0.1 ppm CGA-169374, 0.791 mg injected, 0.085 ng found, 0.107 ppm, 0.107 ppm (corrected for control), 107% recovery. (Response Factor ~ 377761 µV)

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 3) (Continued)
(Multichrom Worksheet: 169GR4)



3. 201464, control + 0.2 ppm CGA-169374, 0.395 mg injected, 0.071 ng found, 0.179 ppm, 0.179 ppm (corrected for control), 89.4% recovery. (Response Factor - 306454 µV)

FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 3) (Continued)
(Multichrom Worksheet: 169GR4)



4. 201465, control + 1.0 ppm CGA-169374; 0.198 mg injected, 0.191 ng found, 0.967 ppm, 0.967 ppm (corrected for control), 96.7% recovery. (Response Factor - 918954 µV)

VIII. REFERENCES

1. Grunenwald, M., Darnow, J., Method No. AG-575B, "Analytical Method for the Determination of CGA-169374 in Wheat Raw Agricultural Commodities by Gas Chromatography with Nitrogen/Phosphorus Detection." MRID No. 42806504

2. Williams, W. L., Ciba Residue Dept. Method No. AG-537A, "Analytical Method for the Determination of CGA-169374 in Wheat Raw Agricultural Commodities by Gas Chromatography." MRID No. 42090051

3. Memorandum (with attachments) dated January 7, 1993 from Cynthia Giles-Parker, PM-22, Fungicide Herbicide Branch, Registration Division (H7505C) to Greg Watson, Ph.D., Ciba re PP#2E4051. CGA-169374 (Difenoconazole, Dividend) Petition Method Validation Trial of Analytical Method AG-575A. MRID No. 420920-52,-53.

4. Memorandum (with attachments) dated September 16, 1996 from Philip V. Errico, Acting Product Manager, Fungicide Herbicide Branch, Registration Division (H7505C) to Greg Peters, Ph.D., Ciba re PP#6f4748. Difenoconazole (Dividend) in or on Barley, Oats, and Triticale. Evaluation of Residue Data and Analytical Methods. MRID No. 440560-01.

5. Memorandum dated November 4, 1992 from Cynthia Giles-Parker, PM-22, Fungicide Herbicide Branch, Registration Division (H7505C) to Eileen D. King-Watson, Ph.D., Ciba re PP#2E4051. CGA-169374 (Difenoconazole, Dividend) in Imported Wheat Barley and Rye Grain.

6. Yokley, R. A., Specificity of Analytical Method AG-575A For The Determination of CGA-169374 in Small Grains. ABR-92084, March 9, 1993. MRID No. 42090052

7. Oakes, T. L., Protocol No. 313-97, Validation of Draft Method AG-676 For Confirmation of CGA-169374 in or on Barley.

VIII. REFERENCES (Continued)

8. Darnow, J. and Sayers, L., Ciba Residue Dept. Method No. AG-575, "Analytical Method for the Determination of CGA-169374 in Wheat Raw Agricultural Commodities by Gas Chromatography with Nitrogen/Phosphorus Detection." MRID No. 42090053

9. Novartis Residue Test Report RI-MV-011-97, Report 1.

APPENDIX I

PREVIOUSLY SUBMITTED DOCUMENTS WITH EPA MRID NUMBERS

1. Grunenwald, M., Darnow, J., Method No. AG-575B, "Analytical Method for the Determination of CGA-169374 in Wheat Raw Agricultural Commodities by Gas Chromatography with Nitrogen/Phosphorus Detection." MRID No. 42806504

2. Williams, W. L., Ciba Residue Dept. Method No. AG-537A, "Analytical Method for the Determination of CGA-169374 in Wheat Raw Agricultural Commodities by Gas Chromatography." MRID No. 42090051

3. Yokley, R. A., Specificity of Analytical Method AG-575A for the Determination of CGA-169374 in Small Grains. ABR-92084, March 9, 1993. MRID No. 42090052

4. Darnow, J. and Sayers, L., Ciba Residue Dept. Method No. AG-575, "Analytical Method for the Determination of CGA-169374 in Wheat Raw Agricultural Commodities by Gas Chromatography with Nitrogen/Phosphorus Detection." MRID No. 42090053

APPENDIX II

HUMAN SAFETY DEPARTMENT RESIDUE TEST REPORTS

Human Safety Department
Novartis Crop Protection, Inc.
Greensboro, North Carolina

RESIDUE TEST REPORT

METHOD VALIDATION TEST NUMBER: RI-MV-011-97
REPORT NUMBER: 1

PROJECT NUMBER:	420011
PROTOCOL NUMBER:	313-97 and Amendment 1
TEST SUBSTANCE:	CGA-169374
TEST SYSTEM:	Barley
NUMBER OF ANALYSES:	58
LABORATORY:	Novartis Residue Analysis

DESCRIPTION: The objective of this study was to validate method AG-676 for the confirmation of residues of CGA-169374 determined in barley using the established tolerance enforcement methodology (AG-575B) with modifications. The validation was performed by analyzed control and fortified control of barley samples. Sample preparation, extraction, and analytical procedures of method AG-575B were used to generate the samples. Controls and fortified controls (recoveries) were first determined using the instrumentation from AG-575B. The results were then confirmed by injecting the same final fraction onto the confirmatory GC/MSD system.

STUDY DIRECTOR: T. L. Oakes

SIGNATURE: Timothy L. Oakes

DATE: 7/17/97

DISTRIBUTION: To be distributed per protocol distribution list.

011971 [S:\77102\77102\313-97\RTR\DOC] sbh/mae:7/16/97

BIOLOGY SECTION

Substrate	Commodity	Residue Test Number	Inventory Number	Project Number
Barley	Hay, Forage, Straw,	RI-MV-011-97	16968.1	420011

CIRCUMSTANCES AFFECTING THE STUDY

None

DEVIATIONS FROM PROTOCOL

None

TEST AND REFERENCE SUBSTANCES

Analytical Standard	Identification No.	Purity	Reanalysis Date
CGA-169374	S93-1669	96.0	4/98

Standards were provided by Ciba Analytical and Product Chemistry Department formerly known as Ciba Production Technical Analytical Services (PTAS).

SAMPLE IDENTIFICATION NUMBERS

Each sample was assigned an unique analytical number in Table I of Protocol 313-97. All sample identification numbers were recorded in the laboratory notebooks throughout the course of the study.

STUDY PERSONNEL

T. L. Oakes (TLO), Chemist IV
J. T. Adair (JTA), Olsten Temporary Services

ANALYTICAL SECTION

Methodology

AG-575B - with the omission of the charcoal cleanup (Section 6.0). The GC conditions of AG-575B were also modified to include a DB-17 (15 meter x 0.53 mm ID, d_f = 1.0 µm) megabore column instead of a packed column.

An additional hexane:acetonitrile partition was added to grain samples as documented in AG-676. The use of polyethylene glycol was also used as documented in AG-676.

FROM THE ANALYSIS OF BARLEY GRAIN

Barley Substrate	Numeric ID	Fortification Level (ppm)	GC/MSD Recovery (%)	GC/NPD Recovery (%)
Hay	200043	control	--	--
Hay	201644	0.05	100	113
Hay	201645	0.1	102	101
Hay	201646	0.5	94.3	108
		Average =	98.9	107
		Standard Deviation =	4.1	6.1
Forage	203792	control	--	--
Forage	203849	0.05	96.1	110
Forage	203850	0.1	116	106
Forage	203851	0.5	103	96.5
		Average =	105	104
		Standard Deviation =	10.2	6.7
Straw	200044	control	--	--
Straw	201647	0.05	104	95.5
Straw	201648	0.1	100	87.9
Straw	201649	0.5	102	102
		Average =	102	95.3
		Standard Deviation =	1.9	7.3
Grain	200045	control	--	--
Grain	201463 (Set 1)	0.01	148	93.4
Grain	201463 (Set 2)	0.01	171	96.0
Grain	201463 (Set 2)	0.01	148	87.5
Grain	201464 (Set 2)	0.04	118	100
Grain	201464 (Set 1)	0.04	106	93.7
Grain	201465 (Set 1)	0.2	108	95.0
Grain	201465 (Set 2)	0.2	97.0	94.2
Grain	201463a	0.1	107	95.5
Grain	201464a	0.2	89.4	101
Grain	201465a	1.0	96.7	93.8
		Average =	119	95.0
		Standard Deviation =	27	3.7

SUMMARY

This method provides a GC/MSD system that will confirm the presence of CGA-169374 residues detected in barley using the established tolerance enforcement methodology (AG-575B). Confirmation is accomplished by analyzing ions unique to CGA-169374 using GC/MSD and rejecting any potential co-eluting or interfering compounds. Confirmation of CGA-169374 residues can be achieved by analysis of the samples by GC/MSD using selected ion monitoring (SIM) to detect the following ions: (m/z = 265, 323, and 325). Confirmation of residue concentration is obtained by quantitating on the target ion for CGA-169374 (m/z = 323). Confirmation of the compound identity is determined by taking a ratio of the qualifying ions (m/z = 265, 325) to the target ion (m/z = 323). Structural confirmation can be made if the sample ion ratios match the ratio obtained from the standard (+/- 25%).

Note that the tolerance enforcement method has been previously validated for accuracy, precision, and extractability utilizing weathered samples. All procedures for sample extractions, extract cleanup, and original GC conditions are the same for this method, except: 1) the addition of a hexane:acetonitrile partition for grain and 2) the use of polyethylene glycol (0.05%) to help minimize the effects of matrix enhancement. The additional confirmatory GC/MSD column and conditions have been validated here.

To validate this method, recovery results were obtained through analysis of fortified control samples by both the original GC/NPD (AG-575B) and the confirmatory GC/MSD system (AG-676). Recovery results were used to calculate accuracy in terms of mean and standard deviation (SD). The mean recoveries for the tolerance enforcement analyses and the corresponding confirmation analyses are shown below.

Substrate	GC/NPD MEAN(SD)	GC/MSD MEAN (SD)
Hay	107 (6.1)	98.9 (4.1)
Forage	104 (6.7)	105 (10.2)
Straw	95.3 (7.3)	102 (1.9)
Grain	95.0 (3.7)	119 (27)

In conclusion, analytical method AG-676 is a valid and accurate confirmatory method for the determination of parent residues of CGA-169374 in barley. This confirmatory technique offers a highly specific method for the verification of potential CGA-169374 residues in barley and other cereal grains at or above the tolerance level.

169374 Barley Straw Using GC/NPD (Tolerance Enforcement Method)

Worksheet ...: 313-97-BC4R
Ana Set Name : 313-97-BC
Analysis ....: BC0623
Subject .....: BARLEY

Limit of Determ : 0.010 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 1.0000

Extracted : 4-Jun-1997
Analyzed .: 23-Jun-1997
Channel ..: 41

Product .: CGA-169374
Analyte .: CGA-169374
Project .: 313-97

Bt Nm	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												2.00	
2	S												2.00	
3*	C	1	RIMV01197	000148	11.00	20.00	300.00	60.00			3.971	2.0	2.00	3.971
4	S												2.00	
5	R	1	"	"	11.00	20.00	300.00	60.00			3.971	4.0	2.00	1.985
6	R	1	"	"	11.00	20.00	300.00	60.00			3.971	10.0	2.00	0.794
7	S												2.00	
8	R	1	"	"	11.00	20.03	300.00	60.00			3.977	20.0	2.00	0.398
9	B				11.00	20.00	300.00	60.00			3.971	2.0	2.00	3.971
10	S												2.00	
11	S												2.00	

Bt Nm	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Comments
1	S		0.20000	0.4000	36281	0.405							standard cga-169374
2	S		0.10000	0.2080	16473	0.184							standard cga-169374
3*	C	1			382	0.004	0.001				95.27	<0.010	control (straw)
4	S		0.02000	0.0400	3397	0.038							standard cga-169374
5	R	1			8677	0.097	0.049	0.048	0.050	95.46			rec. + 0.05ppm cga-169374
6	R	1			6330	0.071	0.089	0.088	0.100	87.92			rec. + 0.10ppm cga-169374
7	S		0.50000	1.0000	89629	1.001							standard cga-169374
8	R	1			18276	0.204	0.513	0.512	0.500	102.43			rec. + 0.50ppm cga-169374
9	B				308	0.003	0.001						reagent blank
10	S		0.01000	0.0200	1658	0.019							standard cga-169374
11	S		0.04000	0.0800	7587	0.085							standard cga-169374

Substrates : STRAW

Notes :

B : Blank C : Control F : Freezer R : Recovery S : Standard V : Solvent X : Sample
* : Control used for this group Negative controls are treated as 0.0 Underline, Strikethrough : Manual override, ~~Rejected~~

169374 Barley Straw Using GC/MSD (Confirmatory Method)

Worksheet ...: PRESSTRAN
Ana Set Name :
Analysis ....:
Subject .....:

Limit of Determ : 0.010 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 1.0000

Extracted : 4-Jun-1997
Analyzed .:
Channel ..: 41

Product .:
Analyte .: CGA-169374
Project .: 313-97

Bt Nm	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												2.00	
2	S												2.00	
3*	C	1	RIMV01197	000148	11.00	20.00	300.00	60.00			3.971	2.0	2.00	3.971
4	S												2.00	
5	R	1	"	"	11.00	20.00	300.00	60.00			3.971	4.0	2.00	1.985
6	R	1	"	"	11.00	20.00	300.00	60.00			3.971	10.0	2.00	0.794
7	S												2.00	
8	R	1	"	"	11.00	20.03	300.00	60.00			3.977	20.0	2.00	0.398
9	B	1	"	"	11.00	20.00	300.00	60.00			3.971	2.0	2.00	3.971
10	S												2.00	
11	S												2.00	

Bt Nm	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Comments
1	S		0.20000	0.4000	2482777	0.379							STANDARD CGA-169374
2	S		0.10000	0.2000	1069574	0.176							STANDARD CGA-169374
3*	C	1			0	0.000	0.000				100.00	<0.010	CONTROL STRAW
4	S		0.02000	0.0400	272824	0.052							STANDARD CGA-169374
5	R	1			620917	0.103	0.052	0.052	0.050	104.15			REC + 0.05 PPM CGA-169374
6	R	1			461097	0.080	0.100	0.100	0.100	100.43			REC + 0.10 PPM CGA-169374
7	S		0.50000	1.0000	6770810	1.013							STANDARD CGA-169374
8	R	1			1299370	0.204	0.512	0.512	0.500	102.45			REC + 0.50 PPM CGA-169374
9	B	1			0	0.000	0.000				100.00	<0.010	REAGENT BLANK
10	S		0.01000	0.0200	153981	0.034							STANDARD CGA-169374
11	S		0.04000	0.0800	547157	0.092							STANDARD CGA-169374

Subetrates :

Notes :

B : Blank C : Control F : Freezer R : Recovery S : Standard V : Solvent X : Sample
* : Control used for this group Negative controls are treated as 0.0 Underline, Strikethrough : Manual override, Rejected

PROTOCOL NUMBER: 313-97
REPORT NUMBER: 1

RESIDUE TEST REPORT RI-MV-011-97

PROJECT NUMBER: 420011
PAGE 1 OF 16

169374 Barley Forage Using GC/NPD (Tolerance Enforcement Method)

Worksheet ...: 313-97-BD2
Ana Set Name : 313-97-BD
Analysis ....: BD703
Subject .....: BARLEY

Limit of Determ : 0.010 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 1.0000

Extracted : 27-Jun-1997
Analyzed .: 3-Jul-1997
Channel ..: 41

Product .: CGA-169374
Analyte .: CGA-169374
Project .: 313-97

Bt Nm	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												2.00	
2	S												2.00	
3*	C	1	RIMV01197	000247	70.00	20.00	200.00	40.00			3.738	2.0	2.00	3.738
4	S												2.00	
5	R	1	"	"	70.00	20.00	200.00	40.00			3.738	4.0	2.00	1.869
6	R	1	"	"	70.00	20.00	200.00	40.00			3.738	10.0	2.00	0.746
7	S												2.00	
8	R	1	"	"	70.00	20.00	200.00	40.00			3.738	20.0	2.00	0.374
9	R	1			70.00	20.00	200.00	40.00			3.738	2.0	2.00	3.738
10	S												2.00	
11	S												2.00-	

Bt Nm	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Comments
1	S		0.20000	0.4000	20278	0.354							Standard CGA-169374
2	S		0.10000	0.2000	10874	0.190							Standard CGA-169374
3*	C	1			180	0.003	0.001				100.00	<0.010	CONTROL (FORAGE)
4	S		0.02000	0.0400	2399	0.042							Standard CGA-169374
5	R	1			5961	0.104	0.056	0.055	0.050	109.54			REC + 0.05 PPM cga-169374
6	R	1			4572	0.080	0.107	0.106	0.100	105.80			REC + 0.10 PPM cga-169374
7	S		0.50000	1.0000	58562	1.021							Standard CGA-169374
8	R	1			10367	0.181	0.484	0.483	0.500	96.54			REC + 0.50 PPM cga-169374
9	R	1			372	0.006	0.002				100.00	<0.010	REAGENT BLANK
10	S		0.01000	0.0200	1243	0.022							Standard CGA-169374
11	S		0.04000	0.0800	4145	0.072							Standard CGA-169374

Substrates : FORAGE

Notes :

B : Blank C : Control F : Freezer R : Recovery S : Standard V : Solvent X : Sample
* : Control used for this group Negative controls are treated as 0.0 Underline, Strikethrough : Manual override, Rejected

169374 Barley Forage Using GC/MSD (Confirmatory Method)

Worksheet ...: TARGETFORAGE4C
Ana Set Name : 313-97-BD
Analysis ....:
Subject .....: BARLEY

Limit of Determ : 0.010 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 1.0000

Extracted : 27-Jun-1997
Analyzed .:
Channel ..: 41

Product .: CGA-169374
Analyte .: CGA-169374
Project .: 313-97

Bt Nm	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												2.00	
2	S												2.00	
3*	C	1	RIHV01197	000247	70.00	20.00	200.00	40.00			3.738	2.0	2.00	3.738
4	S												2.00	
5	R	1	"	"	70.00	20.00	200.00	40.00			3.738	4.0	2.00	1.869
6	R	1	"	"	70.00	20.00	200.00	40.00			3.738	10.0	2.00	0.748
7	S												2.00	
8	R	1	"	"	70.00	20.00	200.00	40.00			3.738	40.0	2.00	0.187
9	B	1			70.00	20.00	200.00	40.00			3.738	2.0	2.00	3.738
10	S												2.00	

Bt Nm	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Comments
1	S		0.20000	0.4000	1808491	0.434							Standard CGA-169374
2	S		0.10000	0.2000	775405	0.183							Standard CGA-169374
3*	C	1			0	0.000	0.000				100.00	<0.011	CONTROL (FORAGE)
4	S		0.02000	0.0400	167471	0.036							Standard CGA-169374
5	R	1			390332	0.090	0.048	0.048	0.050	96.10			REC + 0.05 PPM cga-169374
6	R	1			374405	0.087	0.116	0.116	0.100	116.26			REC + 0.10 PPM cga-169374
7	S		0.50000	1.0000	4101014	0.950							Standard CGA-169374
8	R	1			418623	0.097	0.517	0.517	0.500	103.45			REC + 0.50 PPM cga-169374
9	B	1			0	0.000	0.000				100.00	<0.011	REAGENT BLANK
10	S		0.04000	0.0800	337061	0.077							Standard CGA-169374

Substrates : FORAGE

Notes : This worksheet was originally targetforage1

B : Blank C : Control F : Freezer R : Recovery S : Standard V : Solvent X : Sample
* : Control used for this group Negative controls are treated as 0.0 Underline, Strikethrough : Manual override, Rejected

169374 Barley Hay Using GC/NPD (Tolerance Enforcement Method)

Worksheet ...: 313-97-PEGH
Ana Set Name : 313-97-BB
Analysis ....: PEGH624
Subject .....: BARLEY

Limit of Determ : 0.010 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 1.0000

Extracted : 23-Jun-1997
Analyzed .: 25-Jun-1997
Channel ..: 41

Product .: CGA-169374
Analyte .: CGA-169374
Project .: 313-97

Bt Nm	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												2.00	
2	S												2.00	
3*	C	1	RIMV01197	000147	11.00	20.01	200.00	40.00			3.958	2.0	2.00	3.958
4	S												2.00	
5	R	1	•	•	11.00	20.00	200.00	40.00			3.956	4.0	2.00	1.978
6	R	1	•	•	11.00	20.00	200.00	40.00			3.956	10.0	2.00	0.791
7	S												2.00	
8	R	1	•	•	11.00	20.03	200.00	40.00			3.962	20.0	2.00	0.396
9	B	1			11.00	20.01	200.00	40.00			3.958	2.0	2.00	3.958
10	S												2.00	
11	S												2.00	

Bt Nm	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Comments
1	S		0.20000	0.4000	26908	0.407							standard cga-169374
2	S		0.10000	0.2000	13330	0.202							standard cga-169374
3*	C	1			0	0.000	0.000				100.00	<0.010	control (hay)
4	S		0.02000	0.0400	2451	0.037							standard cga-169374
5	R	1			7386	0.112	0.057	0.057	0.050	113.03			rec. + 0.05ppm cga-169374
6	R	1			5278	0.080	0.101	0.101	0.100	100.96			rec. + 0.10ppm cga-169374
7	S		0.50000	1.0000	65855	0.997							standard cga-169374
8	R	1			14188	0.215	0.542	0.542	0.500	108.39			rec. + 0.50ppm cga-169374
9	B	1			0	0.000	0.000				100.00	<0.010	reagent blank
10	S		0.01000	0.0200	1438	0.022							standard cga-169374
11	S		0.04000	0.0800	5321	0.081							standard cga-169374

Substrates : HAY

Notes :

B : Blank C : Control P : Freezer R : Recovery S : Standard V : Solvent X : Sample
* : Control used for this group Negative controls are treated as 0.0 Underline, Strikethrough : Manual override, Rejected

169374 Barley Hay Using GC/MSD (Confirmatory Method)

Worksheet ...: PRESSHAY
Ana Set Name :
Analysis ....: HAY2
Subject .....:

Limit of Determ : 0.010 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 1.0000

Extracted : 23-Jun-1997
Analyzed .:
Channel ..: 41

Product .:
Analyte .: cga-169374
Project .: 313-97

Bt No	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												2.00	
2	S												2.00	
3*	C	1	RIMV00197	000147	11.00	20.01	200.00	40.00					2.00	
4	S										3.958	2.0	2.00	3.958
5	R	1	"	"	11.00	20.00	200.00	40.00					2.00	
6	R	1	"	"	11.00	20.00	200.00	40.00			3.956	4.0	2.00	1.978
7	S										3.956	10.0	2.00	0.791
8	R	1	"	"	11.00	20.03	200.00	40.00					2.00	
9	R	1	"	"	11.00	20.01	200.00	40.00			3.962	20.0	2.00	0.396
10	S										3.958	2.0	2.00	3.958
11	S												2.00	
													2.00	

Bt No	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Comments
1	S		0.20000	0.4000	1979282	0.380							STANDARD CGA-169374
2	S		0.10000	0.2000	1034495	0.200							STANDARD CGA-169374
3*	C	1			0	0.000	0.000						CONTROL (HAY)
4	S		0.02000	0.0400	239517	0.046					98.93	<0.010	STANDARD CGA-169374
5	R	1			508165	0.099	0.050	0.050	0.050	100.31			REC + 0.05 PPM cga-169374
6	R	1			411723	0.081	0.102	0.102	0.100	102.17			REC + 0.10 PPM cga-169374
7	S		0.50000	1.0000	5276305	1.008							STANDARD CGA-169374
8	R	1			968127	0.187	0.472	0.472	0.500	94.32			REC + 0.5 PPM cga-169374
9	R	1			0	0.000	0.000				98.93	<0.010	REAGENT BLANK
10	S		0.01000	0.0200	114042	0.024							STANDARD CGA-169374
11	S		0.04000	0.0800	423042	0.083							STANDARD CGA-169374

Substrates :

Notes :

B : Blank C : Control F : Freezer R : Recovery S : Standard V : Solvent X : Sample
* : Control used for this group Negative controls are treated as 0.0 Underline, Strikethrough : Manual override, Rejected

169374 Barley Grain Forage Using GC/NPD (Tolerance Enforcement Method)
(SET 1)

Worksheet ...: 313-97-PBGG
Ana Set Name : 313-97-BA
Analysis ....: PPBG624
Subject .....: BARLEY

Limit of Determ : 0.010 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 1.0000

Extracted : 2-Jun-1997
Analyzed .: 24-Jun-1997
Channel ..: 41

Product .: CGA-169374
Analyte .: CGA-169374
Project .: 313-97

Bt Nm	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												2.00	
2	S												2.00	
3*	C	1	RIMV01197	000149	12.00	20.00	200.00	40.00			3.953	2.0	2.00	3.953
4	S												2.00	
5	R	1	"	"	12.00	20.00	200.00	40.00			3.953	2.0	2.00	3.953
6	R	1	"	"	12.00	20.00	200.00	40.00			3.953	3.0	2.00	2.635
7	S												2.00	
8	R	1	"	"	12.00	20.00	200.00	40.00			3.953	6.0	2.00	1.318
9	B	1			12.00	20.00	200.00	40.00			3.953	2.0	2.00	3.953
10	S												2.00	
11	S												2.00	

Bt Nm	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Comments
1	S		0.20000	0.4000	28485	0.394							Standard CGA-169374
2	S		0.10000	0.2000	12495	0.173							Standard CGA-169374
3*	C	1			0	0.000	0.000				94.04	<0.010	Control Barley Grain
4	S		0.02000	0.0400	2739	0.038							Standard CGA-169374
5	R	1			2668	0.037	0.009	0.009	0.010	93.42			Rec + 0.01 ppm CGA-169374
6	R	1			7138	0.099	0.037	0.037	0.040	93.71			Rec + 0.04 ppm CGA-169374
7	S		0.50000	1.0000	72856	1.008							Standard CGA-169374
8	R	1			18086	0.250	0.190	0.190	0.200	94.98			Rec + 0.2 ppm CGA-169374
9	B	1			0	0.000	0.000				94.04	<0.010	Reagent Blank
10	S		0.01000	0.0200	1313	0.018							Standard CGA-169374
11	S		0.04000	0.0800	5481	0.076							Standard CGA-169374

Substrates : GRAIN

Notes :

B : Blank C : Control F : Freezer R : Recovery S : Standard V : Solvent X : Sample
* : Control used for this group Negative controls are treated as 0.0 Underline, Strikethrough : Manual override, Rejected

169374 Barley Grain Using GC/MSD (Confirmatory Method)
(SET 1)

Worksheet ...: PRESSGRAIN
Ana Set Name : 313-97-BA
Analysis ....:
Subject .....: BARLEY

Limit of Determ : 0.010 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 1.0000

Extracted : 2-Jun-1997
Analyzed ..:
Channel ..: 41

Product .: CGA-169374
Analyte .: CGA-169374
Project .: 313-97

Bt No	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												2.00	
2	S												2.00	
3*	C	1	RIMV01197	000149	12.00	20.00	200.00	40.00			3.953	2.0	2.00	3.953
4	S												2.00	
5	R	1	"	"	12.00	20.00	200.00	40.00			3.953	2.0	2.00	3.953
6	R	1	"	"	12.00	20.00	200.00	40.00			3.953	3.0	2.00	2.635
7	S												2.00	
8	R	1	"	"	12.00	20.00	200.00	40.00			3.953	6.0	2.00	1.318
9	B	1			12.00	20.00	200.00	40.00			3.953	2.0	2.00	3.953
10	S												2.00	
11	S												2.00	

Bt No	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Comments
1	S		0.20000	0.4000	1701014	0.338							Standard CGA-169374
2	S		0.10000	0.2000	817296	0.174							Standard CGA-169374
3*	C	1			0	0.000	0.000				100.00	<0.010	Control Barley Grain
4	S		0.02000	0.0400	193681	0.059							Standard CGA-169374
5	R	1			191169	0.058	0.015	0.015	0.010	147.96			Rec + 0.01 ppm CGA-169374
6	R	1			479213	0.113	0.042	0.042	0.040	106.05			Rec + 0.04 ppm CGA-169374
7	S		0.50000	1.0000	5425204	1.027							Standard CGA-169374
8	R	1			1409492	0.284	0.216	0.216	0.200	107.76			Rec + 0.2 ppm CGA-169374
9	B	1			0	0.000	0.000				100.00	<0.010	Reagent Blank
10	S		0.01000	0.0200	94174	0.041							Standard CGA-169374
11	S		0.04000	0.0800	421896	0.101							Standard CGA-169374

Substrates : GRAIN

Notes : PRESSURE PROGRAMMING ON GC/MSD
FILES d:69374
PEOPS001-PEOPS011

B : Blank C : Control F : Freezer R : Recovery S : Standard V : Solvent X : Sample
* : Control used for this group Negative controls are treated as 0.0 Underline, Strikethrough : Manual override, Rejected

169374 Barley Grain Forage Using GC/NPD (Tolerance Enforcement Method)
(SET 2)

Worksheet ....: 313-97-PEGG2
Ana Set Name : 313-97-BA
Analysis .....: PPBG626
Subject .....: BARLEY

Limit of Determ : 0.010 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 1.0000

Extracted : 24-Jun-1997
Analysed .: 26-Jun-1997
Channel ..: 41

Product .: CGA-169374
Analyte .: CGA-169374
Project .: 313-97

Bt No	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												2.00	
2	S												2.00	
3*	C	1	RINV01197	000149	12.00	20.01	200.00	40.00			3.955	2.0	2.00	3.955
4	S												2.00	
5	R	1	"	"	12.00	20.00	200.00	40.00			3.953	2.0	2.00	3.953
6	R	1	"	"	12.00	20.01	200.00	40.00			3.955	2.0	2.00	3.955
7	S												2.00	
8	R	1	"	"	12.00	20.03	200.00	40.00			3.958	3.0	2.00	2.639
9	R	1	"	"	12.00	20.03	200.00	40.00			3.958	6.0	2.00	1.319
10	S												2.00	
11	B	1			12.00	20.00	200.00	40.00	—		3.953	2.0	2.00	3.953
12	S												2.00	

Bt No	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Comments
1	S		0.20000	0.4000	30984	0.433							Standard CGA-169374
2	S		0.10000	0.2000	14589	0.204							Standard CGA-169374
3*	C	1			0	0.000	0.000				94.52	<0.010	Control Barley Grain
4	S		0.02000	0.0400	2680	0.037							Standard CGA-169374
5	R	1			2716	0.038	0.010	0.010	0.010	96.01			Rec + 0.01 ppm CGA-169374
6	R	1			2477	0.035	0.009	0.009	0.010	87.91			Rec. + 0.01 ppm CGA-169374
7	S		0.50000	1.0000	70619	0.987							Standard CGA-169374
8	R	1			7584	0.106	0.040	0.040	0.040	100.38			Rec + 0.04 ppm CGA-169374
9	R	1			17786	0.249	0.188	0.188	0.200	94.17			Rec + 0.2 ppm CGA-169374
10	S		0.01000	0.0200	1582	0.022							Standard CGA-169374
11	B	1			0	0.000	0.000				94.52	<0.010	Reagent Blank
12	S		0.04000	0.0800	5180	0.072							Standard CGA-169374

Subtrates : GRAIN

Notes :

B : Blank C : Control F : Freezer R : Recovery S : Standard V : Solvent X : Sample
* : Control used for this group Negative controls are treated as 0.0 Underline, Strikethrough : Manual override, Rejected

169374 Barley Grain Using GC/MSD (Confirmatory Method)
(SET 2)

Worksheet ...: PREPROGRAIN2
Ana Set Name : 313-97-BA
Analysis ....:
Subject .....: BARLEY

Limit of Determ : 0.010 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 1.0000

Extracted : 24-Jun-1997
Analyzed .:
Channel ...: 41

Product .: CGA-169374
Analyte .: CGA-169374
Project .: 313-97

Bt No	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												2.00	
2	S												2.00	
3*	C	1	RIMV01197	000149	12.00	20.01	200.00	40.00			3.955	2.0	2.00	3.955
4	S												2.00	
5	R	1	"	"	12.00	20.00	200.00	40.00			3.953	2.0	2.00	3.953
6	R	1	"	"	12.00	20.01	200.00	40.00			3.955	2.0	2.00	3.955
7	S												2.00	
8	R	1	"	"	12.00	20.03	200.00	40.00			3.958	3.0	2.00	2.639
9	R	1	"	"	12.00	20.03	200.00	40.00			3.958	6.0	2.00	1.319
10	S												2.00	
11	B	1			12.00	20.00	200.00	40.00			3.953	2.0	2.00	3.953
12	S												2.00	

Bt No	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Comments
1	S		0.20000	0.4000	1634931	0.293							Standard CGA-169374
2	S		0.10000	0.2000	1020902	0.195							Standard CGA-169374
3*	C	1			0	0.000	0.000				100.00	<0.010	Control Barley Grain
4	S		0.02000	0.0400	221942	0.064							Standard CGA-169374
5	R	1			242257	0.068	0.017	0.017	0.010	170.93			Rec + 0.01 ppm CGA-169374
6	R	1			186185	0.058	0.015	0.015	0.010	147.88			Rec + 0.01 ppm CGA-169374
7	S		0.50000	1.0000	6251863	1.041							Standard CGA-169374
8	R	1			593837	0.124	0.047	0.047	0.040	117.94			Rec + 0.04 ppm CGA-169374
9	R	1			1406365	0.256	0.194	0.194	0.200	97.04			Rec + 0.2 ppm CGA-169374
10	S		0.01000	0.0200	126937	0.049							Standard CGA-169374
11	B	1			0	0.000	0.000				100.00	<0.010	Reagent Blank
12	S		0.04000	0.0800	430272	0.098							Standard CGA-169374

Substrates : GRAIN

Notes :

B : Blank C : Control F : Freezer R : Recovery S : Standard V : Solvent X : Sample
* : Control used for this group Negative controls are treated as 0.0 Underline, Strikethrough : Manual override, Rejected

169374 Barley Grain Forage Using GC/NPD (Tolerance Enforcement Method)
(SET 3)

Worksheet ...: 313-97-PRO03 | Limit of Determ : 0.010 ppm | Extracted : 27-Jun-1997 | Product .: CGA-169374
Ana Set Name : 313-97-BA | Corr Factor A ..: 1.0000 | Analyzed .: 1-Jul-1997 | Analyte .: CGA-169374
Analysis ....: PFE0701 | Corr Factor B ..: 1.0000 | Channel ..: 41 | Project .: 313-97
Subject .....: BARLEY

Bt No	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												2.00	
2	S												2.00	
3*	C	1	RIMV01197	000149	12.00	20.03	200.00	40.00			3.958	2.0	2.00	3.958
4	S												2.00	
5	R	1	"	"	12.00	20.01	200.00	40.00			3.955	10.0	2.00	0.791
6	R	1	"	"	12.00	20.00	200.00	40.00			3.953	20.0	2.00	0.395
7	S												2.00	
8	R	1	"	"	12.00	20.00	200.00	40.00			3.953	40.0	2.00	0.198
9	B	1			12.00	20.00	200.00	40.00			3.953	2.0	2.00	3.953
10	S												2.00	
11	S												2.00	
12	S												2.00	

Bt No	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Comments
1	S		0.20000	0.4000	5915	0.460							Standard CGA-169374
2	S		0.10000	0.2000	2801	0.218							Standard CGA-169374
3*	C	1			0	0.000	0.000				96.70	<0.010	Control Barley Grain
4	S		0.02000	0.0400	504	0.039							Standard CGA-169374
5	R	1			971	0.076	0.096	0.096	0.100	95.53			Rec + 0.10 ppm CGA-169374
6	R	1			1024	0.080	0.201	0.201	0.200	100.74			Rec + 0.20 ppm CGA-169374
7	S		0.50000	1.0000	12516	0.974							Standard CGA-169374
8	R	1			2384	0.185	0.938	0.938	1.000	93.83			Rec + 1.0 ppm CGA-169374
9	B	1			0	0.000	0.000				96.70	<0.010	Reagent Blank
10	S		0.01000	0.0200	193	0.015							Standard CGA-169374
11	S		0.04000	0.0800	839	0.065							Standard CGA-169374
~~12~~	~~S~~		~~1.00000~~	~~2.0000~~	~~21473~~	~~1.670~~							Standard CGA-169374

Substrates : GRAIN

Notes :

B : Blank C : Control F : Freezer R : Recovery S : Standard V : Solvent X : Sample
* : Control used for this group Negative controls are treated as 0.0 Underline, Strikethrough : Manual override, ~~Rejected~~

169374 Barley Grain Using GC/MSD (Confirmatory Method)
(SET 3)

Worksheet ...: 169GR4
Ana Set Name : 313-97-BA
Analysis ....:
Subject .....: BARLEY

Limit of Determ : 0.010 ppm
Corr Factor A ..: 1.0000
Corr Factor B ..: 1.0000

Extracted : 27-Jun-1997
Analyzed ..:
Channel ..: 41

Product .: CGA-169374
Analyte .: CGA-169374
Project .: 313-97

Bt No	T	G	Field Test	Smp Code	Moist %	Smp wt ext g	Ext vol ml	Aliq 1 vol ml	Interm vol ml	Aliq 2 vol ml	Final wt g	Final vol ml	Inj vol ul	Smp Wt Inj mg
1	S												2.00	
2	S												2.00	
3*	C	1	RIMV01197	000149	12.00	20.03	200.00	40.00					2.00	
4	S										3.958	2.0	2.00	3.958
5	R	1	"	"	12.00	20.01	200.00	40.00					2.00	
6	R	1	"	"	12.00	20.00	200.00	40.00			3.955	10.0	2.00	0.791
7	S										3.953	20.0	2.00	0.395
8	R	1	"	"	12.00	20.00	200.00	40.00					2.00	
9	R	1			12.00	20.00	200.00	40.00			3.953	40.0	2.00	0.198
10	S										3.953	2.0	2.00	3.953
11	S												2.00	
													2.00	

Bt No	T	G	Std soln ng/ul	Std Wt Inj ng	Peak Height uV	Analyte Found ng	Residue Found ppm	Value corrt for control	Added Amount ppm	Recov %	Avg Recov %	Corrt Residue ppm	Comments
1	S		0.20000	0.4000	1606555	0.326							Standard CGA-169374
2	S		0.10000	0.2000	969917	0.201							Standard CGA-169374
3*	C	1			0	0.000	0.000				97.73	<0.010	Control Barley Grain
4	S		0.02000	0.0480	176809	0.045							Standard CGA-169374
5	R	1			377781	0.085	0.107	0.107	0.100	107.09			Rec + 0.10 ppm CGA-169374
6	R	1			306454	0.071	0.179	0.179	0.200	89.41			Rec + 0.20 ppm CGA-169374
7	S		0.50000	1.0000	5167245	1.026							Standard CGA-169374
8	R	1			918954	0.191	0.967	0.967	1.000	96.70			Rec + 1.0 ppm CGA-169374
9	R	1			0	0.000	0.000				97.73	<0.010	REAGENT BLANK
10	S		0.01000	0.0280	96230	0.029							Standard CGA-169374
11	S		0.04000	0.0800	515304	0.112							Standard CGA-169374

Substrates : GRAIN

Notes :

B : Blank C : Control F : Freezer R : Recovery S : Standard V : Solvent X : Sample
* : Control used for this group Negative controls are treated as 0.0 Underline, Strikethrough : Manual override, Rejected

APPENDIX III

CERTIFICATION OF AUTHENTICITY

The reports included in this submittal volume, Laboratory Project I. D. AG-676 Analytical Method, are certified to be authentic accounts of the experiments and the results of these experiments, described herein.

Darren S. Hackett for Tim Oakes

Tim Oakes, Chemist IV, and Study Director
Human Safety Department
(910) 632-2393

7/18/97
Date

APPENDIX II

ANALYTICAL PHASE REPORT

ENVIRO-TEST LABORATORIES (ETL)
9936 - 67 AVENUE
EDMONTON, ALBERTA T6E 0P5

ANALYTICAL PHASE REPORT

STUDY TITLE:	CGA-293343 COMBI FS-D - MAGNITUDE OF THE RESIDUES IN OR ON CANOLA
DATA REQUIREMENT:	US EPA Pesticide Assessment Guideline, Subdivision O, 171-4
AUTHORS:	Gary Bruns and Susan Nelson Enviro-Test Laboratories
ANALYTICAL PRINCIPAL INVESTIGATOR:	Susan Nelson Enviro-Test Laboratories 9936 - 67 Avenue Edmonton, Alberta T6E 0P5 Ph: (403) 413-5216 Fx: (403) 434-9178
SPONSOR:	Novartis Crop Protection Inc. 410 Swing Road Greensboro, NC 27419
STUDY DIRECTOR:	Timothy P. Vincent, Outside Research II Novartis Crop Protection Inc. 410 Swing Road Greensboro, NC 27419 Ph: (336) 632-7535 Fx: (336) 632-7581
PERFORMING LABORATORY:	Enviro-Test Laboratories 9936 - 67 Avenue Edmonton, Alberta T6E 0P5
STUDY DATES:	Study Initiation Date: September 24, 1998 Experimental Initiation Date: September 16, 1998 Experimental Termination Date: November 4, 1998
STUDY PROTOCOL NO.:	476-97-B
NOVARTIS PROJECT NO.:	346055
ETL REPORT NO.:	98NOV37.REP
REPORT DATE:	November 5, 1998
PAGES OF REPORT:	106

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

"We, the undersigned, hereby certify that this study was generated by Enviro-Test Laboratories in compliance with Good Laboratory Practices according to EPA FIFRA, Section 40 CFR part 160, October 16, 1989."

Gary B Date: Nov 5/98
Gary Bruns
Analytical Laboratory Manager
Enviro-Test Laboratories

Susan Nelson Date: Nov 5/98
Susan Nelson
Analytical Principal Investigator
Enviro-Test Laboratories

STATEMENT OF THE QUALITY ASSURANCE UNIT

ETL Report No.: 98NOV37.REP
Study Protocol No.: 476-97-B

The quality assurance unit of Enviro-Test Laboratories has inspected and/or audited the analytical phase of this study and the report, and has reported its findings to the Study Director and to ETL Management. The raw data is complete, consistent, well documented and accurately reflects the method in which the study was conducted.

AUDIT DATES	REPORTED TO PRINCIPAL INVESTIGATOR	REPORTED TO ETL MANAGEMENT	REPORTED TO STUDY DIRECTOR
In-phase audit: Oct. 2/98 (DMA) Oct.9,13/98 (MS 162.00) Oct.15 (MS 172.00)	Nov. 5/98	Nov. 5/98	Nov. 5/98
Final Report/Data Audit: Nov. 3,4 & 5/98	Nov. 5/98	Nov. 5/98	Nov. 5/98

Anne Beaulieu
Signature of QAU

Date: Nov. 5/98

REF.#: QAD1.OLN

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1.0 METHOD OVERVIEW

The method used for the analysis of CGA-293343 and metabolite CGA-322704 in canola was developed at ETL and based on Novartis method AG-675. Two clean-up steps were eliminated because of the use of a more selective detection system (LC/MS/MS). The analytical procedure is detailed in Section 4.2.

The method for the analysis of Difenoconazole (CGA-169374) in canola was Novartis method AG-676. Modifications are listed in Section 4.1 (Analytical Procedures and Modifications).

The method used for the total residues of Mefenoxam (CGA-329351) as 2,6-Dimethylaniline (2,6-DMA) in canola is based on the Novartis analytical method AG-395 entitled "Improved Method for the Determination of Total Residues of Metalaxyl in Crop as 2,6-Dimethylaniline".

The treated seeds were analyzed based on Novartis method AG-397 with modifications. Surface residues were extracted by sonication and soaking with acetone, which was recommended as the best solvent by Novartis, US.

2.0 SAMPLE RECEIPT AND PREPARATION

A total of 90 canola seed samples were received at Enviro-Test Laboratories on September 9 and 29,1998. All samples were received frozen with ice packs or dry ice and were stored in freezers at -20 ± 5°C until removed for sample processing.

Each sample was given a unique laboratory number generated by Enviro-Test Laboratories Information Management System (LIMS). This number (eg. E8-09-386-01A) was cross referenced with the unique field sample number. E8-09 represents the date received (eg. September of 1998). The last part of the sample number, 386-01A, signifies a LIMS batch number (386) and a unique sample ID (01A).

2.0 SAMPLE RECEIPT AND PREPARATION cont'd

The canola seed samples were ground using a coffee grinder in the presence of dry ice. After grinding, a subsample was weighed for analysis and these samples were returned to the freezer. Treated seed samples were analyzed whole as received.

3.0 PRINCIPLE OF METHOD

3.1 Canola Seed (for CGA-293343 and metabolite CGA-322704)

Analysis of CGA-293343 and metabolite CGA-322704 was based on Novartis method AG-675. The method used a double ACN/water extraction.

3.2 Canola Seed (for CGA-169374 analysis)

Analysis of Difenoconazole (CGA-169374) using method AG-676 involves the extraction of residues by refluxing with 8:2 methanol/conc.NH_4OH, filtration and removal of lipid by hexane/acetonitrile partitioning. A further clean-up step using a silica gel solid phase extraction (SPE) cartridge was done prior to analysis by GC/MS using selected ion monitoring (SIM).

3.3 Canola Seed (for Metalaxyl as 2,6-Dimethylaniline)

The analytical method followed was Novartis Method AG-395. The canola crop tissue samples were extracted by refluxing with a methanol-water mixture. After cooling, an aliquot was taken and evaporated to dryness. The remaining residue was dissolved in water and mixed with methane sulfonic acid. This mixture was refluxed for 15 minutes and diluted with water. The extract was steam distilled after being basified using the improved Nielsen-Kryger apparatus provided by Novartis. The hexane fraction containing the DMA was analyzed by GC/MS using Selected Ion Monitoring (SIM).

3.4 Treated Seed (for CGA-293343, CGA-169374 and CGA-329351 analysis)

Analysis of parent analytes was done by soaking treated seed overnight in acetone followed by a 10 minute sonication. The resulting extract was centrifuged, diluted and run by LC/MS/MS.

4.0 ANALYTICAL PROCEDURE AND MODIFICATIONS

4.1 Extraction and Clean-up of Canola Seed Samples (CGA-293343 and metabolite CGA-322704) (Novartis ref.: AG-675, ETL ref.: MS 162.00):

4.1.1 Weigh a 10 g sample of homogenized seed into a 250 mL centrifuge bottle.

4.1.2 Fortify recovery samples here.

4.1.3 Add 120 mL of acetonitrile (ACN) and 30 mL of hexane and homogenize for approximately 1 minute with a Polytron Homogenizer at high speed.

4.1.4 Centrifuge the bottle at 5000-6000 RPM for approximately 10 minutes.

4.1.5 Decant, through a funnel fitted with a cotton ball, into a 250 mL graduated cylinder.

4.1.6 Repeat the extraction once more with 40 mL of ACN and 40 mL of hexane.

4.1.7 Combine the hexane layers and back extract with 30 mL of ACN.

4.1.8 Combine the ACN in a graduated cylinder and bring the volume to 200 mL with ACN.

4.1.9 Mix well and measure a 40 mL aliquot into a Turbovap tube or 250 mL boiling flask.

4.1.10 Evaporate the sample to about 0.5 mL using a vacuum rotary evaporator (water bath at 30-35°C) or on a Turbovap at 30-35°C.

4.1.11 Rinse down the Turbovap tube or boiling flask with a small volume (~0.5 mL) of ACN and add 19 mL of water.

4.1.12 Attach a 75 mL reservoir with adapter to the top of a 1 g Varian C_{18} SPE cartridge.

4.1 Extraction and Clean-up of Canola Seed Samples (CGA-293343 and metabolite CGA-322704): cont'd

4.1.13 Condition the cartridge with 5 mL of methanol followed by 5 mL of 5:95 methanol:0.1% acetic acid solution (do not allow the sorbent to go dry here nor in any of the subsequent steps).

4.1.14 Apply the extract to the cartridge and elute at 1-2 drops per second.

4.1.15 Rinse the boiling flask with 5 mL of 5:95 methanol:0.1% acetic acid in water and elute at one drop per second.

4.1.16 Elute 12 mL of methanol through the cartridge and collect in a 15 mL culture tube.

4.1.17 Evaporate to less than 2.5 mL using a N-evaporator with a 25-30°C water bath.

4.1.18 Bring to a 5 mL volume with water and vortex at medium speed to mix.

4.1.19 Dilute 800 µL of extract to 4 mL with acetonitrile in a 4 mL vial.

4.1.20 Store the extract in a freezer until analysis by LC/MS/MS.

4.2 Extraction and Clean-up of Canola Seed Samples for Difenoconazole (CGA-169374) (Novartis ref.: AG-676, ETL ref.: MS 171.00):

4.2.1 Weigh a 20 g sample of finely chopped seed into a 500 mL boiling flask.

4.2.2 Fortify recovery sample prior to the extraction and allow sample to equilibrate.

4.2.3 Add 200 mL of 80% MeOH/Conc. ammonium hydroxide and boiling chips.

4.2.4 Reflux for 2 hours.

4.2.5 Let cool, and transfer to a 250 mL centrifuge bottle.

4.2.6 Centrifuge and remove a 40.0 mL aliquot and place into a 250 mL separatory funnel.

4.2.7 To the 40.0 mL aliquot add 100 mL of water and 4 mL of saturated NaCl.

4.2 Extraction and Clean-up of Canola Seed Samples for Difenoconazole (CGA-169374): cont'd

4.2.8 Partition with 50 mL of hexane. Add more sat. NaCl if needed to break emulsions.

4.2.9 Transfer the hexane into a second separatory funnel.

4.2.10 Partition the aqueous layer a second time with 50 mL of hexane.

4.2.11 Combine the hexane with the first hexane partition.

4.2.12 Back partition the hexane twice with 50 mL of CH_3CN.

4.2.13 Combine the CH_3CN into a 250 mL flask.

4.2.14 Evaporate the sample to dryness on a rotary evaporator (~40°C).

4.2.15 Dissolve the residue in the flask with 5 mL of toluene.

4.2.16 The following steps are done using gravity flow. Do not allow column to go dry between elution.

4.2.17 Pre-wash the silica gel sep-pack with 5 mL of toluene.

4.2.18 Load the 5 mL sample extract into the sep-pack.

4.2.19 Discard the eluent.

4.2.20 Elute CGA-169374 with 15 mL of 85:15 toluene/acetone.

4.2.21 Evaporate the toluene:acetone to dryness.

4.2.22 Add 1.0 mL of toluene and transfer to a 4 mL vial.

4.2.23 Store vial in a freezer until analysis by GC/MS.

Modifications of AG-676: The phenyl bond-elut solid phase extraction clean-up was not performed due to the selectivity of the GC/MS system. Controls were free of any interferences.

4.3 Extraction and clean-up of Canola Seed Samples (Total Mefenoxam as 2,6-DMA) (Novartis ref.: AG-395, ETL ref.: MS 170.00):

4.3.1 Weigh 10 g of finely ground canola sample into a 500 mL boiling flask.

4.3.2 Fortify recovery samples prior to the extraction and allow the sample to absorb the standard.

4.3.3 Add 100 mL of 20:80 water:methanol (v/v) and add a few boiling chips.

4.3 Extraction and clean-up of Canola Seed Samples (Total Metenoxam as 2,6-DMA) cont'd

4.3.4 Place the flask in an electrothermal apparatus wrapped in glass wool, attach a reflux condenser, and reflux for 2 hours.

4.3.5 Allow the solution to cool and transfer the sample to a 250 mL centrifuge bottle.

4.3.6 Centrifuge the bottle at 5000-6000 RPM for approximately 10 minutes.

4.3.7 Decant into a 100 mL amber bottle.

4.3.8 Measure a 20 mL aliquot of the extract and transfer to a 500 mL boiling flask.

4.3.9 Evaporate the sample to dryness using a rotary evaporator (bath temperature at 30-40°C).

4.3.10 Add 1.5 mL of water to the residue and swirl to dissolve.

4.3.11 Add some boiling chips and 25 mL of methanesulfonic acid reagent to the flask, swirl to completely dissolve the residue, and place in an electrothermal apparatus and wrap with glass wool (add a glass wool plug to the top of the condenser).

4.3.12 Attach a reflux condenser and reflux for 15 minutes (refluxing for more than 20 minutes may cause losses through degradation). Start timer when condensation starts.

4.3.13 Remove from heating mantle and allow the solution to cool for about 20 minutes. Add 100 mL of water, 15 mL of hexane, 80 mL of 25% NaOH and another 100 mL of water through the top of the condenser.

4.3.14 Connect the 500 mL boiling flask to the Nielson-Kryger Steam distillation apparatus.

4.3.15 Ensure the pH is basic and reflux for 1 hour and cool.

4.3.16 Cool and drain excess water and collect hexane layer in a scintillation vial.

4.3.17 Place in a freezer at -20°C±5°C for more than 2 hours to freeze any water in the vial.

4.3.18 Remove from freezer, measure and record the hexane volume.

4.3.19 Analyze an aliquot of the hexane by GC/MS for 2,6-Dimethylanaline (DMA) using Selected Ion Monitoring (SIM).

4.4 Extraction for Treated Canola Seed (CGA-293343, CGA-169374 and CGA-329351) (Novartis ref.: AG-397, ETL ref.: MS 172.00):

4.4.1 Weigh 2.0 g of seed sample into a 50 mL disposable tube.

4.4.2 Fortify recovery sample prior to the extraction and allow sample to equilibrate.

4.4.3 Add 40.0 mL of acetone.

4.4.4 Let it soak overnight at room temperature.

4.4.5 Sonicate for 10 minutes.

4.4.6 Centrifuge and filter if particulates are present.

4.4.7 Dilute in the range of linearity in the same solvent system as the standards.

4.4.8 Analyze by LC/MS/MS (SIM).

5.0 MATERIALS AND EQUIPMENT (equivalent equipment may be substituted)

5.1 Apparatus

Balance, analytical - 1) Mettler (model AE163) and 2) AND (model ER182A)

Balance, top-loading - 1) Sartorius (model 1206MP), 2) Mettler (model PJ6000), and 3) AND (model FX-4000)

Boiling chips - Chemware PTFE boiling stones

Bottles, centrifuge - Nalgene®

Centrifuge - 1) International Equipment Co. (model HN-S), and 2) Sorvall®, Superspeed RC2-B

Condensor, Reflux - Kimble Glass Inc.

Cotton balls - London Drugs

Electrothermal apparatus - Electromantle ME

Evaporator, Rotary - Buchi 461 water bath

Filter paper - Whatman #4

Flasks, Boiling - 250 and 500 mL

Flasks, Erlenmeyer - Kimble Glass Inc., Kimax

Flasks, volumetric - Pyrex®

Food chopper - Hobart

Food processor - Braun, Multipractic 100

Funnels, filter - Nalgene®

Glass Wool - Fisher

Graduated cylinders - Kimble Glass Inc., Kimax

Micropipettes - Microman

Nitrogen evaporator - Organomation Assoc. Inc. (model 111)

Oven - Precision Scientific Co., Thelco (model 27)

Pipette - 1) Microman and 2) Socorex, Swiss

Polytron homogenizer - Kinematica

Scoopulas, stainless steel - VWR Scientific Products

Shaker, mechanical - Burrell, wrist-action

Sonicator - Fisher Scientific FS-28

5.1 Apparatus cont'd

Spatulas, micro - VWR Scientific Products
SPE cartridges - Varian, octadecyl
SPE cartridges - ENV, 1 g
SPE cartridges - SCX, 1 g
SPE reservoirs (with adapter)
Steam Distillation Apparatus, Nielson-Kryger, Improved Version, Supplied by Novartis Canada and Novartis US
Trays, aluminium - VWR Scientific
Tubes, culture - Kimble Glass Inc., Kimax
TurboVap - Zymark
Vacuum manifold, SPE - Supelco, Visiprep 12-port model
Vacuum pump - Sanborn Manufacturing Co., Magna Force
Vials, autosampler - National Scientific Co.
Vortex - Fisher Scientific

5.2 Reagents (equivalent reagents may be substituted.)

Acetic acid, glacial - BDH, AnalaR®
Acetonitrile - EM Science, OmniSolv®
Argon - Praxair, Zero Gas
Dry ice - Liquid Carbonic Inc.
Hexane - OmniSolv®
Methanesulfonic acid - Lancaster 98+%
Methanol - EM Science, OmniSolv®
Nitrogen - Praxair, Pre Pure
Potassium chloride - BDH, ACS grade
Toluene - EM Science, OmniSolv®
Water - EM Science, OmniSolv®

5.3 Solutions

20:80 water: acetonitrile (v/v) -

Mix 200 mL of water with 800 mL of acetonitrile

1% acetic acid in water (v/v) -

Add 10 mL of glacial acetic acid to 1 L of water and mix.

0.1% acetic acid in water (v/v) -

Add 0.1 mL of glacial acetic acid to 100 mL of water and mix.

5:95 methanol: 0.1% acetic acid in water -

Mix 5 mL of methanol and 95 mL of 0.1% acetic acid in water

10:90 methanol: 1% acetic acid in water

Mix 100 mL of methanol and 900 mL of 1% acetic acid in water.

0.1 M acetic acid in water -

Mix 5.7 mL of glacial acetic acid in water and dilute to 1 L with water.

20:80 methanol: water

Mix 200 mL of methanol and 800 mL of water.

1:24:75 glacial acetic acid:water:acetonitrile

Mix 10 mL of glacial acetic acid, 240 mL of water and 750 mL of acetonitrile.

5.4 Instrumentation (equivalent hardware may be used.)

5.4.1 Instrument (LC/MS/MS)

Instrument: PE SCIEX API III Biomolecular Mass Analyzer
HPLC: Varian Solvent Delivery System 9012
Autoinjector: Waters 715 Ultra WISP Sample Processor
Data System: Macintosh IIci

5.4.1.1 HPLC Operating Conditions

For CGA-293343 and CGA-322704 analysis of Canola Seed:

Analytical Column: Supelcosil LC-18, 4.6 × 25.0 cm, 5µm particle size, Supelco Inc., Bellefonte, PA

Guard Column: Bondapak™ C Guard-Pak™, Waters Corporation, Milford, MA.

Mobile Phase A: 0.1 M acetic acid in water

Mobile Phase B: acetonitrile

Flow Rate: 0.80 mL/min (5:1 split)

Gradient: (A/B): Isocratic 25% A/75% B

Injection Vol.: 25 µL

For CGA-329351, CGA-293343 and CGA-169374 analysis of Treated Canola Seed:

Analytical Column: Zorbax 300-SCX, 4.6 × 150 mm, 5µm particle size, Hewlett-Packard Company

Guard Column: Zorbax SCX, 4.6 × 12.5 mm, 5µm particle size, Hewlett-Packard Company

Mobile Phase A: 25mm ammonium acetate in water

Mobile Phase B: acetonitrile

Flow Rate: 1.5 mL/min

Gradient: (A/B): Isocratic 50% A/50% B

Injection Vol.: 100 µL

5.4.1.2 MS/MS Operating Conditions

Typical Retention Times and Monitored Masses			
Analyte	Mass Transition	R.T. (min.)	Ion Mode
CGA-293343	292.2→210.6	3.46	positive
CGA-329351	280.0→159.6	3.52	positive
CGA-169374	406.0→250.6	3.12	positive
CGA-322704	249.8→168.6	3.52	positive

5.4.1.2 MS/MS Operating Conditions cont'd

Note: The following recommended instrument parameters were found to be optimal for the instrument used for the method development. The exact values used must be optimized for each instrument.

	TIS
Curtain gas: Nitrogen @	1.2 L/min.
Nebulizer gas: Nitrogen @	60 psi
Auxiliary gas: Nitrogen @	4.5 L/min.
Interface setpoint:	50°C
Collision gas: Argon @	225 cgt
Turbo ion spray temp.:	450°C

Positive Ion Mode:	Period 1
Ion spray voltage:	4500 V*
Orifice potential:	49 V
Collision offset (R0/R2):	30 V/19 V
Acquisition delay:	1 min.
Acquire:	5 min.
Dwell time:	200 ms.

5.4.1.3 LC/MS/MS Instrumental Analysis Summary

Chromatographic separation of the analytes was achieved by elution through an octadecyl and strong cation exchange bonded analytical columns in the reverse phase which were connected to a Turbo Ion Spray (TIS) Atmospheric Pressure Ionization (API): The analysis was performed by isocratic elution. The liquid flow was introduced from the column to a splitter and then to the ion source region.

5.4.1.3 LC/MS/MS Instrumental Analysis Summary cont'd

CGA-293343, CGA-322704, CGA-329351 and CGA-169374 were analyzed by positive ionization MS/MS. The positive ion MS/MS used the protonated molecular ions as precursors. Product ions were formed by collision induced dissociation (CID) of the precursors in the collision cell of the mass spectrometer. The predominant product ions were mass analyzed in the third quadrupole filter.

5.4.2 Instrument (GC/MSD for 2,6-DMA Analysis)

Autosampler: HP 18596A
Gas chromatograph: HP 5890A
Mass spectrometer: HP 5971A/HP 5970

5.4.2.1 Operating Conditions (GC/MSD)

Gas Chromatograph Column: Stabilmax (30 m × 0.25 μm × 0.25 mm)
(Supplied by Restek)
Injection port: 230°C (frosted silanized liner)
Oven Program: 100°C for 1 min.
10°C/min. to 230°C (hold 0 min.)
GC/MS Interface temperature: 280°C
2,6-DMA was analyzed using ion: m/z 121, 120, 106
Dwell time: 100 ms
Solvent delay: 7 min.
Head pressure: 10 psi

5.4.2.1 Operating Conditions (GC/MSD) cont'd

Purge valve on: 0.75 min.
Carrier gas: Helium
Injection volume: 3 μL

Note: Prior to analyzing samples, it is important to "condition" the inlet system of the GC/MS by injecting several control matrix samples prior to initializing a sequence.

5.4.3 Instrument (GC/MSD for CGA-169374 Difenoconazole Analysis)

Autosampler: HP 18596A
Gas chromatograph: HP 5890A
Mass spectrometer: HP 5971A/HP 5970

5.4.3.1 Operating Conditions (GC/MSD)

Gas Chromatograph Column: DB1301 (30 m × 0.25 μm × 0.25 mm)
Injection port: 240°C (frosted silanized liner)
Oven Program: 100°C for 1 min. to 290°C at 20°C/min. hold 9.5 min.
GC/MS Interface temperature: 280°C
CGA-169374 was analyzed using ion: m/z 323, 325
Dwell time: 100 ms
Solvent delay: 7 min.
Head pressure: 10 psi
Purge valve on: 0.75 min.
Carrier gas: Helium
Injection volume: 3 μL

5.5 LC/MS/MS Instrumental Analysis Summary:

All new analytical columns were conditioned according to the manufacturer's specifications. Further conditioning of the injector port system and the analytical column was done by the injection of sample extracts prior to analysis of a sequence. A deactivated fused silica pre-column was used to prolong the lifetime of the analytical column.

The analysis was performed by electron impact ionization and subsequent fragmentation followed by a quadrupole mass filter. The most predominant and interference free ion (m/z 121) was selected to monitor 2,6-DMA and quantitated based on area response relative to external calibration standards.

Positive confirmation was obtained through retention time, peak shape and presence of this ion relative to an external calibration standard.

6.0 STANDARD (REFERENCE SUBSTANCE)

Reference Substance	Supplier	Received (m/d/y)	Purity (%)	Lot/ Batch No.	Expiry Date (m/y)
CGA-293343	Novartis	6/01/98	98.9	S96-1883	05/2001
CGA-322704	Novartis	6/03/98	>99.9	DAH-XXIV-93	11/1998
CGA-169374	Novartis	6/01/98	96.0	S93-1669	04/2000
2,6-DMA	Novartis	9/17/98	99.6	DAH-X-73	01/2000
CGA-329351	Novartis	9/24/98	98.1	S97-2087	08/2000

7.0 FORTIFICATION SUMMARY

7.1 CGA-293343 and CGA-322704 in Canola Seed:

This method was validated previously at Enviro-Test Labs. Fortifications were performed in the range of 0.01 ppm to 0.5 ppm.

7.2 CGA-169374 in Canola Seed:

This modified method was verified by spiking at the LOQ (0.01 ppm) and 10 × LOQ (0.10 ppm) in duplicate along with 2 controls. In-phase fortifications ranged from 0.01 ppm to 0.1 ppm.

7.3 CGA-329351 (as 2,6-DMA) in Canola Seed:

The method was verified at the LOQ (0.05 ppm) and 10 × LOQ (0.50 ppm) in duplicate along with 2 controls. In-phase fortifications ranged from 0.05 ppm to 0.1 ppm.

7.4 CGA-293343, CGA-169374 and CGA-329351 in Treated Seed:

In-phase spikes were analyzed with these samples, since no verification was required (by protocol). Fortifications ranged from 5.0 ppm to 5000 ppm for CGA-293343, from 5.0 ppm to 1000 ppm for CGA-169374, and from 5.0 ppm to 300 ppm for CGA-329351.

8.0 LIMIT OF QUANTITATION

A LOQ of 50 ppb was demonstrated for canola seed samples for the analysis of CGA-329351 (as 2,6-DMA), CGA-293343, and CGA-322704 by analyzing fortified canola seed at this level for those analytes.

8.0 LIMIT OF QUANTITATION cont'd

A LOQ of 10 ppb was demonstrated for canola seed samples for CGA-169374 by analysis of fortified canola seed at this level.

A LOQ of 500 ppb was demonstrated for the treated canola seed samples for the analysis of CGA-169374, CGA-293343, and CGA-322704.

9.0 CALCULATIONS

Quantitation was performed using a weighted linear regression plot of matrix standards injected intermittently between samples of an analytical sample set.

9.1 Concentration of an Analyte from the LC/MS/MS (for CGA-293343 and CGA-322704)

$$Result\ (ppb) = \left[\frac{PA - (y\text{-}Intercept)}{Slope}\right] \times \frac{AF \times FV}{Sample\ Mass\ (g)}$$

Where:
PA = peak area of the analyte of interest
AF = aliquot factor [extraction volume (mL)/aliquot volume (mL)]
FV = final volume (mL)

9.2 Recovery of Fortified Control Samples:

$$Recovery\ (\%) = \frac{Result\ (ppb)}{Fortification\ Level\ (ppb)} \times 100$$

9.3 Example of Calculation:

Compound: CGA-293343
Matrix: Canola seed
Analysis date: Oct. 23/98
Chrom.I.D.: NM102298008
E8-09-E19-21A+11; 0S-SR-/858-97/GA 1-4-A Cont. fortified at 0.0540 ppm

$$\text{Result} = \left[\frac{2521 - (0)}{5997883}\right] \times \frac{(200/40\ \text{mL}) \times 250\ \text{mL}}{10\ \text{g}} = 0.0525\ \text{ppb}$$

$$\%\ \text{Recovery} = \frac{0.0525\ \text{ppm}}{0.0540\ \text{ppm}} \times 100 = 97\%$$

9.4 Concentration of Mefenoxam as 2,6-DMA

$$\textit{Response Factor (R.F.)} = \frac{\textit{Standard Concentration (ppm)}}{\textit{Peak Area}}$$

$$\textit{Aliquot Factor (A.F.)} = \frac{100\ \textit{mL Extraction Volume}}{20\ \textit{mL Aliquot Volume}} = 5$$

$$\textit{Conversion Factor (C.F.)} = \frac{\textit{Mol. Wt. Mefenoxam} = 279.3}{\textit{Mol. Wt. 2,6-DMA} = 121} = 2.31$$

$$\textit{Mefenoxam (ppm)} = \frac{\textit{Peak Area} \times \textit{R.F.} \times \textit{F.V.} \times \textit{A.F.}}{\textit{Sample Wt. (g)}} \times \textit{C.F.}$$

9.5 Example of Mefenoxam Calculation

Lab Sample #: E8-09-E19-21A+5
Client I.D.: 0S-SR-858-97/GA 1-4-A Cont. fortified at 0.0530 ppm

$$= \frac{6.22 \times (4.79 \times 10^{-4}) \times 15.0\ \text{mL} \times 5.0}{10.0\ \text{g}} \times 2.31 = 0.0516\ \text{ppm}$$

$$\%\ \text{Recovery} = \frac{0.0516}{0.0530} \times 100 = 97\%$$

9.6 Concentration of CGA-169374

$$Result\ (ppm) = \frac{Peak\ Area \times R.F. \times FV\ (mL)}{Sample\ Weight\ (g) \times A.F.}$$

$$A.F = \frac{40\ mL\ aliquot}{200\ mL\ extraction\ volume} = 0.2$$

9.7 Example of CGA-169374 Calculation

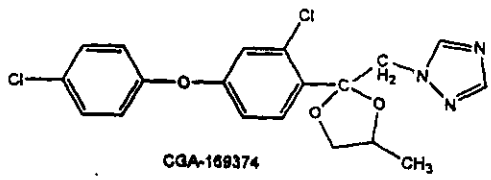
Lab Sample #: E8-09-E19-21A+1C
Client I.D.: 0S-SR-858-97 Seed

$$= \frac{138 \times (2.65 \times 10^{-4}) \times 1.0\ mL}{20\ g \times 0.20} = 0.00914\ ppm$$

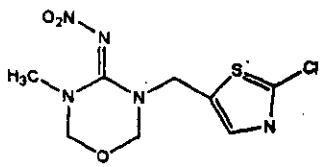
$$\%\ Recovery = \frac{0.00914}{0.0101} \times 100 = 90\%$$

10.0 FIGURES:

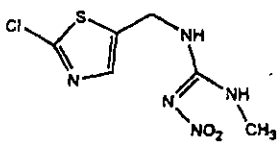
10.1 Figure 1 - Structure of Analytes



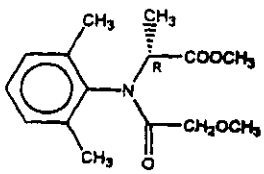
CGA-169374



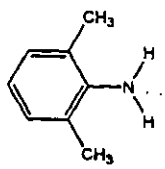
CGA-293343



CGA-322704



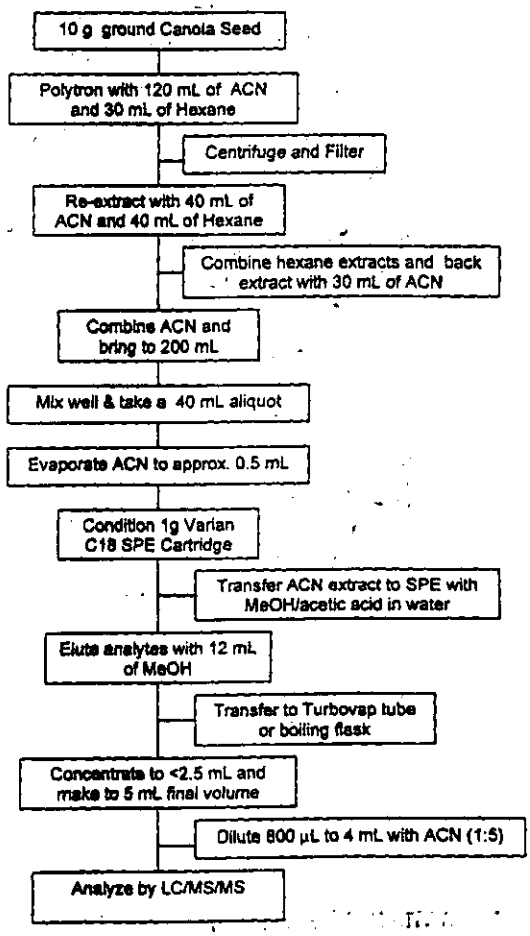
CGA-329351 active R isomer



DMA

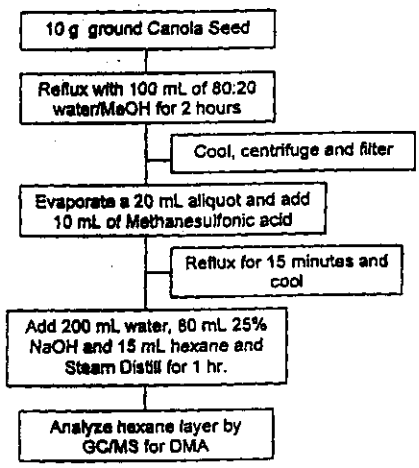
10.0 FIGURES: cont'd

10.2 Figure 2A - Canola Method Flow Diagram (CGA-293343 and CGA-322704)



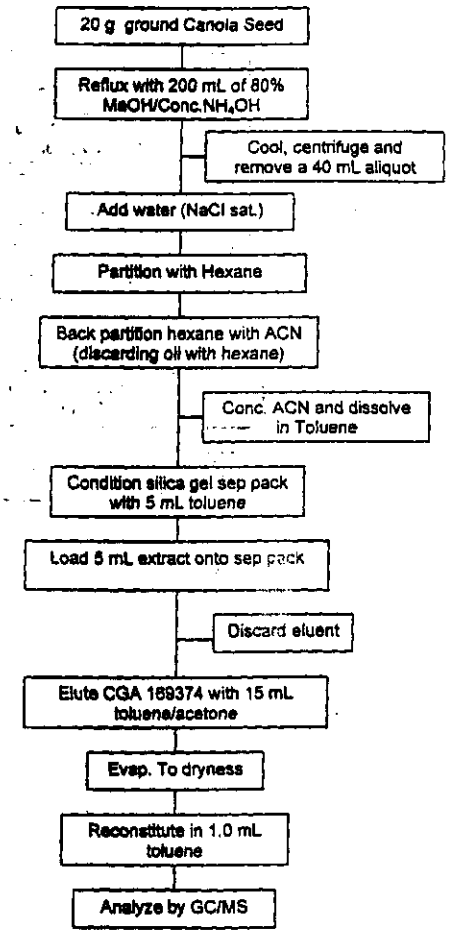
10.0 FIGURES: cont'd

10.2 Figure 2B - Canola Seed Method Flow Diagram (Mefenoxam as 2,6-DMA)



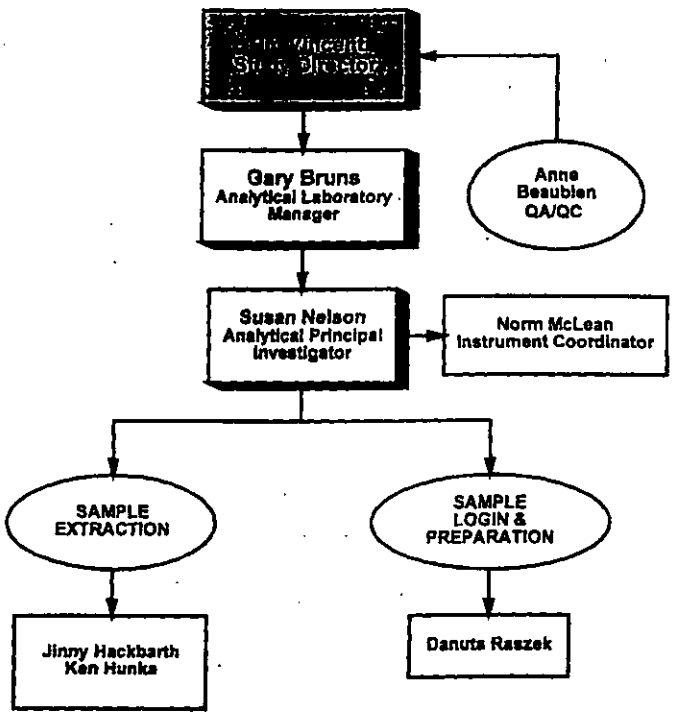
10.0 FIGURES: cont'd

10.2 Figure 2C - Canola Seed Method Flow Diagram for Difenoconazole (CGA-169374)



10.0 FIGURES: cont'd

10.3 Figure 3 - Personnel Organizational Chart



11.0 DISCUSSION

The LC/MS/MS method for the analysis of CGA-293343 and metabolite CGA-322704, based on the method AG-675, worked well with recoveries ranging from 76% to 106% for CGA-293343 and from 75% to 103% for CGA-322704 (see Table 3). The GC/MS method for the analysis of CGA-169374, based on method AG-676, worked well with recoveries ranging from 71% to 114% (see Tables 1 and 4).

The GC/MS method (AG-395) for the analysis of CGA-329351, (based on total 2,6-DMA), worked well with recoveries ranging from 71% to 117% (see Tables 2 and 5).

The quantitation of CGA-169374 and 2,6-DMA by GC/MS was done using response factors rather than regression curves, since the response of these compounds can vary with time.

The quantitation of the high-level storage treated canola seeds was done using regression curves. Due to the varying response of the 3 analytes by LC/MS/MS, there was some recoveries less than 70% and greater than 120%. There was a spike with each small set and these spikes were done at the same level as the treated seeds. Thus, the results in Table 6 are corrected for recoveries.

No residues of CGA-293343, CGA-322704, CGA-169374 or CGA-329351 2,6-DMA) were detected in any of the canola see samples.

The final report and all original study specific raw data will be transferred to Novartis Crop Protection Inc., for permanent archival. Enviro-Test Labs will retain a copy of the final report and raw data.

12.0 RECOVERY AND RESULTS DATA

TABLE 1: METHOD TRIAL AG-675 FOR CGA-169374 IN CANOLA SEED BY GC/MS

LAB SAMPLE #	DATE EXTR. (m/d/y)	DATE ANAL. (m/d/y)	AMOUNT SPIKED (ppm)	AMOUNT FOUND (ppm)	% RECOVERY
0W-SR-204-98/SD 1-4-A Cont.					
E8-09-E19-42A-	10/2/98	10/13/98	—	<0.010	—
E8-09-E19-42A+1	10/2/98	10/13/98	0.0101	0.00748	74
E8-09-E19-42A+2	10/2/98	10/13/98	0.0101	0.00718	71
E8-09-E19-42A+3	10/2/98	10/13/98	0.101	0.106	105
E8-09-E19-42A+4	10/2/98	10/13/98	0.101	0.101	100

Avg. % Recovery - 88%

SD - ±17

12.0 RECOVERY AND RESULTS DATA

TABLE 2: METHOD TRIAL AG-395 FOR CGA-329351 as 2,6-DMA IN CANOLA SEED BY GC/MS

LAB SAMPLE #	DATE EXTR. (m/d/y)	DATE ANAL. (m/d/y)	AMOUNT SPIKED (ppm)	AMOUNT FOUND (ppm)	% RECOVERY
0S-SR-858-97/GA 1-4-A Cont.					
E8-09-E19-21A-	10/5/98	10/20/98	—	<0.010	—
E8-09-E19-21A+1	10/5/98	10/20/98	0.0530	0.0450	85
E8-09-E19-21A+2	10/5/98	10/20/98	0.0530	0.0517	98
E8-09-E19-21A+3	10/5/98	10/20/98	0.530	0.478	90
E8-09-E19-21A+4	10/5/98	10/20/98	0.530	0.375	71

Avg. % Recovery - 86%

SD - ±11

12.0 RECOVERY AND RESULTS DATA

TABLE 3: ANALYTICAL SETS FOR DETERMINATION OF RESIDUES CGA-293343 AND CGA-322704 IN CANOLA SEED AND PROCESSED CANOLA FRACTIONS ACQUIRED FROM PLANTS GROWN FROM CONTROL AND TREATED SEED (BY NOVARTIS METHOD AG-675)

Lab Sample #	Sample I.D.	PHI (Days)	Nominal Tr. Rates (ga.i./100kg)	Formulation	Nominal Fortification* (ppm)	Date Extracted (m/d/y)	Date Analyzed (m/d/y)	Recovery/Residues (% / ppm) CGA-293343	CGA-322704
	0S-SR-858-97/GA Seed (at maturity)								
E8-09-E19-21A-5	1-4-A	—	—	Control	—	10/9/98	10/23/98	<0.01	<0.01
E8-09-E19-21A+11	1-4-A	—	—	Control+	0.05	10/9/98	10/23/98	97%	103%
E8-09-E19-22A	2-4-A	—	500	Combi FS-D	—	10/9/98	10/23/98	<0.01	<0.01
E8-09-E19-23A	2-4-B	—	500	Combi FS-D	—	10/9/98	10/23/98	<0.01	<0.01
	0W-SR-201-98/MN Seed (at maturity)								
E8-09-386-11A-3	1-4-A	—	—	Control	—	10/9/98	10/23/98	<0.01	<0.01
E8-09-386-11A+3	1-4-A	—	—	Control+	0.50	10/9/98	10/23/98	82%	89%
E8-09-386-13A	2-4-A	—	500	Combi FS-D	—	10/9/98	10/23/98	<0.01	<0.01
E8-09-386-14A	2-4-B	—	500	Combi FS-D	—	10/9/98	10/23/98	<0.01	<0.01
E8-09-386-15A	3-4-A	—	1500	Combi FS-D	—	10/9/98	10/23/98	<0.01	<0.01
	0W-SR-301-98/ID Seed (at maturity)								
E8-09-E19-45A-3	1-4-A	—	—	Control	—	10/9/98	10/23/98	<0.01	<0.01
E8-09-E19-45A+3	1-4-A	—	—	Control+	0.02	10/9/98	10/23/98	76%	75%
E8-09-E19-46A	2-4-A		500	Combi FS-D	—	10/9/98	10/23/98	<0.01	<0.01
E8-09-E19-47A	2-4-B		500	Combi FS-D	—	10/9/98	10/23/98	<0.01	<0.01

* Fortification value for each of CGA-293343 and CGA-322704.

12.0 RECOVERY AND RESULTS DATA

TABLE 3: ANALYTICAL SETS FOR DETERMINATION OF RESIDUES CGA-293343 AND CGA-322704 IN CANOLA SEED AND PROCESSED CANOLA FRACTIONS ACQUIRED FROM PLANTS GROWN FROM CONTROL AND TREATED SEED (BY NOVARTIS METHOD AG-675) cont'd

Lab Sample #	Sample I.D.	PHI (Days)	Nominal Tr. Rates (ga.i./100kg)	Formulation	Nominal Fortification* (ppm)	Date Extracted (m/d/y)	Date Analyzed (m/d/y)	Recovery/Residues (% / ppm) CGA-293343	CGA-322704
	0W-SR-202-98/ND Seed (at maturity)								
E8-09-387-11A-3	1-4-A	—	—	Control	—	10/9/98	10/23/98	<0.01	<0.01
E8-09-387-11A+3	1-4-A	—	—	Control+	0.01	10/9/98	10/23/98	106%	95%
E8-09-387-13A	2-4-A		500	Combi FS-D	—	10/9/98	10/23/98	<0.01	<0.01
E8-09-387-14A	2-4-B		500	Combi FS-D	—	10/9/98	10/23/98	<0.01	<0.01
E8-09-387-15A	3-4-A		1500	Combi FS-D	—	10/9/98	10/23/98	<0.01	<0.01
	0W-SR-204-98/SD Seed (at maturity)								
E8-09-E19-42A-	1-4-A	—	—	Control	—	10/13/98	10/23/98	<0.01	<0.01
E8-09-E19-42A+1	1-4-A	—	—	Control+	0.01	10/13/98	10/23/98	99%	85%
E8-09-E19-43A	2-4-A		500	Combi FS-D	—	10/13/98	10/23/98	<0.01	<0.01
E8-09-E19-44A	2-4-B		500	Combi FS-D	—	10/13/98	10/23/98	<0.01	<0.01
	0W-SR-302-98/WA Seed (at maturity)								
E8-09-E19-48A-3	1-4-A	—	—	Control	—	10/9/98	10/23/98	<0.01	<0.01
E8-09-E19-48A+3	1-4-A	—	—	Control+	0.05	10/9/98	10/23/98	84%	81%
E8-09-E19-49A	2-4-A		500	Combi FS-D	—	10/9/98	10/23/98	<0.01	<0.01
E8-09-E19-50A	2-4-B		500	Combi FS-D	—	10/9/98	10/23/98	<0.01	<0.01

* Fortification value for each of CGA-293343 and CGA-322704.

12.0 RECOVERY AND RESULTS DATA

TABLE 4: ANALYTICAL SETS FOR DETERMINATION OF RESIDUES CGA-169374 IN CANOLA SEED AND PROCESSED CANOLA FRACTIONS ACQUIRED FROM PLANTS GROWN FROM CONTROL AND TREATED SEED (BY NOVARTIS METHOD AG-676)

Lab Sample #	Sample I.D.	PHI (Days)	Nominal Tr. Rates (ga.i./100kg)	Formulation	Nominal Fortification* (ppm)	Date Extracted (m/d/y)	Date Analyzed (m/d/y)	Recovery/Residues (% / ppm) CGA-169374
	0S-SR-858-97/GA Seed (at maturity)							
E8-09-E19-21A-4	1-4-A	—	—	Control	—	10/5-6/98	10/21/98	<0.010
E8-09-E19-21A+10	1-4-A	—	—	Control+	0.01	10/5-6/98	10/21/98	90%
E8-09-E19-22A	2-4-A		30	Combi FS-D	—	10/5-6/98	10/21/98	<0.010
E8-09-E19-23A	2-4-B		30	Combi FS-D	—	10/5-6/98	10/21/98	<0.010
	0W-SR-201-98/MN Seed (at maturity)							
E8-09-386-11A-2	1-4-A	—	—	Control	—	10/5-6/98	10/15/98	<0.010
E8-09-386-11A+2	1-4-A	—	—	Control+	0.10	10/5-6/98	10/15/98	114%
E8-09-386-13A	2-4-A		30	Combi FS-D	—	10/5-6/98	10/15/98	<0.010
E8-09-386-14A	2-4-B		30	Combi FS-D	—	10/5-6/98	10/15/98	<0.010
E8-09-386-15A	3-4-A		90	Combi FS-D	—	10/5-6/98	10/15/98	<0.010
	0W-SR-301-98/ID Seed (at maturity)							
E8-09-E19-45A-2	1-4-A	—	—	Control	—	10/5-6/98	10/15/98	<0.010
E8-09-E19-45A+2	1-4-A	—	—	Control+	0.01	10/5-6/98	10/15/98	70%
E8-09-E19-46A	2-4-A		30	Combi FS-D	—	10/5-6/98	10/15/98	<0.010
E8-09-E19-47A	2-4-B		30	Combi FS-D	—	10/5-6/98	10/15/98	<0.010

12.0 RECOVERY AND RESULTS DATA

TABLE 4: ANALYTICAL SETS FOR DETERMINATION OF RESIDUES CGA-169374 IN CANOLA SEED AND PROCESSED CANOLA FRACTIONS ACQUIRED FROM PLANTS GROWN FROM CONTROL AND TREATED SEED (BY NOVARTIS METHOD AG-676) cont'd

Lab Sample #	Sample I.D.	PHI (Days)	Nominal Tr. Rates (ga.i./100kg)	Formulation	Nominal Fortification* (ppm)	Date Extracted (m/d/y)	Date Analyzed (m/d/y)	Recovery/Residues (% / ppm) CGA-169374
	0W-SR-202-98/ND Seed (at maturity)							
E8-09-387-11A-2	1-4-A	—	—	Control	—	10/5-6/98	10/15/98	<0.010
E8-09-387-11A+2	1-4-A	—	—	Control+	0.01	10/5-6/98	10/15/98	99%
E8-09-387-13A	2-4-A		30	Combi FS-D	—	10/5-6/98	10/15/98	<0.010
E8-09-387-14A	2-4-B		30	Combi FS-D	—	10/5-6/98	10/15/98	<0.010
E8-09-387-15A	3-4-A		90	Combi FS-D	—	10/5-6/98	10/15/98	<0.010
	0W-SR-204-98/SD Seed (at maturity)							
E8-09-E19-42A-	1-4-A	—	—	Control	—	10/13/98	10/15/98	<0.010
E8-09-E19-42A+1	1-4-A	—	—	Control+	0.01	10/13/98	10/15/98	79%
E8-09-E19-43A	2-4-A		30	Combi FS-D	—	10/13/98	10/15/98	<0.010
E8-09-E19-44A	2-4-B		30	Combi FS-D	—	10/13/98	10/15/98	<0.010
	0W-SR-302-98/WA Seed (at maturity)							
E8-09-E19-48A-2	1-4-A	—	—	Control-	—	10/5-6/98	10/15/98	<0.010
E8-09-E19-48A+2	1-4-A	—	—	Control+	0.10	10/5-6/98	10/15/98	73%
E8-09-E19-49A	2-4-A		30	Combi FS-D	—	10/5-6/98	10/15/98	<0.010
E8-09-E19-50A	2-4-B		30	Combi FS-D	—	10/5-6/98	10/15/98	<0.010

12.0 RECOVERY AND RESULTS DATA

TABLE 5: ANALYTICAL SETS FOR DETERMINATION OF RESIDUES CGA-329351 IN CANOLA SEED AND PROCESSED CANOLA FRACTIONS ACQUIRED FROM PLANTS GROWN FROM CONTROL AND TREATED SEED (BY NOVARTIS METHOD AG-395)

Lab Sample #	Sample I.D.	PHI (Days)	Nominal Tr. Rates (gai./100kg)	Formulation	Nominal Fortification (ppm)	Date Extracted (m/d/y)	Date Analyzed (m/d/y)	Recovery/Residues (% / ppm) CGA-329351*
	0S-SR-858-97/GA Seed (at maturity)							
E8-09-E19-21A-2	1-4-A	—	—	Control	—	10/14/98	10/22/98	<0.050
E8-09-E19-21A+5	1-4-A	—	—	Control+	0.05	10/14/98	10/22/98	97%
E8-09-E19-22A	2-4-A		10	Combi FS-D	—	10/14/98	10/22/98	<0.050
E8-09-E19-23A	2-4-B		10	Combi FS-D	—	10/14/98	10/22/98	<0.050
	0W-SR-301-98/ID Seed (at maturity)							
E8-09-E19-45A-	1-4-A	—	—	Control	—	10/14/98	10/22/98	<0.050
E8-09-E19-45A+1	1-4-A	—	—	Control+	0.10	10/14/98	10/22/98	94%
E8-09-E19-46A	2-4-A		10	Combi FS-D	—	10/14/98	10/22/98	<0.050
E8-09-E19-47A	2-4-B		10	Combi FS-D	—	10/14/98	10/22/98	<0.050
	0W-SR-201-98/MN Seed (at maturity)							
E8-09-386-11A-	1-4-A	—	—	Control	—	10/14/98	10/22/98	<0.050
E8-09-386-11A+1	1-4-A	—	—	Control+	0.10	10/14/98	10/22/98	87%
E8-09-386-13A	2-4-A		10	Combi FS-D	—	10/14/98	10/22/98	<0.050
E8-09-386-14A	2-4-B		10	Combi FS-D	—	10/14/98	10/22/98	<0.050
E8-09-386-15A	3-4-A		30	Combi FS-D	—	10/14/98	10/22/98	<0.050

* Detected as 2,6-DMA and converted to metanoxam equivalents by a wt/wt conversion factor of 2.308.

12.0 RECOVERY AND RESULTS DATA

TABLE 5: ANALYTICAL SETS FOR DETERMINATION OF RESIDUES CGA-329351 IN CANOLA SEED AND PROCESSED CANOLA FRACTIONS ACQUIRED FROM PLANTS GROWN FROM CONTROL AND TREATED SEED (BY NOVARTIS METHOD AG-395)

Lab Sample #	Sample I.D.	PHI (Days)	Nominal Tr. Rates (ga.i./100kg)	Formulation	Nominal Fortification (ppm)	Date Extracted (m/d/y)	Date Analyzed (m/d/y)	Recovery/Residues (% / ppm) CGA-329351*
	0W-SR-202-98/ND Seed (at maturity)							
E8-09-387-11A-	1-4-A	—	—	Control	—	10/14/98	10/22/98	<0.050
E8-09-387-11A+1	1-4-A	—	—	Control+	0.05	10/14/98	10/22/98	72%
E8-09-387-13A	2-4-A		10	Combi FS-D	—	10/14/98	10/22/98	<0.050
E8-09-387-14A	2-4-B		10	Combi FS-D	—	10/14/98	10/22/98	<0.050
E8-09-387-15A	3-4-A		30	Combi FS-D	—	10/14/98	10/22/98	<0.050
	0W-SR-204-98/SD Seed (at maturity)							
E8-09-E19-42A-	1-4-A	---	---	Control	---	10/14/98	10/22/98	<0.050
E8-09-E19-42A+1	1-4-A	---	---	Control+	0.05	10/14/98	10/22/98	82%
E8-09-E19-43A	2-4-A		10	Combi FS-D	---	10/14/98	10/22/98	<0.050
E8-09-E19-44A	2-4-B		10	Combi FS-D	---	10/14/98	10/22/98	<0.050
	0W-SR-302-98/WA Seed (at maturity)							
E8-09-E19-48A-	1-4-A	---	---	Control	---	10/14/98	10/22/98	<0.050
E8-09-E19-48A+1	1-4-A	---	---	Control+	0.10	10/14/98	10/22/98	117%
E8-09-E19-49A	2-4-A		10	Combi FS-D	---	10/14/98	10/22/98	<0.050
E8-09-E19-50A	2-4-B		10	Combi FS-D	---	10/14/98	10/22/98	<0.050

* Detected as 2,6-DMA and converted to metanoxam equivalents by a wt/wt conversion factor of 2.308.

12.0 RECOVERY AND RESULTS DATA

TABLE 6: ANALYTICAL SETS FOR DETERMINATION OF RESIDUES CGA-293343, CGA-169374, AND CGA-329351 IN CONTROL AND TREATED CANOLA SEED (Corrected for Recoveries)

Lab Sample #	Sample I.D.	Ambient Storage (Days)	Nominal Tr. Rates (ga.i./100kg)	Formulation	Nominal Fortifications* (ppm)	Date Extracted (m/d/y)	Date Analyzed (m/d/y)	Recovery/Residues (% / ppm) CGA-293343	CGA-169374	CGA-329351
	0S-SR-858-97/GA Seed (FCA)									
E8-09-E19-01A-	1-1-AB	—	—	Control		10/16/98	10/26/98	<5.0	<5.0	<5.0
E8-09-E19-01A+	1-1-AB	—	—	Control+	5000,300,100	10/16/98	10/26/98	109%	72%	60%
E8-09-E19-02A	2-1-AB	0	500,30,10	Combi FS-D		10/16/98	10/26/98	55/2741	108/324	31/31.2
	0S-SR-858-97/GA Seed (FSS)									
E8-09-E19-03A-1	1-3-AB	—	—	Control		10/16/98	10/26/98	<5.0	<5.0	<5.0
E8-09-E19-03A+	1-3-AB	—	—	Control+	5.0,5.0,5.0	10/16/98	10/26/98	97%	114%	105%
E8-09-E19-04A	2-3-AB	23	500,30,10	Combi FS-D		10/16/98	10/26/98	61/3068	75/225	41/40.9
	0W-SR-201-98/MN Seed (FCA)									
E8-09-386-01A-	1-1-AB	—	—	Control		10/16/98	10/26/98	<5.0	<5.0	<5.0
E8-09-386-01A+	1-1-AB	—	—	Control+	300,1000,300	10/16/98	10/26/98	75%	120%	118%
E8-09-386-02A	2-1-AB	0	500,30,10	Combi FS-D		10/16/98	10/26/98	122/6119	123/369	52/51.9
E8-09-386-03A	3-1-AB	0	1500,90,30	Combi FS-D		10/16/98	10/26/98	109/16343	120/1084	43/128
E8-09-386-04A	4-1-AB	0	500,30,10	Combi FS-D		10/16/98	10/26/98	115/5727	128/384	44/43.6
E8-09-386-05A	5-1-AB	0	1500,90,30	Combi FS-D		10/16/98	10/26/98	102/15280	125/1124	62/186
	0W-SR-201-98/MN Seed (FSS)									
E8-09-386-06A-	1-3-AB	—	—	Control		10/16/98	10/26/98	<5.0	<5.0	<5.0
E8-09-386-06A+	1-3-AB	—	—	Control+	5000,300,100	10/16/98	10/26/98	95%	148%	117%
E8-09-386-07A	2-3-AB	20	500,30,10	Combi FS-D		10/16/98	10/26/98	93/4628	108/324	42/41.9
E8-09-386-08A	3-3-AB	20	1500,90,30	Combi FS-D		10/16/98	10/26/98	82/12358	112/1009	58/175
E8-09-386-09A	4-3-AB	20	500,30,10	Combi FS-D		10/16/98	10/26/98	100/4985	121/364	56/56.1
E8-09-386-10A	5-3-AB	20	1500,90,30	Combi FS-D		10/16/98	10/26/98	92/13782	107/963	68/203

FCA - For Confirmatory Analysis; FSS - For Storage Stability; * Rates and Fortifications in order: CGA-293343, CGA-169374, CGA-329351

12.0 RECOVERY AND RESULTS DATA

TABLE 6: ANALYTICAL SETS FOR DETERMINATION OF RESIDUES CGA-293343, CGA-169374, AND CGA-329351 IN CONTROL AND TREATED CANOLA SEED cont'd (Corrected for Recoveries)

Lab Sample #	Sample I.D.	Ambient Storage (Days)	Nominal Tr. Rates (ga.i./100kg)	Formulation	Nominal Fortifications* (ppm)	Date Extracted (m/d/y)	Date Analyzed (m/d/y)	Recovery/Residues (% / ppm) CGA-293343	CGA-169374	CGA-329351
	0W-SR-202-98/ND Seed (FCA)									
E8-09-387-01A-	1-1-AB	—	—	Control		10/16/98	10/26/98	<5.0	<5.0	<5.0
E8-09-387-01A+	1-1-AB	—	—	Control+	300,1000,300	10/16/98	10/26/98	81%	133%	124%
E8-09-387-02A	2-1-AB	0	500,30,10	Combi FS-D		10/16/98	10/26/98	87/4354	111/332	44/44.3
E8-09-387-03A	3-1-AB	0	1500,90,30	Combi FS-D		10/16/98	10/26/98	85/12895	123/1111	47/140
E8-09-387-04A	4-1-AB	0	500,30,10	Combi FS-D		10/16/98	10/26/98	88/4419	123/368	44/44.4
E8-09-387-05A	5-1-AB	0	1500,90,30	Combi FS-D		10/16/98	10/26/98	79/11779	112/1004	55/164
	0W-SR-202-98/ND Seed (FSS)									
E8-09-387-06A-	1-3-AB	—	—	Control		10/16/98	10/26/98	<5.0	<5.0	<5.0
E8-09-387-06A+	1-3-AB	—	—	Control+	5000,300,100	10/16/98	10/26/98	81%	150%	107%
E8-09-387-07A	2-3-AB	23	500,30,10	Combi FS-D		10/16/98	10/26/98	83/4164	95/285	32/31.9
E8-09-387-08A	3-3-AB	20	1500,90,30	Combi FS-D		10/16/98	10/26/98	46/6878	104/939	49/148
E8-09-387-09A	4-3-AB	20	500,30,10	Combi FS-D		10/16/98	10/26/98	94/4685	110/329	57/57.0
E8-09-387-10A	5-3-AB	20	1500,90,30	Combi FS-D		10/16/98	10/26/98	48/7140	78/699	53/159
	0W-SR-204-98/SD Seed (FCA)									
E8-09-E19-11A-	1-1-AB	—	—	Control		10/16/98	10/26/98	<5.0	<5.0	<5.0
E8-09-E19-11A+	1-1-AB	—	—	Control+	5000,300,100	10/16/98	10/26/98	85%	139%	71%
E8-09-E19-12A	2-1-AB	0	500,30,10	Combi FS-D		10/16/98	10/26/98	116/5817	98/294	104/104

FCA - For Confirmatory Analysis; FSS - For Storage Stability; * Rates and Fortifications in order: CGA-293343, CGA-169374, CGA-329351