Test Material:	Cyproconazole
MRID:	49863306
Title:	Cyproconazole – Analytical Method GRM033.01A for the Determination of Cyproconazole, 1,2,4-Triazole and Triazole Acetic Acid in Soil Using Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry (Including Validation Data) – Method
MRID:	49863305
Title:	Cyproconazole – Cyproconazole – Independent Laboratory Validation of the Analytical Method GRM033.01A, Determination of Cyproconazole, 1,2,4-Triazole and Triazole Acetic Acid in Soil Using Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry – Final Report
EPA PC Code:	128993
OCSPP Guideline:	850.6100

For CDM/CSS-Dynamac JV

Primary Reviewer: Lisa Muto **Signature:** 

Date: 10/13/16

Secondary Reviewer: Kathleen Ferguson **Signature:** 

Date: 10/13/16

Quality Assurance Manager: Joan Gaidos **Signature:** 

Date: 10/13/16

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Analytical method for cyproconazole, and its metabolites, 1,2,4-triazole and triazole acetic acid, in soil

Reports: ECM: EPA MRID No.: 49863306. Huang, S-B. 2007. Cyproconazole – Analytical Method GRM033.01A for the Determination of Cyproconazole, 1,2,4-Triazole and Triazole Acetic Acid in Soil Using Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry (Including Validation Data) – Method. Syngenta Report and Task No. T004701-06. Report prepared, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 41 pages. Final report issued August 7, 2007.

ILV: EPA MRID No. 49863305. Thomas, C.A. 2009. Cyproconazole – Cyproconazole – Independent Laboratory Validation of the Analytical Method GRM033.01A, Determination of Cyproconazole, 1,2,4-Triazole and Triazole Acetic Acid in Soil Using Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry – Final Report. Syngenta Report and Task No. T004702-06. NCL Study No.: 110.029. Report prepared by North Coast Laboratories, Ltd. (NCL), Arcata, California, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 155 pages. Final report issued February 5, 2009.

Document No.: MRIDs 49863306 & 49863305

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with the USEPA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 49863306). Signed and dated No Data Confidentiality, Quality Assurance and GLP statements were provided (pp. 2-4). A certification of authenticity was not included.

ILV: The study was conducted in accordance with the USEPA FIFRA GLP standards (40 CFR Part 160), except for a few instances of late data entries, which have been noted in the study report (p. 3 of MRID 49863305). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A certification of authenticity was not included.

Classification: This analytical method is classified as **Acceptable**. In the ECM, no samples were fortified at 10×LOQ. The purities of the test materials were not reported in the ECM.

PC Code: 128993

Reviewer: Jerrett Fowler, Physical Scientist **Signature:** 2018.09.24 14:48:18 -04'00'
Date: 9/24/2018

Stephen P. Wente, Senior Scientist **Signature:** STEPHEN WENTE Digitally signed by STEPHEN WENTE Date: 2018.09.24 14:16:36 -04'00'
Date: 9/24/2018

Executive Summary

This analytical method, Syngenta Residue Method GRM033.01A, is designed for the quantitative determination of cyproconazole and its metabolites, 1,2,4-triazole and triazole acetic acid, in soil using LC/MS/MS. The method is quantitative for all three analytes at the stated LOQ of 1.0 ng/g (1.0 ppb). The LOQ is less than the lowest toxicological level of concern in soil for all three analytes. The ECM validated the method using sandy loam soil. The ILV validated the method using sandy loam soil with the first trial with minor modifications to the analytical method; however, the ILV study author requested optimization of a few steps of the method. The sources for the soil matrices of the ECM and ILV appeared to be the same. One ion transition was monitored in the ECM and ILV; a confirmatory method is not usually required when GC/MS or LC/MS is the primary identification method. In the ECM, no samples were fortified at 10×LOQ, and the purities of the test materials were not reported. In the ECM and ILV, the interferences were <50% of the LOD; sample recoveries were only corrected in the ECM.

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						
Cyproconazole	49863306	49863305		Soil[2,3]	07/08/2007	Syngenta Crop Protection, LLC	LC/MS/MS	1.0 ng/g (1.0 ppb)
1,2,4-Triazole								
Triazole acetic acid								

1 Cyproconazole = (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol; 2-(4-Chloro-phenyl)-3-cyclopropyl-1-[1,2,4]triazol-1-yl-butan-2-ol.

2 In the ECM, the sandy loam soil matrix (47% sand, 30% silt, 23% clay, pH 7.5, percent organic matter 3.4) was collected from Iowa obtained from an on-going Syngenta field dissipation study (USDA soil textural classification; p. 20 of MRID 49863306). The soil characterization was performed at Agvise Laboratories located in Northwood, North Dakota.

3 In the ILV, the sandy loam soil matrix (54% sand, 26% silt, 20% clay, pH 7.3, percent organic matter 2.6) was collected from Greene County, Iowa, and obtained from Syngenta (USDA soil textural classification; Sample ID RIEN00707-0002; p. 16 of MRID 49863305). The soil characterization was performed at Agvise Laboratories located in Northwood, North Dakota. The soil sample appeared to be sourced from the same bulk control soil sample which was used in the ECM.

I. Principle of the Method

The method contained the following precautions due to the low detection limit of the method: 1) new plastic-ware/glassware should be used for each batch; 2) each solvent should be checked to verify that it is free from contamination (if contamination is suspected); and 3) existing glassware should be solvent rinsed, after washing and before use in the method (p. 11 of MRID 49863306).

Samples of soil (10 ± 0.1 g) were transferred to 50-mL disposable plastic centrifuge tubes and fortified, as necessary (pp. 12-13; Appendix 5, p. 41 of MRID 49863306). After five minutes of equilibration with the fortification solution, the samples were extracted twice with 25 mL of methanol:water (80:20, v:v) via shaking on a mechanical shaker at a unspecified speed (a speed which provided visible agitation) for a minimum of 20 minutes; samples should be in a flat or horizontal orientation. After centrifugation at 5000 rpm for five minutes, the supernatant was decanted and collected in a 250-mL flat bottom flask via a Whatman 2V filter paper lined funnel. After the second extraction supernatant was collected, the filter paper was rinsed with 10 mL of methanol which was combined with the supernatants. The combined supernatants and rinse were concentrated to aqueous (*ca.* 3 mL) under vacuum with a rotary evaporator at a bath temperature of 35°C. The residue was transferred to a clean 15-mL plastic centrifuge tube using de-ionized or HPLC grade water to rinse the concentration flask. The volume of the residue was adjusted to 10 mL using de-ionized or HPLC grade water. The method noted that this point was a method stopping point. The analytes were isolated from the soil extract via two different solid phase extraction (SPE) procedures. During the SPE clean-ups, the cartridges were to remain moist, the solvents were to be added after the prior solvent had completely entered cartridge, and the flow rate should be less than 20 drops per minute, with the aid of vacuum if necessary.

To isolate triazole acetic acid (TAA), the soil extract was purified via Waters Oasis MAX SPE cartridge (150 mg/6-mL, 60 µm; the method noted that no substitution was allowed; p. 13; Appendix 1, p. 25; Appendix 5, p. 41 of MRID 49863306). The SPE column was pre-conditioned with methanol (one full cartridge amount x 1), 2% formic acid in methanol (5 mL x 1), de-ionized or HPLC grade water (5 mL x 2), 1% ammonium hydroxide in methanol (freshly prepared, 5 mL x 2), 1% ammonium hydroxide in water (freshly prepared, 3 mL x 2) and 1% ammonium hydroxide in water (400 µL). After 2.0-mL of the soil extract was loaded onto the column by gravity, the cartridge was washed with de-ionized or HPLC grade water (2 mL x 2), de-ionized or HPLC grade water (5 mL x 1), methanol:water (50:50, v:v; 5 mL x 1), 1% ammonium hydroxide in methanol (freshly prepared, 2 mL x 3) and methanol (2 mL x 1). The analyte was eluted with 2% formic acid in methanol (2 mL x 4) into a clean 15-mL plastic centrifuge tube. The solvent was evaporated to dryness under a gentle stream of nitrogen at a bath temperature of 40°C. The residue was reconstituted to a final volume of 2 mL (or higher volumes for higher concentrations) with methanol:water (5:95, v:v). After vortex mixing, the sample was analyzed via LC/MS/MS.

To isolate cyproconazole (CCZ) and 1,2,4-triazole (T), the soil extract was purified via Varian Bond Elut Certify SPE cartridge (300 mg/3-mL; the method noted that no substitution was allowed; pp. 13-14; Appendix 1, p. 25; Appendix 5, p. 41 of MRID 49863306). The SPE column was pre-conditioned with methanol (one full cartridge amount x 2), 0.5% ammonium hydroxide

in methanol:water (90:10, v:v; freshly prepared, 2 mL x 2), de-ionized or HPLC grade water (2 mL x 2), 5% formic acid in methanol (2 mL x 2) and 2% formic acid in methanol (2 mL x 1). After 2.0-mL of the soil extract was loaded onto the column by gravity, the cartridge was washed with de-ionized or HPLC grade water (2 mL x 2) and methanol (2 mL x 2). The analyte was eluted with 0.5% ammonium hydroxide in methanol:water (90:10, v:v; freshly prepared, 2 mL x 3) into a clean 15-mL plastic centrifuge tube. The solvent was evaporated to aqueous (< 0.6 mL) under a gentle stream of nitrogen at a bath temperature of 40°C. The residue was reconstituted to a final volume of 2 mL (or higher volumes for higher concentrations) with methanol:water (5:95, v:v). After vortex mixing, the sample was analyzed via LC/MS/MS.

Samples were analyzed for cyproconazole (CCZ), 1,2,4-triazole (T) and triazole acetic acid (TAA) using a Thermo Electron Surveyor Plus LC (pp. 15-17 of MRID 49863306). The following LC conditions were used: Zorbax SB-Aq column (75 x 4.6 mm, 3.5 µm; column temperature 25°C), ColumnSaver column filter, mobile phase of (A) 0.1% formic acid in HPLC grade water and (B) 0.1% formic acid in HPLC grade methanol [TAA: percent A:B (v:v) at 0.0-5.0 min. 98:2; CCZ and T: percent A:B (v:v) at 0.0-2.0 min. 98:2, 3.0-8.0 min. 10:90, 8.1-11.0 min. 98:2], and injection volume of 50 µL. The following MS/MS conditions were used: ESI negative ion polarity and multiple reaction monitoring (MRM) for TAA and ESI positive ion polarity and multiple reaction monitoring (MRM) for CCZ and T. One ion pair transition was monitored for each analyte: *m/z* 126.1 → 82.2 for TAA, *m/z* 70.1 → 43.2 for T and *m/z* 292.1 → 125.0 for CCZ. Expected retention times were *ca*. 2.8, 2.6 and 6.0 minutes for TAA, T and CCZ, respectively.

In the ILV, the method was performed as written (pp. 15, 18-20; Appendix 4, pp. 109-153 of MIRD 49863305). An Applied Biosciences/MDS Sciex API 4000 LC/MS/MS triple quadrupole mass spectrometer was used for all analyses. The following analytical parameters were used in the ILV: an Agilent ZORBAX SB-Aq Rapid Resolution column (4.6 x 75 mm, 3.5 µm; column temperature unreported), Phenomenex MAX-RP "Security Guard" cartridge guard column, mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in methanol [TAA: percent A:B (v:v) at 3.51 min. 98:2, 3.60 min. 2:98, 6.70 min. 98:2; CCZ and T: percent A:B (v:v) at 2.00 min. 98:2, 2.50 min. 10:90, 8.11 min. 98:2], and injection volume of 60 µL. One ion pair transition was monitored for each analyte: *m/z* 125.8 → 82.2 for TAA, *m/z* 70.1 → 43.1 for T and *m/z* 292.0 → 125.1 for CCZ. Expected retention times were *ca*. 2.56, 3.00 and 4.47 minutes for TAA, T and CCZ, respectively.

The Limit of Quantification (LOQ) for cyproconazole, 1,2,4-triazole and triazole acetic acid was reported as 1.0 ng/g (1.0 ppb) in the ECM and the ILV (pp. 8, 21-22; Figure 2, p. 33 of MRID 49863306; pp. 12, 17, 29 of MRID 49863305). The Limit of Detection (LOD) was reported as 25 pg on-column based on a 50-µL LC injection volume in the ECM, which was equivalent to the 0.5 ppb calibration standard. The LOD was not reported in the ILV, but appeared to be 0.5 ppb based on the data in the recovery tables.

II. Recovery Findings

ECM (MRID 49863306): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of cyproconazole, 1,2,4-triazole and triazole acetic acid at the LOQ (1.0 ppb, 1.0 ng/g), 50×LOQ (50 ppb, 50 ng/g), and 100×LOQ (100 ppb, 100 ng/g) in one soil matrix (Tables 1-4, pp. 28-31). No samples were prepared at 10×LOQ. Sample recovery results were corrected when residues were quantified in the controls; however, residues were only identified in the control samples of 1,2,4-triazole (1.840-2.660 ppb, *ca*. 4-6% of the LOQ; pp. 18-19; Tables 2-4, pp. 29-31). One ion transition was monitored for each analyte; a confirmatory method is not usually required when GC/MS or LC/MS is the primary identification method. The sandy loam soil matrix (47% sand, 30% silt, 23% clay, pH 7.5, percent organic matter 3.4) was collected from Iowa obtained from an on-going Syngenta field dissipation study (USDA soil textural classification; p. 20). The soil characterization was performed at Agvise Laboratories located in Northwood, North Dakota.

ILV (MRID 49863305): Mean recoveries and RSDs were within guideline requirements for analysis of cyproconazole, 1,2,4-triazole and triazole acetic acid at the LOQ and 10×LOQ in one soil matrix (Table 1, pp. 31-32). One ion pair was monitored for each analyte. The sandy loam soil matrix (54% sand, 26% silt, 20% clay, pH 7.3, percent organic matter 2.6) was collected from Greene County, Iowa, and obtained from Syngenta (USDA soil textural classification; Sample ID RIEN00707-0002; p. 16). The soil characterization was performed at Agvise Laboratories located in Northwood, North Dakota. The soil sample appeared to be sourced from the same bulk control soil sample which was used in the ECM. The method was validated with the first trial with insignificant modifications to the analytical method; however, the ILV study author requested optimization of a few steps of the method (pp. 12, 18-20, 23; Table 1, pp. 31-32).

Table 2. Initial Validation Method Recoveries for Cyproconazole, 1,2,4-Triazole and Triazole Acetic Acid in Soil

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil[1,2]						
Cyproconazole[3]	1.0 (LOQ)	5	99.0-106	102	3.0	3.0
	50	5	103-108	105	2.2	2.1
	100	5	99.6-102	101	0.9	0.89
1,2,4-Triazole	1.0 (LOQ)	3	84.5-90.7	86.7	2.6	3.0
	50	5	78.9-82.7	80.5	1.7	2.1
	100	5	77.6-83.0	80.3	2.1	2.6
Triazole acetic acid	1.0 (LOQ)	3	81.6-91.8	86.3	4.6	5.3
	50	3	79.6-81.9	80.7	0.9	1.1
	100	5	77.9-82.8	79.7	1.8	2.3

Data (recovery results were corrected when residues were quantified in the controls; pp. 18-19) were obtained from p. 21; Appendix 3, Tables 1-4, pp. 28-31 of MRID 49863306.

1 One ion pair transition was monitored for each analyte: *m/z* 126.1 → 82.2 for triazole acetic acid, *m/z* 70.1 → 43.2 for 1,2,4-triazole and *m/z* 292.1 → 125.0 for cyproconazole (pp. 15-17).

2 The sandy loam soil matrix (47% sand, 30% silt, 23% clay, pH 7.5, percent organic matter 3.4) was collected from Iowa obtained from an on-going Syngenta field dissipation study (USDA soil textural classification; p. 20). The soil characterization was performed at Agvise Laboratories located in Northwood, North Dakota.

3 Cyproconazole = (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol; 2-(4-Chloro-phenyl)-3-cyclopropyl-1-[1,2,4]triazol-1-yl-butan-2-ol.

Table 3. Independent Validation Method Recoveries for Cyproconazole, 1,2,4-Triazole and Triazole Acetic Acid in Soil

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Soil[1,2]						
Cyproconazole[3]	1.0 (LOQ)	5	78.3-85.6	82.3	2.6	3.2
	10	5	79.3-88.5	83.6	3.5	4.2
1,2,4-Triazole	1.0 (LOQ)	5	96.3-106	101	3.5	3.5
	10	5	82.0-91.0	86.4	3.4	3.9
Triazole acetic acid	1.0 (LOQ)	5	97.7-108	104	4.8	4.6
	10	5	86.4-95.1	90.2	3.8	4.2

Data (uncorrected recovery results, pp. 26-27) were obtained from Table 1, pp. 31-32 of MRID 49863305.

1 One ion pair transition was monitored for each analyte: *m/z* 125.8 → 82.2 for triazole acetic acid, *m/z* 70.1 → 43.1 for 1,2,4-triazole and *m/z* 292.0 → 125.1 for cyproconazole (pp. 18-20).

2 The sandy loam soil matrix (54% sand, 26% silt, 20% clay, pH 7.3, percent organic matter 2.6) was collected from Greene County, Iowa, and obtained from Syngenta (USDA soil textural classification; Sample ID RIEN00707-0002; p. 16). The soil characterization was performed at Agvise Laboratories located in Northwood, North Dakota. The soil sample appeared to be sourced from the same bulk control soil sample which was used in the ECM.

3 Cyproconazole = (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol; 2-(4-Chloro-phenyl)-3-cyclopropyl-1-[1,2,4]triazol-1-yl-butan-2-ol.

III. Method Characteristics

The LOQ for cyproconazole, 1,2,4-triazole and triazole acetic acid was reported as 1.0 ng/g (1.0 ppb) in the ECM and the ILV (pp. 8, 21-22; Figure 2, p. 33 of MRID 49863306; pp. 12, 17, 29; Table 1, pp. 31-32 of MRID 49863305). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, i.e. which yielded a mean recovery of 70-120% and relative standard deviation of ≤20%. No LOQ calculations were provided in the ECM. No justifications of the LOQ were provided in the ILV. The LOD was reported as 25 pg on-column based on a 50-µL LC injection volume in the ECM, which was equivalent to the 0.5 ppb calibration standard. The LOD was not reported in the ILV, but appeared to be 0.5 ppb based on the data in the recovery tables. In the ECM, the LOD was defined as the smallest standard amount injected during the chromatographic run and typically corresponds to an amount of analyte equivalent to *ca*. one-half of the theoretical amount for a recovery sample at the method LOQ. The ECM study author noted that the LOD may vary from instrument to instrument depending on the injection volume and concentrations needed to obtain adequate analyte response.

Table 4. Method Characteristics

Analyte		Cyproconazole[1]	1,2,4-Triazole	Triazole acetic acid
Limit of Quantitation (LOQ)		1.0 ng/g (1.0 ppb)		
Limit of Detection (LOD)	ECM	0.5 ppb (25 pg on-column based on a 50-µL LC injection volume)		
	ILV	0.5 ppb[2]		
Linearity (calibration curve r^2 and concentration range)	ECM	r^2 = 0.9996	r^2 = 0.9994	r^2 = 0.9997
		(0.5-60 pg/µL)		
	ILV[3]	r^2 = 0.9998	r^2 = 1.0000	r^2 = 1.0000
		(0.5-20 ng/g)		
Repeatable	ECM[4]	Yes at LOQ, 50×LOQ and 100×LOQ, but **no** samples were prepared at 10×LOQ.		
	ILV[5]	Yes at LOQ and 10×LOQ.		
Reproducible		Yes at LOQ and 10×LOQ.		
Specific	ECM	Yes, no matrix interferences were observed.	Yes. Matrix interferences were <10% of the LOQ.	Yes, no matrix interferences were observed.
	ILV	Yes. No matrix interferences were <5% of the LOQ.	Yes, matrix interferences were 12-14% of the LOQ.[6]	Yes, matrix interferences were 13-15% of the LOQ.[7] Non-ideal peak shapes were observed at the LOQ.

Data were obtained from pp. 8, 21-22; Appendix 3, Tables 1-4, pp. 28-31 (recovery results); Appendix 4, Figures 1-6, pp. 32-37 (chromatograms); Appendix 4, Figures 7-9, pp. 38-40 (calibration curves) of MRID 49863306; pp. 12, 17, 29; Table 1, pp. 31-32 (recovery results); Figures 1-45, pp. 35-79 (calibration curves and chromatograms) of MRID 49863305; DER Attachment 2.

1 Cyproconazole = (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol; 2-(4-Chloro-phenyl)-3-cyclopropyl-1-[1,2,4]triazol-1-yl-butan-2-ol.

2 The LOD was not reported in the ILV, but appeared to be 0.5 ppb based on the data in the recovery tables (pp. 12, 17, 29; Table 1, pp. 31-32 of MRID 49863305).

3 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (Figure 1, p. 35; Figure 16, p. 50; Figure 31, p. 65 of MRID 49863305; see DER Attachment 2).

4 In the ECM, the sandy loam soil matrix (47% sand, 30% silt, 23% clay, pH 7.5, percent organic matter 3.4) was collected from Iowa obtained from an on-going Syngenta field dissipation study (USDA soil textural classification; p. 20 of MRID 49863306). The soil characterization was performed at Agvise Laboratories located in Northwood, North Dakota.

5 In the ILV, the sandy loam soil matrix (54% sand, 26% silt, 20% clay, pH 7.3, percent organic matter 2.6) was collected from Greene County, Iowa, and obtained from Syngenta (USDA soil textural classification; Sample ID RIEN00707-0002; p. 16 of MRID 49863305). The soil characterization was performed at Agvise Laboratories located in Northwood, North Dakota. The soil sample appeared to be sourced from the same bulk control soil sample which was used in the ECM.

6 Based on Figures 24-27, pp. 58-61 of MRID 49863305.

7 Based on Figures 39-42, pp. 73-76 of MRID 49863305.

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

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM analysis, no samples were fortified at 10×LOQ (Tables 1-4, pp. 28-31 of MRID 49863306). 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.

2. The purities of the test materials were not reported in the ECM (pp. 8-9 of MRID 49863306).

   In the EMC, the reviewer noted that the CAS # of cyproconazole was reported as 94361-06-5, which corresponds to the (2RS, 3RS)-isomers (p. 8 of MRID 49863306). (p. 17 of MRID 49863305). In the ILV, the reviewer noted that the CAS #s of cyproconazole were reported as 94361-06-5, see above, and 94361-07-6, which corresponds to the (2RS, 3SR)-isomers.

3. The ECM soil matrix was sandy loam (47% sand, 30% silt, 23% clay, pH 7.5, percent organic matter 3.4) was collected from Iowa obtained from an on-going Syngenta field dissipation study (USDA soil textural classification; p. 20 of MRID 49863306), and ILV soil matrix was sandy loam (54% sand, 26% silt, 20% clay, pH 7.3, percent organic matter 2.6) was collected from Greene County, Iowa, and obtained from Syngenta (USDA soil textural classification; Sample ID RIEN00707-0002; p. 16 of MRID 49863305). The ILV soil sample appeared to be sourced from the same bulk control soil sample which was used in the ECM; however, more details about the soil sources would need to be provided in order to make an accurate judgement.

4. The estimations of the LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 21-22; Figure 2, p. 33 of MRID 49863306; pp. 12, 17, 29; Table 1, pp. 31-32 of MRID 49863305). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, i.e. which yielded a mean recovery of 70-120% and relative standard deviation of ≤20%. No LOQ calculations were provided in the ECM or ILV. No justifications of the LOQ were provided in the ILV. In the ECM, the LOD was defined as the smallest standard amount injected during the chromatographic run and typically corresponds to an amount of analyte equivalent to *ca*. one-half of the theoretical amount for a recovery sample at the method LOQ. The ECM study author noted that the LOD may vary from instrument to instrument depending on the injection volume and concentrations needed to obtain adequate analyte response). No LOD calculations were reported in ECM or ILV.

   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.

5. The ILV study author noted the following suggestions to improve the method: 1) more details about the evaporation time and conditions should be provided for concentration of

the extracts after each SPE column clean-up; and 2) the SPE clean-up flow rate of less than 20 drops per minute was considered time consuming and difficult to achieve, and more guidance was requested to discuss the flow rate optimization (p. 23 of MRID 49863305).

6. The ECM method was validated by the ILV with the second analysis of the extracts of the first trial (pp. 12, 23-24 of MRID 49863305). For the analysis of the first extracts, the ILV study author used an acetonitrile-based organic mobile phase, which was a sponsor-approved modification from the methanol-based organic mobile phase specified in the method. The ILV study author noted that this modification "appeared to be allowed by the flexibility of the method as written" (p. 24). The ILV first analysis yielded shaper peaks for the analytes, but unacceptable results. When the ILV used a methanol-based organic mobile phase for the second analysis of the extracts of the first trial, acceptable results were achieved. Therefore, the reviewer concluded that the methanol-based organic phase for the analytical method cannot be modified.

7. In the ECM, the method calculations allowed for recoveries to be corrected for residues quantified in the controls; however, residues were only identified in the control samples of 1,2,4-triazole (1.840-2.660 ppb, *ca*. 4-6% of the LOQ; pp. 18-19; Tables 2-4, pp. 29-31 of MRID 49863306).

   In the ILV, the study author noted that analyte interference peaks were observed in the controls for 1,2,4-triazole and triazole acetic acid (pp. 12, 22 of MRID 49863305). The interferences were considered negligible because the area count was <50% of the LOD. No values for the control residues were reported in the recovery data tables in the study report (Table 1, pp. 31-32 of MRID 49863305).

8. In ECM and ILV representative LOQ and 10×LOQ chromatograms showed minor matrix interferences (<15% of the LOQ) in the controls at the retention times of the analytes (Appendix 4, Figures 1-6, pp. 32-37 of MRID 49863306; Figures 1-45, pp. 35-79 of MRID 49863305).

9. The ILV study author provided communication details between the ILV laboratory personnel and the Study Sponsor (pp. 23-24 of MRID 49863305). These communications included typical questions about the laboratory materials for the method and method clarifications.

10. It was reported for the ILV that a sample set consisting of approximately 13 samples required *ca*. 12 hours (*ca*. 1.5 person days) to complete (p. 22 of MRID 49863305).

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

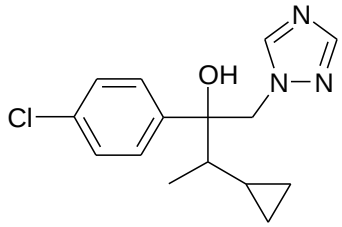
Cyproconazole (SAN619; CCZ)

IUPAC Name: (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol.
2-(4-Chloro-phenyl)-3-cyclopropyl-1-[1,2,4]triazol-1-yl-butan-2-ol.

CAS Name: α-(4-Chlorophenyl)-α-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol.

CAS Number: 94361-06-5 (2RS, 3RS)-isomers.
94361-07-6 (2RS, 3SR)-isomers.
113096-99-4 (unstated stereo chemistry).

SMILES String: Clc1ccc(cc1)C(O)(C(C1CC1)C)Cn1ncnc1 (ISIS v2.3/Universal SMILES).
c1cc(Cl)ccc1C(O)(C(C)C2CC2)Cn3ncnc3 (EPI Suite, v3.12 SMILES).



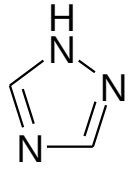
1,2,4-Triazole (CGA 71019; T)

IUPAC Name: 4H-[1,2,4]Triazole.

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

CAS Number: 288-88-0.

SMILES String: n1cnnc1 (ISIS v2.3/Universal SMILES).
n1ncnc1 (EPI Suite, v3.12).



Triazole Acetic Acid (CGA-142856; CSAA131731; TAA)

IUPAC Name: [1,2,4]Triazol-1-yl-acetic acid.

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

CAS Number: 28711-29-7

SMILES String: Not found