1.0 INTRODUCTION

This report describes the independent laboratory validation (ILV) of Analytical Method No. T003103-03 as performed by Enviro-Test Laboratories for the determination of the CGA-293343 and its degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406 and NOA-459602 in well and surface water by direct injection high performance liquid chromatography with mass spectrometric detection.

This study was conducted to satisfy guideline requirements described in the US EPA FIFRA Pesticide Assessment Guidelines for Subdivisions N, E, and K, and addenda for Data Reporting Guideline for Environmental Methods [2]. It also satisfies the requirements outlined in the harmonized guidelines from the OPPTS, "Public Draft" - Data Reporting for Environmental Chemistry Methods, OPPTS 850.7100 [3].

2.0 STUDY PERSONNEL

The following personnel from Enviro-Test Laboratories participated in the conduct of this study.

Susan Nelson	Study Director
Connie Blenkinsop, Paxton Saramaga Meselu Abetew	Residue Analysts
Danuta Raszek	Log-In and Sample Control

3.0 MATERIALS

3.1 Test and Reference Substances

Reference substances were shipped from Syngenta US to Enviro-Test Laboratories and were received from March 18, 2004 to October 8, 2004 (see the Standard disposition sheets in the Raw Data package). The following substances were used:

Compound	Lot Number	Purity (%)	Expiration Date
CGA-293343	S01-2536	99.2	June 2007
CGA-322704	DAH-XXVII-52	96.6	May 31, 2005
CGA-355190	DAH-XXXI-49	98.2	Sep. 30, 2006
CGA-353042	JAK-XX-52	99.1	July 31, 2006
NOA-404617	DAH-XXXI-50	99.6	Sep. 30, 2006
NOA-407475	DAH-XXVIII-46	97.1	Aug. 31, 2006
SYN-501406	CDC-XIII-85-2	96.3	Mar. 31, 2006
NOA-459602	CDC-VIII-87-1	90.6	Nov. 30, 2005

The reference substances were logged in and then kept stored in a freezer after arrival at ETL. Syngenta Crop Protection, Inc. maintains the characterization and stability data for the reference substances.

On October 19, 2004 stock standards were prepared from the neat reference substances for use in preparing instrument calibration and fortification solutions. All stock standards were prepared as per the method. Fortification and working solutions were prepared from the stock standards on October 20, 2004. The stock standards and working solutions were kept stored in a refrigerator when not in use.

3.2 Control Water

Well Water:

Control ground well water, from a rural well near Rimbey, Alberta, was used to validate the method. The control water sample was characterized by Enviro-Test Laboratories in Edmonton.

The control well water was characterized for selected inorganic parameters as specified in the protocol. The complete characterization report can be found in Appendix 1.

Ground Well Water Characterization	
pH	7.6
Sodium	263 ppm
Calcium	40 ppm
Magnesium	14 ppm
Hardness mg equivalent CaCO3/L	159 ppm
Conductivity	1.360 mmhos/cm
Sodium Absorbtion Ratio (SAR)	9.1
Total Dissolved Solids	470 ppm
Total Suspended Solids	<3 ppm
Turbidity	0.20 NTU
Alkalinity	572 mg CaCO3/L

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Surface Water:

Control surface water, from Gull Lake, Alberta, was used to validate the method. The control water sample was characterized by Enviro-Test Laboratories in Edmonton.

The control surface water was characterized for selected inorganic parameters as specified in the protocol. The complete characterization report can be found in Appendix 2.

Surface Water Characterization	
pH	9.1
Sodium	250 ppm
Calcium	12.1 ppm
Magnesium	79.3 ppm
Hardness mg equivalent CaCO3/L	357 ppm
Conductivity	1.540 mmhos/cm
Sodium Absorbtion Ratio (SAR)	5.8
Total Dissolved Solids	1070 ppm
Total Suspended Solids	<3 ppm
Turbidity	0.80 NTU
Alkalinity	830 mg CaCO3/L

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3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in T003103-03 (Section 2.0 Materials and Methods). Identical or equivalent apparatus and materials were used.

4.0 METHOD AND METHOD MODIFICATIONS

4.1 Modifications

No modifications were made to the extraction section of the method. It was performed exactly as written. As a result of the difference in LC/MS/MS systems the following specific modifications to the method are noted:

1. A PE Sciex API 4000 MS/MS system was used in place of the PE Sciex API-III+ Mass Spectrometer. The instrument specifications are listed in Table 1 and Table 2.

2. Individual stock standard solutions were made up in 100 mL volumetric flasks.

3. An Aquasil C18 analytical column was used instead of a Devosil C18 analytical column for the analysis of CGA-293343, CGA-322704, CGA-355190, NOA-404617, SYN-501406 and NOA-459602.

4. Due to modification 3, NOA-404617 was analyzed in period 3 with CGA-293343, CGA-322704 and CGA-355190 instead of individually in a fourth period.

4.2 Sample Preparation, Fortification, and Extraction

Both validation trials consisted of one analytical set. This set consisted of 13 samples: one reagent blank, two matrix blanks, five matrix blanks fortified at the LOQ (0.05 ppb) and five matrix blanks fortified at 10X LOQ (0.5ppb).

Twelve (10) mL portions of water were used as samples. Samples designated as spikes were fortified with either 50 µL of a 10 µg/mL a mixed standard fortification solution (for LOQ fortifications) or 50 µL of 100 µg/mL (for 10X LOQ fortifications). See detailed method below:

Extraction:

1. Pipette representative amounts of water (10 ± 0.05 mL) into separate 15 mL disposable test tubes. From the fortified samples, 60 µL of sample were removed prior to acidifying and fortifying.

2. Add 10 µL of glacial acetic acid, cap and shake.

3. Fortify appropriate samples with either 50 µL of the 10 ppb solution mix or 50 µL of the 100 ppb solution mix. The samples were capped and mixed.

4. Transfer an aliquot of sample to a glass autosampler vial for analysis by LC with triple quadrupole mass spectrometric detection (LC/MS/MS).

4.3 LC/MS/MS Instrumentation

All samples were analyzed using an Applied Biosystems API-4000 Triple Quadrupole Mass Spectrometer with Turbo Ion Spray Interface. The following components completed the system:

HPLC: Two Perkin Elmer Series 200 Micropumps
Autoinjector: CTC HTS PAL
Column Heater: Waters Temp. Control module Millipore
Data System: Dell Precision 360, Intel(R) Pentium Computer, 4 CPU 3.00 GHz running Microsoft Windows 2000 Version 5 and Analyst Version 1.4

The HPLC operating parameters are shown in Table 1. The API 4000 MS/MS operating parameters are shown in Table 2.

4.4 Data Acquisition and Reporting

Peak integration and quantitation were performed by using Analyst, Version 1.4 (Applied Biosystems). Analytes were quantitated by external calibration. The MS detector response (peak area) versus the standard concentration was used to generate calibration curves for the analytes. Best-fit weighted 1/x linear regression equation for the curves were derived and these equations were used to calculate the concentration of analytes in the samples. Recovery results were computed for each sample. The equations used for quantitation are presented in Table 5.

Statistical treatment of the data includes calculation of averages, standard deviations, relative

standard deviations and confidence limits. The calculations were performed using Excel 97 SR-2. Results were rounded off for reporting purposes but not during calculations.

6.0 CONCLUSIONS

Syngenta Analytical Method No. T003103-03 is well written, concise and clear. The independent validation of the method was completed successfully on the first trial for both matrices. The validation recoveries were excellent indicating that the method is rugged. The method is suitable for determining residues of CGA-293343 and its degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406 and NOA-459602 down to a level of 0.05 ppb.

7.0 REFERENCES

1. Marlow, D. A., McDaniel, D. D., Dupuy, Jr. A. E., and Leovey, E. M. 1995. Data Reporting Guideline for Environmental Chemistry Methods - Pesticide Assessment Guidelines, Subdivisions N, E, and K. U.S. Environmental Protection Agency, Office of Pesticide Programs. EPA 733-B-95-001.

2. U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxic Substances (OPPTS). 1996. "Public Draft" - Data Reporting for Environmental Chemistry Methods, OPPTS 850.7100. EPA 712-C-96-348.

3. U.S. Environmental Protection Agency, Office of Compliance Monitoring. 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule, 40 CFR, Part 160. Federal Register, Vol. 54, No. 158: pp. 34052-34074.

4. U.S. Environmental Protection Agency. 1986. PR Notice 86-5. Standard format for data submitted under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and certain provisions of the Federal Food, Drug and Cosmetic Act (FFDCA).

5. Mayer, T. J., March 17, 2004. Syngenta Method No. T003103-03: “Analytical Method for the Determination of CGA-293343 and its Degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406 and NOA-459602 in Water by Direct Injection High Performance Liquid Chromatography with Mass Spectrometric Detection.”

8.0 TABLES

Table 1. HPLC System (Well Water)

(NOA-459602, SYN-501406, CGA-293343, CGA355190, CGA-322704, and NOA-404617)

Analytical Column:	Aquasil C18, 3 x 150 mm, 3 µm, Part No. #77503-153031
Guard Column:	Phenomenex Security Guard C 18 (3.0 x 4 mm), Part No.: AJO-4287
Mobile Phase Flow Rate:	500 µL/min
Mobile Phase A:	0.1% acetic acid in water
Mobile Phase B:	0.1% acetic acid in methanol
Run time:	16 minutes
Injection Volume:	100 µL

	Period 1 & 2	Period 3
Interface:	Turbo Ion-Spray	Turbo Ion Spray
Polarity:	Negative	Positive
Nebuliser Gas (GS1):	60	60
Turbo Gas (GS2):	60	60
Curtain Gas (CUR):	25 (arbitrary units)	15
Temperature (TEM):	600.0°C	600°C
Ion-Spray voltage:	-3500	5500
Collision gas (CAD):	Nitrogen 4 (arbitrary units)	3
Scan type:	MRM	MRM

Mobile Phase Program:

Duration (min.)	%A	%B
0.10	95.0	5.0
1.0	95.0	5.0
7.0	0	100.0
10.0	0	100.0
15.0	98.0	2.0
16.0	95.0	5.0

Analyte Retention times:

Analyte	Min.
NOA-459602	3.13
SYN-501406	4.31
NOA-404617	6.85
CGA-293343	6.97
CGA-322704	7.22
CGA-355190	7.66

Table 2. LC/MS/MS Operating Parameters (Well Water)

NOA-459602 (Period 1 Experiment 1) was analyzed using negative ion detection. The MRM (multiple reaction monitoring) scan mode was used for the signal acquisition.

	NOA-459602
Q1 Mass	335.90
Q3 Mass	204.80
Dwell time (msec)	1300
Resolution Q1	UNIT
Resolution Q3	UNIT
Declustering potential (DP)	-50
Entrance potential (EP)	-10
Collision energy (CE)	-28
Collision cell exit potential (CXP)	-7

Table 3. LC/MS/MS Operating Parameters (Well Water)

SYN-501406 (Period 2 Experiment 1) was analyzed using negative ion detection. The MRM (multiple reaction monitoring) scan mode was used for the signal acquisition.

	SYN-501406
Q1 Mass	293.80
Q3 Mass	192.70
Dwell time (msec)	600
Resolution Q1	UNIT
Resolution Q3	UNIT
Declustering potential (DP)	-55
Entrance potential (EP)	-10
Collision energy (CE)	-34
Collision cell exit potential (CXP)	-11

Table 4. LC/MS/MS Operating Parameters (Well Water)

NOA-404617, CGA-293343, CGA-322704 and CGA-355190 (Period 3 Experiment 1) were analyzed using positive ion detection. The MRM (multiple reaction monitoring) scan mode was used for the signal acquisition.

	CGA-293343	CGA-322704
Q1 Mass	291.90	249.80
Q3 Mass	211.00	132.10
Dwell time (msec)	150	150
Resolution Q1	UNIT	UNIT
Resolution Q3	UNIT	UNIT
Declustering potential (DP)	41	51
Entrance potential (EP)	-10	-10
Collision energy (CE)	17	21
Collision cell exit potential (CXP)	18	12

	CGA-355190	NOA-404617
Q1 Mass	247.80	236.90
Q3 Mass	175.00	175.00
Dwell time (msec)	150	150
Resolution Q1	UNIT	UNIT
Resolution Q3	UNIT	UNIT
Declustering potential (DP)	56	31
Entrance potential (EP)	-10	-10
Collision energy (CE)	29	17
Collision cell exit potential (CXP)	16	16

Table 5. HPLC System (Well Water)

(CGA-353042, and NOA-407475)

Analytical Column:	Zorbax 300-SCX, 2.1 x 150 mm, 5 μm, Part No. #883700-704
Guard Column:	Zorbax SCX guard column (4.6 x 12.5 mm, 5 μm), Part No.: 850950-904
Mobile Phase Flow Rate:	800 μL/min
Mobile Phase A:	50% acetonitrile/25 mM Ammonium Acetate in bottled water
Run time:	7 minutes
Injection Volume:	100 μL
Interface:	Turbo Ion-Spray
Polarity:	Positive
Scan type:	MRM

Mobile Phase Isocratic Program:

Duration (min.)	%A	%B
0.10	100.0	0
5.00	100.0	0
7.00	100.0	0

Analyte Retention times:

Analyte	Min.
CGA-353042	4.29
NOA-407475	4.46

Table 6. LC/MS/MS Operating Parameters (Well Water)

CGA-353042 and NOA-407475 (Period 1 Experiment 1) was analyzed using positive ion detection. The MRM (multiple reaction monitoring) scan mode was used for the signal acquisition.

	CGA-353042	NOA-407475
Q1 Mass	116.00	247.10
Q3 Mass	86.00	132.00
Dwell time (msec)	500	500
Resolution Q1	UNIT	UNIT
Resolution Q3	UNIT	UNIT
Declustering potential (DP)	45	55
Entrance potential (EP)	-10	-10
Collision energy (CE)	16	37
Collision cell exit potential (CXP)	8	12

Table 7. HPLC System (Surface Water)

(NOA-459602, SYN-501406, CGA-293343, CGA355190, CGA-322704, and NOA-404617)

Analytical Column:	Aquasil C18, 3 x 150 mm, 3 µm, Part No. #77503-153031
Guard Column:	Phenomenex Security Guard C 18 (3.0 x 4 mm), Part No.: AJO-4287
Mobile Phase Flow Rate:	500 µL/min
Mobile Phase A:	0.1% acetic acid in water
Mobile Phase B:	0.1% acetic acid in methanol
Run time:	16 minutes
Injection Volume:	50 µL

	Period 1 & 2	Period 3
Interface:	Turbo Ion-Spray	Turbo Ion Spray
Polarity:	Negative	Positive
Nebuliser Gas (GS1):	60	60
Turbo Gas (GS2):	60	60
Curtain Gas (CUR):	10 (arbitrary units)	15
Temperature (TEM):	600.0°C	600°C
Ion-Spray voltage:	-4000	5500
Collision gas (CAD):	Nitrogen 6 (arbitrary units)	3
Scan type:	MRM	MRM

Mobile Phase Program:

Duration (min.)	%A	%B
0.10	95.0	5.0
1.0	95.0	5.0
7.0	0	100.0
10.0	0	100.0
15.0	98.0	2.0
16.0	95.0	5.0

Analyte Retention times:

Analyte	Min.
NOA-459602	3.09
SYN-501406	4.04
NOA-404617	6.36
CGA-293343	6.38
CGA-322704	6.64
CGA-355190	7.07

Table 8. LC/MS/MS Operating Parameters (Surface Water)

NOA-459602 (Period 1 Experiment 1) was analyzed using negative ion detection. The MRM (multiple reaction monitoring) scan mode was used for the signal acquisition.

	NOA-459602
Q1 Mass	335.90
Q3 Mass	204.80
Dwell time (msec)	1300
Resolution Q1	UNIT
Resolution Q3	UNIT
Declustering potential (DP)	-50
Entrance potential (EP)	-10
Collision energy (CE)	-28
Collision cell exit potential (CXP)	-7

Table 9. LC/MS/MS Operating Parameters (Surface Water)

SYN-501406 (Period 2 Experiment 1) was analyzed using negative ion detection. The MRM (multiple reaction monitoring) scan mode was used for the signal acquisition.

	SYN-501406
Q1 Mass	293.80
Q3 Mass	192.70
Dwell time (msec)	600
Resolution Q1	UNIT
Resolution Q3	UNIT
Declustering potential (DP)	-55
Entrance potential (EP)	-10
Collision energy (CE)	-34
Collision cell exit potential (CXP)	-11

Table 10. LC/MS/MS Operating Parameters (Surface Water)

NOA-404617, CGA-293343, CGA-322704 and CGA-355190 (Period 3 Experiment 1) were analyzed using positive ion detection. The MRM (multiple reaction monitoring) scan mode was used for the signal acquisition.

	CGA-293343	CGA-322704
Q1 Mass	291.90	249.80
Q3 Mass	211.00	132.10
Dwell time (msec)	150	150
Resolution Q1	UNIT	UNIT
Resolution Q3	UNIT	UNIT
Declustering potential (DP)	41	51
Entrance potential (EP)	-10	-10
Collision energy (CE)	17	21
Collision cell exit potential (CXP)	18	12

	CGA-355190	NOA-404617
Q1 Mass	247.80	236.90
Q3 Mass	175.00	175.00
Dwell time (msec)	150	150
Resolution Q1	UNIT	UNIT
Resolution Q3	UNIT	UNIT
Declustering potential (DP)	56	31
Entrance potential (EP)	-10	-10
Collision energy (CE)	29	17
Collision cell exit potential (CXP)	16	16

Table 11. HPLC System (Surface Water)

(CGA-353042, and NOA-407475)

Analytical Column:	Zorbax 300-SCX, 2.1 x 150 mm, 3 μm, Part No. #883700-704
Guard Column:	Zorbax SCX guard column (4.6 x 12.5 mm, 5 μm), Part No.: 850950-904
Mobile Phase Flow Rate:	800 μL/min
Mobile Phase A:	50% acetonitrile/25 mM Ammonium Acetate in bottled water
Run time:	7 minutes
Injection Volume:	20 μL
Interface:	Turbo Ion-Spray
Polarity:	Positive
Scan type:	MRM

Mobile Phase Isocratic Program:

Duration (min.)	%A	%B
0.10	100.0	0
5.00	100.0	0
7.00	100.0	0

Analyte Retention times:

Analyte	Min.
CGA-353042	3.91
NOA-407475	4.13

Table 12. LC/MS/MS Operating Parameters (Surface Water)

CGA-353042 and NOA-407475 (Period 1 Experiment 1) was analyzed using positive ion detection. The MRM (multiple reaction monitoring) scan mode was used for the signal acquisition.

	CGA-353042	NOA-407475
Q1 Mass	116.00	247.10
Q3 Mass	86.00	132.00
Dwell time (msec)	500	500
Resolution Q1	UNIT	UNIT
Resolution Q3	UNIT	UNIT
Declustering potential (DP)	45	55
Entrance potential (EP)	-10	-10
Collision energy (CE)	16	37
Collision cell exit potential (CXP)	8	12

Table 17. Clarifications, Communication, and Recommendations to perform Analytical

Method No. T003103-03
Minimal communication was required for this ILV. The method was clearly written and easy to follow.

Table 18. Calculations

Peak areas and external calibrations were used for data analysis. The Analyst Version 1.4 quantitation software package was used to calculate a best fit, 1/x weighted line of the standards. Extract concentration found was determined from the analyte peak area versus the calibration.

a) Calculated Concentration in Samples:

$$\text{Calc.Conc.(ppb)} = \frac{(x - b)}{m} \times \text{D.F.}$$

Where:
x = Peak Area of the analyte
b = Intercept from weighted 1/x regression analysis (Peak Area)
m= Slope from weighted 1/x regression analysis (response per concentration)
D.F. = Dilution Factor

$$\text{D.F.} = \frac{\text{Final Volume (mL)}}{\text{Sample Volume (mL)}} = \frac{10.0\,\text{mL}}{10\,\text{mL}} = 1.0$$

The Analyst data processing software generates both the slope and intercept.

The calculation of averages, standard deviations, relative standard deviations and 95% confidence limits were performed in Excel.

The report percent recoveries shown on Table 13 and Table 15 may not exactly match the corresponding recoveries on the Analyst Result tables shown in Appendix 3. This is because Analyst uses a large string of un-rounded numbers to calculate the percent recoveries.