Addendum #1 to Data Evaluation Record of MRIDs 46950128 and 46950214

MRIDs: 46950128 / 46950214
PC Code: 128847
OCSPP Guideline: 835.6100. Environmental Chemistry Method (ECM) and Associated Independent Laboratory Validation (ILV)

Date: May 19, 2017

Study Title: Determination of Difenoconazole and Its Metabolites CGA-205375, CGA-142856 and CGA-71019 in Soil, Using Liquid Chromatography-Electrospray ionization Tandem Mass Spectrometry.

Changes Made: Study classification is being upgraded to **supplemental** (from the former classification of unacceptable).

Rationale for Upgrade: Syngenta provided a copy of a letter from Anne Beaubien, QA officier of ALS laboratory at Edmonton, Alberta and the personnel sheet obtained from raw data archives of the people who were involved with the ILV of this analytical method (MRID 46950128). Ms. Beaubien confirmed that the participants involved in conducting the ILV in 2006 were independent of the method development that was performed by Enviro-Test Laboratories in 2005 by Russell Gottschalk (MRID 46950214).

The submitted information addresses one of the deficiencies listed in the Data Evaluation Record (DER) for MRIDs 46950128 and 46950214 (USEPA, 2016; DP 434978). Therefore, the study classifications were upgraded from "**unacceptable**" to "**supplemental**".

Reference

Difenoconazole: Transmittal of an Environmental Fate DER on Analytical Method for the Determination of Difenoconazole and Its Metabolites CGA-205375, CGA-142856 and CGA-71019 in Soil. Environmental Fate and Effects Division, Office of Pesticide Programs, United States Environmental Protection Agency (D434978; August 10, 2016).

Revised by: Faruque Khan **Date:** May 19, 2017

Secondary reviewed by: Greg Orrick **Date:** May 19, 2017

Analytical method for difenoconazole, and its metabolites, CGA-205375, CGA-142856 and CGA-71019, in soil

Reports: ECM: EPA MRID No.: 46950128. Gottschalk, R. 2005. DIFENOCONAZOLE: METHOD: Determination of Difenoconazole and Its Metabolites CGA-205375, CGA-142856 and CGA-71019 in Soil, Using Liquid Chromatography-Electrospray ionization Tandem Mass Spectrometry. Enviro-Test Laboratories Method M 314. Syngenta No. T013656-05. Report prepared by Enviro-Test Laboratories, Alberta, Canada, and sponsored and submitted by Syngenta Crop Protection, Inc., Greensboro, North Carolina; 70 pages. Final report issued December 15, 2005.

ILV: EPA MRID No. 46950214. McLean, N. 2006. DIFENOCONAZOLE: INDEPENDENT LABORATORY VALIDATION: DETERMINATION OF DIFENOCONAZOLE AND ITS METABOLITES CGA-205375, CGA-142856 AND CGA-71019 IN SOIL, USING LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY: ENVIRO-TEST LABORATORIES METHOD M 314: SYNGENTA METHOD T013656-05: FINAL REPORT. Study No.: 05ILV11SYN. ETL Report No.: 06SYN181.REP. Syngenta No. T002596-05. Report prepared by ALS Laboratory Group – Environmental Division, Formerly Enviro-Test Laboratories, Alberta, Canada, sponsored and submitted by Syngenta Crop Protection, Inc., Greensboro, North Carolina; 74 pages. Final report issued June 22, 2006.

Document No.: MRIDs 46950128 & 46950214

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with the USEPA FIFRA Good Laboratory Practice (GLP) standards (p. 3 of MRID 46950128). Signed and dated No Data Confidentiality, GLP, Authenticity and Quality Assurance statements were provided (pp. 2-3, 5). A certification of authenticity was included with the Quality Assurance statement.

ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (p. 3 of MRID 46950214). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5, 7 of MRID 46950214). An authenticity statement was included with the GLP and Quality Assurance statements.

Classification: This analytical method is classified as Unacceptable. It could not be determined if the ILV was independent of the ECM due to lack of personnel and communication information. In the ECM, the number of samples was insufficient (n = 3) for all analyses. All ILV linear regressions were unsatisfactory. Representative chromatograms and raw data were not provided for two of the three soil matrices in the ECM. This analytical method is upgradeable with the submission of personnel and communication information that confirms the analysts, study director, equipment, instruments, and supplies of the two laboratories (which were part of the same facility) were distinct and operated separately and without collusion, and that the analysts and study director of the ILV were unfamiliar with the

method both in its development and subsequent use in field studies. The reporting standard for this analytical method is high because the laboratories conducting both validations were located at the same facility.

PC Code: 128847
Reviewer: Lewis Ross Brown
Environmental Biologist
Signature: Brown, Lewis 2016.08.11 10:41:27 -04'00'
Date: August 3, 2016

All page citations for MRID 46950214 refer to those listed at the right, bottom-most portion of the pages.

Executive Summary

This analytical method, Enviro-Test Laboratories Method M 314 and Syngenta Method T013656-05, is designed for the quantitative determination of difenoconazole and its metabolites, CGA-205375, CGA-142856 and CGA-71019, in soil using LC/MS/MS. The method is quantitative for all three analytes at the stated LOQ of 1.00 ng/g (1.0 ppb). The LOQ is less than the lowest toxicological level of concern in soil for all four analytes. The ECM validated the method using sandy clay loam, loam and loamy sand soils; the ILV validated the method using sandy clay loam soil, the same soil sample as the ECM. The number of trials was not specified, but the reviewer assumed that method was validated by the ILV with the first trial with minor modifications to the analytical method for optimization. However, it could not be determined if the ILV was independent of the ECM due to lack of personnel and communication information; the same laboratory performed the ECM and ILV. In the ECM, the number of samples was insufficient (n = 3) for all analyses and chromatograms and raw data were not provided for two of the three soil matrices. All ILV linear regressions were unsatisfactory (r^2 <0.995), and non-optimal peak shapes were observed in the ILV chromatograms for CGA-71019 and CGA-142856 at the LOQ.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Difenoconazole (CGA-169374)	46950128	46950214		Soil[2,3]	15/12/2005	Syngenta Crop Protection, Inc.	LC/MS/MS	1.00 ng/g; 1.00 ppb
CGA-205375								
CGA-142856								
CGA-71019								

1 Difenoconazole (CGA-169374) = Cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether. CGA-205375 = 1-[2-Chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanol. CGA-142856 = 4-(Methylsulfonyl)-2-nitro-benzoic acid. CGA-71019 = 1,2,4-Triazole.

2 Characterized sandy clay loam soil (56% sand, 22% silt, 22% sand; pH 6.0; 3.5% organic matter) from Grand Forks County, North Dakota, loam soil (48% sand, 40% silt, 12% clay; pH 8.2; 0.8% organic matter) from Tulare County, California, and loamy sand soil (85% sand, 10% silt, 5% clay; pH 7.1; 0.9% organic matter) were used in the ECM validation (USDA soil texture classification; Table 1, p. 36; Appendix 3, pp. 68-70 of MRID 46950128). The data for the sandy clay loam, loam and loamy sand soils was taken from Syngenta Study No.s T002983-03, T002984-03 and T002985-03, respectively.

3 Characterized sandy clay loam soil (56% sand, 22% silt, 22% sand; pH 6.0; 3.5% organic matter) from North Dakota (0-6") was used in the ILV validation (USDA soil texture classification; p. 13; Appendix 3, p. 69 of MRID

46950214). The data for the sandy clay loam soil was taken from Syngenta Study No. T002983-03; this was the same soil as the ECM sandy clay loam soil.

I. Principle of the Method

The method contained the following precautions: 1) disposable glassware should be used, if specified, clean glassware/plasticware should always be used; 2) high-purity solvents should be used; and 3) glassware should be washed between batches (pp. 16-17 of MRID 46950128).

Soil samples were homogenized prior to experiment (p. 17; Figure 1, p. 45; of MRID 46950128). Samples of soil (10.0 g) were weighed into 250-mL round bottom flasks. After fortification, as necessary, the soil was extracted with 100 mL of acetonitrile:0.3% formic acid in water (70:30, v:v) by refuxing on a heating mantle for 1 hour. After cooling to room temperature, 45 mL of the mixture was transferred to a 50-mL polypropylene centrifuge tube; the remainder of the solution was discarded. After centrifugation (3500 rpm for 5 minutes), the sample was stored cool.

For difenoconazole and CGA-205375 analysis, 0.5 mL of the supernatant of the centrifuged sample (equivalent to 0.050 g of soil) was mixed with 0.5 mL water in an autosampler vial (p. 17; Figure 1, p. 45 of MRID 46950128). After capping and vortexing, the sample was analyzed via LC/MS/MS.

For CGA-71079 analysis, 1.0 mL of the supernatant of the centrifuged sample (equivalent to 0.1 g of soil) was transferred to a screw-capped glass test tube (15-mL; pp. 17-18; Figure 1, pp. 45-46 of MRID 46950128). Derivatization was carried-out by adding 1 mL of 0.1 M sodium bicarbonate solution, 20 µL of 10% ammonium hydroxide, 100 µL of 10% EDTA and 100 µL of 50 mM dansyl chloride in acetone solution. The mixture was heated to 40°C on a heating block for 30 minutes, protected from direct light with aluminium foil. After cooling for 10 minutes, 2 mL of dichloromethane was added. After vortexing for 30 seconds, 5 mL of water was added. After centrifugation (1000 rpm for 1 minute), the lower layer was transferred to a 4-mL test tube. The dichloromethane was evaporated completely under a stream of clean, dry nitrogen. The residue was reconstituted in 1.0 mL of pH 11 water:acetonitrile (60:40, v:v) and analyzed by LC/MS/MS. The method noted that samples need to be stored deep-frozen if not analyzed immediately. Also, the sample tray of the LC/MS/MS should be maintained at 3-7°C.

For CGA-71079 analysis, 20 mL of the supernatant of the centrifuged sample (equivalent to 2.0 g of soil) was transferred to a disposable, screw-capped glass test tube (40-mL) and acidified with 400 µL concentrated acetic acid (pp. 15-16, 18-19; Figure 1, pp. 46-47 of MRID 46950128). After mixing the sample via inversion, the sample was transferred to a prepared cation exchange column (Bio-Rad AG 50W-X4, 200-400 mesh size). The column was prepared by mixing *ca*. 200 g of the resin with 1 L of high purity water and 1.0 mL concentrated formic acid. This resin slurry was transferred to a 10 mL polypropylene column (16 mm ID x 80 mm) for a resin bed height of 2.5 cm (5 mL). The water was drained from the resin via gravity, and the column was washed using gravity with 10 mL of water and 10 mL of acetonitrile:2% formic acid in water (70:30, v:v). After the sample was transferred to the prepared cation exchange column, the sample tube was rinsed with 5 mL of water which was then added to the cation exchange column. After the rinse drained under gravity, the analyte was eluted using 20 mL of methanol:concentrated ammonium (75:25, v:v) under gravity into a 125-mL round bottom flask. The eluate was reduced to dryness under reduced pressure at 35-40°C (water bath). The residue was reconstituted in 4.0 mL acetonitrile:0.3% formic

acid in water (50:50, v:v). After ultrasonication, 1.0 mL of the sample was transferred to an autosampler vial for analysis via LC/MS/MS.

Samples were analyzed for difenoconazole and CGA-205375 by reversed-phase silica-based HPLC, using a Perkin Elmer Series 200 coupled with an Applied Biosystems Sciex API 4000 Triple Quad in Turbo Spray Ionization Mode (600°C; pp. 21-23 of MRID 46950128). The following LC conditions were used: Aquasil C18 column (150 x 3.0 mm, 3 µm; column temperature 40°C), mobile phase of (A) 0.2% formic acid in water and (B) acetonitrile [percent A:B (v:v) at 0.0-1.0 min. 50:50, 4.0-7.0 min. 10:90, 7.1-10 min. 50:50], and injection volume of 25 µL. The following MS/MS conditions were used: positive ion polarity and multiple reaction monitoring (MRM). One ion pair transition was monitored for each analyte: *m/z* 406.0 → 251.1 for difenoconazole and *m/z* 350.1 → 69.9 for CGA-205375. Expected retention times were *ca*. 6.0 (doublet) and 4.9 minutes for difenoconazole and CGA-205375, respectively.

Samples were analyzed for CGA-71019 (as the dansyl derivative) by reversed-phase silica-based HPLC, using a Perkin Elmer Series 200 coupled with an Applied Biosystems Sciex API 4000 Triple Quad in Turbo Spray Ionization Mode (600°C; pp. 21, 23-25 of MRID 46950128). The following LC conditions were used: Aquasil C18 column (150 x 3.0 mm, 3 µm; column temperature 40°C), mobile phase of (A) 0.2% acetic acid in water and (B) acetonitrile [percent A:B (v:v) at 0.0-1.0 min. 60:40, 4.0-8.0 min. 10:90, 8.1-10 min. 60:40], and injection volume of 50 µL. The following MS/MS conditions were used: positive ion polarity and multiple reaction monitoring (MRM). One ion pair transition was monitored for CGA-71019 dansyl derivative: *m/z* 303.0 → 181.00. Expected retention time was *ca*. 5.3 minutes for CGA-71019 dansyl derivative.

Samples were analyzed for CGA-142856 by normal-phase HPLC on a pentafluorophenyl phase with a propyl spacer (pPFP), using a Perkin Elmer Series 200 coupled with an Applied Biosystems Sciex API 4000 Triple Quad in Turbo Spray Ionization Mode (600°C; pp. 21, 25-27 of MRID 46950128). The following LC conditions were used: Allure PFP Propyl column (250 x 3.2 mm, 5 µm; column temperature 40°C), mobile phase of (A) 20% 5 mM ammonium acetate at pH 4.5 in acetonitrile and (B) acetonitrile [percent A:B (v:v) at 0.0-2.0 min. 0:100, 6.0-8.0 min. 100:0, 10 min. 0:100], and injection volume of 50 µL. The following MS/MS conditions were used: negative ion polarity and multiple reaction monitoring (MRM). One ion pair transition was monitored for CGA-142856: *m/z* 125.80 → 81.9. Expected retention time was *ca*. 4.2 (3.5-6) minutes for CGA-142856. The method recommended equilibrating the Allure pPFP column prior to use with 100% acetonitrile at 1.0 mL/min for 1 hour, followed by 20% 5 mM ammonium acetate at pH 4.5 in acetonitrile at 0.5 mL/min for 1 hour, followed by 5% ammonium acetate at pH 4.5 in acetonitrile at 1.0 mL/min for 1 hour.

The method noted the following potential problems with the procedure: 1) preparation of the dansyl triazole derivative can be problematic due to the sensitivity of the dansyl chloride; 2) CGA-71019 contamination in the soil is common; 3) optimization of the cation exchange cartridge or elution solvent may be required if the recoveries are low; 4) the resin must not leak into the collection flask; 5) optimization of LC/MS/MS sample concentration may be required for CGA-142856 due to low sensitivity; and 6) optimization of the Allure pPFP column may be required by altering the aqueous percentage of the mobile phase (p. 30 of MRID 46950128).

In the ILV, the method was performed as written, except for minor modifications of the analytical parameters: the difenoconazole and CGA-205375 HPLC gradient final LC step was extended from 10 to 12 minutes, the dansyl triazole HPLC gradient final LC step was extended from 10 to 13

minutes, the injection volumes of the dansyl triazole and CGA-142856 autosampler were reduced from 50 to 25 µL, and the maximum flow rates of the CGA-142856 HPLC gradient were reduced from 1.0 to 0.8 mL/min (pp. 13-15, 17; Tables 5-10, pp. 24-29 of MIRD 46950214). The Perkin Elmer Series 200 coupled with a PE-Applied Biosystems Sciex API 3000 was used for all analyses. Also, for difenoconazole, CGA-205375 and CGA-71019 analysis, a Keystone Aquasil C18 column (150 x 3.0 mm, 3 µm) with guard column was specified. The ILV study author noted the following critical step: the dansyl derivative of CGA-71019 is unstable and must be kept in the freezer pending analysis. The ILV study author also noted the following potential interference of soil containing background amounts of CGA-71019 and/or CGA-142856.

The LOQ for difenoconazole, CGA-205375, CGA-142856 and CGA-71019 was reported as 1.0 ng/g (1.0 ppb) in the ECM and the ILV (pp. 32, 34 of MRID 46950128; p. 11; Tables 1-4, pp. 20-23 of MRID 46950214). The LOD for all analytes was estimated as 0.50 ppb in the ECM and ILV. In the ECM, the method LOD for each analyte based on the lowest external standard concentration run was 0.625 pg injected for difenoconazole and CGA-205375, 2.5 pg injected for CGA-71019 and 12.5 pg injected for CGA-142856. The LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 46950128): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of difenoconazole, CGA-205375, CGA-142856 and CGA-71019 at the LOQ, 10×LOQ and 100×LOQ in three soil matrices (Tables 2-9, pp. 37-42). The number of samples was insufficient for all analyses (n = 3). Only one ion transition was monitored for each analyte; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Procedural recoveries were corrected for residues quantified in the controls at > 1/3 of the LOQ (pp. 27-29; Tables 6-9, pp. 39-42). Data for controls and chromatograms were only provided for one of the three soil matrices, North Dakota sandy clay loam; no residues in the controls were quantified at > 1/3 of the LOQ. Standard deviations for the analytes in loam and loamy sand soils were reviewer-calculated based on data provided in Tables 2-5, pp. 37-38 since the study author did not provide the s.d. values for these soils (see DER Attachment 2). The soils were fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; Table 1, p. 36; Appendix 3, pp. 68-70). Sandy clay loam soil (56% sand, 22% silt, 22% sand; pH 6.0; 3.5% organic matter) from Grand Forks County, North Dakota, loam soil (48% sand, 40% silt, 12% clay; pH 8.2; 0.8% organic matter) from Tulare County, California, and loamy sand soil (85% sand, 10% silt, 5% clay; pH 7.1; 0.9% organic matter) were used in the study. The data for the sandy clay loam, loam and loamy sand soils was taken from Syngenta Study No.s T002983-03, T002984-03 and T002985-03, respectively.

ILV (MRID 46950214): Mean recoveries and RSDs were within guideline requirements for analysis of difenoconazole, CGA-205375, CGA-142856 and CGA-71019 at the LOQ and 10×LOQ in one soil matrix (Tables 1-4, pp. 20-23). Fully characterized sandy clay loam soil (56% sand, 22% silt, 22% sand; pH 6.0; 3.5% organic matter) from North Dakota (0-6") was used in the study (USDA soil texture classification; p. 13; Appendix 3, p. 69). The data for the sandy clay loam soil was taken from Syngenta Study No. T002983-03; this was the same soil as the ECM sandy clay loam soil. The number of trials was not specified, but the reviewer assumed that method was validated with the first trial (pp. 11, 17).

Table 2. Initial Validation Method Recoveries for Difenoconazole, CGA-205375, CGA-142856 and CGA-71019 in Soil

Analyte[1]	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[2]	Relative Standard Deviation (%)
Sandy Clay Loam Soil[3]						
Difenoconazole (CGA-169374)	1.00 (LOQ)	3	104-108	107	2.3	2.2
	10.0	3	103-108	105	2.6	2.5
	100	3	82-107	96	13	13
CGA-2053375	1.00 (LOQ)	3	83-91	87	4.0	4.6
	10.0	3	97-100	99	1.5	1.5
	100	3	81-116	98	18	18
CGA-71019	1.00 (LOQ)	3	91-102	97	5.6	5.7
	10.0	3	94-106	100	6.0	6.0
	100	3	93-114	107	12	11
CGA-142856	1.00 (LOQ)	3	76-102	90	13	15
	10.0	3	74-86	78	6.7	8.5
	100	3	76-83	79	3.5	4.4
Loam Soil[3]						
Difenoconazole (CGA-169374)	1.00 (LOQ)	3	81-113	95	17	17
	10.0	3	77-81	80	2	2.9
	100	3	88-90	89	1	1.1
CGA-2053375	1.00 (LOQ)	3	78-89	85	6	6.9
	10.0	3	81-90	86	5	5.3
	100	3	85-90	88	3	3.0
CGA-71019	1.00 (LOQ)	3	95-111	101	9	8.4
	10.0	3	73-98	89	14	16
	100	3	75-109	96	18	19
CGA-142856	1.00 (LOQ)	3	87-111	98	12	13
	10.0	3	87-104	96	9	8.9
	100	3	91-96	94	3	2.7
Loamy Sand Soil[3]						
Difenoconazole (CGA-169374)	1.00 (LOQ)	3	109-120	113	6	5.2
	10.0	3	96-116	106	10	9.4
	100	3	105-118	112	7	5.8
CGA-2053375	1.00 (LOQ)	3	114-119	116	3	2.2
	10.0	3	93-114	102	11	11
	100	3	103-108	105	3	2.5
CGA-71019	1.00 (LOQ)	3	98-119	109	11	9.7
	10.0	3	102-105	103	2	1.5
	100	3	105-109	106	2	2.2
CGA-142856	1.00 (LOQ)	3	98-103	101	3	2.6
	10.0	3	98-104	101	3	3.0
	100	3	84-109	95	13	13

Data (recoveries were corrected for residues quantified in the controls at > 1/3 of the LOQ; pp. 27-29; Tables 6-9, pp. 39-42) were obtained from Tables 2-9, pp. 37-42 of MRID 46950128 and DER Attachment 2.

1 Difenoconazole (CGA-169374) = Cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether. CGA-205375 = 1-[2-Chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanol. CGA-142856 = 4-(Methylsulfonyl)-2-nitro-benzoic acid. CGA-71019 = 1,2,4-Triazole.

2 Standard deviations for the analytes in loam and loamy sand soils were reviewer-calculated based on data provided in Tables 2-5, pp. 37-38 since the study author did not provide the s.d. values for these soils (see DER Attachment 2).

3 Sandy clay loam soil (56% sand, 22% silt, 22% sand; pH 6.0; 3.5% organic matter) from Grand Forks County, North

Dakota, loam soil (48% sand, 40% silt, 12% clay; pH 8.2; 0.8% organic matter) from Tulare County, California, and loamy sand soil (85% sand, 10% silt, 5% clay; pH 7.1; 0.9% organic matter) were used in the study (USDA soil texture classification; Table 1, p. 36; Appendix 3, pp. 68-70). The data for the sandy clay loam, loam and loamy sand soils was taken from Syngenta Study No.s T002983-03, T002984-03 and T002985-03, respectively.

Table 3. Independent Validation Method Recoveries for Difenoconazole, CGA-205375, CGA-142856 and CGA-71019 in Soil

Analyte[1]	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil[2]						
Difenoconazole (CGA-169374)	1 (LOQ)	5	71-86	79	5.9	7.3
	10	5	71-87	80	7.2	9.0
CGA-2053375	1 (LOQ)	5	76-102	87	10.4	12.0
	10	5	88-96	92	3.2	3.5
CGA-71019	1 (LOQ)	5	88-120	108	13.7	12.7
	10	5	94-114	101	7.8	7.7
CGA-142856	1 (LOQ)	5	75-117	88	17.1	19.4
	10	5	81-90	86	4.1	4.8

Data (uncorrected recovery results, Appendix 4, pp. 71-74) were obtained from Tables 1-4, pp. 20-23 of MRID 46950214.

1 Difenoconazole (CGA-169374) = Cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether. CGA-205375 = 1-[2-Chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanol. CGA-142856 = 4-(Methylsulfonyl)-2-nitro-benzoic acid. CGA-71019 = 1,2,4-Triazole.

2 Sandy clay loam soil (56% sand, 22% silt, 22% sand; pH 6.0; 3.5% organic matter) from North Dakota (0-6") was used in the study (USDA soil texture classification; p. 13; Appendix 3, p. 69). The data for the sandy clay loam soil was taken from Syngenta Study No. T002983-03; this was the same soil as one of the ECM soils.

III. Method Characteristics

The LOQ for difenoconazole, CGA-205375, CGA-142856 and CGA-71019 was reported as 1.0 ng/g (1.0 ppb) in the ECM and the ILV (pp. 32, 34 of MRID 46950128; p. 11; Tables 1-4, pp. 20-23 of MRID 46950214). In the ECM, the LOQ was defined as the lowest analyte concentration which yielded a mean recovery of 70-120% and relative standard deviation of ≤20%. No justifications of the LOQ were provided in the ILV. The LOD for all analytes was estimated as 0.50 ppb in the ECM and ILV. In the ECM, the method LOD for each analyte based on the lowest external standard concentration run was 0.625 pg injected for difenoconazole and CGA-205375, 2.5 pg injected for CGA-71019 and 12.5 pg injected for CGA-142856. In the ECM, the LOD was defined as the smallest standard amount injected during the chromatographic run. The ECM study author noted that the LOD was approximately equivalent to half of the theoretical amount for a recovery sample at the method LOQ.

Table 4. Method Characteristics

Analyte[1]		Difenoconazole	CGA-205375	CGA-71019	CGA-142856
Limit of Quantitation (LOQ)		1.0 ng/g (1.0 ppb)			
Limit of Detection (LOD)	ECM/ILV	0.50 ppb (*ca*. 50% of the LOQ)			
	ECM	0.625 pg[2]		2.5 pg[2]	12.5 pg[2]
Linearity (calibration curve r^2 and concentration range)	ECM[3]	r^2 = 0.9992 (0.025-5.00 ppb)	r^2 = 0.9996 (0.025-5.00 ppb)	r^2 = 0.9998 (0.05-20.0 ppb)	r^2 = 0.9990 (0.25-100 ppb)
	ILV[4]	r^2 = **0.9948** (0.025-0.25 pg/µL)	r^2 = **0.9839** (0.025-0.25 pg/µL)	r^2 = **0.9809** (0.05-2 pg/µL)	r^2 = **0.9920** (0.25-2.5 pg/µL)
Repeatable	ECM[5]	Yes at LOQ, 10×LOQ and 100×LOQ, but n = **3**.			
	ILV[6]	Yes at LOQ and 10×LOQ.			
Reproducible		Yes at LOQ and 10×LOQ.			
Specific	ECM	Yes. Interferences were quantified as 23% of LOQ. Baseline noise was noted at LOQ.	Yes. No matrix interferences were observed.	Yes. Interferences were quantified as 7% of LOQ.	Yes. No matrix interferences were observed. Baseline noise was noted at LOQ.
		Only chromatograms for sandy clay loam soil were provided.			
	ILV	Yes. No matrix interferences were observed.		Yes, no matrix interferences were noted; however, peak shape was very choppy at LOQ, and baseline noise was noted.[7]	Yes, no matrix interferences were noted; however, peak shape was very broad at LOQ.[8]

Data were obtained from pp. 32, 34; Tables 2-9, pp. 37-42 (recovery results); Table 10, pp. 43-44 (calibration data); Figures 3-10, pp. 50-57 (sandy clay loam chromatograms); Figures 11-14, pp. 58-61 (calibration curves) of MRID 46950128; p. 11; Tables 1-4, pp. 20-23 (recovery results); Appendix 1, pp. 32-35 (raw data); Appendix 2, Figures 1-4, pp. 37-40 (calibration curves); Appendix 2, Figures 5-22, pp. 41-58 (chromatograms) of MRID 46950214; DER Attachment 2.

1 Difenoconazole (CGA-169374) = Cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether. CGA-205375 = 1-[2-Chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanol. CGA-142856 = 4-(Methylsulfonyl)-2-nitro-benzoic acid. CGA-71019 = 1,2,4-Triazole.

2 The method LOD for each analyte based on the lowest external standard concentration run (p. 32 of MRID 46950128).

3 ECM correlation coefficients for all analytes were reviewer-calculated from r values of 0.9995-0.9999 (analytes combined; Figures 11-14, pp. 58-61 of MRID 46950128; DER Attachment 2). The calibration data and curves were only provided for the ND sandy clay loam soil matrix.

4 ILV correlation coefficients for all analytes were reviewer-calculated from r values of 0.9904-9974 (analytes combined; Appendix 1, pp. 32-35; Figures 1-4, pp. 37-40 of MRID 46950214; DER Attachment 2).

5 Characterized sandy clay loam soil (56% sand, 22% silt, 22% sand; pH 6.0; 3.5% organic matter) from Grand Forks County, North Dakota, loam soil (48% sand, 40% silt, 12% clay; pH 8.2; 0.8% organic matter) from Tulare County, California, and loamy sand soil (85% sand, 10% silt, 5% clay; pH 7.1; 0.9% organic matter) were used in the ECM validation (USDA soil texture classification; Table 1, p. 36; Appendix 3, pp. 68-70 of MRID 46950128). The data for the sandy clay loam, loam and loamy sand soils was taken from Syngenta Study No.s T002983-03, T002984-03 and T002985-03, respectively.

6 Characterized sandy clay loam soil (56% sand, 22% silt, 22% sand; pH 6.0; 3.5% organic matter) from North Dakota (0-6") was used in the ILV validation (USDA soil texture classification; p. 13; Appendix 3, p. 69 of MRID 46950214). The data for the sandy clay loam soil was taken from Syngenta Study No. T002983-03; this was the same soil as the ECM sandy clay loam soil.

7 Based on Appendix 2, Figure 12, p. 48 and Figure 15, p. 51 of MRID 46950214.

8 Based on Appendix 2, Figure 13, p. 49 and Figure 16, p. 52 of MRID 46950214.

A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. It could not be determined if the ILV was independent of the ECM due to lack of personnel and communication information. According to the OCSPP guidelines, if the laboratory that conducted the validation belonged to the same organization as the originating laboratory, the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion, and the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies. The laboratory which performed the ILV, ALS Laboratory Group – Environmental Division, Formerly Enviro-Test Laboratories, Alberta, Canada, was the same as that which performed the ECM, Enviro-Test Laboratories, Alberta, Canada (pp. 1, 9 of MRID 46950128; pp. 1, 8 of MRID 46950214). The study authors of the ECM and ILV were distinct, as were the analytical laboratory equipment; however, a full list of the laboratory personnel was not listed in the ECM for comparison. Also, the communication between the ECM and ILV was not reported or discussed in the ILV. The ILV study author only reported that communication with the Study Sponsor, Syngenta Crop Protection, Inc., was limited to updates on progress.

   Additionally, the North Dakota sandy clay loam soil which was used in the ILV study was the same soil as the ECM North Dakota sandy clay loam soil; both soil matrices were reportedly taken from Syngenta Study No. T002983-03 (Table 1, p. 36; Appendix 3, pp. 68-70 of MRID 46950128; p. 13; Appendix 3, p. 69 of MRID 46950214). This soil matrix was the most difficult soil matrix tested by the ECM.

2. In the ECM analysis, the number of samples was insufficient (n = 3) for all analyses at the LOQ, 10×LOQ and 100×LOQ (Tables 2-9, pp. 37-42 of MRID 46950128). OCSPP guidelines recommend that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

3. All of the ILV linear regressions were unsatisfactory (r^2 <0.995): 0.9809-9948 (analytes combined; Appendix 1, pp. 32-35; Figures 1-4, pp. 37-40 of MRID 46950214; DER Attachment 2).

4. In the ECM, sample chromatograms are only provided for one of the three soil matrices, North Dakota sandy clay loam. Also, chromatograms of the reagent blanks were not included. OCSPP guidelines states that representative chromatograms should be provided for reagent blanks, matrix blanks, standard curves, and spiked samples at the LOQ and 10× LOQ for all analytes in each matrix.

5. In the ILV chromatograms, non-optimal peak shapes were observed for CGA-71019 and CGA-142856 at the LOQ (Appendix 2, Figures 12-13, pp. 48-49 and Figures 15-16, pp. 51-52 of MRID 46950214).

6. The estimations of the LOQ in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 32, 34 of MRID 46950128; p. 11; Tables 1-4, pp. 20-23 of MRID 46950214). No calculations were reported in ECM or ILV to support the method LOQ. In the ECM, the LOQ was defined as the lowest analyte concentration which yielded a mean recovery of 70-120% and relative standard deviation of ≤20%. No justifications of the LOQ were provided in the ILV. The LOD for all analytes was estimated

as 0.50 ppb in the ECM and ILV. The ECM study author noted that the LOD was approximately equivalent to half of the theoretical amount for a recovery sample at the method LOQ.

Additionally, the lowest toxicological level of concern in soil for the analytes was not reported in the ECM and ILV. An LOQ above toxicological levels of concern results in an unacceptable method classification.

7. The reviewer noted that the LOQ and LOD were reported as different values in the ILV report. In Tables 1-4, the LOQ and LOD were reported as 1.0 ppb and 0.5 ppb, respectively, which were the same values as those of the ECM; however, in Appendix 2, Figures 23-, the analytical standards which represented the LOQ and LOD were reported as 0.05 ppb and 0.025 ppb, respectively, for difenoconazole and CGA-205375, 0.1 ppb and 0.5 ppb, respectively, for CGA-71019, 0.5 ppb and 0.25 ppb, respectively, for CGA-142856 (Tables 1-4, pp. 20-23; Appendix 2, Figures 23-28, pp. 59-64 of MRID 46950214).

8. The method calculations reported that procedural recoveries were corrected for residues quantified in the controls at > 1/3 of the LOQ; however, no residues in the controls were quantified at > 1/3 of the LOQ (pp. 27-29; Tables 6-9, pp. 39-42 of MRID 46950128). The reviewer noted that raw data for controls were only provided for one of the three soil matrices, North Dakota sandy clay loam. Procedural recoveries were not corrected in the ILV (Appendix 4, pp. 71-74 of MRID 46950214).

9. ILV modifications of analytical method were minor: the difenoconazole and CGA-205375 and the dansyl triazole HPLC gradient final LC steps were extended, the injection volumes of the dansyl triazole and CGA-142856 autosampler were reduced, and the maximum flow rates of the CGA-142856 HPLC gradient were reduced (pp. 13-15, 17; Tables 5-10, pp. 24-29 of MIRD 46950214). The ILV study author noted that some of these modificaitons were optimizations since the API 3000 was used for all analyses, instead of the API 4000 since the API 4000 instrument was not available. Also, for difenoconazole, CGA-205375 and CGA-71019 analysis, a Keystone Aquasil C18 column (150 x 3.0 mm, 3 µm) with guard column was specified. An updated ECM was not recommended to incorporate these ILV modifications.

10. The ILV study author reported that the API 3000 and API 4000 were both used in the method (p. 17 of MRID 46950214). The reviewer found both instruments listed in the ECM Appendix, but only found the API 4000 instrument listed in the study report "LC/MS/MS System Description and Operating Conditions" portions (pp. 21-27; Appendix 1, p. 63 of MRID 46950128).

11. It was reported for the ILV that a single analyst completed a sample set consisting of approximately 12 samples in 12 hours, not including LC/MS/MS (p. 17 of MRID 46950214). The time required for LC/MS/MS analysis was reported as "three separate days, due to the fact that each analysis requires unique HPLC conditions" (p. 17).

V. References

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

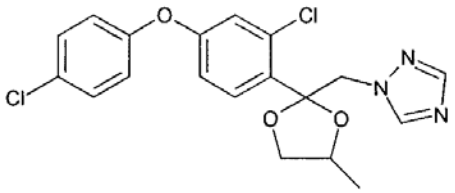
Difenoconazole (CGA-169374)

IUPAC Name: Cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether

CAS Name: 1-[[2-[2-Chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxan-2-yl]methyl-1H-1,2,4-triazole

CAS Number: 119446-68-3

SMILES String: Not found



CGA-205375

IUPAC Name: 1-[2-Chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanol

CAS Name: Alpha-[2-chloro-4-(4-chlorophenoxy)phenyl]-1H-1,2,4-triazole-1-ethanol

CAS Number: 117018-19-6

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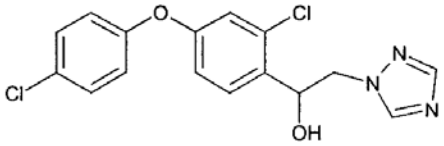

CGA-142856

IUPAC Name: 4-(Methylsulfonyl)-2-nitro-benzoic acid

CAS Name: 1H-1,2,4-Triazole-1-acetic acid

CAS Number: 110964-79-9

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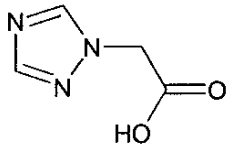

CGA-71019

IUPAC Name: 1,2,4-Triazole

CAS Name: 1H-1,2,4-Triazole

CAS Number: 288-88-0

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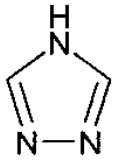

Test Material:	Difenoconazole
MRID:	46950128
Title:	DIFENOCONAZOLE: METHOD: Determination of Difenoconazole and Its Metabolites CGA-205375, CGA-142856 and CGA-71019 in Soil, Using Liquid Chromatography-Electrospray ionization Tandem Mass Spectrometry
MRID:	46950214
Title:	DIFENOCONAZOLE: INDEPENDENT LABORATORY VALIDATION: DETERMINATION OF DIFENOCONAZOLE AND ITS METABOLITES CGA-205375, CGA-142856 AND CGA-71019 IN SOIL, USING LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY: ENVIRO-TEST LABORATORIES METHOD M 314: SYNGENTA METHOD T013656-05: FINAL REPORT
EPA PC Code:	128847
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** Lisa Muto

Date: 7/21/16

Secondary Reviewer: Kathleen Ferguson **Signature:** Kathleen P. Ferguson

Date: 7/21/16

QC/QA Manager: Joan Gaidos **Signature:**

Date: 7/21/16