Analytical method for Cyclobutrifluram (SYN549522) in water

Reports: ECM: EPA MRID No. 51459319. Hewa, K.T. 2021. Cyclobutrifluram. SYN549522 – Analytical Method GRM076.04A for the Determination of SYN549522 in Water. Analytical Method. PASC Project ID: 141-6426. PASC Report No.: PASC-TMS-1564, V03. Task No.: TK0540379. Report prepared by Primera Analytical Solutions Corp., Cranbury, New Jersey, and sponsored and submitted by Syngenta Crop Protection, LLC. Greensboro, North Carolina; 42 pages. Final report issued January 28, 2021.

ILV: EPA MRID No. 51459325. Jutson, J.I. 2021. Cyclobutrifluram. Independent Laboratory Validation - SYN549522 - Analytical Method GRM076.04A for the Determination of SYN549522 in Water. Final Report. Smithers Report and Study No. 1781-7389. Task No.: TK0592066. Report prepared by Smithers, Wareham, Massachusetts, and sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 140 pages. Final report issued April 30, 2021.

Document No.: MRIDs 51459319 & 51459325

Guideline: 850.6100

Statements: ECM: This study makes no claim of compliance with USEPA FIFRA Good Laboratory Practices (40 CFR Part 160, 1989; p. 3 of MRID 51459319). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included. A Statement of Previous Versions was included (p. 4).
ILV: The study was conducted in accordance with USEPA FIFRA GLP (40 CFR Part 160, 1989; p. 3 of MRID 51459325). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was not included.

Classification: This analytical method is classified as acceptable. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ. Note that as of the date of this review the residues of concern for both ecological and human health risk assessments have been based upon cyclobutrifluram parent alone, therefore, there is no level of concern to compare analytical minimum detection or quantification limits with for any degradation products.

PC Code: 126002

EFED Final Reviewer:	Dena Barrett, Chemist	Signature: Date:	Dena Barrett 3/25/2025
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CDM/CSS-Dynamac JV Reviewers:	Summer Piantes, M.S., Environmental Scientist	Signature Date:	Summer Piantes 04/27/2022
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Environmental Scientist Date: 04/29/2022

Executive Summary

This analytical method, GRM076.04A, is designed for the quantitative determination of cyclobutrifluram (SYN549522) in water at the LOQ of 0.025 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for cyclobutrifluram parent. As of the date of this review the residues of concern in EFED's ecological and human health risk assessments have been based upon cyclobutrifluram parent alone. Therefore, there is no level of concern to compare to analytical minimum detection or quantification limits for any degradation products. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ILV reported method LOQ for all analytes in water (0.025 µg/L).

The ILV validated the method, GRM076.04A, with the first trial as written, except for the use of a LC column with a stationary phase particle size of 2.1 µm stationary phase rather than a 1.7 µm particle size and an injection volume of 15 µL rather than 50 µL. An updated ECM is not required. The ILV test matrices were characterized ground and surface water matrices, while uncharacterized surface and ground water matrices were used in the ECM.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for cyclobutrifluram (SYN549522) at 0.025 µg/L and 0.25 µg/L.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Cyclobutrifluram (SYN549522)	51459319[1]	51459325[2]		Water	28/01/2021	Syngenta Crop Protection, LLC	LC/MS/MS	0.025 µg/L

[1] In the ECM, source of the surface (pond) and ground water was not reported. The water characterization analyses were not reported.

[2] In the ILV, surface and ground water matrices were use. The surface water matrix was from the Weweantic River in West Wareham, Massachusetts and had the following characteristics: pH 6.6, total hardness 20 mg equivalent $CaCO_3$/L, total dissolved solids 438 ppm, turbidity 1.17 NTU, calcium 5.1 ppm, magnesium 1.8 ppm, and sodium 34 ppm (p. 12; Table 1, p. 24, and Table 3, p. 26 of MRID 51459325). The ground water was collected from a 100-meter bedrock well located at Smithers facility in Wareham, Massachusetts and had the following characteristics: pH 6.7, total hardness 94 mg equivalent $CaCO_3$/L, total dissolved solids 162 ppm, turbidity 0.15 NTU, calcium 24 ppm, magnesium 8.2 ppm, and sodium 107 ppm (p. 12; Tables 1-2, pp. 24-25. The water characterization was performed by Agvise Laboratories in Northwood, North Dakota.

I. Principle of the Method

Surface water and ground water samples were centrifuged and transferred into HPLC vials for analysis by ultra-high performance liquid chromatography using a C8 UPLC column separation with triple quadrupole mass spectrometric detection (LC-MS/MS; p. 10 of MRID 51459319).

If frozen, water samples should be allowed to defrost completely at room temperature (p. 13 of MRID 51459319). The samples (1.5 mL) should be transferred to a centrifuge vial and centrifuged at greater than 10,000 rpm for two minutes to remove any suspended solids. Taking care not to disturb or re-suspend solid particles, the upper portion of the sample should be transferred to an auto-sampler vial for LC-MS/MS analysis.

Stock solutions of (100 µg/L) were prepared by one of two methods (pp. 10-11 of MRID 51459319). The first method consisted of weighing sufficient analytical standard into a 50-mL "Class A" volumetric flask and diluting to the mark with the solvent (acetonitrile). The second method consisted of weight a known amount of analytical standard material in an appropriate volume of solvent. The analytical standard or volume of solvent should be adjusted for purity based on the certificate of analysis and salt content, if applicable (p. 13 of MRID 51459319). Specific sample preparation and stock solution procedures for the ECM method verification were not reported in the MRID 51459319.

Water samples were fortified to concentrations of 25 ng/mL and 2.5 ng/mL with serial dilution with deionized (Milli-Q) water (p. 11 of MRID 51459319). Details of the serial dilutions were not reported in the MRID.

Samples were analyzed using a Shimadzu ultra-high performance liquid chromatography (UPLC) system with a SHIMADZU LC-30AD HPLC pump coupled to AB Sciex API 6500 MS (pp. 14-15 of MRID 51459319). The following LC conditions were used: Waters Acquity UPLC C8 (2.1 mm x 50 mm column; 1.7 µm particle size; column temperature ambient/30°C), mobile phase of (A) 0.1% acetic acid in water, and (B) 0.1% acetic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0-1.0 min. 90:10, 2.5-4.0 10:90, 4.1-5.0 min. 90:10], flow 0.6 mL/min., and injection volume of 50 µL. The following MS/MS conditions were used: negative mode (source temperature 500°C), Turbo Ion Spray interface, and multiple reaction monitoring (MRM). Cyclobutrifluram was identified using one ion pair transition: *m/z* 386 →146 and a confirmation ion pair transition: *m/z* 386 → 215. Expected retention time was 2.5 minutes.

The independent laboratory performed the ECM as written, with the following modifications: 1) The equipment used differed from the equipment in the ECM. An AB MDS Sciex API 6500+ QTRAP LC-MS/MS was used (pp. 16-17 of MRID 51459325). 2) A Shimadzu LC-20ADXR HPLC pump was used in the ILV. 3) A Waters^{TM} Xbridge BEH C8, 2.1 × 50 mm, 2.5 µm particle size column was used. The stationary phase of the column was kept the same as ECM, but the different particle size was chosen to make the ILV method more compatible with the LC-

MS/MS system used in the ILV. In addition, the mobile phase gradient was A:B (v:v) at 0-1.0 min. 90:10, 2.5-4.0 min. 10:90, 5 min. 90:10. 4) An injection volume of 15 µL was used instead of the 50 µL used in the ECM. The ILV lists acetonitrile:methanol:purified reagent water (30:30:40, v:v:v) as the autosampler rinse solution. The ECM did not report the rinse solution.

The following MS/MS conditions were used in the ILV: negative mode (source temperature 500°C), Turbo Ion Spray interface, and multiple reaction monitoring (MRM; p. 17 of MRID 51459325). Cyclobutrifluram was identified using two ion pair transitions (quantitation and confirmation, respectively): *m/z* 386 →146 and *m/z* 386 → 215; quantitation ion transition was the same as that of the ECM. Observed retention time was *ca*. 3.1 minutes (Figures 5-6, pp. 36-37; Figures 13-14, pp. 44-45; Appendix 6, p. 125)

The Limit of Quantification (LOQ) for cyclobutrifluram in surface water and ground water was reported as 0.025 µg/L in the ECM and the ILV (pp. 10 and 18 of MRID 51459319; pp. 9, 10, 19 and 21 of MRID 51459325). The Limit of Detection (LOD) for cyclobutrifluram in surface water and ground water was reported as 0.008 µg/L in the ECM (p. 18 of MRID 51459319) and 0.004 µg/L in the ILV (pp. 9, 10 and 19 of MRID 51459325).

Since the LOQs were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQs are the lowest levels of method validation (LLMVs) rather than LOQs.

II. Recovery Findings

ECM (MRID 51459319): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for surface water and ground water matrix samples were fortified with cyclobutrifluram at the LOQ (0.025 µg/L) and 10×LOQ (0.25 µg/L; Tables 1-4, pp. 22-23). Two untreated control samples and five samples for each matrix and each fortification level were analyzed for cyclobutrifluram which was identified using one ion pair transition: *m/z* 386 →146 and a confirmation ion pair transition: *m/z* 386 → 215. Performance data for the quantification and confirmation ion pair transitions were comparable. Cyclobutrifluram was not detected in the control samples.

The source of ground water samples was not reported in the ECM. The surface water matrix was collected from an unspecified pond (p. 24). Water characterization analyses were not reported in the ECM.

ILV (MRID 51459325): Mean recoveries and RSDs met requirements for surface water and ground water matrix samples were fortified with cyclobutrifluram at the LOQ (0.025 µg/L) and 10×LOQ (0.25 µg/L; Tables 4-5, pp. 27-28). Two untreated control samples and five samples for each matrix and each fortification level were analyzed for cyclobutrifluram which was identified using one ion pair transition: *m/z* 386 →146 and a confirmation ion pair transition: *m/z* 386 → 215. Performance data for the quantification and confirmation ion pair transitions were comparable. Cyclobutrifluram was not detected in the control samples.

The surface water samples were collected from the Weweantic River, West Wareham, Massachusetts on 17 February 2021 (p. 12). Ground water samples were collected from a 100-meter bedrock well on 3 March 2021 at Smithers facility in Wareham, Massachusetts and filtered to remove any organic material.

The surface water had the following characteristics: pH 6.6, total hardness 20 mg equivalent $CaCO_3$/L, total dissolved solids 438 ppm, turbidity 1.17 NTU, calcium 5.1 ppm, magnesium 1.8 ppm, and sodium 34 ppm (p. 12; Table 1, p. 24, and Table 3, p. 26). The ground water had the following characteristics: pH 6.7, total hardness 94 mg equivalent $CaCO_3$/L, total dissolved solids 162 ppm, turbidity 0.15 NTU, calcium 24 ppm, magnesium 8.2 ppm, and sodium 107 ppm (p. 12; Tables 1-2, pp. 24-25 of MRID 51459325). The water characterization was performed by Agvise Laboratories in Northwood, North Dakota.

The method was validated by the ILV in the surface water matrix and the ground water matrix with modifications to the analytical method (see Reviewer's Comment #2). The ILV validated the method in one trial and no matrix suppression or enhancement was observed (pp. 9-10). The ILV method was based on the ECM method; however, the ILV was modified to make the method compatible with the LC-MS/MS equipment at the ILV (p. 21). An updated ECM is not required.

Table 2. Initial Validation Method Recoveries for Cyclobutrifluram (SYN549522) in Water[1,2]

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground water quantitation ion pair transition (Q): *m/z* 386→146						
Cyclobutrifluram (SYN549522)	0.025 (LOQ)	5	89.2-98.0	94.1	3.65	3.88
	0.25 (10x LOQ)	5	100.0-103.2	101.5	1.15	1.13
Ground water quantitation ion pair transition (C): *m/z* 386→215						
Cyclobutrifluram (SYN549522)	0.025 (LOQ)	5	90.8-100.0	95.0	3.88	4.09
	0.25 (10x LOQ)	5	99.2-102.0	101.1	1.15	1.13
Surface (Pond) Water quantitation ion pair transition (Q): *m/z* 386→146						
Cyclobutrifluram (SYN549522)	0.025 (LOQ)	5	81.6-94.8	85.4	5.44	6.37
	0.25 (10x LOQ)	5	79.2-85.6	82.2	2.84	3.45
Surface (Pond) Water quantitation ion pair transition (C): *m/z* 386→215						
Cyclobutrifluram (SYN549522)	0.025 (LOQ)	5	81.2-90.0	85.4	3.84	4.5
	0.25 (10x LOQ)	5	77.6-85.6	82.2	2.96	3.60

Data (uncorrected recovery results; p. 16; Tables 1-4, pp. 22-23) were obtained from Tables 1-4, pp. 22-23 of MRID 51459319. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 Cyclobutrifluram was identified using one quantitation ion pair transition (Q): *m/z* 386→146 and one confirmatory ion pair transition (C) *m/z* 386→215.

2 The surface (pond) water was collected from an unspecified pond. Surface water quality analysis was not reported in the ECM. The source of the ground water matrix was not reported in the ECM.

Table 3. Independent Validation Method Recoveries for Cyclobutrifluram (SYN549522) in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground water quantitation ion pair transition (Q): *m/z* 386→146						
Cyclobutrifluram (SYN549522)	0.025 (LOQ)	5	87.8-96.0	92.8	3.10	3.34
	0.25 (10x LOQ)	5	91.3-95.6	93.7	1.84	1.97
Ground water quantitation ion pair transition (C): *m/z* 386→215						
Cyclobutrifluram (SYN549522)	0.025 (LOQ)	5	90.9-95.3	92.0	1.87	2.03
	0.25 (10x LOQ)	5	91.2-95.1	93.4	1.62	1.73
Surface (Pond) Water quantitation ion pair transition (Q): *m/z* 386→146						
Cyclobutrifluram (SYN549522)	0.025 (LOQ)	5	83.6-87.6	85.3	1.58	1.85
	0.25 (10x LOQ)	5	83.5-91.4	87.0	3.37	3.87
Surface (Pond) Water quantitation ion pair transition (C): *m/z* 386→215						
Cyclobutrifluram (SYN549522)	0.025 (LOQ)	5	85.4-94.2	88.5	3.53	3.98
	0.25 (10x LOQ)	5	82.9-89.5	86.9	2.99	3.44

Data (uncorrected recovery results; Appendix 4, pp. 110-111) were obtained from Tables 4-5, pp. 27-28 of MRID 51459325. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The reported LOQ of the ECM differed from that of the ILV.

[1] Cyclobutrifluram was identified using one quantitation ion pair transition (Q): *m/z* 386→146 and one confirmatory ion pair transition (C) *m/z* 386→215.

[2] The surface water matrix was from the Weweantic River in West Wareham, Massachusetts and had the following characteristics: pH 6.6, total hardness 20 mg equivalent $CaCO_3$/L, total dissolved solids 438 ppm, turbidity 1.17 NTU, calcium 5.1 ppm, magnesium 1.8 ppm, and sodium 34 ppm (p. 12; Table 1, p. 24, and Table 3, p. 26 of MRID 51459325). The ground water was collected from a 100-meter bedrock well located at Smithers facility in Wareham, Massachusetts and had the following characteristics: pH 6.7, total hardness 94 mg equivalent $CaCO_3$/L, total dissolved solids 162 ppm, turbidity 0.15 NTU, calcium 24 ppm, magnesium 8.2 ppm, and sodium 107 ppm (p. 12; Tables 1-2, pp. 24-25 of MRID 51459325). The water characterization was performed by Agvise Laboratories in Northwood, North Dakota.

III. Method Characteristics

The LOQ for cyclobutrifluram in surface water and ground water was reported as 0.025 µg/L in the ECM and the ILV (pp. 10 and 18 of MRID 51459319; pp. 9, 10, 19 and 21 of MRID 51459325).

In the ECM, the LOQ was justified as the lowest analyte concentration in a sample at which the methodology has been developed. The estimated LOQ was taken as not less than ten times of the background noise (NLT 10; p. 18 of MRID 51459319). The LOD in the ECM was reported as 0.008 µg/L and was based on an estimate of 3 times the background noise in an untreated sample (p. 18 of MRID 51459319).

The LOQ in the ILV was justified using external solvent calibration (p. 9 of MRID 51459325). The LOD in the ILV was 0.004 µg/L and was calculated using the following equation (pp. 9, 10; Appendix 4, p. 113):

$LOD = (3x(N_{ctl})/(Resp_{LS}) \text{ x } Conc_{LS} \text{ x } DF_{CNTL}$

Where, LOD is the limit of detection of the analysis, N_{ctl} is the mean noise in height of the control samples (or blanks), $Resp_{LS}$ is the mean response in height of the two low calibration standards, $Conc_{LS}$ is the concentration of the low calibration standard, and DF_{CNTL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 1.00).

Since the LOQs were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQs are the lowest levels of method validation (LLMVs) rather than LOQs.

Table 4. Method Characteristics –Water[1,2]

		Cyclobutrifluram (SYN549522)
Limit of Quantitation (LOQ)*	ECM	0.025 µg/L (ppb)[1]
	ILV	
Limit of Detection (LOD)	ECM	0.008 µg/L (ppb) (3x background noise) [SW and GW]
	ILV	0.004 µg/L (3x background noise relative to calibration responses) [SW and GW]
Linearity (calibration curve r and concentration range)	ECM[3]	r = 0.9990 (Q); r = 0.9991 (C)
		0.008-1 µg/L
	ILV[4]	[SW] r = 0.9999 (Q); r = 0.9998 (C) [GW] r = 0.9998 (Q); r = 0.9998 (C)
		0.008-1 µg/L
Repeatable	ECM	Yes for 0.025 µg/L and 0.25 µg/L in one uncharacterized surface water matrix and one uncharacterized ground water matrix where n = 5.
	ILV[5]	Yes for 0.025 µg/L and 0.25 µg/L in one characterized surface water matrix and one characterized ground water matrix where n = 5.
Reproducible		Yes for 0.025 µg/L (LLMV) and 0.25 µg/L in surface water and ground water matrices.
Specific	ECM	Yes, no matrix interferences were observed.
	ILV	Yes, no matrix interferences were observed.

ECM (MRID 51459319) data obtained from: LOQ/LOD from p. 18; Recovery from Tables 1-4, pp. 22-23; Calibration from p. 11 and Figures 20-21, p. 36. ILV (MRID 51459325) data obtained from: LOQ/LOD pp. 9, 10, and 19; Recovery from Tables 4-5, pp. 27-28; Calibration from Tables 7-8, p. 30 and Figures 21-24, pp. 52-53.

Q = quantitative ion transition; C = confirmatory ion transition; SW = Surface water; GW = Ground water.

* Since the LOQs were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQs are the lowest levels of method validation (LLMVs) rather than LOQs. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

[1] Cyclobutrifluram was identified using one quantitation ion pair transition (Q): *m/z* 386→146 and one confirmatory ion pair transition (C) *m/z* 386→215.

[2] The surface (pond) water was collected from an unspecified pond. Surface water quality analysis was not reported in the ECM. The source of the ground water matrix was not reported in the ECM. The surface water matrix in the ILV was from the Weweantic River in West Wareham, Massachusetts and had the following characteristics: pH 6.6, total hardness 20 mg equivalent $CaCO_3$/L, total dissolved solids 438 ppm, turbidity 1.17 NTU, calcium 5.1 ppm, magnesium 1.8 ppm, and sodium 34 ppm (p. 12; Table 1, p. 24, and Table 3, p. 26 of MRID 51459325). The ground water matrix in the ILV was from a well located on the Smithers facility in Wareham, Massachusetts. The ILV ground water was collected from a 100-meter bedrock well located at Smithers facility in Wareham, Massachusetts and had the following characteristics: pH 6.7, total hardness 94 mg equivalent $CaCO_3$/L, total dissolved solids 162 ppm, turbidity 0.15 NTU, calcium 24 ppm, magnesium 8.2 ppm, and sodium 107 ppm (p. 12; Tables 1-2, pp. 24-25 of MRID 51459325). The water characterization was performed by Agvise Laboratories in Northwood, North Dakota.

[3] The ECM calibration used reagent-pure water and thus was not a matrix-matched calibration.

[4] The ILV reported the coefficient of determination (R^2). The reviewer converted ILV values to r (Tables 7-8, p. 30 of MRID 51459325; DER Excel Attachment). Values were reported to four significant figures.

[5] The ILV validated the method, GRM076.04A, in the first trial, as written with modifications to make the method compatible with the LC-MS/MS equipment at the ILV (pp. 9-10, 21 of MRID 51459325). An updated ECM is not required.

IV. Method Deficiencies and Reviewer's Comments

1. Since the reported method LOQs of the ECM and ILV were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQs of the ECM and ILV are the lowest levels of method validation (LLMVs) rather than LOQs (pp. 10 and 18 of MRID 51459319; pp. 9, 10, 19 and 21 of MRID 51459325). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

2. The submitted ILV MRID 51459325 was conducted to develop and validate a confirmatory method for cyclobutrifluram (SYN549522) analysis in water using ground water and surface water matrices. The ILV was based on the ECM with some insignificant modifications. The use of drinking/finished water was not addressed as a test matrix.

3. The ILV made the following modifications to the ECM (Primera Report No: GRM076.04A): 1) The equipment used differed from the equipment in the ECM. An AB MDS Sciex API 6500+ QTRAP LC-MS/MS was used (pp. 16-17 of MRID 51459325). 2) A Shimadzu LC-20ADXR HPLC pump was used in the ILV. 3) A Waters^{TM} Xbridge BEH C8, 2.1 × 50 mm, 2.5 µm particle size column was used. The stationary phase of the column was kept the same as ECM, but the different particle size was chosen to make the ILV method more compatible with the LC-MS/MS system used in the ILV. In addition, the mobile phase gradient was percent A:B (v:v) at 0-1.0 min. 90:10, 2.5-4.0 min. 10:90, 5 min. 90:10. 4) An injection volume of 15 µL was used instead of the 50 µL used in the ECM (p. 16 of MRID 51459325). The ILV lists an acetonitrile:methanol:purified reagent water (30:30:40, v:v:v) as the autosampler rinse solution. The ECM did not report the rinse solution.

   The analytical instrumentation and equipment modifications included the following: 1) changing the particle size of the stationary phase in the UPLC column; and 2) injection volume change. Cyclobutrifluram had a later retention time in the ILV which would minimize interference from possible polar contaminants. Overall, the ILV method (Smithers Study No.: 1781-7389) was a suitable version of the ECM. The ECM allows for use of different instrumentation than that specified as long as performance and sensitivity requirements are met (p. 14 of MRID51459319).

4. In the ECM, the final extracts in the ground water and surface water matrices were determined to be stable for up to 3 days when refrigerated (temperature not reported; p. 18; Table 5, p. 24 of MRID 51459319). The extracts should be allowed to equilibrate to room temperature prior to use. Stock and standard solutions should be refrigerated to prevent decomposition; the temperature and stability were not reported in the ECM (p. 12 of MRID 51459319).

5. The ECM requires that the method be performed using instruments with suitable performance and sensitivity (p. 14 of MRID 51459319). The difference in equipment and associated in the ILV meets the performance and sensitivity requirements of the ECM.

6. ILV communications were summarized and provided in the study report (p. 20 of MRID 51459325). The performing laboratories and personnel differed between the ECM and ILV (pp. 1, 3 of MRID 512459319; pp. 1, 5 of MRID 51459325). E-mail communications prior to study initiation (February 25, 2021) indicated that the ILV laboratory contacted the study sponsor (Louis Mayer, Syngenta) proposing an alternate column particle size (2.1 µm versus 1.7 µm) in order to control the pressure within the UPLC equipment available at the ILV laboratory (p. 5; Appendix 6, p. 139 of MRID 51459325). A representative for the sponsor approved this modification (Appendix 6, p. 138 of MRID 51459325). This communication was compliant with the OCSPP 850.6100 guidelines regarding independent laboratory verification since it was not a method issue and occurred before the ILV trial initiation. The reviewer noted that this type of communication did not need to occur since the ECM allows for use of different instrumentation than that specified as long as performance and sensitivity requirements are met (p. 14 of MRID51459319).

7. The reviewer noted the following typographical errors: the ECM calibration curve title in Figure 21 is labeled as the Primary Transition for ion pair transition *m/z* 386 → 215. This ion pair is the Confirmatory Transition pair (p. 36 of MRID 51459319). The calibration procedure incorrectly references the standard preparation section as "2.4.3" while it is Section 2.3.3 which actually references the preparation of calibration standards (pp. 11, 16 of MRID 51459319). An updated ECM which corrects these typographical errors is not required but is preferred.

8. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 10, 18 of MRID 51459319; pp. 9, 14, 19, and 21 of MRID 51459325). In the ECM, the LOQ was justified as the lowest concentration at which the method was developed. In the ILV, the LOQ was justified using external solvent calibration. Representative calculations LOD were not included in the ECM. Representative LOD calculations based on a calculation of the baseline noise of the control samples and the response heights of calibration standards are included the ILV (Appendix 4, p. 113 of MRID 51459325). Detection and quantitation limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

   Since the LOQs were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQs are the lowest levels of method validation (LLMVs) rather than LOQs.

9. In the ECM, no significant matrix effects ((<20%) were observed (p. 18 of MRID 51459319). There were no significant matrix effects observed in the ILV p. 20, Table 6 p. 29 of MRID 51459325). Solvent-based calibrations were used in the ECM and ILV (p. 11 of MRID 51459319; pp. 13, 14, Tables 7-8, p. 30 of MRID 51459325).

10. The ILV reported that a single analyst could complete two sets of 13 samples in 1 working day (8 hours; p. 21 of MRID 51459325). The time requirement for the method was not reported in the ECM. The ECM reported that the analytical procedure can be

stopped overnight or for the weekend, if necessary. The results from recovery samples can be used to validate work-flow interruptions (p. 14 of MRID 51459319). The ECM reported that samples should be stored refrigerated in sealed containers when analysis cannot be completed in a single day.

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.

USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319, and Revision 2; 1994 and 2016.

Attachment 1: Chemical Names and Structures

Cyclobutrifluram (SYN549522)

IUPAC Name: Mixture comprised of 80–100% N-[(1S,2S)-2-(2,4-dichlorophenyl)cyclobutyl]-2-(trifluoromethyl)nicotinamide and 20–0% of the (1R,2R)-enantiomer

CAS Name: rel-N-[(1R,2R)-2-(2,4-dichlorophenyl)cyclobutyl]-2-(trifluoromethyl)-3-pyridinecarboxamide

CAS Number: 1460292-16-3

SMILES String: O=C(C1=C(C(F)(F)F)N=CC=C1)NC2C(C3=CC=C(Cl)C=C3Cl)CC2