Analytical method for isocycloseram (SYN547407) and three degradates SYN549107, SYN549431, and SYN550738 in soil

Reports: ECM 1: EPA MRID No. 51228135. Mayer, L.C. 2020. Isocycloseram. SYN547407- Analytical Method GRM072.12A for the Determination of SYN547407 and its Metabolites SYN549107, SYN549431 and SYN550738 in Soil. Analytical Method. Report No.: GRM072.12A. Task No.: TK0279960. Report prepared, sponsored, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina (p. 3); 125 pages. Final report issued February 5, 2020.

ECM 2: EPA MRID No. 51228136. Wabbel, C. 2019. Isocycloseram. SYN547407- Validation of Analytical Method GRM072.12A for the Determination of SYN547407 and its Metabolites SYN549107, SYN549431 and SYN550738 in Soil. Final Report Amendment 1. Study and Report No.: S17-08610. Task No.: TK0081937. Report prepared by Eurofins Agroscience Services EcoChem GmbH (EAS EcoChem GmbH), Niefem-Öschelbronn, Germany, sponsored by Syngenta Ltd., Berkshire, United Kingdom, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina (p. 2); 137 pages. Final report issued December 5, 2019. Report Amendment 1 dated December 19, 2019.

ILV: EPA MRID No. 51228137. Silinski, M. 2021. Isocycloseram. SYN547407- Independent Laboratory Validation of Residue Method (GRM072.12A) for the Determination of SYN547407 and its Metabolites SYN549107, SYN549431, and SYN550738 in Soil by LC-MS/MS. Final Report. RTI Study and Report No.: RTI-1279. Task No.: TK0081938. Report prepared by RTI International, Research Triangle Park, North Carolina, and sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina (p. 3); 302 pages. Final report issued February 9, 2021.

Document No.: MRIDs 51228135 & 51228136 & 51228137

Guideline: 850.6100

Statements: ECM 1: No claim of compliance with USEPA FIFRA Good Laboratory Practices (GLP; 1989) is made for the study (p. 3 of MRID 51228135). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included. A Summary of Revisions to Previous Version was included (p. 4).
ECM 2: The study was conducted in accordance with OECD GLP standards (1997; ENV/MC/CHEM(98)17), which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA), and Japan (MHW, MAFF, and METI), as well as German GPL (p. 3; Appendix 4, p. 137 of MRID 51228136). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4; Appendix 4, p. 137). A statement of the authenticity of the study report was included with the Quality Assurance and GLP statements.

	ILV: The study was conducted in accordance with USEPA FIFRA GLP (40 CFR Part 160 (p. 3 of MRID 51228137). 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. Sponsor/Submitter signatures were dated May 4, 2021 (pp. 2-3).		
Classification:	This analytical method is classified as **supplemental but upgradable**. There was communication between the ILV study personnel and the "method author" (not specified). During this communication, the ILV authors were given updated method instructions contrary to what as described in the ECM, including switching from polypropylene to HDPE bottles for extraction, as well as clarifications on using glass sampler vials, the solvent composition of the matrix-matched calibration standards and chilling the autosampler. An updated ECM should be submitted with the updated method information. 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 a true LOQ. The LOD was not reported in the ILV.		
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This Data Evaluation Record has been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. The CDM/CSS-Dynamac JV role does not include establishing Agency policies.

Executive Summary

This analytical method, GRM072.12A, is designed for the quantitative determination of isocycloseram (SYN547407) and three degradates (SYN549107, SYN549431, and SYN550738) at 0.001 mg/kg in soil using LC/MS/MS. Since the reported method limit of quantitation (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 a true LOQ. Based on the performance data submitted for the ILV, ECM 1, and ECM 2, the LLMV was equivalent to the ILV reported method LOQ for all analytes in soil (0.001 mg/kg). The LLMV is less (more sensitive) than the lowest toxicological level of concern in soil for all analytes. In this case, the lowest toxicological level of concern is the No Observed Adverse Effect Concentration (NOAEC) at 0.095 mg/kg for the non-target arthropod collembolans (*Folsomia candida*).

MRID 51228135 (dated 2020) is an updated version of MRID 51228136 (ECM 2; dated 2019). MRID 51228135 contains the same methodology and performance data; however, the methodology was updated with an optional Solid Phase Extraction (SPE) procedure. No new performance data were submitted for the method using the optional SPE procedure.

The ILV validated the method using the characterized silty clay loam and sandy loam soil matrices; the ECMs (ECM 1 and 2) validated the method using the characterized loamy sand and sandy loam soil matrices. The ILV was based on MRID 51228135 (ECM 1) and validated GRM072.12A with and without the optional Solid Phase Extraction procedure; MRID 51228136 (ECM 2) was included in the DER as the original internal validation.

The ILV validated the method, GRM072.12A (with and without the optional SPE procedure), with the first trial as written, except for the use of HDPE bottles for extraction rather than polypropylene (per correspondence with the method author), the sample was only evaporated to 1.5 mL instead of 0.5 mL, and insignificant modifications to the analytical parameters and equipment. The ILV was not conducted independently of the internal validation (ECM 1 or 2) since there was communication between the ILV study personnel and the method author. The ECM should allow for the complete conduct of the method without needing to refer to the DER/ILV.

All ILV and ECM data (without SPE procedure) regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for parent isocycloseram (SYN547407) and its metabolites SYN549107, SYN549431, and SYN550738 at 0.001 mg/kg and 0.01 mg/kg.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	LLMV
	ECM	ILV					
Isocycloseram (SYN547407)	51228135[1,2] & 51228136[2,3]	51228137[4,5]	Soil	05/02/2020 (ECM 1) 05/12/2019 (ECM 2 Final Report) 19/12/2019 (ECM 2 Amendment 1)	Syngenta Crop Protection, LLC	LC/MS/MS	0.001 mg/kg
SYN549107							
SYN549431							
SYN550738							

ECM= Environmental Chemistry Method; Independent Laboratory Validation; LLMV= Lowest Level of Method Validation

1 ECM 1 = MRID 51228135 (GRM072.12A). MRID 51228135 (dated 2020) was an updated version of MRID 51228136 (dated 2019). MRID 51228135 contained the same methodology and performance data; however, the methodology was updated with an optional Solid Phase Extraction (SPE) procedure. No new performance data was submitted for the method using the optional SPE procedure.

2 In the ECM 1 and ECM 2, the soil matrices were loamy sand soil (LUFA F2.1; pH 5.32 (0.01M $CaCl_2$); 86.3% sand, 10.8% silt, 2.9% clay, 0.99% organic matter, 0.58% organic carbon) and sandy loam soil (LUFA 5M; pH 7.34 (0.01M $CaCl_2$); 57.4% sand, 32.3% silt, 10.3% clay, 1.11% organic matter (reported as 1.11% organic carbon in Table 1, p. 34 of MRID 51228135); matrices were obtained from Germany and characterized by the supplier (USDA texture classification; Table 1, p. 34 of MRID 51228135; Appendix 3, pp. 135-136 of MRID 51228136). The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

3 ECM 2 = MRID 51228136.

4 In the ILV, the matrices were silty clay loam soil (Soil 1; Sample ID: PA.IA.K.CHAR; pH 6.1 (1:1 soil:water ratio); 16% sand, 54% silt, 30% clay, 3.0% organic matter-Walkley Black) and sandy loam soil (Soil 2; Sample ID: PA.CA.K.BS.CHAR; pH 7.8 (1:1 soil:water ratio); 63% sand, 28% silt, 9% clay, 0.89% organic matter-Walkley Black); matrices were characterized by Agvise Laboratories, Northwood, North Dakota (USDA texture classification; p. 15; Table 1, p. 26 of MRID 51228137). The soil textures were verified by the reviewer using USDA-NRCS technical support tools. Soil sources were not reported.

5 The ILV was based on MRID 51228135 and validated GRM072.12A with and without the optional Solid Phase Extraction procedure (pp. 18, 24 of MRID 51228137).

I. Principle of the Method

Samples (10 g, dry weight) of soil in a 250-mL polypropylene bottle were fortified with 1 or 0.1 µg/L mixed fortification solutions (isocycloseram, SYN549107, SYN549431, and SYN550738), as necessary, then allowed to stand for at least five minutes (pp. 17, 20-21; Appendix 4, p. 125 of MRID 51228135; pp. 28-29; Appendix 1, p. 130 of MRID 51228136). The sample was extracted with 50 mL of acetonitrile:water (80:20, v:v) for 60 minutes on a flatbed shaker (250 rpm) then centrifuged (3500 rpm for 3 minutes) and decanted. The residual sample was extracted with 50 mL of acetonitrile:ultra-pure water:acetic acid (80:20:0.1, v:v:v) for 60 minutes on a flatbed shaker (250 rpm), centrifuged (3500 rpm for 3 minutes), then decanted. An aliquot was transferred to a centrifuge tube and centrifuged (10000 rpm for 5 minutes), then 500 µL of the supernatant was diluted with 500 µL ultra-pure water prior to LC/MS/MS analysis.

An optional Solid Phase Extraction (SPE) procedure was included in MRID 51228135 (pp. 21-22 of MRID 51228135). An Oasis HLB cartridge (3 mL, 60 mg) was pre-conditioned with 2 mL

each of methanol followed by ultra-pure water under vacuum (*ca*. 1 mL/min. flow rate). The cartridge was not allowed to become dry. The soil extract (5 mL aliquot) was diluted with 10 mL of ultra-pure water then loaded onto the cartridge. The cartridge was again not allowed to become dry. The sample container was rinsed with 3 mL of ultra-pure water:acetonitrile (70:30, v:v) which was added to the column. High vacuum was applied for 3 minutes to remove water then the analytes were eluted with 5 mL acetonitrile under gravity or slight vacuum. High vacuum was applied for 10 seconds to elute any remaining volume. The samples were evaporated to 0.5 mL under a gentle stream of nitrogen or air in a sample concentrator with a heating block set to 40°C. The residue was reconstituted to 1 mL using 0.5 mL of ultra-pure water. The method noted that if the samples were evaporated to dryness, low recoveries may be observed. The samples were vortexed and analyzed by LC/MS/MS. The method noted that SPE should only be performed if the concentration of the samples results in significant matrix effect (>20%) which impacts residue determination using matrix-matched standards. The method cautioned to dilute high-fortification samples so that contamination from carryover does not occur.

Samples were analyzed for isocycloseram (SYN547407), SYN549107, SYN549431, and SYN550738 using a Sciex QTRAP 6500+ coupled with an Agilent Infinity equipped with a TurboIonSpray interface, in multiple reaction monitoring (MRM) mode (pp. 22-26 of MRID 51228135; pp. 30-32 MRID 51228136). The following LC conditions were used: Kinetex C18 column (2.1 mm x 50 mm, 2.6 µm; column temperature 30°C), Phenomenex C18 pre-column (4 mm), mobile phase of (A) 0.1% acetic acid + ultra-pure water and (B) 0.1% acetic acid + acetonitrile [mobile gradient phase of percent A:B (v:v) at 0-1.0 min. 60:40, 6.0-7.0 min. 5:95, 7.1-8.0 min. 60:40], MS temperature 550°C, and injection volume of 30 µL. For SYN547407 and SYN549431, positive polarity was used; for SYN549107 and SYN550738, polarity was positive/negative switching (0.0-4.5 min.) then negative (4.5-8.0 min.). Expected retention times were 3.9, 4.3, 3.5, and 5.2 minutes for isocycloseram (SYN547407), SYN549107, SYN549431, and SYN550738, respectively. Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 548→418 and *m/z* 548→160 for isocycloseram (SYN547407), *m/z* 434→238 and *m/z* 434→354 for SYN549107, *m/z* 435→392 and *m/z* 435→160 for SYN549431, and *m/z* 448→378 and *m/z* 448→69 for SYN550738.

The ILV was based on MRID 51228135 and validated GRM072.12A with and without the optional Solid Phase Extraction procedure (pp. 18, 24 of MRID 51228137). The ILV performed the ECM method, GRM072.12A, as written, except for the use of HDPE bottles for extraction rather than polypropylene (per correspondence with the method author), the sample was only evaporated to 1.5 mL instead of 0.5 mL, and insignificant modifications to the analytical parameters and equipment (pp. 15-20). Samples were analyzed for isocycloseram (SYN547407) and SYN549107, SYN549431, and SYN550738 using Waters Acquity (Classic) UPLC system coupled with an Applied Biosystems API-5000 MS detector equipped with ESI interface, MRM mode. The LC/MS/MS parameters were the same as those of the ECM, except that a SecurityGuard ULTRA C18 pre-column was used and MS TEM was 500°C (positive) and 400°C (negative). Expected retention times were 3.2, 3.6, 2.8, and 4.6 minutes for isocycloseram (SYN547407), SYN549107, SYN549431, and SYN550738, respectively. Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 548.1→418.0 and *m/z* 548.1→160.1 for isocycloseram (SYN547407), *m/z* 434.1→238.0 and *m/z* 434.1→354.0 for

SYN549107, *m/z* 435.1→392.1 and *m/z* 435.1→160.1 for SYN549431, and *m/z* 448.1→378.0 and *m/z* 448.1→69.0 for SYN550738; the monitored ion transitions of the ILV were similar to those of the ECM.

Since the Limit of Quantitation (LOQ) was not based on scientifically acceptable procedures as defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than a true LOQ. The Lowest Level of Method Validation (LLMV) in soil was reported as 0.001 mg/kg for isocycloseram (SYN547407), SYN549107, SYN549431, and SYN550738 in the ECM 1, ECM 2, and ILV (pp. 17, 29 of MRID 51228135; pp. 22, 35 of MRID 51228136; pp. 20, 23 of MRID 51228137). The Limit of Detection (LOD) in soil was reported as 0.0003 mg/kg for all four analytes in the ECM 1 and ECM 2. The LOD was not determined in the ILV.

II. Recovery Findings

ECMs 1 & 2 (MRID 51228135 & MRID 51228136): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of isocycloseram (SYN547407), SYN549107, SYN549431, and SYN550738 in two soil matrices at the LLMV (0.001 mg/kg) and 10×LLMV (0.01 mg/kg; Tables 2-9, pp. 35-38 of MRID 51228135; Tables 1-8, pp. 40-43 of MRID 51228136). Recovery results of the quantitative and confirmatory ion transitions were comparable. Identical performance data was reported in ECM 1 & 2; therefore, the performance data was generated from the method without the optional SPE clean-up procedure since only ECM 1 contained the optional SPE clean-up procedure. The soil matrices were loamy sand soil (LUFA F2.1; pH 5.32 (0.01M $CaCl_2$); 86.3% sand, 10.8% silt, 2.9% clay, 0.99% organic matter, 0.58% organic carbon) and sandy loam soil (LUFA 5M; pH 7.34 (0.01M $CaCl_2$); 57.4% sand, 32.3% silt, 10.3% clay, 1.11% organic matter (reported as 1.11% organic carbon in Table 1, p. 34 of MRID 51228135); matrices were obtained from Germany and characterized by the supplier (USDA texture classification; Table 1, p. 34 of MRID 51228135; Appendix 3, pp. 135-136 of MRID 51228136). The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

ILV (MRID 51228137): The ILV was based on MRID 51228135 and validated method GRM072.12A with and without the optional SPE procedure (pp. 18, 24; Appendix 4, pp. 143-267).

Mean recoveries and RSDs met requirements for analysis of isocycloseram (SYN547407), SYN549107, SYN549431, and SYN550738 in two soil matrices at the LLMV (0.001 mg/kg) and 10×LLMV (0.01 mg/kg) with and without the optional SPE procedure (Tables 2-9, pp. 27-34). Recovery results of the quantitative and confirmatory ion transitions were comparable. Recovery results were comparable with and without the optional SPE procedure for all analytes/transitions/fortifications, except for the confirmation ion transition analysis of SYN550738 at the LLMV. The matrices were silty clay loam soil (Soil 1; Sample ID: PA.IA.K.CHAR; pH 6.1 (1:1 soil:water ratio); 16% sand, 54% silt, 30% clay, 3.0% organic matter-Walkley Black) and sandy loam soil (Soil 2; Sample ID: PA.CA.K.BS.CHAR; pH 7.8 (1:1 soil:water ratio); 63% sand, 28% silt, 9% clay, 0.89% organic matter-Walkley Black); matrices were characterized by Agvise Laboratories, Northwood, North Dakota (USDA texture

classification; p. 15; Table 1, p. 26). The soil textures were verified by the reviewer using USDA-NRCS technical support tools. Soil sources were not reported.

Table 2. Initial Validation Method Recoveries for Isocycloseram (SYN547407), SYN549107, SYN549431, and SYN550738 in Soil[1,2,3,4]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[5]	Relative Standard Deviation (%)
Method Without Optional SPE						
Sandy Loam Soil (LUFA 5M)						
Quantitation ion transition						
Isocycloseram (SYN547407)	0.001 (LLMV)	5	100-105	103	2	2
	0.01	5	101-106	103	2	2
SYN549107	0.001 (LLMV)	5	92-98	95	2	2
	0.01	5	96-101	98	2	2
SYN549431	0.001 (LLMV)	5	94-110	104	6	6
	0.01	5	102-104	103	1	1
SYN550738	0.001 (LLMV)	5	98-105	102	3	3
	0.01	5	101-105	103	2	2
Confirmation ion transition						
Isocycloseram (SYN547407)	0.001 (LLMV)	5	90-103	97	5	5
	0.01	5	103-106	104	1	1
SYN549107	0.001 (LLMV)	5	96-102	98	2	2
	0.01	5	97-100	99	1	1
SYN549431	0.001 (LLMV)	5	95-109	103	5	5
	0.01	5	100-105	102	2	2
SYN550738	0.001 (LLMV)	5	103-107	104	2	2
	0.01	5	104-110	106	3	3
Loamy Sand Soil (LUFA F2.1)						
Quantitation ion transition						
Isocycloseram (SYN547407)	0.001 (LLMV)	5	96-99	97	1	1
	0.01	5	98-101	99	1	1
SYN549107	0.001 (LLMV)	5	94-98	96	2	2
	0.01	5	95-98	96	1	1
SYN549431	0.001 (LLMV)	5	96-109	101	5	5
	0.01	5	100-103	101	1	1
SYN550738	0.001 (LLMV)	5	98-102	101	2	2
	0.01	5	97-101	99	2	2
Confirmation ion transition						
Isocycloseram (SYN547407)	0.001 (LLMV)	5	98-106	102	3	3

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[5]	Relative Standard Deviation (%)
	0.01	5	98-100	99	1	1
SYN549107	0.001 (LLMV)	5	95-102	98	3	3
	0.01	5	95-97	96	1	1
SYN549431	0.001 (LLMV)	5	98-106	102	4	4
	0.01	5	100-103	101	1	1
SYN550738	0.001 (LLMV)	5	91-105	99	5	5
	0.01	5	94-99	97	2	2

Data (uncorrected results, pp. 27-28 of MRID 51228135; p. 33 of MRID 51228136) were obtained from Tables 2-9, pp. 35-38 of MRID 51228135; Tables 1-8, pp. 40-43 of MRID 51228136; DER Excel Attachment. 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 ECM 1 = MRID 51228135 (GRM072.12A). MRID 51228135 (dated 2020) was an updated version of MRID 51228136 (dated 2019). MRID 51228135 contained the same methodology and performance data; however, the methodology was updated with an optional Solid Phase Extraction (SPE) procedure. No new performance data was submitted for the method using the optional SPE procedure.

2 In the ECM 1 and ECM 2, the soil matrices were loamy sand soil (LUFA F2.1; pH 5.32 (0.01M $CaCl_2$); 86.3% sand, 10.8% silt, 2.9% clay, 0.99% organic matter, 0.58% organic carbon) and sandy loam soil (LUFA 5M; pH 7.34 (0.01M $CaCl_2$); 57.4% sand, 32.3% silt, 10.3% clay, 1.11% organic matter (reported as 1.11% organic carbon in Table 1, p. 34 of MRID 51228135); matrices were obtained from Germany and characterized by the supplier (USDA texture classification; Table 1, p. 34 of MRID 51228135; Appendix 3, pp. 135-136 of MRID 51228136). The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

3 ECM 2 = MRID 51228136.

4 Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 548→418 and *m/z* 548→160 for isocycloseram (SYN547407), *m/z* 434→238 and *m/z* 434→354 for SYN549107, *m/z* 435→392 and *m/z* 435→160 for SYN549431, and *m/z* 448→378 and *m/z* 448→69 for SYN550738.

5 Standard deviations were reviewer-calculated (see DER Excel Attachment) based on data provided in the study report because standard deviations were not calculated by the study authors.

Table 3. Independent Validation Method Recoveries for Isocycloseram (SYN547407), SYN549107, SYN549431, and SYN550738 in Soil[1,2,3]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Method Without Optional SPE						
Silty Clay Loam Soil (Soil 1)						
Quantitation ion transition						
Isocycloseram (SYN547407)	0.001 (LLMV)	5	83.4-113	100	15.2	15.2
	0.01	5	91.6-107	101	5.9	5.9
SYN549107	0.001 (LLMV)	5	85.8-109	99.4	12.2	12.3
	0.01	5	89.7-98.2	95.4	3.4	3.6
SYN549431	0.001 (LLMV)	5	96.5-104	101	2.6	2.6
	0.01	5	93.9-101	96.7	2.9	3.0
SYN550738	0.001 (LLMV)	5	102-112	104	4.4	4.2
	0.01	5	98.6-105	103	2.5	2.4
Confirmation ion transition						
Isocycloseram (SYN547407)	0.001 (LLMV)	5	81.8-111	98.9	15.3	15.4
	0.01	5	91.5-105	100	5.3	5.3
SYN549107	0.001 (LLMV)	5	91.6-105	98.3	5.3	5.4
	0.01	5	87.6-96.8	93.7	3.7	4.0
SYN549431	0.001 (LLMV)	5	81.1-112	99.4	16.1	16.2
	0.01	5	89.0-103	98.6	5.6	5.7
SYN550738	0.001 (LLMV)	5	92.8-107	100	6.8	6.8
	0.01	5	95.6-100	98.3	1.7	1.7
Sandy Loam Soil (Soil 2)						
Quantitation ion transition						
Isocycloseram (SYN547407)	0.001 (LLMV)	5	97.9-103	99.9	1.9	1.9
	0.01	5	93.3-103	97.9	3.4	3.4
SYN549107	0.001 (LLMV)	5	98.5-108	103	3.6	3.5
	0.01	5	89.5-96.4	93.3	2.5	2.7
SYN549431	0.001 (LLMV)	5	98.4-111	102	5.0	4.9
	0.01	5	92.1-99.8	97.3	3.2	3.3
SYN550738	0.001 (LLMV)	5	86.6-107	95.9	8.5	8.8
	0.01	5	85.3-100	93.4	5.5	5.9
Confirmation ion transition						
Isocycloseram (SYN547407)	0.001 (LLMV)	5	92.2-103	99.2	4.2	4.2

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.01	5	90.8-101	97.8	4.2	4.3
SYN549107	0.001 (LLMV)	5	83.5-123	101	19.1	18.8
	0.01	5	90.4-95.3	92.4	2.0	2.2
SYN549431	0.001 (LLMV)	5	83.8-110	99.2	10.3	10.3
	0.01	5	94.4-97.8	96.1	1.6	1.6
SYN550738	0.001 (LLMV)	5	68.5-101	81.1	13.0	16.1
	0.01	5	85.1-96.5	90.4	4.2	4.6
Method With Optional SPE						
Silty Clay Loam Soil (Soil 1)						
Quantitation ion transition						
Isocycloseram (SYN547407)	0.001 (LLMV)	5	97.5-111	103	6.0	5.8
	0.01	5	101-118	108	8.1	7.5
SYN549107	0.001 (LLMV)	5	93.1-102	97.9	3.8	3.9
	0.01	5	91.8-99.2	95.6	3.0	3.2
SYN549431	0.001 (LLMV)	5	98.0-102	99.8	1.4	1.4
	0.01	5	96.1-102	98.7	2.9	2.9
SYN550738	0.001 (LLMV)	5	97.2-100	99.5	1.7	1.7
	0.01	5	88.2-111	99.6	9.3	9.3
Confirmation ion transition						
Isocycloseram (SYN547407)	0.001 (LLMV)	5	99.8-106	102	2.7	2.6
	0.01	5	98.3-105	101	3.1	3.1
SYN549107	0.001 (LLMV)	5	89.3-103	97.3	4.9	5.0
	0.01	5	91.3-102	95.6	4.5	4.7
SYN549431	0.001 (LLMV)	5	94.4-102	99.9	3.2	3.2
	0.01	5	96.0-101	98.1	2.2	2.3
SYN550738	0.001 (LLMV)	5	84.7-105	97.7	8.8	9.0
	0.01	5	88.5-107	98.5	7.0	7.1
Sandy Loam Soil (Soil 2)						
Quantitation ion transition						
Isocycloseram (SYN547407)	0.001 (LLMV)	5	89.0-112	98.0	10.8	11.0
	0.01	5	94.6-118	108	10.0	9.3
SYN549107	0.001 (LLMV)	5	101-105	103	1.9	1.8
	0.01	5	93.6-105	99.3	5.1	5.1

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
SYN549431	0.001 (LLMV)	5	97.0-104	101	2.7	2.7
	0.01	5	96.6-104	100	3.1	3.1
SYN550738	0.001 (LLMV)	5	87.6-105	96.6	7.6	7.8
	0.01	5	83.2-100	93.4	6.9	7.3
Confirmation ion transition						
Isocycloseram (SYN547407)	0.001 (LLMV)	5	92.1-102	97.1	3.9	4.0
	0.01	5	96.4-104	99.8	3.9	3.9
SYN549107	0.001 (LLMV)	5	98.8-103	101	1.5	1.5
	0.01	5	92.8-105	99.7	5.2	5.2
SYN549431	0.001 (LLMV)	5	97.9-104	100	2.6	2.6
	0.01	5	96.1-104	99.9	3.4	3.4
SYN550738	0.001 (LLMV)	5	81.0-104	95.4	8.5	8.9
	0.01	5	80.1-97.8	89.0	6.4	7.2

Data (uncorrected results, p. 18; Appendix 4, pp. 169-170) were obtained from Tables 2-9, pp. 27-34 of MRID 51228137. 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 In the ILV, the matrices were silty clay loam soil (Soil 1; Sample ID: PA.IA.K.CHAR; pH 6.1 (1:1 soil:water ratio); 16% sand, 54% silt, 30% clay, 3.0% organic matter-Walkley Black) and sandy loam soil (Soil 2; Sample ID: PA.CA.K.BS.CHAR; pH 7.8 (1:1 soil:water ratio); 63% sand, 28% silt, 9% clay, 0.89% organic matter-Walkley Black); matrices were characterized by Agvise Laboratories, Northwood, North Dakota (USDA texture classification; p. 15; Table 1, p. 26 of MRID 51228137). The soil textures were verified by the reviewer using USDA-NRCS technical support tools. Soil sources were not reported.

2 The ILV was based on MRID 51228135 and validated GRM072.12A with and without the optional Solid Phase Extraction procedure (pp. 18, 24 of MRID 51228137).

3 Two ion pair transitions were monitored (primary and confirmatory, respectively *m/z* 548.1→418.0 and *m/z* 548.1→160.1 for isocycloseram (SYN547407), *m/z* 434.1→238.0 and *m/z* 434.1→354.0 for SYN549107, *m/z* 435.1→392.1 and *m/z* 435.1→160.1 for SYN549431, and *m/z* 448.1→378.0 and *m/z* 448.1→69.0 for SYN550738; the monitored ion transitions of the ILV were similar to those of the ECM.

III. Method Characteristics

Since the LOQ was not based on scientifically acceptable procedures as defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than a true LOQ. The LLMV in soil was reported as 0.001 mg/kg for isocycloseram (SYN547407), SYN549107, SYN549431, and SYN550738, respectively, in the ECM 1, ECM 2, and ILV (pp. 17, 29 of MRID 51228135; pp. 22, 35 of MRID 51228136; pp. 20, 23 of MRID 51228137). In the ECM 1 and ECM 2, the LLMV was defined as the lowest fortification level in which the method was validated where mean recovery was 70-110% and relative standard deviation was ≤20%. No justifications for the LLMV were reported in the ILV. No calculations for the LLMV were reported in the ILV or ECM. The limit of detection (LOD) of the method is defined as 30 % of the LOQ. The LOD in soil was reported as 0.0003 mg/kg (30% of the LLMV) for all four

analytes in the ECM 1 and ECM 2. In ECM 2, the LOD was determined as 0.45 pg injected on column, equivalent to 0.015 ng/mL when using a 30-µL injection volume. No calculations for LOD were reported in the ECM 1 and ECM 2. The LOD was not reported in the ILV.

Table 4. Method Characteristics

			Isocycloseram (SYN547407)	SYN549107	SYN549431	SYN550738
Lowest Level of Method Validation (LOQ)*	ECM 1 & ECM 2[1,2]		0.001 mg/kg			
	ILV					
Limit of Detection (LOD)	ECM 1 & ECM 2		0.0003 mg/kg (30% of the LLMV)			
	ILV		**Not reported**			
Linearity (calibration curve r and concentration range)	ECM 1 & ECM 2	No SPE	r^2 = 1 (LS, Q & C) r^2 = 0.9998 (SL, Q & C)	r^2 = 0.9998 (LS, Q & C)r^2 = 0.9998 (SL, Q & C)	r^2 = 0.9998 (LS, Q) r^2 = 1 (LS, C) r^2 = 1 (SL, Q) r^2 = 0.9996 (SL, C)	r^2 = 0.9994 (LS, Q & C) r^2 = 0.9996 (SL, Q & C)
		SPE	**Not performed**			
		Range	0.015-10 ng/mL (0.0003-0.2 mg/kg; 0.045-300 pg when using 30 µL injection)			
	ILV[2]	No SPE	r^2 = 0.9996 (SCL, Q) r^2 = 0.9994 (SCL, C) r^2 = 1 (SL, Q) r^2 = 0.9999 (SL, C)	r^2 = 0.9998 (SCL, Q) r^2 = 0.9999 (SCL, C) r^2 = 1 (SL, Q & C)	r^2 = 1 (SCL, Q) r^2 = 0.9994 (SCL, C) r^2 = 1 (SL, Q & C)	r^2 = 1 (SCL, Q & C) r^2 = 0.9998 (SL, Q) r^2 = 1 (SL, C)
		SPE	r^2 = 1 (SCL, Q & C) r^2 = 1 (SL, Q & C)	r = 1 (SCL, Q & C) r = 1 (SL, Q & C)	r^2 = 1 (SCL, Q & C) r^2 = 1 (SL, Q & C)	r^2 = 1 (SCL, Q & C) r^2 = 0.9998 (SL, Q) r^2 = 1 (SL, C)
		Range	0.015-10 ng/mL			
Repeatable	ECM 1 & ECM 2	No SPE[3]	Yes for LLMV (0.001 mg/kg) and 10×LLMV (0.01 mg/kg) in two characterized soil matrices (sandy loam and loamy sand)			
		SPE	**Not performed**			
	ILV[4,5,6]	No SPE	Yes for LLMV (0.001 mg/kg) and 10×LLMV (0.01 mg/kg) in two characterized soil matrices (sandy loam and silty clay loam)			
		SPE				
Reproducible[7]		No SPE	Yes for 0.001 mg/kg (LLMV)* and 0.01 mg/kg in soil matrices			
		SPE	**Could not be determined; only one set of performance data submitted in ECM.**			

Specific	ECM	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed. Some minor baseline noise was noted at the LLMV.	Yes, no matrix interferences were observed.
	ILV	Yes, matrix interferences were <5% of the LLMV (based on quantified residues).[7]	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed.	Yes, matrix interferences were <2% of the LLMV (based on quantified residues).[7]

Data were obtained from pp. 17, 29 (LLMV/LOD); Tables 2-9, pp. 35-38 (recovery data); Tables 17-24, pp. 43-46 (linearity coefficients); p. 30; Figures 117-132, pp. 108-115 (calibration curves); Figures 5-116, pp. 52-107 (chromatograms) of MRID 51228135; pp. 22, 35 (LLMV/LOD); Tables 1-8, pp. 40-43 (recovery data); Tables 15-22, pp. 47-50 (linearity coefficients); p. 35; Figures 113-143, pp. 109-124 (calibration curves); Figures 1-112, pp. 53-108 (chromatograms) of MRID 51228136; pp. 20, 23 (LLMV/LOD); Tables 2-9, pp. 27-34 (recovery data); Figures 17-32, pp. 84-99 (calibration curves); Figures 1-16, pp. 36-83 (chromatograms) of MRID 51228137; DER Excel Attachment. Q = quantitative ion transition; C = confirmatory ion transition; LS = Loamy and soil; SL = Sandy loam soil; SCL = Silty clay loam soil.

* 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 lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 ECM 1 = MRID 51228135 (GRM072.12A). MRID 51228135 (dated 2020) was an updated version of MRID 51228136 (dated 2019). MRID 51228135 contained the same methodology and performance data; however, the methodology was updated with an optional Solid Phase Extraction (SPE) procedure. No new performance data was submitted for the method using the optional SPE procedure.

2 ECM 2 = MRID 51228136.

3 In the ECM 1 and ECM 2, the soil matrices were loamy sand soil (LUFA F2.1; pH 5.32 (0.01M $CaCl_2$); 86.3% sand, 10.8% silt, 2.9% clay, 0.99% organic matter, 0.58% organic carbon) and sandy loam soil (LUFA 5M; pH 7.34 (0.01M $CaCl_2$); 57.4% sand, 32.3% silt, 10.3% clay, 1.11% organic matter (reported as 1.11% organic carbon in Table 1, p. 34 of MRID 51228135); matrices were obtained from Germany and characterized by the supplier (USDA texture classification; Table 1, p. 34 of MRID 51228135; Appendix 3, pp. 135-136 of MRID 51228136). The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

4 In the ILV, the matrices were silty clay loam soil (Soil 1; Sample ID: PA.IA.K.CHAR; pH 6.1 (1:1 soil:water ratio); 16% sand, 54% silt, 30% clay, 3.0% organic matter-Walkley Black) and sandy loam soil (Soil 2; Sample ID: PA.CA.K.BS.CHAR; pH 7.8 (1:1 soil:water ratio); 63% sand, 28% silt, 9% clay, 0.89% organic matter-Walkley Black); matrices were characterized by Agvise Laboratories, Northwood, North Dakota (USDA texture classification; p. 15; Table 1, p. 26 of MRID 51228137). The soil textures were verified by the reviewer using USDA-NRCS technical support tools. Soil sources were not reported.

5 The ILV was based on MRID 51228135 and validated GRM072.12A with and without the optional Solid Phase Extraction procedure (pp. 18, 24 of MRID 51228137).

6 The ILV validated the method, GRM072.12A (with and without the optional SPE procedure), with the first trial as written, except for the use of HDPE bottles for extraction rather than polypropylene (per correspondence with the method author), the sample was only evaporated to 1.5 mL instead of 0.5 mL, and insignificant modifications to the analytical parameters and equipment (pp. 18, 20; Appendix 6, p. 302 of MRID 51228137). More information regarding the ILV modifications is required to determine if an updated method GRM072.12A should be submitted.

7 Data contained in Appendix 5, pp. 268-300 of MRID 51228137.

IV. Method Deficiencies and Reviewer's Comments

1. Since the reported method LOQ was not based on the scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than a true LOQ (pp. 17, 29 of MRID 51228135; pp. 22, 35 of MRID 51228136; pp. 20, 23 of MRID 51228137). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV, ECM 1, and ECM 2, the LLMV was equivalent to the ILV reported method LLMV for all analytes in soil (0.001 mg/kg). No justifications for the LLMV were reported in the ILV. No calculations for the LOQ/LLMV were reported in the ILV or ECM.

2. The LLMV is less (more sensitive) than the lowest toxicological level of concern in soil for all analytes. In this case, the lowest toxicological level of concern is the No Observed Adverse Effect Concentration (NOAEC) at 0.095 mg/kg for the non-target arthropod collembolans (*Folsomia candida*) (Geary 2015; MRID 51229448).

3. This DER was prepared with two MRIDs as the internal laboratory method validations (ECMs): MRID 51228135 & MRID 51228136. ECM 1 = MRID 51228135 (GRM072.12A). ECM 2 = MRID 51228136. MRID 51228135 (dated 2020) was an updated version of MRID 51228136 (dated 2019). MRID 51228135 contained the same methodology and performance data; however, the methodology was updated with an optional Solid Phase Extraction (SPE) procedure. No new performance data were submitted for the method using the optional SPE procedure. In ECM 1 (MRID 51228135), the method noted that SPE should only be performed if the concentration of the samples results in significant matrix effect (>20%) which impacts residue determination using matrix-matched standards (pp. 21-22 of MRID 51228135). No significant matrix effect was observed in ECMs 1 or 2, and matrix-matched calibration standards were used (p. 30; Table 10, p. 39 of MRID 51228135; p. 35; Table 23, p. 51 of MRID 51228136).

4. The ILV was based on MRID 51228135 and validated method GRM072.12A with and without the optional Solid Phase Extraction procedure (pp. 18, 24 of MRID 51228137). Matrix effects were not studied in the ILV. No significant matrix interferences were observed, and matrix-matched calibration standards were used. Recovery results were comparable with and without the optional SPE procedure for all analytes/transitions/ fortifications, except for the confirmation ion transition analysis of SYN550738 at the LLMV (81.1% mean 16.1% RSD without SPE and 95.4% mean 8.9% RSD with SPE; Tables 2-9, pp. 27-34).

5. There was communication between the ILV study personnel and the "method author" (not specified). During this communication, the ILV authors were instructed to use HDPE bottles for extraction rather than polypropylene, as was used in the ECM. Additional clarifications included instructions to use glass sampler vials, the solvent composition of the matrix-matched calibration standards and chilling the autosampler. An updated ECM should be submitted with these additional instructions.

6. The ILV was not conducted completely independently of the internal method validation (ECM 1 or 2) since there was communication between the ILV study personnel and the method author (not specified; p. 18 of MRID 51228137). OCSPP guidelines specify that the ILV must be conducted independently of the internal validation without collusion between the laboratory personnel or equipment. The communications between the ILV Study Director/Author (Melanie Silinski, RTI International), Study Sponsor (Lula Ghebremicahe, Syngenta Crop Protection, LLC) and "others familiar with the study" (not specified but reported as "method author") were summarized and listed but not detailed (pp. 5, 18; Appendix 6, p. 302). Communications (emails and teleconference) involved protocol and report template issue, exchange of information regarding test materials and soils, instructions to use HDPE bottles for extraction rather than polypropylene and to conduct the procedure with and without SPE procedure, and method clarifications involving the solvent composition of the matrix-matched calibration standards and chilling the autosampler.

7. The ILV validated the method, GRM072.12A (with and without the optional SPE procedure), with the first trial as written, except for the use of HDPE bottles for extraction rather than polypropylene (per correspondence with the method author). The sample was only evaporated to 1.5 mL instead of 0.5 mL, with insignificant modifications to the analytical parameters and equipment (pp. 18, 20; Appendix 6, p. 302 of MRID 51228137). Also, the fact that the sample reduction was amended to have a higher volume could indicate that sample loss occurs when the sample is reduced to 0.5 mL, as specified in method GRM072.12A (p. 22 of MRID 51228135).

   In the ECM and ILV, soil property ranges are as follows: pH 5.32- 7.8, sand 16-87%, silt 11-54%, clay 3-35%, and organic carbon 0.58-3.1%. The sand, silt, and clay quantities in the method validation cover the ranges evaluated in the twelve submitted terrestrial field dissipation (TFD) studies (MRIDs 51228406, 51228407, 51228408, 51228409, 51228410, 51228411, 51228412, 51228413, 51228414, 51228415, 51228416, and 51228417). The pH range in the field dissipation studies is 4.8 to 8.8. Submitted sorption studies (MRIDs 51228421 and 51228422) show no to weak correlation between pH and sorption coefficient, so this parameter may be less significant. The upper bound of the organic carbon, 3.1%, evaluated in the ECM and ILV is greater than that in all the field dissipation studies, therefore adequately covering sorption on-field. Therefore, the ECM and ILV cover the range of soils used in the TFDs.

8. In the ECM 1 and ECM 2, the characterization of the soil matrices is inconsistent for the sandy loam soil (LUFA 5M), where 1.11% organic carbon was reported in Table 1, p. 34 of MRID 51228135 (% organic matter was not reported) while 1.11% organic matter was reported in Appendix 3, pp. 135-136 of MRID 51228136 (% organic carbon was not reported).

9. The LOD in soil was reported as 30% of the LLMV for all four analytes in the ECM 1 and ECM 2. In ECM 2, the LOD was determined as 0.45 pg injected on column, equivalent to 0.015 ng/mL when using a 30-µL injection volume. No calculations for

LOD were reported in the ECM 1 and ECM 2. The LOD was not reported in the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

10. The time requirement for the method was reported in the ILV as *ca*. 10 hours (one extended workday) for sample preparation and processing (with and without SPE) with UPLC/MS/MS analysis performed overnight (p. 23 of MRID 51228137). In the ECM 1, the time requirement for the method was reported as 1 day (8 hours) for a batch of 12 samples (p. 22 of MRID 51228135). No time requirement was reported in ECM 2.

V. References

Geary, N. 2015. Isocycloseram: SYN547407 - A Laboratory Test to Determine the Effects of Fresh Residues on the Springtail *Folsomia candida* (Collembola, Isotomidae): Final Report. Project Number: SYN/15/18, TK0226046, MRID 51229448. Unpublished Study Prepared by Mambo-Tox Limited.

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. 344-347, and Revision 2; 2015 and 2016.

Attachment 1: Chemical Names and Structures

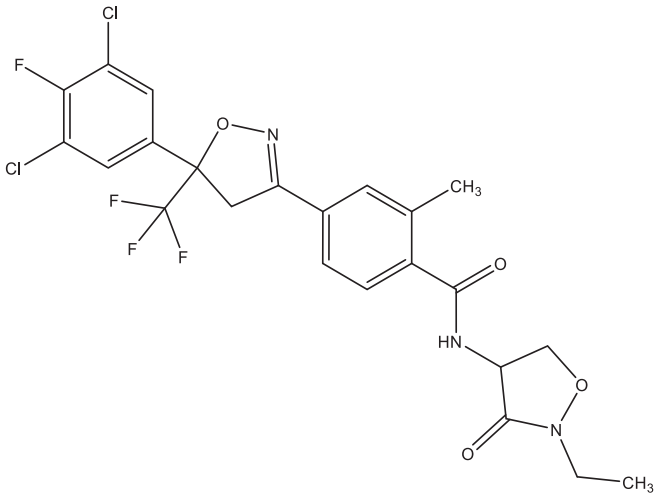
Isocycloseram (SYN547407)

IUPAC Name: 4-[5-(3,5-Dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl]-N-(2-ethyl-3-oxozolidin-4-yl)-2-methylbenzamide

CAS Name: 4-[5-(3,5-Dichloro-4-fluorophenyl)-4,5-dihydro-5-(trifluoromethyl)-3-isoxazolyl]-N-(2-ethyl-3-oxo-4-isoxazolidinyl)-2-methylbenzamide

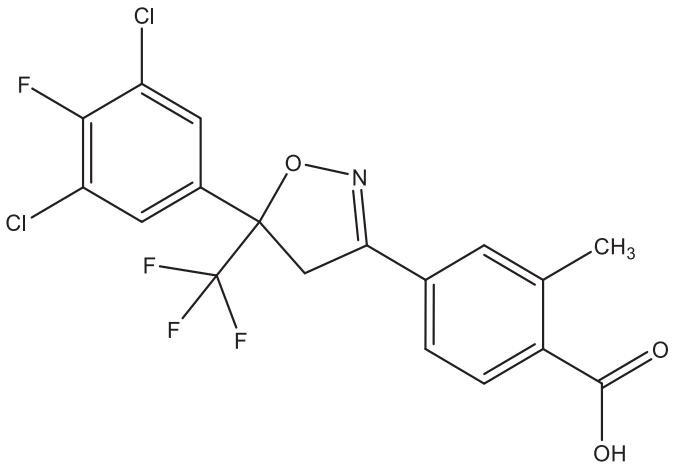
CAS Number: 2061933-85-3

SMILES String: ClC1=C(F)C(Cl)=CC(C2(C(F)(F)F)CC(C3=CC=C(C(NC4C(N(CC)OC4)=O)=O)C(C)=C3)=NO2)=C1



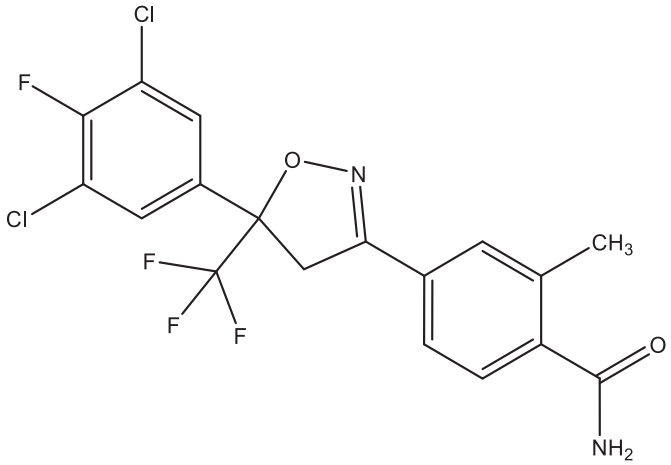
SYN549107

IUPAC Name:	4-[5-(3,5-Dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl]-2-methylbenzoic acid
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	ClC1=C(F)C(Cl)=CC(C2(C(F)(F)F)CC(C3=CC=C(C(O)=O)C(C)=C3)=NO2)=C1



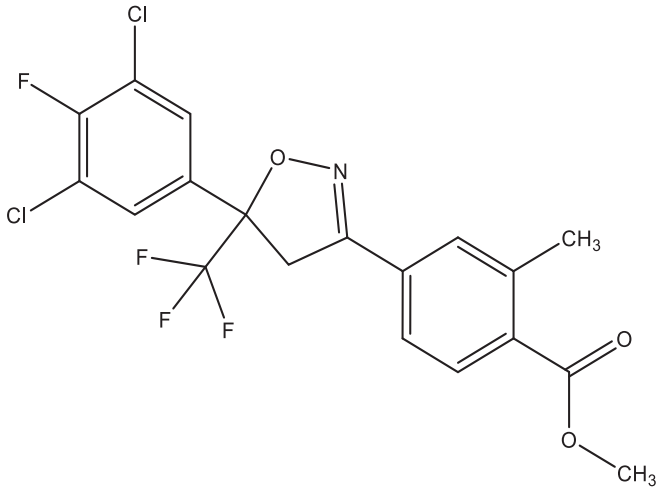
SYN549431

IUPAC Name:	4-(5-(3,5-Dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl)-2-methylbenzamide
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	ClC1=C(F)C(Cl)=CC(C2(C(F)(F)F)CC(C3=CC=C(C(N)=O)C(C)=C3)=NO2)=C1



SYN550738

IUPAC Name:	Methyl 4-[5-(3,5-dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl]-2-methylbenzoate
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	ClC1=C(F)C(Cl)=CC(C2(C(F)(F)F)CC(C3=CC=C(C(OC)=O)C(C)=C3)=NO2)=C1



DER Excel Attachment- Please see **attached Excel file** for additional information about calculations referenced in the main text.