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

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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
STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d)(1)(A), (B) or (C).

Company: Novartis Crop Protection, Inc.
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Title: Senior Regulatory Manager

[Signature] 7/21/82 [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.

[Signature]
Robert K. Williams
Manager of Residue Chemistry and
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[Signature] 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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Study Initiation Date: May 12, 1997

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I. SUMMARY AND INTRODUCTION

A. SCOPE

Ciba Analytical Method AG-575B is used for the determination of parent residues of CGA-169374 (1-[[2-(2-chloro-4-(4-chloro-phenoxo)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 as a result of an EPA data review and method trial 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# 5904748 dated June 14, 1996 for difenoconazole (Dividend) on Barley, Cereals, and Triticale. In this review of petition PP# 5904748 (received by Dr. Gregory T. Peters on September 26, 1996), 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 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/MS) 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 COA-150374 in barley by AG-676 utilizes gas chromatography and nitrogen/phosphorous (GC/NPD) with a DB-1 megabore column (15 meter x 0.53 mm ID, df = 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, df = 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-675B 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).


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

B. REAGENTS

1.0. Acetone, reagent grade (Fisher Cat. #A185K-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. 95:15 (v/v) Toluene:acetone.

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

13.0. Water, distilled.

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

15.0 Polyethylene glycol (Sigma Catalog) CAS #25322-69-3.

16.0 1:1 Polyethylene glycol:toluene
C. ANALYTICAL PROCEDURE

All analytical procedures described in this section are identical to those described in AOAC 975.0 except for the omission of the charcoal column cleanup and the addition of an extra partition step in Section 6.0 (for grain only). Polyethyleneglycol 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 an 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 50-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 C6A-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. Partition Cleanup (grain only)

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 ml 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 mL 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 each sample injection vial. (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 FPD (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\(^1\), EPA requested that the specificity of the method (AG-575B) be demonstrated. A method specificity study\(^4\) was conducted where 58 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 of 6-8 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 mg/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 13.

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

\[
\text{PPM CGA-169374} = \frac{\text{CGA-169374 found (mg)}}{\text{mg sample injected}} \times \frac{100}{R\%}
\]

where mg sample injected is calculated as follows:

\[
G = \text{milligrams of sample extracted} \\
V = \text{volume of the extraction solvent (mL)} \\
V_f = \text{total volume of final injection solution (mL)} \\
N = \text{recovery ratio given by equation (3)} \\
V_i = \text{injection volume (mL)} \\
V_a = \text{aliquot volume (mL)} \\
W = \text{weight of sample extracted (g)} \\
M = \text{moisture content of sample (%)}
\]

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:

\[(1) \quad R = \frac{\text{CGA-169374 found (mg) ppm CGA-169374 added}}{100} \times 100\%\]

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, mg recovered, and percent recovery for each sample.

To calculate the mg 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 mg (mV/mg), the Y-intercept \(b = 0\) mV, and the peak height = 7386 \(\mu\)V.
X = (7386 μV - 0)/6068 = 0.112 mg found.

To convert to ppm, divide the ng determined in the sample by the ng 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 323 to the target ion at m/z = 323. Structural confirmation can be made if the same ion ratios match the ratios obtained from the standard (+/- 25%).

This method validation was performed under Protocol 313-97. 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-575B.
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 RT-MV-011-97.

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-5758. Recovery and control values from the confirmatory analyses compare well with the initial analyses of these samples (below).

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

Figure 7 shows representative reagent blank chromatograms obtained from AG-5758 (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-5758).

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 CQA-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-Methoc) 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.

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

7/17/97

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.

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

7/17/97

Robert K. Williams
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
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.

<table>
<thead>
<tr>
<th>AUDIT TYPE</th>
<th>INSPECTION/AUDIT DATES</th>
<th>REPORTING DATE</th>
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</thead>
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<tr>
<td>Protocol</td>
<td>5/12/97</td>
<td>5/12/97</td>
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<tr>
<td>In-Progress</td>
<td>6/19/97</td>
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<tr>
<td>Final Report</td>
<td>7/10-11/97</td>
<td>7/11/97</td>
</tr>
</tbody>
</table>

Prepared by: Barbara S. North

Date: 7/11/97
VII. TABLES AND FIGURES

TABLE I. GC/MS OPERATING PARAMETERS FOR CGA-169374 RESIDUES BY AG-5755E

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 = 1.0um

Gases:
Carrier: Helium at 90°C, constant flow mode.

Injection: 2 ul, 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
| 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, df = 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.  
| BRW offset: 400 |
| Timetable Events: | Time 0.0 Purge-Off  
| Time 0.75 min Purge On |
| CGA-169754 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**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Numeric ID</th>
<th>Fortification Level (ppm)</th>
<th>GC/MSD Recovery (%)</th>
<th>GC/NPD Recovery (%)</th>
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<tbody>
<tr>
<td>Hay</td>
<td>200043</td>
<td>control</td>
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<tr>
<td>Hay</td>
<td>201644</td>
<td>0.05</td>
<td>100</td>
<td>113</td>
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<tr>
<td>Hay</td>
<td>201645</td>
<td>0.1</td>
<td>102</td>
<td>101</td>
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<td>Hay</td>
<td>201646</td>
<td>0.2</td>
<td>94.3</td>
<td>108</td>
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<tr>
<td></td>
<td></td>
<td><strong>Average =</strong></td>
<td><strong>98.9</strong></td>
<td><strong>107</strong></td>
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<tr>
<td></td>
<td></td>
<td><strong>Standard Deviation =</strong></td>
<td><strong>4.1</strong></td>
<td><strong>6.1</strong></td>
</tr>
<tr>
<td>Forage</td>
<td>203792</td>
<td>control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forage</td>
<td>203849</td>
<td>0.05</td>
<td>96.1</td>
<td>110</td>
</tr>
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<td>Forage</td>
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<td>0.1</td>
<td>116</td>
<td>106</td>
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<td>0.5</td>
<td>103</td>
<td>96.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Average =</strong></td>
<td><strong>105</strong></td>
<td><strong>104</strong></td>
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<tr>
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<td></td>
<td><strong>Standard Deviation =</strong></td>
<td><strong>10.2</strong></td>
<td><strong>6.7</strong></td>
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<tr>
<td>Straw</td>
<td>200044</td>
<td>control</td>
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<td></td>
</tr>
<tr>
<td>Straw</td>
<td>201647</td>
<td>0.05</td>
<td>104</td>
<td>95.5</td>
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<tr>
<td>Straw</td>
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<td>0.2</td>
<td>100</td>
<td>87.9</td>
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<tr>
<td>Straw</td>
<td>201649</td>
<td>0.5</td>
<td>102</td>
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<tr>
<td></td>
<td></td>
<td><strong>Average =</strong></td>
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<tr>
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<td><strong>Standard Deviation =</strong></td>
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<td>Grain</td>
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<td>(Set 1)</td>
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<td>Grain</td>
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<td>(Set 2)</td>
<td>148</td>
<td>93.4</td>
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<tr>
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<td>201463</td>
<td>(Set 2)</td>
<td>148</td>
<td>93.4</td>
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<td>(Set 2)</td>
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<td>0.2</td>
<td>89.4</td>
<td>101</td>
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<tr>
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<td>1.0</td>
<td>96.7</td>
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<td><strong>Standard Deviation =</strong></td>
<td><strong>27</strong></td>
<td><strong>-3.7</strong></td>
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</table>
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 this residue in 3.0 mL of hexane

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

(cont.)

Load onto a phenyl Bond-elut - discard

1) Wash 2x3 mL hexane - discard
2) Wash 4x3 mL 9:1 hexane:ether - discard
3) Elute with 4 x 2 mL methanol into scintillation vial

Quantitatively transfer to 50 mL round bottom

Evaporate to dryness

Grain Only

Potage, Hay, Straw

Dissolve residue with 25 mL ACN

Partition with 2x25 mL hexane

ACN - Discard

Hexane - Discard

Evaporate ACN to dryness

Dissolve residue in toluene for GC analysis

Add 1000 µl of each sample and standard in injection vial

Add 10 µl of 50% ethylene glycol to each sample and standard vial
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 (AQ-575B)
(Multichrom Worksheet: 31J-97-Pegg)

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

2. 0.2 ng standard CGA-169174 (Response Factor - 12495 µV)
FIGURE 3.  TYPICAL STANDARD CHROMATOGRAMS OF CGA-169374 USING GC/NPD (AG-575B). (Continued)
(Multichrom Worksheet: 313-97-PSSG)

3.  0.04 mg standard CGA-169374 (Response Factor = 3739 μV)

4.  1.0 mg standard CGA-169374 (Response Factor = 72856 μV)
FIGURE 3. TYPICAL STANDARD CHROMATOGRAMS OF CGA-169374 USING GC/NDP (AG-575B) (Continued)
(Multichrom Worksheet... 313-97-PEOG)

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

6. 0.08 ng standard CGA-169374 (Response Factor - 5461 μV)
Figure 4. Typical calibration plot of CGA-169374 corresponding to Figure 1 obtained using AG-575B (Multichrom worksheet: 313-97-PBGG)
FIGURE 5. TYPICAL STANDARD CHROMATOGRAMS OF CGA-169374 USING GC/MSD (AG-676)
(Multichrom Worksheet: PRESSTRAM)

1. 1.0 ng standard CGA-169374 (Response Factor = 6770480 µV)

2. 0.4 ng standard CGA-169374 (Response Factor = 2462777 µV)
FIGURE 5. TYPICAL STANDARD CHROMATOGRAMS OF CGA-169374 USING GC/MSD (AG-676) (Continued)
(Multichrom Worksheet: PBESTRAW)

3. 0.1 ng standard CGA-169374 (Response Factor = 1059574 µV)

4. 0.08 ng standard CGA-169374 (Response Factor = 547157 µV)
FIGURE 5. TYPICAL STANDARD CHROMATOGRAMS OF CGA-169374 USING GC/MSD (49-676) (Continued)
(Multichrom Worksheet: PRESSRAW)

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 CSA-169374
CORRESPONDING TO FIGURE 5 OBTAINED USING AG-676
(Multichrom Worksheet: FREESTRAM)
FIGURE 7. CHROMATOGRAMS OF THE REAGENT BLANKS FROM ANALYSIS OF COA-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-575B).

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: 312-97-BC4R)

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

1. 200044. control. 3.97 mg injected. 0.006 mg found, <0.01 ppm (0.003 ppm). (Response Factor = 385 μV)

2. 201647. control = 0.05 ppm CGA-169374. 1.09 mg injected. 0.097 mg found, 0.049 ppm found. 0.048 ppm (corrected for control). 35.5% recovery. (Response Factor = 8677 μV)
3. 2014A, control = 0.15 ppm CGA-169374, 0.794 mg injected, 0.071 mg found, 0.089 ppm, 0.087 ppm (corrected for control) 87.9% recovery. (Response Factor = 6330 uV)

4. 2016A, control = 0.5 ppm CGA-169374, 0.398 mg injected, 0.204 mg found, 0.513 ppm, 0.512 ppm (corrected for control) 102% recovery. (Response Factor = 18376 uV)
Figure 9. Representative chromatograms from the analysis of CGA-159374 in barley straw using Ag-676
(Multichrom Worksheet: PRESHTRAW)

CGA-159374: Slope = 6762482 μV/ng Y-intercept = -78266 μV

1. 200044, control, 3.97 mg injected, 0.00 mg found, 40.61 ppm.
   (Response Factor = 0 μV)
Human Safety Department  
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FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CCA-169374 IN BARLEY STRAW USING AG-676  
(Continued)  
(Multichrom Worksheet: PESSSTRAW)

Abundance

Ion 325.00 (324.70 to 325.30): PEG62316.wmf

Time [min]  Abundance

0 10 11 12 13 14 15 16 17 18 19

14.22

Abundance

Ion 323.00 (322.70 to 323.30): PEG62316.D

Time [min]  Abundance

0 10 11 12 13 14 15 16 17 18 19

14.22

Abundance

Ion 265.00 (264.70 to 265.30): PEG62316.D

Time [min]  Abundance

0 10 11 12 13 14 15 16 17 18 19

14.22

2. 201647, control + 0.05 ppm CCA-169374, 1.99 mg injected, 0.103 mg found, 0.052 ppm, 0.052 ppm (corrected for control), 1048 recovery. (Response Factor = 620317 UV)
FIGURE 9. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-165374 IN BARLEY STRAW USING AG-576
(Continued)
(Multichrom Worksheet: PRESSTRAW)

3. 201648, control = 0.10 ppm CGA-165374, 0.794 ng injected, 0.080 ng found, 0.100 ppm, 0.100 ppm (corrected for control), 100% recovery.
(Response Factor = 461.297 UF)
FIGURE 9. REPRESENTATIVE CHROMATROGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY STRAW USING AG-676
(Continued)
(Multichrom Worksheet: PRESSTRAW)

4. 201.649, control, 0.5 ppm CGA-169374, 0.398 ppm injected, 0.204 ppm found, 0.512 ppm (corrected for control), 100% recovery.
(Response Factor = 1299374 µV)
FIGURE 10. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CCA-159374 IN BARLEY HAY USING AC-575B
(Multichrom Workbench: 313-97-PESH)

CCA-159374: Slope = 66066 µV/ng Y-intercept = 0

1. 200043, control, 3.95 mg injected, 0.000 ng found, <0.01 ppm (0.000 ppm). (Response Factor = 0 µV)

2. 201644, control = 0.025 ppm CCA-159374, 1.98 mg injected, 0.112 ng found, 0.057 ppm, 0.057 ppm (corrected for control), 113% recovery. (Response Factor = 7386 µV)
3. 201645. control + 0.1 ppm CGA-169374. 0.781 mg injected, 0.08 mg found. 0.101 ppm, 0.101 ppm (corrected for control), 100% recovery. (Response Factor - 5278 µV)

4. 201646. control + 0.5 ppm CGA-169374. 0.396 mg injected, 0.232 mg found. 0.562 ppm, 0.562 ppm (corrected for control), 100% recovery. (Response Factor - 1618 µV)
**FIGURE 11. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-159374 IN BARLEY HAY USING AG-676**
(Multichrom Worksheet: PRESSHAY)

**CGA-159374:** Slope = 5248423 µV/ng  Y-intercept = -12582 µV

<table>
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<th>Time</th>
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<tr>
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<td>14.00</td>
<td>50000</td>
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</tr>
<tr>
<td>17.00</td>
<td>20000</td>
</tr>
<tr>
<td>18.00</td>
<td>10000</td>
</tr>
</tbody>
</table>

- Ion 325.00 (324.7 to 325.3): PGPFRHG3.OM

- Ion 323.00 (322.7 to 323.3): PGPFRHG3.L

- Ion 265.00 (264.7 to 265.3): PGPFRHG3.D

1. 200403: Control, 3.96 mg injected, 0.00 ng found, <0.01 ppm (0.000 ppm). (Response Factor = 0 µV)

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

2. 201644. control = 0.95 ppm COA-169374, 1.98 mg injected, 0.999 mg found, 0.050 ppm, 0.050 ppm (corrected for control), 100% recovery. 
(Response Factor - 508155 πV)
FIGURE 11: REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CDA-169374 IN BARLEY HAY USING AO-676 (Continued)
(Multichrom Worksheet: FODSMAY)

3. 201645; control: 0.1 ppm CDA-169374, 0.79% mg injected, 0.081 mg found, 0.10% ppm, 0.102 ppm (corrected for control), 102% recovery. (Response Factor = 411723 uV)
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, 0.398 mg injected, 0.187 mg
found, 0.472 ppm. 0.472 ppm (corrected for control), 94.3% recovery.
(Response Factor = 968127 UV)
1. 203792, control, 3.74 mg injected, 0.003 mg found, 40.01 ppm (0.011 ppm). (Response Factor = 180 uV)

2. 203849, control + 0.05 ppm CGA-169374, 1.87 mg injected. 0.104 mg found. 0.055 ppm; 0.055 ppm (corrected for control), 110% recovery. (Response Factor = 5985 uV)
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 mg found, 0.107 ppm, 0.105 ppm (corrected for control), 106% recovery. (Response Factor - 4571 μV)

4. 203851, control + 0.50 ppm CGA-169374, 0.374 mg injected, 0.181 mg found, 0.684 ppm, 0.683 ppm (corrected for control), 98.5% 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 uV/ng  Y-intercept = -20189 uV

1. 203792, control; 3.74 mg injected, 0.00 ng found, <0.01 ppm,
   (Response Factor = 0 uV)
2. 203849, control + 0.05 ppm C8A-169374, 1.87 mg injected, 0.090 ng found, 0.048 ppm, 0.048 ppm (corrected for control), 96.1% recovery. (Response Factor = 39031U UV)
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FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF
CGA-169374 IN BARLEY FORAGE USING AG-676
(Continued)
(Multichrom Worksheet: TARGETFORAGE4C)

Ion 325.00 (324.70 to 325.30): PFP70305.m/z!

Ion 323.00 (322.70 to 323.30): PFP70306.D

Ion 265.00 (264.70 to 265.30): PFP70308.D

3. 203850. control + 0.1 ppm CGA-169374, 0.748 mg injected, 0.087 mg
found. 0.116 ppm, 0.116 ppm (corrected for control), 116% recovery.
(Response Factor = 378405 µV)
FIGURE 13. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CPA-159374 IN BARLEY FORAGE USING AG-576
(Continued)
[Multi-chrom Worksheet: TARGETFORAGE4C]

Abundance

Ion 325.00 (324.70 to 325.30): PPF70308.wmf

Time -> Abundance

Ion 322.00 (322.70 to 322.30): PPF70309.D

Time -> Abundance

Ion 265.00 (264.70 to 265.30): PPF70206.D

Time ->

4. 203551, control + 0.59 ppm CPA-169374, 0.187 mg injected, 0.097 mg found, 0.517 ppm, 0.517 ppm (corrected for control), 1034 recovery.
(Response Factor - 418623 µV)

PAGE 59 OF 100
FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-575E (SEP 1)
(Multichrom Worksheet: 313-97-P000)

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

1. 2000.45 mg control, 3.95 mg injected, 0.00 mg found, <0.01 ppm. (Response Factor = 0 µV)

2. 2014.63 mg control + 0.01 ppm CGA-169374, 3.95 mg injected, 0.037 mg found, 0.009 ppm, 0.009 ppm (corrected for control), 93.4% recovery. (Response Factor = 2668 µV)
3. 201464, control + 0.04 ppm CGA-169374, 2.64 mg injected, 0.099 mg found, 0.037 ppm, 0.037 ppm (corrected for control), 93.7% recovery. (Response Factor - 7138 μl)
FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CQA-168374 IN BARLEY GRAIN USING AG-5758 (SET 2)

(Continued)

(Multichrom Worksheet: 313-97-PGEG2)

CQA-168374: 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. 201443, control + 0.01 ppm CQA-168374, 3.95 mg injected, 0.038 ng found, 0.015 ppm, 0.016 ppm (corrected for control), 98.0% recovery.
   (Response Factor = 714 µV)
Human Safety Department
Analytical Method 676
Novartis Crop Protection, Inc.
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FIGURE 14. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-163374 IN BARLEY GRAIN USING AG-575B (SET 2) (CONTINUED) (Multichrom Worksheet: 313-97-MX5G2)

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), 97.5% recovery. (Response Factor = 2477 μV)

4. 201464, control ≈ 0.04 ppm CGA-169374, 2.64 mg injected, 0.108 ng found, 0.04 ppm, 0.04 ppm (corrected for control), 100% recovery. (Response Factor = 7584 μV)

PAGE 63 OF 100
5. 201665, control - 0.20 ppm CGA-169374; 1.32 mg injected, 0.249 ng found, 0.188 ppm, 0.186 ppm (corrected for control); 94.2% recovery. (Response Factor - 17786 µV)
Human Safety Department
Analytical Method 676
Novartis Crop Protection, Inc.
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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 mg found, <0.01 ppm (MD).
(Response Factor = 0 μV)

2. 201463, control + 0.10 ppm CGA-169374, 0.791 mg injected, 0.076 mg
found, 0.096 ppm, 0.096 ppm (corrected for control), 95.5% recovery.
(Response Factor = 0.971 μV)
3. 201464, control + 0.2 ppm CGA-169974, 0.395 mg injected. 0.38 ng found. 0.201 ppm, 0.201 ppm (corrected for control), 191% recovery. (Response Factor - 1.024 uV)

4. 201465, control + 1.0 ppm CGA-169974, 0.198 mg injected. 0.185 ng found. 0.938 ppm, 0.938 ppm (corrected for control), 93.8% recovery. (Response Factor - 2384 uV)
FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF
CGA-169374 IN BARLEY GRAIN USING AG-676 (SET 1)
(Multichrom Worksheet: PRESSGRAIN)

CGA-169374: Slope = 5403830 μV/ng Y-intercept = -124813 μV

Ion 325.00 (324.70 to 325.30): PEPSG03.wmf

Ion 323.00 (322.70 to 323.30): PEPSG03.D

Ion 265.00 (264.70 to 265.30): PEPSG03.D

1. 200045, control: 3.96 ng injected, 0.09 ng found, <0.01 ppm.
   [Response Factor - 0 μV]
FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-876 (SET 1) (Continued)
(Multichrom Worksheet: PRESSGRAIN)

Abundance

Ion 325.00 (324.70 to 325.30): PEGPSC05.W

Time→ Abundance

Ion 329.00 (322.70 to 323.30): PEGPSC05.D

Time→ Abundance

Ion 265.00 (264.70 to 265.30): PEGPSC05.D

Time→

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

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

Abundance

Ion 323.00 (324.70 to 325.30): PEGPSG06.msf

Time—> Abundance

Ion 323.00 (322.70 to 323.30): PEGPSG06.D

Time—> Abundance

Ion 285.00 (284.70 to 285.30): PEGPSG06.D

Time—>

3. 20144, control = 0.04 ppm CGA-169374, 2.54 mg injected; 0.112 ng found, 0.043 ppm, 0.045 ppm (corrected for control); 1064 recovery. (Response Factor = 479213 µV)

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FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-576 (SET 1) (Continued) (Multichrom Worksheet: PRESSGRAIN)

Abundance

Ion 325.00 (324.70 to 325.39); PEGPSGO

Time -> Abundance

Ion 323.00 (322.70 to 323.30); PEGPSGO

14.21

Time -> Abundance

Ion 255.00 (254.70 to 256.30); PEGPSGO

14.21

Time -> Abundance

4. 201685, control + 0.2 ppm CGA-169374, 1.32 mg injected, 0.284 ng found. 0.216 ppm, 0.216 ppm (corrected for control), 100% recovery. (Response Factor: 1.469492 µV)
Human Safety Department
Analytical Method 676
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FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF
CGA-169374 IN BARLEY GRAIN USING AG-676 (SEPT. 2)
(Continued)
(Multichrom Worksheet: PREPEGRAIN2)

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

Abundance

Time ->

Abundance

Time ->

Abundance

1. 200445, control. 3.96 mg injected. 0.00 mg found. 0.00 ppm (ND).
(Linear Response Factor = 0 µV)

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FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-678 (SET 2) (Continued) (Multichrom Worksheet: PREPPEGRAIN2)

Abundance

Ion 325.00 (324.70 to 325.30): PPG982605.wmf

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Ion 323.00 (322.70 to 323.30): PPG982605.D

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Ion 265.00 (264.70 to 265.30): PPG982605.D

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</table>

2. 201463, control: 0.01 ppm CGA-169374, 1.95 mg injected, 0.088 mg found, 0.017 ppm, 0.017 ppm (corrected for control), 171% recovery. (Response Factor - .242257 µV)
3. 201463, control + 0.01 ppm COA-169374, 3.96 mg injected, 0.058 mg found, 0.015 ppm, 0.013 ppm (corrected for control), 146% recovery. (Response Factor - 136195 µV)
4. 201464, control + 0.04 ppm CCA-159374, 2.64 ng injected, 0.124 ng found, 0.847 ppm, 0.747 ppm (corrected for control), 118% recovery.
(Response Factor - 553357 µV)
5. 201465, control + 0.2 ppm CGA-169374, 1.32 mg injected. 0.298 mg 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

Ion 325.00 (324.70 to 325.30): GR070103.wmf

Ion 323.00 (322.70 to 323.30): GR070103.D

Ion 285.00 (284.70 to 285.30): GR070103.D

1. 200045, control, 3.96 ng 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-476 (SET 3).
(Continued)
(Multichrom Worksheet: 169GR4)

2. 201463, control + 0.2 ppm CGA-169374, 0.791 mg injected, 0.085 mg found, 0.107 ppm, 0.307 ppm (corrected for control), 107% recovery. (Response Factor = 377761 µV)
FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-159374 IN BARLEY GRAIN USING AG-676 (SET 3)
(Continued)
(Multichrom Worksheet: 169GR4)

3. 201464, control = 0.7 ppm CGA-159374, 0.395 mg injected, 0.071 mg found, 0.179 ppm, 0.179 ppm (corrected for control), 89.4% recovery.
(Response Factor = 306654 µV)
FIGURE 15. REPRESENTATIVE CHROMATOGRAMS FROM THE ANALYSIS OF CGA-169374 IN BARLEY GRAIN USING AG-276 (SET 3)
(Continued)
(Multichrom Worksheet: 169GR4)

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4. 201465, control + 1.0 ppm CGA-169374; 0.198 mg injected, 0.191 mg found, 0.967 ppm, 0.987 ppm (corrected for control), 98.7% recovery. (Response Factor - 918964 µ0)

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VIII. REFERENCES


4. Memorandum (with attachments) dated September 16, 1996 from Philip V. Errico, Acting Product Manager, Fungicide Herbicide Branch, Registration Division (H750SC) to Greg Peters, Ph.D., Ciba re PP#5E4748. Difenoconazole (Dividend) in or on Barley, Cots, 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 (H750SC) to Eileen D. Kling-Watson, Ph.D., Ciba re PP#3E4051. CGA-169374 (Difenoconazole, Dividend) in Imported Wheat Barley and Rye Grain.


VIII. REFERENCES (Continued)


APPENDIX I

PREVIOUSLY SUBMITTED DOCUMENTS WITH EPA MRID NUMBERS


SUBMITTER/SPONSOR: Nevatis Crop Protection, Inc., P. O. Box 18500, Greensboro, NC 27411-8500

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APPENDIX II

HUMAN SAFETY DEPARTMENT RESIDUE TEST REPORTS

SUBMITTER/SPONSOR: Neuvita Crop Protection, Inc. Post Office Box 13299, Greensboro, NC 27413

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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: Amyly L. Oakes

DATE: 3/4/97

DISTRIBUTION: To be distributed per protocol distribution list.
**BIOLOGY SECTION**

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**CIRCUMSTANCES AFFECTING THE STUDY**

None

**DEVATIONS 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 a 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. Oakas (TLO), Chemist IV
J. C. Adair (TSA), Olsen Temporary Services

**ANALYTICAL SECTION**

Methodology

AO-5758 - with the omission of the charcoal cleanup (Section 6.0). The QC conditions of AO-5758 were also modified to include a DB-17 (15 meter x 0.33 mm ID, df = 1.0 um megabore column instead of a packed column.

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

01/1971 [5:\77102\77102\313-97\MTRD001] - abb/mnl: 7/15/97

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FROM THE ANALYSIS OF BARLEY GRAIN

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**SUMMARY**

This method provides a GC/MSD system that will confirm the presence of CQA-169374 residues detected in barley using the established tolerance enforcement methodology (AG-575B). Confirmation is accomplished by analyzing ions unique to CQA-169374 using GC/MSD and rejecting any potential co-eluting or interfering compounds. Confirmation of CQA-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, 322, and 325). Confirmation of residue concentration is obtained by quantitating on the target ion for CQA-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 (+/- 2%).

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:acetone:tritile 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-5756). 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.

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In conclusion, analytical method AG-576 is a valid and accurate confirmatory method for the determination of parent residues of CQA-169374 in barley. This confirmatory technique offers a highly specific method for the verification of potential CQA-169374 residues in barley and other cereal grains at or above the tolerance level.
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**Substrates:** STEIN

**Notes:**
- B: Blank
- C: Control
- F: Filler
- R: Recovery
- S: Standard
- V: Solvent
- *: Sample

*Control used for this group. Negative controls are treated as 0.0. Underline: Strike through: Manual override: Rejected
## Worksheet: PREDRAN

### Limit of Detection:
0.010 ppm

### Corrected:
4-Jun-1997

### Product:
CWA-165794

### Analyte:
CWA-165794

### Chem.
41

### Project:
313-91

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### Standards:

- Blank
- Control
- Recovery
- Standard
- Solvent
- Sample

### Notes:

- Control used for this group
- Negative controls are treated as 0.0
- Underline: Strikethrough: Manual override: Ignored
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**Substrates:** FORAGE

**Notes:**

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- Control
- Control used for this group
- Negative controls are treated as 0.0
- Underline - Strike-through - Manual override - Rejected
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Substrates: FORAGEN

Notes: This worksheet was originally targeted/processed.
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* Control used for this group; negative controls are treated as 0.0
** Overhead, stir through; manual override, rejected
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**Substrates:** G17W

**Notes:**
- B: Blank
- C: Control
- P: Preset
- S: Standard
- V: Solvent
- X: Sample

Control used for this group. Negative controls are treated as 0.0.
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Notes:

- B: Blank
- C: Control
- F: Fray
- R: Recovery
- S: Standard
- V: Solvent
- X: Sample

*Control used for this group: Note: Negative controls are treated as 0.0
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**Notes:**

- B: Blank
- C: Control
- F: Present
- R: Recovery
- S: Standard
- V: Solvent
- I: Sample
- Control used for this group
- Negative controls are treated as 0.0
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**Notes:** Pressure Programming on Q3/RED

**Fields:** 04/1976, FED/ERA111

- **B**: Blank
- **C**: Control
- **F**: Frassor
- **K**: Recovery
- **S**: Standard
- **V**: Solvent
- **X**: Sample
- **X**: Control used for this group
- Negative controls are treated as 0.0
- Underline: Strikethrough: Manual entry: Rejected
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**Substrates:** OATHE

**Notes:**

- B: Blank
- C: Control
- F: Reservoir
- R: Recovery
- S: Sample
- Underline: No sample
- Underline: Sample rejected

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### PAGE 97 OF 100

**Substrates:** CHWay

**Notes:**
- B: Blm
- C: Control
- F: Fmnet
- H: Recovery
- N: Standard
- S: Sample
- V: Wetted

Control used for this group. Negative controls are treated as 0.0. Underline: Strike-through: Manual override: bracketed
| No | T | 0 Field Test | Exp Code | Limit b | Exp wt ml | Net vol ml | Aliq 1 vol ml | Inter vol ml | Aliq 2 vol ml | Final vol ml | Final wt | Inj vol ml | Emp wt | Emp vol ml | Emp wt | Emp vol ml | Emp wt | Emp vol ml |
| 1 | a | 12.00 | 20.61 | 200.00 | 40.00 | 3.955 | 20.0 | 4.20 | 3.958 | 3.955 | 20.0 | 4.00 | 3.955 | 3.955 | 20.0 | 4.00 | 3.958 |
| 2 | a | 12.00 | 20.61 | 200.00 | 40.00 | 3.955 | 20.0 | 4.20 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 |
| 3 | a | 12.00 | 20.61 | 200.00 | 40.00 | 3.955 | 20.0 | 4.20 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 |
| 4 | a | 12.00 | 20.61 | 200.00 | 40.00 | 3.955 | 20.0 | 4.20 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 |
| 5 | a | 12.00 | 20.61 | 200.00 | 40.00 | 3.955 | 20.0 | 4.20 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 |
| 6 | a | 12.00 | 20.61 | 200.00 | 40.00 | 3.955 | 20.0 | 4.20 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 |
| 7 | a | 12.00 | 20.61 | 200.00 | 40.00 | 3.955 | 20.0 | 4.20 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 |
| 8 | a | 12.00 | 20.61 | 200.00 | 40.00 | 3.955 | 20.0 | 4.20 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 |
| 9 | a | 12.00 | 20.61 | 200.00 | 40.00 | 3.955 | 20.0 | 4.20 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 |
| 10 | a | 12.00 | 20.61 | 200.00 | 40.00 | 3.955 | 20.0 | 4.20 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 | 3.955 | 20.0 | 4.00 | 3.955 |

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### Notes
- Black: C = Control
- Control used for this group
- Negative controls are treated as 0.0
- Underline: Strikethrough: Manual override: Rejected
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Analyzed... 313-97-RM
Analysis... 12000
Subject... BASIL

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Notes:
B: Blank  C: Control  F: Filler  R: Recovery  S: Standard  V: Solvent  P: Sample
* Control used for this group
Negative controls are treated as 0.0
Underline, strikethrough = manual override, depressed
APPENDIX III

CERTIFICATION OF AUTHENTICITY

The reports included in this submittal volume, Laboratory Project 1, D. AG-676 Analytical Method, are certified to be authentic accounts of the experiments and the results of these experiments, described herein.

Tim Oakes, Chemist IV, and Study Director
Human Safety Department
(910) 632-1393

7/18/97

Date

SUBMITTER/SPONSOR: Novartis Crop Protection, Inc., P. O. Box 13360, Greensboro, NC 27418-4360

PAGE 100 OF 100
APPENDIX II

ANALYTICAL PHASE REPORT
ENViro-TEST LABORATORIES (ETL)
9936 - 67 Avenue
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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

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STUDY DATES: Study Initiation Date: September 24, 1996
Experimental Initiation Date: September 16, 1996
Experimental Termination Date: November 4, 1996

STUDY PROTOCOL NO.: 476-97-B
NOVARTIS PROJECT NO.: 346655
ETL REPORT NO.: 96NOV37.REP
REPORT DATE: November 5, 1996
PAGES OF REPORT: 106
NOVARTIS NUMBER: 476-87
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 18, 1989."

Gary Brune
Analytical Laboratory Manager
Enviro-Test Laboratories

Date: Nov 5/98

Susen Nelson
Analytical Principal Investigator
Enviro-Test Laboratories

Date: Jan 5/97
STATEMENT OF THE QUALITY ASSURANCE UNIT

ETL Report No.: 98NOV37.REP
Study Protocol No.: 478-97-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.

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<th>AUDIT DATES</th>
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Final Report/Data Audit:
Nov. 3, 4 & 5/98 | Nov. 5/98 | Nov. 5/98 | Nov. 5/98

Signature of QAU: [Signature]
Date: Nov. 5/98

NOVARTIS NUMBER: 478-97
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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 Difenconazole (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 Metanoxam (CGA-329351) as 2,6-Dimethylaniline (2,6-DMA) in canola is based on the Novartis analytical method AG-365 entitled "Improved Method for the Determination of Total Residues of Metanoxyl 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 26, 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 (UMS). This number (e.g., E8-09-396-01A) was cross referenced with the unique field sample number. E8-09 represents the date received (e.g., September of 1998). The last part of the sample number, 396-01A, signifies a UMS batch number (396) and a unique sample ID (01A).

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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-186374 analysis)

Analysis of Difenocoumazole (CGA-186374) using method AG-676 involves the extraction of residues by refluxing with 8:2 methanol/conc. NH₄OH, 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 Mefenoxam 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-Kyger 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-189374 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 152.00):

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 lined 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 Turbomix 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 Turbomix at 30-35°C.

4.1.11 Rinse down the Turbomix tube or boiling flask with a small volume (~0.5 mL) of ACN and add 10 mL of water.

4.1.12 Attach a 75 mL reservoir with adapter to the top of a 1 g Varian C18 SPE cartridge.
4.1 Extraction and Clean-up of Canola Seed Samples (CGA-233343 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-576, ETL ref.: MS 171.90):

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 Difenconazole (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$_3$CN.
4.2.13 Combine the CH$_3$CN 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 eluant.
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-876: The phenyl bond-out 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-396, 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 Mefenoxam 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 5000-8000 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-Dimethylaniline (DMA) using Selected Ion Monitoring (SIM).
4.4 Extraction for Treated Canola Seed (CGA-293343, CGA-169374 and CGA-326351) (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
Condenser, 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, Multipracta 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-2B

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5.1 Apparatus cont'd

Spatulas, micro - WWR 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 - WWR 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®

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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

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5.4.1.1 HPLC Operating Conditions

For CGA-293343 and CGA-322704 analysis of Canola Seed:
Analytical Column: Supelcoil LC-18, 4.6 x 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-322351, CGA-293343 and CGA-169374 analysis of Treated Canola Seed:
Analytical Column: Zorbax 300-SCX, 4.6 x 150 mm, 5μm particle size.
Hewlett-Packard Company
Guard Column: Zorbax SCX, 4.6 x 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

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<td>3.52</td>
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<td>CGA-169374</td>
<td>406.0-250.6</td>
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<tr>
<td>CGA-322704</td>
<td>249.8-169.6</td>
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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.

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<th>Parameter</th>
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<tr>
<td>Auxiliary gas: Nitrogen @</td>
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<td>Interface setpoint:</td>
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<td>Collision gas: Argon @</td>
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<td>Turbo ion spray temp.:</td>
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Positive ion Mode:

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<td>Orifice potential:</td>
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</tr>
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<td>Acquire:</td>
<td>5 min.</td>
</tr>
<tr>
<td>Dwell time:</td>
<td>200 ms.</td>
</tr>
</tbody>
</table>

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-189374 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 18590A
Gas chromatograph: HP 5890A
Mass spectrometer: HP 5971A/HP 5970

5.4.2.1 Operating Conditions (GC/MSD)

Gas Chromatograph Column: Stabilwax (30 m x 0.25 μm x 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, 108
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-189374 Difenconazole Analysis)

Autosampler: HP 18506A
Gas chromatograph: HP 5890A
Mass spectrometer: HP 5971A/HP 5970

5.4.3.1 Operating Conditions (GC/MSD)

Gas Chromatograph Column: DB1301 (30 m x 0.25 μm x 0.25 mm)
Injection port: 240°C (frosted aluminized 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)

<table>
<thead>
<tr>
<th>Reference Substance</th>
<th>Supplier</th>
<th>Received (mo/ey)</th>
<th>Purity (%)</th>
<th>Lot/ Batch No.</th>
<th>Expiry Date (m/y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGA-293343</td>
<td>Novartis</td>
<td>6/01/98</td>
<td>98.5</td>
<td>S98-1283</td>
<td>05/2001</td>
</tr>
<tr>
<td>CGA-169374</td>
<td>Novartis</td>
<td>6/01/98</td>
<td>98.0</td>
<td>S98-1669</td>
<td>04/2000</td>
</tr>
<tr>
<td>2,6-DMA</td>
<td>Novartis</td>
<td>8/17/98</td>
<td>99.6</td>
<td>DAH-X73</td>
<td>01/2000</td>
</tr>
<tr>
<td>CGA-329351</td>
<td>Novartis</td>
<td>8/34/98</td>
<td>98.1</td>
<td>S97-2087</td>
<td>05/2000</td>
</tr>
</tbody>
</table>
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)

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

Where:
- \( P_A \) = 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:

\[
\text{Recovery (\%)} = \left( \frac{\text{Result (ppb)}}{\text{Fortification Level (ppb)}} \right) \times 100
\]
9.3 Example of Calculation:

Compound: CGA-203349
Matrix: Canola seed
Analysis date: Oct. 23/96
Chrom. I.D.: NM102256008
EB-09-E19-21A+11; CS-SR-858-97/GA 1-4-A Cont. fortified at 0.0540 ppm

\[
\text{Result} = \frac{(25.21 - 0.0)}{(200/40 \text{ ml}) \times 250 \text{ ml}} \times \frac{10 \text{ g}}{5967883} = 0.0525 \text{ ppb}
\]

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

9.4 Concentration of Mefenoxam as 2,6-DMA

\[
\text{Response Factor (R.F)} = \frac{\text{Standard Concentration (ppm)}}{\text{Peak Area}}
\]

\[
\text{Aliquot Factor (A.F)} = \frac{100 \text{ ml Extraction Volume}}{20 \text{ ml Aliquot Volume}} = 5
\]

\[
\text{Conversion Factor (C.F)} = \frac{\text{Mol. Wt. Mefenoxam} \times 279.3}{\text{Mol. Wt. 2,6-DMA} \times 2.31} = \frac{121}{2}
\]

\[
\text{Mefenoxam (ppm)} = \frac{\text{Peak Area} \times \text{R.F} \times \text{E.V.} \times \text{A.F.} \times \text{C.F.}}{\text{Sample Wt. (g)}}
\]

9.5 Example of Mefenoxam Calculation

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

\[
= \frac{6.22 \times 4.78 \times 10^{-4} \times 15.0 \text{ ml} \times 5.9}{10.0 \text{ g}} \times 2.31 = 0.0516 \text{ ppm}
\]

\[
\text{% Recovery} = \frac{0.0516 \times 100}{0.0530} = 97\%
\]
9.6 Concentration of CQA-183374

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

\( A.F = \frac{40 \text{ mL aliquot}}{200 \text{ mL extraction volume}} = 0.2 \)

9.7 Example of CQA-183374 Calculation

Lab Sample #: E8-00-E19-21A+1C
Client ID.: 05-598-97 Seed:

\( = \frac{138 \times (2.65 \times 10^{-5}) \times 1.0 \text{ ml}}{20 \text{ g} \times 0.20} \) = 0.00914 ppm

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

---

NOVARTIS NUMBER: 476-97
10.1 Figure 1 - Structure of Analytes

CGA-188374

CGA-283343

CGA-322704

CGA-329351 active R isomer

DMA

NOVARTIS NUMBER: 476-97
10.0 FIGURES: cont'd

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

10 g ground Canola Seed

Polytron with 120 mL of ACN and 30 mL of Hexane

Centrifuge and Filter

Re-extract with 40 mL of ACN and 40 mL of Hexane

Combine hexane extracts and back extract with 30 mL of ACN

Combine ACN and bring to 200 mL

Mix well & take a 40 mL aliquot

Evaporate ACN to approx. 0.5 mL

Condition 1g Varian C18 SPE Cartridge

Transfer ACN extract to SPE with MeOH/acetic acid in water

Elute analyte with 12 mL of MeOH

Transfer to Turbovap tube or boiling flask

Concentrate to <2.5 mL and make to 5 mL final volume

Dilute 800 μL to 4 mL with ACN (1:5)

Analyze by LC/MS/MS
10.0 FIGURES: cont'd

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

10 g ground Canola Seed

Reflux with 100 mL of 80:20 water/MeOH for 2 hours

Cool, centrifuge and filter

Evaporate a 20 mL aliquot and add
10 mL of Methanesulfonic acid

Reflux for 15 minutes and cool

Add 200 mL water, 60 mL 25% NaOH and 15 mL hexane and Steam Distill for 1 hr.

Analyse hexane layer by GC/MS for DMA
10.2 Figure 2C - Canola Seed Method Flow Diagram for Difenoconazole (CGA-169374)

- 20 g ground Canola Seed
- Reflux with 200 mL of 80% MeCN/Conc. NH₄OH
  - Cool, centrifuge and remove a 40 mL aliquot
  - Add water (NaCl sat.)
  - Partition with Hexane
- Back partition hexane with ACN (discarding oil with hexane)
  - Conc. ACN and dissolve in Toluene
  - Condition silica gel sep pack with 5 mL toluene
  - Load 5 mL extract onto sep pack
  - Discard eluent
  - Elute CGA 169374 with 15 mL toulene/acetone
  - Evap. To dryness
  - Reconstitute in 1.0 mL toluene
  - Analyze by GC/MS
10.0 FIGURES: cont'd

10.3 Figure 3 - Personnel Organizational Chart

- Gary Bruns: Analytical Laboratory Manager
- Anne Beubien: QA/QC
- Susan Nelson: Analytical Principal Investigator
- Norm McLean: Instrument Coordinator

SAMPLE EXTRACTION:
- Jimmy Hackbart
- Ken Hunke

SAMPLE LOGIN & PREPARATION:
- Danuta Rezek
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 were 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 CDA-159374 IN CANOLA SEED BY GC/MS

<table>
<thead>
<tr>
<th>LAB SAMPLE #</th>
<th>DATE EXTR.</th>
<th>DATE ANAL.</th>
<th>AMOUNT SPIKED (ppm)</th>
<th>AMOUNT FOUND (ppm)</th>
<th>% RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>0W-SR-204-99/SD 1-4-A Cont.</td>
<td>10/2/98</td>
<td>10/3/98</td>
<td>--</td>
<td>&lt;0.010</td>
<td>--</td>
</tr>
<tr>
<td>E8-09-E19-42A-</td>
<td>10/2/98</td>
<td>10/3/98</td>
<td>0.0101</td>
<td>0.00748</td>
<td>74</td>
</tr>
<tr>
<td>E8-09-E19-42A+1</td>
<td>10/2/98</td>
<td>10/3/98</td>
<td>0.0101</td>
<td>0.00718</td>
<td>71</td>
</tr>
<tr>
<td>E8-09-E19-42A+2</td>
<td>10/2/98</td>
<td>10/3/98</td>
<td>0.101</td>
<td>0.105</td>
<td>105</td>
</tr>
<tr>
<td>E8-09-E19-42A+3</td>
<td>10/2/98</td>
<td>10/3/98</td>
<td>0.101</td>
<td>0.101</td>
<td>100</td>
</tr>
<tr>
<td>E8-09-E19-42A+4</td>
<td>10/2/98</td>
<td>10/3/98</td>
<td>0.101</td>
<td>0.101</td>
<td>100</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>LAB SAMPLE #</th>
<th>DATE EXTR. (m/d/y)</th>
<th>DATE ANAL. (m/d/y)</th>
<th>AMOUNT SPIKED (ppm)</th>
<th>AMOUNT FOUND (ppm)</th>
<th>% RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>08-SR-059-97/GA 1-4-A Cont.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E8-09-E19-21A-1</td>
<td>10/5/98</td>
<td>10/20/98</td>
<td>0.0530</td>
<td>0.0450</td>
<td>85</td>
</tr>
<tr>
<td>E8-09-E19-21A+1</td>
<td>10/5/98</td>
<td>10/20/98</td>
<td>0.0530</td>
<td>0.0517</td>
<td>98</td>
</tr>
<tr>
<td>E8-09-E19-21A+2</td>
<td>10/5/98</td>
<td>10/20/98</td>
<td>0.530</td>
<td>0.476</td>
<td>90</td>
</tr>
<tr>
<td>E8-09-E19-21A+3</td>
<td>10/5/98</td>
<td>10/20/98</td>
<td>0.530</td>
<td>0.375</td>
<td>71</td>
</tr>
</tbody>
</table>

Avg. % Recovery - 88%
SD - ±11

NOVARTIS NUMBER: 476-97
### 12.0 RECOVERY AND RESULTS DATA

#### TABLE 2: 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)

<table>
<thead>
<tr>
<th>Lab Sample #</th>
<th>Sample I.D.</th>
<th>PHI (ppb)</th>
<th>Nominal Tr. Rate (ppm)</th>
<th>Nominal Fortification* (ppm)</th>
<th>Data Extracted (mLV)</th>
<th>Data Analyzed (mLV)</th>
<th>Recovery/Residues (%)</th>
<th>CGA-293343</th>
<th>CGA-322704</th>
</tr>
</thead>
<tbody>
<tr>
<td>05-SR-058-97/SA Seed (at maturity)</td>
<td>1-A-A</td>
<td>0.14</td>
<td>Control</td>
<td>0.05</td>
<td>10/6/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>05-SR-058-97/TA1 Seed (at maturity)</td>
<td>1-A-A</td>
<td>0.14</td>
<td>Control</td>
<td>0.05</td>
<td>10/6/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>05-SR-058-97/B Seed (at maturity)</td>
<td>1-A-A</td>
<td>0.14</td>
<td>Control</td>
<td>0.05</td>
<td>10/6/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

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-478) cont'd

<table>
<thead>
<tr>
<th>Lab Sample #</th>
<th>Sample ID</th>
<th>pH</th>
<th>Tr Rates (g/L/000ha)</th>
<th>Formulation Form (ppm)</th>
<th>Date Extracted</th>
<th>Date Analyzed</th>
<th>Recovery/Residues (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OW-SIR-202-98/NO Seed (at maturity)</td>
<td>EB-09-397-11A-3</td>
<td>1-4-A</td>
<td>—</td>
<td>Control</td>
<td>10/9/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>EB-09-397-11A+3</td>
<td>1-4-A</td>
<td>—</td>
<td>Control+</td>
<td>10/9/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>EB-09-397-13A</td>
<td>2-4-A</td>
<td>500</td>
<td>Combi FS-D</td>
<td>10/9/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>EB-09-397-14A</td>
<td>2-4-B</td>
<td>500</td>
<td>Combi FS-D</td>
<td>10/9/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>EB-09-397-15A</td>
<td>3-4-A</td>
<td>1500</td>
<td>Combi FS-D</td>
<td>10/9/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>EB-09-E19-42A-3</td>
<td>1-4-A</td>
<td>—</td>
<td>Control</td>
<td>10/13/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>EB-09-E19-42A+1</td>
<td>1-4-A</td>
<td>—</td>
<td>Control+</td>
<td>10/13/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>EB-09-E19-43A</td>
<td>2-4-A</td>
<td>500</td>
<td>Combi FS-D</td>
<td>10/13/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>EB-09-E19-44A</td>
<td>2-4-B</td>
<td>500</td>
<td>Combi FS-D</td>
<td>10/13/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>EB-09-SIR-302-98/WA Seed (at maturity)</td>
<td>EB-09-E19-48A-3</td>
<td>1-4-A</td>
<td>—</td>
<td>Control</td>
<td>10/9/98</td>
<td>10/23/98</td>
</tr>
<tr>
<td></td>
<td>EB-09-E19-48A+3</td>
<td>1-4-A</td>
<td>—</td>
<td>Control+</td>
<td>10/9/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>EB-09-E19-49A</td>
<td>2-4-A</td>
<td>500</td>
<td>Combi FS-D</td>
<td>10/9/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>EB-09-E19-50A</td>
<td>2-4-B</td>
<td>500</td>
<td>Combi FS-D</td>
<td>10/9/98</td>
<td>10/23/98</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* 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-189374 IN CANOLA SEED AND PROCESSED CANOLA FRACTIONS ACQUIRED FROM PLANTS GROWN FROM CONTROL AND TREATED SEED (BY NOVARTIS METHOD AG-676)

<table>
<thead>
<tr>
<th>Lab Sample #</th>
<th>Sample ID</th>
<th>Nominal Pellet (Days)</th>
<th>Nominal Tr. Rate (mg/100kg)</th>
<th>Nominal Fortification (ppm)</th>
<th>Data Extracted (mm/dd)</th>
<th>Data Analyzed (mm/dd)</th>
<th>Recovery/Residue (%) / ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0S-SR-855-97GA Seed (at maturity)</td>
<td>E8-08-E19-21A-4</td>
<td>1-4-A</td>
<td>105-6/98</td>
<td>10/21/98</td>
<td>&lt;0.010</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E8-08-E19-21A+10</td>
<td>1-4-A</td>
<td>105-6/98</td>
<td>10/21/98</td>
<td>0.01</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E8-08-E19-22A</td>
<td>2-4-A</td>
<td>105-6/98</td>
<td>10/21/98</td>
<td>0.01</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E8-08-E19-23A</td>
<td>2-4-B</td>
<td>105-6/98</td>
<td>10/21/98</td>
<td>0.01</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td>0W-SR-201-98/MN Seed (at maturity)</td>
<td>E8-08-385-11A-2</td>
<td>1-4-A</td>
<td>105-6/98</td>
<td>10/15/98</td>
<td>&lt;0.010</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E8-08-385-11A+2</td>
<td>1-4-A</td>
<td>105-6/98</td>
<td>10/15/98</td>
<td>0.10</td>
<td>114%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E8-08-385-15A</td>
<td>2-4-B</td>
<td>105-6/98</td>
<td>10/15/98</td>
<td>0.01</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E8-08-385-15A</td>
<td>2-4-A</td>
<td>105-6/98</td>
<td>10/15/98</td>
<td>0.01</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td>0W-SR-201-98/3D Seed (at maturity)</td>
<td>E8-08-E19-45A-2</td>
<td>1-4-A</td>
<td>105-6/98</td>
<td>10/15/98</td>
<td>&lt;0.010</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E8-08-E19-45A+2</td>
<td>1-4-A</td>
<td>105-6/98</td>
<td>10/15/98</td>
<td>0.01</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E8-08-E19-48A</td>
<td>2-4-A</td>
<td>105-6/98</td>
<td>10/15/98</td>
<td>0.01</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E8-08-E19-47A</td>
<td>2-4-B</td>
<td>105-6/98</td>
<td>10/15/98</td>
<td>0.01</td>
<td>80%</td>
</tr>
</tbody>
</table>
### Table 4: Analytical Sets for Determination of Residues CGA-19374 in Canola Seed and Processed Canola Fractions Acquired from Plants Grown from Control and Treated Seed (By Novartis Method AG-674) cont’d

<table>
<thead>
<tr>
<th>Lab</th>
<th>Sample ID</th>
<th>Sample #</th>
<th>Lab D</th>
<th>pH</th>
<th>Nominal Tr. Rate (ppm)</th>
<th>Nominal Fortification* (ppm)</th>
<th>Data Extracted (m/d/y)</th>
<th>Data Analyzed (m/d/y)</th>
<th>Recovery/Residue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OW-SR-202-98/BD Seed (at maturity)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-09-387-11A</td>
<td>1-4-A</td>
<td>Control</td>
<td>0.01</td>
<td>10/5-6/98</td>
<td>10/15/98</td>
<td>&lt;0.010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-09-387-11A +2</td>
<td>1-4-A</td>
<td>Control</td>
<td>0.01</td>
<td>10/5-6/98</td>
<td>10/15/98</td>
<td>98%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-09-387-13A</td>
<td>2-4-A</td>
<td>30 Combii FS-D</td>
<td>10/5-6/98</td>
<td>10/15/98</td>
<td>&lt;0.010</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-09-387-14A</td>
<td>2-4-B</td>
<td>30 Combii FS-D</td>
<td>10/5-6/98</td>
<td>10/15/98</td>
<td>&lt;0.010</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-09-387-15A</td>
<td>3-4-A</td>
<td>90 Combii FS-D</td>
<td>10/5-6/98</td>
<td>10/15/98</td>
<td>&lt;0.010</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OW-SR-204-98/BD Seed (at maturity)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-09-E19-42A</td>
<td>1-4-A</td>
<td>Control</td>
<td>0.01</td>
<td>10/13/98</td>
<td>10/15/98</td>
<td>&lt;0.010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-09-E19-42A +1</td>
<td>1-4-A</td>
<td>Control</td>
<td>0.01</td>
<td>10/13/98</td>
<td>10/15/98</td>
<td>99%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'EB-09-E19-43A</td>
<td>2-4-A</td>
<td>30 Combii FS-D</td>
<td>10/13/98</td>
<td>10/15/98</td>
<td>&lt;0.010</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-09-E19-44A</td>
<td>2-4-B</td>
<td>30 Combii FS-D</td>
<td>10/13/98</td>
<td>10/15/98</td>
<td>&lt;0.010</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OW-SR-302-98/BD Seed (at maturity)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-09-E19-48A</td>
<td>1-4-A</td>
<td>Control</td>
<td>0.01</td>
<td>10/5-6/98</td>
<td>10/15/98</td>
<td>&lt;0.010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-09-E19-48A +2</td>
<td>1-4-A</td>
<td>Control</td>
<td>0.10</td>
<td>10/5-6/98</td>
<td>10/15/98</td>
<td>79%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-09-E19-48A</td>
<td>2-4-A</td>
<td>30 Combii FS-D</td>
<td>10/5-6/98</td>
<td>10/15/98</td>
<td>&lt;0.010</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB-09-E19-50A</td>
<td>2-4-B</td>
<td>30 Combii FS-D</td>
<td>10/5-6/98</td>
<td>10/15/98</td>
<td>&lt;0.010</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 12.0 RECOVERY AND RESULTS DATA

**TABLE 6: ANALYTICAL SETS FOR DETERMINATION OF RESIDUES CGA-320351 IN CANOLA SEED AND PROCESSED CANOLA FRACTIONS ACQUIRED FROM PLANTS GROWN FROM CONTROL AND TREATED SEED (BY NOVARTIS METHOD AG-386)**

<table>
<thead>
<tr>
<th>Lab Sample #</th>
<th>Sample LD</th>
<th>PHI (Days)</th>
<th>Nominal Tr. Rates (mg/kg)</th>
<th>Nominal Fortification (ppm)</th>
<th>Date Extracted</th>
<th>Date Analyzed</th>
<th>Recovery/Residues (mg/l ppm)</th>
<th>CGA-320351*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E8-08-E18-21A-2</td>
<td>1-4-A</td>
<td>---</td>
<td>Control</td>
<td>---</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
<td></td>
</tr>
<tr>
<td>E8-08-E18-21A+5</td>
<td>1-4-A</td>
<td>---</td>
<td>Control+</td>
<td>0.05</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>97%</td>
<td></td>
</tr>
<tr>
<td>E8-08-E18-22A</td>
<td>2-4-A</td>
<td>10</td>
<td>Combi FS-D</td>
<td>---</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
<td></td>
</tr>
<tr>
<td>E8-08-E18-23A</td>
<td>2-4-B</td>
<td>10</td>
<td>Combi FS-D</td>
<td>---</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
<td></td>
</tr>
</tbody>
</table>

**GW-SR-301-98/ID Seed (at maturity)**

<table>
<thead>
<tr>
<th>Lab Sample #</th>
<th>Sample LD</th>
<th>PHI (Days)</th>
<th>Nominal Tr. Rates (mg/kg)</th>
<th>Nominal Fortification (ppm)</th>
<th>Date Extracted</th>
<th>Date Analyzed</th>
<th>Recovery/Residues (mg/l ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E8-08-E19-45A+</td>
<td>1-4-A</td>
<td>---</td>
<td>Control</td>
<td>---</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>E8-08-E19-45A+1</td>
<td>1-4-A</td>
<td>---</td>
<td>Control+</td>
<td>0.10</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>94%</td>
</tr>
<tr>
<td>E8-08-E19-46A</td>
<td>2-4-A</td>
<td>10</td>
<td>Combi FS-D</td>
<td>---</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>E8-08-E19-47A</td>
<td>2-4-B</td>
<td>10</td>
<td>Combi FS-D</td>
<td>---</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
</tbody>
</table>

**GW-SR-201-98/MN Seed (at maturity)**

<table>
<thead>
<tr>
<th>Lab Sample #</th>
<th>Sample LD</th>
<th>PHI (Days)</th>
<th>Nominal Tr. Rates (mg/kg)</th>
<th>Nominal Fortification (ppm)</th>
<th>Date Extracted</th>
<th>Date Analyzed</th>
<th>Recovery/Residues (mg/l ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E8-08-308-11A</td>
<td>1-4-A</td>
<td>---</td>
<td>Control</td>
<td>---</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>E8-08-308-11A+1</td>
<td>1-4-A</td>
<td>---</td>
<td>Control+</td>
<td>0.10</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>67%</td>
</tr>
<tr>
<td>E8-08-308-13A</td>
<td>2-4-A</td>
<td>10</td>
<td>Combi FS-D</td>
<td>---</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>E8-08-308-14A</td>
<td>2-4-B</td>
<td>10</td>
<td>Combi FS-D</td>
<td>---</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>E8-08-308-15A</td>
<td>3-4-A</td>
<td>30</td>
<td>Combi FS-D</td>
<td>---</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
</tbody>
</table>

*Detected as 2,6-DMA and converted to metoxuron 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-329351 IN CANOLA SEED AND PROCESSED CANOLA FRACTIONS ACQUIRED FROM PLANTS GROWN FROM CONTROL AND TREATED SEED (BY NOVARTIS METHOD AG-395)**

<table>
<thead>
<tr>
<th>Lab Sample #</th>
<th>Sample</th>
<th>Nominal Tr Rates (μg/LOD)</th>
<th>Nominal Fortification (ppm)</th>
<th>Data Extracted (m/d/y)</th>
<th>Data Analyzed (m/d/y)</th>
<th>Recovery Residues (% / ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E9-08-397-11A</td>
<td>1-A</td>
<td>Control</td>
<td>0.05</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>E9-08-397-11A+1</td>
<td>1-A</td>
<td>Control</td>
<td>0.05</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>72%</td>
</tr>
<tr>
<td>E9-08-397-13A</td>
<td>2-4-A</td>
<td>Combi FS-D</td>
<td>10</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>E9-08-397-14A</td>
<td>2-4-B</td>
<td>Combi FS-D</td>
<td>10</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>E9-08-397-15A</td>
<td>3-4-A</td>
<td>Combi FS-D</td>
<td>30</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
</tbody>
</table>

**OW-SR-204-96 SD Seed (at maturity)**

<table>
<thead>
<tr>
<th>Lab Sample #</th>
<th>Sample</th>
<th>Nominal Tr Rates (μg/LOD)</th>
<th>Nominal Fortification (ppm)</th>
<th>Data Extracted (m/d/y)</th>
<th>Data Analyzed (m/d/y)</th>
<th>Recovery Residues (% / ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E9-08-E19-42A</td>
<td>1-A</td>
<td>Control</td>
<td>0.05</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>E9-08-E19-42A+1</td>
<td>1-A</td>
<td>Control</td>
<td>0.05</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>82%</td>
</tr>
<tr>
<td>E9-08-E19-43A</td>
<td>2-4-A</td>
<td>Combi FS-D</td>
<td>10</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>E9-08-E19-44A</td>
<td>2-4-B</td>
<td>Combi FS-D</td>
<td>10</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
</tbody>
</table>

**OW-SR-302 96 WA Seed (at maturity)**

<table>
<thead>
<tr>
<th>Lab Sample #</th>
<th>Sample</th>
<th>Nominal Tr Rates (μg/LOD)</th>
<th>Nominal Fortification (ppm)</th>
<th>Data Extracted (m/d/y)</th>
<th>Data Analyzed (m/d/y)</th>
<th>Recovery Residues (% / ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E9-08-E19-49A</td>
<td>1-A</td>
<td>Control</td>
<td>0.10</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>117%</td>
</tr>
<tr>
<td>E9-08-E19-49A+1</td>
<td>1-A</td>
<td>Control</td>
<td>0.10</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>E9-08-E19-50A</td>
<td>2-4-A</td>
<td>Combi FS-D</td>
<td>10</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
<tr>
<td>E9-08-E19-50A</td>
<td>2-4-B</td>
<td>Combi FS-D</td>
<td>10</td>
<td>10/14/98</td>
<td>10/22/98</td>
<td>&lt;0.050</td>
</tr>
</tbody>
</table>

* Detected as 2,6-DMA and converted to metanoxen equivalents by a w/w conversion factor of 2.308.
12.0 RECOVERY AND RESULTS DATA

<table>
<thead>
<tr>
<th>Sample #</th>
<th>L.D.</th>
<th>Storage (Densi.)</th>
<th>Nominal Tr. Rates (g/ha)</th>
<th>Nominal Fortifications (ppm)</th>
<th>Date Extracted (m/d/y)</th>
<th>Date Analyzed (m/d/y)</th>
<th>Recovery/Residues % (ppm)</th>
<th>CGA-293343</th>
<th>CGA-169374</th>
<th>CGA-329351</th>
</tr>
</thead>
<tbody>
<tr>
<td>05-SR-856-27/SA Seed (FCA)</td>
<td>1-1-AB</td>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>10/15/98 10/25/98</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-SR-856-27/SA Seed (FCA)</td>
<td>1-1-AB</td>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>10/15/98 10/25/98</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-SR-856-27/SA Seed (FCA)</td>
<td>2-1-AB</td>
<td>300,300,300</td>
<td>Combi FS-D</td>
<td>0</td>
<td>10/15/98 10/25/98</td>
<td>552771</td>
<td>1093534</td>
<td>31312</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-SR-856-27/SA Seed (FCA)</td>
<td>1-3-AB</td>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>10/15/98 10/25/98</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-SR-856-27/SA Seed (FCA)</td>
<td>1-3-AB</td>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>10/15/98 10/25/98</td>
<td>97</td>
<td>11.4%</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-SR-856-27/SA Seed (FCA)</td>
<td>2-3-AB</td>
<td>300,300,300</td>
<td>Combi FS-D</td>
<td>0</td>
<td>10/15/98 10/25/98</td>
<td>6130095</td>
<td>752225</td>
<td>41.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-SR-201-06/MN Seed (FCA)</td>
<td>1-1-AB</td>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>10/15/98 10/25/98</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td>&lt;5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-SR-201-06/MN Seed (FCA)</td>
<td>1-1-AB</td>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>10/15/98 10/25/98</td>
<td>78</td>
<td>120%</td>
<td>118%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-SR-201-06/MN Seed (FCA)</td>
<td>2-1-AB</td>
<td>300,300,300</td>
<td>Combi FS-D</td>
<td>0</td>
<td>10/15/98 10/25/98</td>
<td>1228119</td>
<td>120369</td>
<td>52519</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-SR-201-06/MN Seed (FCA)</td>
<td>3-1-AB</td>
<td>300,300,300</td>
<td>Combi FS-D</td>
<td>0</td>
<td>10/15/98 10/25/98</td>
<td>10916343</td>
<td>1201084</td>
<td>43128</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-SR-201-06/MN Seed (FCA)</td>
<td>4-1-AB</td>
<td>300,300,300</td>
<td>Combi FS-D</td>
<td>0</td>
<td>10/15/98 10/25/98</td>
<td>1156727</td>
<td>126384</td>
<td>4443.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-SR-201-06/MN Seed (FCA)</td>
<td>5-1-AB</td>
<td>300,300,300</td>
<td>Combi FS-D</td>
<td>0</td>
<td>10/15/98 10/25/98</td>
<td>10916343</td>
<td>1201084</td>
<td>43128</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 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)

<table>
<thead>
<tr>
<th>Lab</th>
<th>Sample I.D.</th>
<th>Ambient</th>
<th>Storage</th>
<th>Tr. Rates</th>
<th>Formulation</th>
<th>Date</th>
<th>Date</th>
<th>Recovery/Residues (%) ppm</th>
<th>CGA-CGA-CGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>EB-09-387-01A</td>
<td>1-1-AB</td>
<td>—</td>
<td>—</td>
<td>Control</td>
<td>—</td>
<td>10/16/08</td>
<td>10/26/08</td>
<td>5.0</td>
<td>&lt;5.0 &lt;5.0 &lt;5.0</td>
</tr>
<tr>
<td>EB-09-387-01A+</td>
<td>1-1-AB</td>
<td>—</td>
<td>—</td>
<td>Control+</td>
<td>500,1000,200</td>
<td>10/16/08</td>
<td>10/26/08</td>
<td>81%</td>
<td>124% 124%</td>
</tr>
<tr>
<td>EB-09-387-20A</td>
<td>2-1-AB</td>
<td>0</td>
<td>500,30,10</td>
<td>Combi FS-D</td>
<td>—</td>
<td>10/16/08</td>
<td>10/26/08</td>
<td>67/4354</td>
<td>111/652 44/44.3</td>
</tr>
<tr>
<td>EB-09-387-01A</td>
<td>5-1-AB</td>
<td>0</td>
<td>1500,60,30</td>
<td>Combi FS-D</td>
<td>—</td>
<td>10/16/08</td>
<td>10/26/08</td>
<td>85/1297</td>
<td>122/111</td>
</tr>
<tr>
<td>EB-09-387-04A</td>
<td>4-1-AB</td>
<td>0</td>
<td>500,30,10</td>
<td>Combi FS-D</td>
<td>—</td>
<td>10/16/08</td>
<td>10/26/08</td>
<td>89/4419</td>
<td>123/569</td>
</tr>
<tr>
<td>EB-09-387-05A</td>
<td>5-1-AB</td>
<td>0</td>
<td>1500,90,30</td>
<td>Combi FS-D</td>
<td>—</td>
<td>10/16/08</td>
<td>10/26/08</td>
<td>78/1177</td>
<td>112/1004</td>
</tr>
<tr>
<td>EB-09-387-04A</td>
<td>1-3-AB</td>
<td>—</td>
<td>—</td>
<td>Control</td>
<td>—</td>
<td>10/16/08</td>
<td>10/26/08</td>
<td>5.0</td>
<td>&lt;5.0 &lt;5.0 &lt;5.0</td>
</tr>
<tr>
<td>EB-09-387-06A+</td>
<td>1-3-AB</td>
<td>—</td>
<td>—</td>
<td>Control+</td>
<td>5000,300,100</td>
<td>10/16/08</td>
<td>10/26/08</td>
<td>81%</td>
<td>107% 107%</td>
</tr>
<tr>
<td>EB-09-387-07A</td>
<td>2-3-AB</td>
<td>23</td>
<td>500,30,10</td>
<td>Combi FS-D</td>
<td>—</td>
<td>10/16/08</td>
<td>10/26/08</td>
<td>83/464</td>
<td>95/285</td>
</tr>
<tr>
<td>EB-09-387-05A</td>
<td>3-3-AB</td>
<td>20</td>
<td>1500,60,30</td>
<td>Combi FS-D</td>
<td>—</td>
<td>10/16/08</td>
<td>10/26/08</td>
<td>45/678</td>
<td>104/539</td>
</tr>
<tr>
<td>EB-09-387-05A</td>
<td>4-3-AB</td>
<td>20</td>
<td>500,30,10</td>
<td>Combi FS-D</td>
<td>—</td>
<td>10/16/08</td>
<td>10/26/08</td>
<td>94/685</td>
<td>110/529</td>
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<tr>
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<td>5-3-AB</td>
<td>20</td>
<td>1500,90,30</td>
<td>Combi FS-D</td>
<td>—</td>
<td>10/16/08</td>
<td>10/26/08</td>
<td>48/7140</td>
<td>78/699</td>
</tr>
</tbody>
</table>

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