Analytical method for Isocycloseram (SYN547407) and four degradates SYN549107, SYN549431, SYN550455 and SYN551485 in water

Reports: ECM: EPA MRID No. 51228142. Mayer, L.C. 2021. Isocycloseram. SYN547407 - Residue Method GRM072.17A for the Determination of SYN547407 and Metabolites SYN549107, SYN549431, SYN550455 and SYN551485 in Water. Includes Validation Data. Analytical Method. Syngenta Report No.: GRM072.17A. Task No.: TK0603012. Report prepared, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 98 pages. Final report issued March 23, 2021.

ILV: EPA MRID No. 51228148. Draghi, A., Yi J. 2021. Isocycloseram. SYN547407 – Independent Laboratory Validation of Residue Method GRM072.17A for the Determination of SYN547407 and Metabolites SYN549107, SYN549431, SYN550455 and SYN551485 in Water. Final Report Amendment 1. PASC Report No. PASC-REP-3882. PASC Project No. 141-7143. Task No.: TK0081939. Report prepared by Primera Analytical Solutions Corp., Cranbury, New Jersey; sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 110 pages. Final report issued May 3, 2021.

Document No.: MRIDs 51228142 & 51228148

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 51228142). Signed and dated No Data Confidentiality and GLP statements were provided (p. 2-3 of MRID 51228142). Quality Assurance and Authenticity statements were not included.

ILV: The study was conducted in accordance with USEPA FIFRA GLP (40 CFR Part 160, 1989; p. 3 of MRID 51228148). 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 **supplemental**. 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. The LLMV is greater (less sensitive) than the most sensitive aquatic toxicity endpoint. The specificity of the method for SYN550455 was not supported by ECM representative chromatograms since two peaks were observed for SYN550455.

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EFED Final Reviewer:	Sarah Brazeau, Ph.D. Physical Scientist	Signature:	SARAH BRAZEAU Digitally signed by SARAH BRAZEAU Date: 2025.05.01 17:32:29 -04'00'
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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, GRM072.17A, is designed for the quantitative determination of isocycloseram (SYN547407) and metabolites SYN549107, SYN549431, SYN550455 and SYN551485 at 0.05 µg/L in water using LC/MS/MS. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the LLMV rather than an LOQ. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for all analytes in water (0.05 µg/L). The LLMV is greater (less sensitive) than the lowest toxicological level of concern in water for all analytes. For aquatic invertebrates, the most sensitive toxicity endpoint available was determined to the be the No Observed Adverse Effect Concentration (NOAEC) for mysid. The value of this endpoint is 0.00098 µg active ingredient/liter (a.i./L) (Marini 2019; MRID 51229455).

The ILV validated the method, GRM072.17A, with the first trial as written, except for minor modifications to optimize the instrumentation used in the ILV as allowed by the ECM. These modifications included: 1) changing the LC instrumentation; 2) mobile phase and gradient change; and dwell time change for three of the analytes. An updated ECM is not required. The ECM and ILV test matrices were characterized ground and surface water matrices.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for isocycloseram (SYN547407) and metabolites SYN549107, SYN549431, SYN550455 and SYN551485 at 0.05 µg/L and 0.5 µg/L, except for SYN550455 for which the specificity of the method was not supported by ECM representative chromatograms since two

peaks were observed for SYN550455. Only one peak was observed in ILV representative chromatograms for SYN550455; the same LC column was used, but different LC mobile phase and gradient was employed.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Lowest Level of Method Validation (LLMV)
	Environmental Chemistry Method	Independent Laboratory Validation						
Isocycloseram (SYN547407)	51228142[1]	51228148[2]	Supplemental	Water	23/03/2021	Syngenta Crop Protection, LLC	LC/MS/MS	0.05 µg/L
SYN549107								
SYN549431								
SYN550455								
SYN551485								

1 In the ECM, the specific source of the surface water was reported as a local lake. The source of the ground water was reported as residential (Table 1, p. 28 of MRID 51228142). The surface water matrix had the following characteristics: pH: 7.5; conductivity: 0.13 mmhos/cm; hardness: 33 mg; SAR 0.51. The ground water matrix had the following characteristics: pH: 7.3; conductivity: 0.10 mmhos/cm; hardness: 31 mg; SAR 0.53 (Table 1, p. 28 of MRID 51228142). The water characterization report was not provided.

2 The surface and ground water matrices were provided by the study sponsor (pp. 8 and 11 of MRID 51228148). The source of the samples was not reported. The surface water matrix had the following characteristics: pH: 6.3; conductivity: 0.24 mmhos/cm; hardness: 73 mg as $CaCO_3$/L; SAR 0.26; Turbidity 20.3 NTU, Alkalinity 64 mg $CaCO_3$/L (Appendix 9, p. 107 of MRID 51228148). The ground (well) water matrix had the following characteristics: pH: 7.8; conductivity: 0.02 mmhos/cm; hardness: 325 mg as $CaCO_3$/L; SAR 0.58; Turbidity 0.22 NTU (Appendix 9, p. 109). The water characterization was performed by Agvise Laboratories in Northwood, North Dakota.

6. Principle of the Method

Surface water and ground (drinking) water samples were shaken and direct diluted with acetonitrile and transferred into HPLC vials for analysis by high performance liquid chromatography using a C18 HPLC column separation with triple quadrupole mass spectrometric detection (LC-MS/MS; pp. 14 and 19 of MRID 51228142).

Water matrix samples should be collected and stored in glass due to the adsorption of compounds to plastic (e.g., HDPE and PP). If frozen, water samples should be allowed to defrost completely at room temperature (p. 17 of MRID 51228142). The samples should be shaken to thoroughly to ensure homogeneity prior to analysis.

Stock solutions of (100 µg/L) were prepared by one of two methods (p. 16 of MRID 51228142). 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. The stock solutions in the ECM were prepared by weighing the standard material directly into an appropriate storage vessel.

Combined fortification solutions of water samples were fortified to concentrations of 0.10, 0.010 and 0.001 µg/mL with serial dilution with acetonitrile (p. 16 of MRID 51228142). Details of the serial dilutions were not reported in the MRID.

Samples were analyzed using a Sciex ExionLC™ high performance liquid chromatography (HPLC) system coupled to Applied Biosystems QTRAP® 6500 MS (p. 19 of MRID 51228142). The HPLC pump information was not reported. The following LC conditions were used: Phenomenex Kinetex C18 (50 mm x 4.6 mm column; 2.6 µm particle size; column temperature ambient/30°C), mobile phase of (A) 0.01% acetic acid in water, and (B) 0.01% acetic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0-0.5 min. 90:10, 3.0 min. 30:70, 5.0-6.0 min. 5:95, 6.1-7.0 min. 90:10], flow 1 mL/min., and injection volume of 30 µL. The following MS/MS conditions were used: 5500V positive mode/-4500V negative mode, (source temperature 600°C), Turbo Ion Spray interface, and multiple reaction monitoring (MRM; p. 20 of MRID 51228142). Two ion pair transitions were monitored for isocycloseram (SYN547407), SYN549107, SYN549431, SYN550455, and SYN551485. Separate injections in negative and positive mode were required to provide enough data points across the target peak(s). The following MS settings were reported in the ECM (pp. 20-21 of MRID 51228142).

Analyte	Primary	Confirmatory	Mode	Expected Retention Times (minutes)
SYN547407	*m/z* 548→418	*m/z* 548→160	Positive	4.1
SYN549107	*m/z* 434→354	*m/z* 434→238	Negative	4.3
SYN549431	*m/z* 435→392	*m/z* 435→160	Positive	3.9
SYN550455	*m/z* 553→277	*m/z* 553→247	Negative	3.4
SYN551485	*m/z* 503→390	*m/z* 503→431	Negative	4.1

The independent laboratory performed the ECM as written, with the following modifications: 1) The equipment used differed from the equipment in the ECM. A Shimadzu LC-30AD UHPLC LC system was used coupled with an AB MDS Sciex API 6500 triple quadrupole MS LC-MS/MS (pp. 11-12; Figure 7, pp. 45-46 of MRID 51228148). 2) The mobile phase and gradient differed from the ECM. Mobile phase A was 0.1% formic acid in water. Mobile phase B was 0.1% formic acid in acetonitrile. The mobile phase gradient was A:B (v:v) at 0-0.5 min. 100:0, 1.2-3.0 min. 20:80, 3.0-3.5 min. 0:100, 4.0 min 100:0. The Pump B concentration was changed from 10% to 0%. The column specifications, the column temperature, the injection volume, and flow rate were the same as used in the ECM. The ILV lists a strong wash solution as the autosampler rinse solution. The ECM did not report the rinse solution.

The MS parameters of the ILV were the same as those of the ECM (p. 12, Figure 7, pp. 45-46 of MRID 51228148). However, dwell times of the transitions of SYN547407, SYN549431 and SYN551485 were changed from 25 msec to 50 msec**.**

The Limit of Quantification (LOQ) for isocycloseram (SYN547407), SYN549107, SYN549431, SYN550455, and SYN551485 in surface water and groundwater was reported as 0.05 µg/L in the ECM and the ILV (pp. 14 and 24 of MRID 51228142; pp. 8 and 12 of MRID 51228148). The

Limit of Detection (LOD) for isocycloseram (SYN547407), SYN549107, SYN549431, SYN550455, and SYN551485 in surface water and ground water was reported as 0.005 µg/L in the ECM and ILV (p. 24 of MRID 51228142; and p. 12 of MRID 51228148).

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 51228142): 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 isocycloseram (SYN547407) and metabolites SYN549107, SYN549431, SYN550455 and SYN551485 at the LLMV (0.05 µg/L) and 10×LLMV (0.5 µg/L; Tables 2-5, pp. 29-32 of MRID 51228142). One reagent blank, one control sample, and five samples for each matrix and each fortification level were analyzed (Figures 10-17, pp. 43-46; Figures 24-31; pp. 50-53 of MRID 51228142). Performance data for the quantification and confirmation ion pair transitions were evaluated and comparable. Residues of isocycloseram (SYN547407) and metabolites SYN549107, SYN549431, SYN550455 and SYN551485 were less than 30% of the LLMV in the control samples and reagent blanks (Tables 6, pp. 33 of MRID 51228142).

In the ECM, the specific source of the surface water was reported as a local lake. The source of the ground water was reported as residential (Table 1, p. 28 of MRID 51228142). The surface water matrix had the following characteristics: pH: 7.5; conductivity: 0.13 mmhos/cm; hardness: 33 mg; SAR 0.51. The ground water matrix had the following characteristics: pH: 7.3; conductivity: 0.1 mmhos/cm; hardness: 31 mg; SAR 0.53 (Table 1, p. 28 of MRID 51228142). The water characterization report was not provided.

ILV (MRID 51228148): Mean recoveries and RSDs met requirements for surface water and ground water matrix samples were fortified with isocycloseram (SYN547407) and metabolites SYN549107, SYN549431, SYN550455 and SYN551485 at the LLMV (0.05 µg/L) and 10×LLMV (0.5 µg/L; pp. 8, 14-15, Tables 4-5, pp. 21-30 of MRID 51228148). One control sample, one reagent blank, and five samples for each matrix and each fortification level were analyzed for isocycloseram and the target metabolites. The analytes were quantified one ion pair transition and confirmatory ion pair transition. The quantitation and confirmatory ion pair transitions are listed in Section I. Performance data for the quantification and confirmation ion pair transitions were comparable. Isocycloseram and the metabolites were not detected in the control samples.

The surface and ground water matrices were provided by the study sponsor (pp. 8 and 11 of MRID 51228148). The source of the samples was not reported. The surface water matrix had the following characteristics: pH: 6.3; conductivity: 0.24 mmhos/cm; hardness: 73 mg as $CaCO_3$/L; SAR 0.26; Turbidity 20.3 NTU; Alkalinity 64 mg $CaCO_3$/L (Appendix 9, p. 107 of MRID 51228148). The ground (well) water matrix had the following characteristics: pH: 7.8; conductivity: 0.02 mmhos/cm; hardness: 325 mg as $CaCO_3$/L; SAR 0.58; Turbidity 0.22 NTU (Appendix 9, p. 109). The water characterization was performed by Agvise Laboratories in Northwood, North Dakota.

Each bulk water sample was checked for possible contamination or background levels of isocycloseram (SYN547407) and metabolites SYN549107, SYN549431, SYN550455 and SYN551485 prior to use in the ILV.

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 #3). The ILV validated the method in one trial and no matrix suppression or enhancement was observed (pp. 16 and 17 of MRID 51228148). The ILV method was based on the ECM method; however, the ILV was modified to optimize the LC-MS/MS equipment at the ILV. An updated ECM is not required.

Table 2. Initial Validation Method Recoveries for Isocycloseram (SYN547407), SYN549107, SYN549431, SYN550455, and SYN551485 in Water[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface water						
Quantitation ion transition						
Isocycloseram (SYN547407)	0.05 (LOQ)	5	85-99	88	5.9	6.7
	0.5 (10x LOQ)	5	90-113	101	8.6	8.5
SYN549107	0.05 (LOQ)	5	95-100	98	2.4	2.4
	0.5 (10x LOQ)	5	93-102	98	3.4	3.5
SYN549431	0.05 (LOQ)	5	81-91	87	3.7	4.2
	0.5 (10x LOQ)	5	92-98	94	2.4	2.5
SYN550455	0.05 (LOQ)	5	95-104	99	3.2	3.2
	0.5 (10x LOQ)	5	90-100	94	3.7	3.9
SYN551485	0.05 (LOQ)	5	86-99	93	5.5	5.9
	0.5 (10x LOQ)	5	89-102	94	5.3	5.6
Confirmation ion transition						
Isocycloseram (SYN547407)	0.05 (LOQ)	5	84-98	89	5.1	5.8
	0.5 (10x LOQ)	5	87-101	94	5.4	5.8
SYN549107	0.05 (LOQ)	5	88-96	93	3.0	3.2
	0.5 (10x LOQ)	5	95-101	97	2.6	2.7
SYN549431	0.05 (LOQ)	5	87-98	93	4.6	4.9
	0.5 (10x LOQ)	5	93-97	96	1.9	2.0
SYN550455	0.05 (LOQ)	5	91-100	96	4.1	4.3
	0.5 (10x LOQ)	5	91-98	94	2.7	2.9
SYN551485	0.05 (LOQ)	5	97-110	103	4.7	4.6
	0.5 (10x LOQ)	5	87-99	92	4.8	5.2
Ground water						
Quantitation ion transition						
Isocycloseram (SYN547407)	0.05 (LOQ)	5	87-107	98	9.2	9.4
	0.5 (10x LOQ)	5	86-115	101	12.0	11.9
SYN549107	0.05 (LOQ)	5	89-98	95	3.9	4.1
	0.5 (10x LOQ)	5	93-107	99	5.2	5.3
SYN549431	0.05 (LOQ)	5	90-108	100	7.2	7.2
	0.5 (10x LOQ)	5	95-105	100	3.8	3.8
SYN550455	0.05 (LOQ)	5	88-108	99	8.3	8.4
	0.5 (10x LOQ)	5	90-102	98	4.5	4.6
SYN551485	0.05 (LOQ)	5	92-102	99	3.8	3.8
	0.5 (10x LOQ)	5	88-105	97	6.9	7.2
Confirmation ion transition						
Isocycloseram (SYN547407)	0.05 (LOQ)	5	90-100	93	3.6	3.8
	0.5 (10x LOQ)	5	80-97	88	8.0	9.2
SYN549107	0.05 (LOQ)	5	91-103	97	4.8	4.9
	0.5 (10x LOQ)	5	95-105	101	4.4	4.4
SYN549431	0.05 (LOQ)	5	82-103	95	8.6	9.0
	0.5 (10x LOQ)	5	90-103	98	5.0	5.1
SYN550455	0.05 (LOQ)	5	89-111	101	8.2	8.2
	0.5 (10x LOQ)	5	92-102	97	4.9	5.0

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
SYN551485	0.05 (LOQ)	5	88-109	97	8.3	8.6
	0.5 (10x LOQ)	5	84-102	95	7.2	7.6

Data (uncorrected recovery results; p. 22) were obtained from Tables 2 and 4 (quantitation ion pair transition) and Tables 3 and 5 (confirmatory ion pair transition); Tables 2-5, pp. 29-32 of MRID 51228142). 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 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→354 and *m/z* 434→238 for SYN549107, *m/z* 435→392 and *m/z* 435→160 for SYN549431, *m/z* 553→277 and *m/z* 553→247 for SYN550455 and *m/z* 503→390 and *m/z* 503→431 for SYN551485 (pp. 20-21 of MRID 51228142).

2 The specific source of the surface water was reported as a local lake. The source of the ground water was reported as residential (Table 1, p. 28 of MRID 51228142). The surface water matrix had the following characteristics: pH: 7.5; conductivity: 0.13 mmhos/cm; hardness: 33 mg; SAR 0.51. The ground water matrix had the following characteristics: pH: 7.3; conductivity: 0.1 mmhos/cm; hardness: 31 mg; SAR 0.53 (Table 1, p. 28 of MRID 51228142). The water characterization report was not provided.

Table 3. Independent Validation Method Recoveries for Isocycloseram (SYN547407), SYN549107, SYN549431, SYN550455, and SYN551485 in Water[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
Surface water						
Quantitation ion transition						
Isocycloseram (SYN547407)	0.05 (LOQ)	5	101-104	102	1	1.26
	0.5 (10x LOQ)	5	94.8-100	98.9	2	2.40
SYN549107	0.05 (LOQ)	5	94.4-106	100	4	4.26
	0.5 (10x LOQ)	5	98.4-103	101	2	2.15
SYN549431	0.05 (LOQ)	5	92.0-102	98.7	4	4.00
	0.5 (10x LOQ)	5	95.2-102	100	3	3.06
SYN550455	0.05 (LOQ)	5	102-112	106	4	3.69
	0.5 (10x LOQ)	5	102-106	105	2	1.52
SYN551485	0.05 (LOQ)	5	82.8-94.8	87.2	4.7	5.39
	0.5 (10x LOQ)	5	82.0-86.8	84.8	1.8	2.11
Confirmation ion transition						
Isocycloseram (SYN547407)	0.05 (LOQ)	5	98.4-102	101	2	1.64
	0.5 (10x LOQ)	5	96.8-103	100	3	2.42
SYN549107	0.05 (LOQ)	5	98.8-105	102	3	2.69
	0.5 (10x LOQ)	5	97.6-103	101	2	2.04
SYN549431	0.05 (LOQ)	5	98.8-106	102	3	2.73
	0.5 (10x LOQ)	5	94.8-103	99.4	3	3.12
SYN550455	0.05 (LOQ)	5	100-110	105	4	3.93
	0.5 (10x LOQ)	5	103-109	106	3	2.60
SYN551485	0.05 (LOQ)	5	85.2-94.0	90.7	3.3	3.63
	0.5 (10x LOQ)	5	86.4-88.8	87.4	1.0	1.15
Ground water						
Quantitation ion transition						
Isocycloseram (SYN547407)	0.05 (LOQ)	5	98.8-103	99.9	2	1.64
	0.5 (10x LOQ)	5	96.8-101	99.3	2	1.72
SYN549107	0.05 (LOQ)	5	96.4-101	98.9	2	2.17
	0.5 (10x LOQ)	5	98.4-105	101	3	2.59
SYN549431	0.05 (LOQ)	5	100-105	102	2	2.02
	0.5 (10x LOQ)	5	99.6-106	104	3	2.58
SYN550455	0.05 (LOQ)	5	101-106	103	2	1.75
	0.5 (10x LOQ)	5	98.4-104	102	2	2.14
SYN551485	0.05 (LOQ)	5	82.0-87.6	84.3	2.2	2.56
	0.5 (10x LOQ)	5	82.4-84.8	84.2	1.2	1.19
Confirmation ion transition						
Isocycloseram (SYN547407)	0.05 (LOQ)	5	98.8-101	100	1	0.769
	0.5 (10x LOQ)	5	97.2-102	100	2	2.04
SYN549107	0.05 (LOQ)	5	94.4-102	98.2	3	2.77
	0.5 (10x LOQ)	5	100-102	101	1	0.722
SYN549431	0.05 (LOQ)	5	99.2-108	103	3	3.01
	0.5 (10x LOQ)	5	98.0-106	103	3	2.89
SYN550455	0.05 (LOQ)	5	99.2-107	104	3	2.72
	0.5 (10x LOQ)	5	101-103	102	1	1.13

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
SYN551485	0.05 (LOQ)	5	78.4-89.2	85.2	4.0	4.73
	0.5 (10x LOQ)	5	83.2-88.8	86.2	2.0	2.31

Data (uncorrected recovery results; Figure 8, pp. 47-48) were obtained from Table 4 (quantitation ion pair transition) and Table 5 (confirmatory ion pair transition); pp. 8, 14-15, Tables 4-5 pp. 21-30 of MRID 51228148). 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 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→354 and *m/z* 434→238 for SYN549107, *m/z* 435→392 and *m/z* 435→160 for SYN549431, *m/z* 553→277 and *m/z* 553→247 for SYN550455 and *m/z* 503→390 and *m/z* 503→431 for SYN551485 (Figure 7, p. 46 of MRID 51228148).

2 The surface and ground water matrices were provided by the study sponsor (pp. 8 and 11 of MRID 51228148). The source of the samples was not reported. The surface water matrix had the following characteristics: pH: 6.3; conductivity: 0.24 mmhos/cm; hardness: 73 mg as $CaCO_3$/L; SAR 0.26; Turbidity 20.3 NTU, Alkalinity 64 mg $CaCO_3$/L (Appendix 9, p. 107 of MRID 51228148). The ground (well) water matrix had the following characteristics: pH: 7.8; conductivity: 0.02 mmhos/cm; hardness: 325 mg as $CaCO_3$/L; SAR 0.58; Turbidity 0.22 NTU (Appendix 9, p. 109). The water characterization was performed by Agvise Laboratories in Northwood, North Dakota.

3 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. Rules of significant figures were followed.

III. Method Characteristics

Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the LLMV rather than LOQ. The LLMV for parent isocycloseram (SYN547407), SYN549107, SYN549431, SYN550455, and SYN551485 in surface water and groundwater was reported as 0.05 µg/L in the ECM and the ILV (pp. 14 and 24 of MRID 51228142; pp. 8 and 12 of MRID 51228148). In the ECM and the ILV, the LLMV was justified as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-120% with a relative standard deviation of ≤20% has been obtained (p. 24 of MRID 51228142; p. 12 of MRID 51228148).

The Limit of Detection (LOD) for isocycloseram (SYN547407), SYN549107, SYN549431, SYN550455, and SYN551485 in surface water and ground water was reported as 0.005 µg/L in the ECM and ILV (p. 24 of MRID 51228142; and p. 12 of MRID 51228148). The LOD in the ECM was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. The LOD was determined as 0.15 pg injected on column, equivalent to 0.005 µg/mL when using a 30 µL injection volume. The LOD was based on the lowest concentration sample injected (p. 24 of MRID 51228142).

The LOD in the ILV was not independently validated. The ILV reported the LOD as specified in the Syngenta analytical method GRM072.17A has been used as 0.005 ng/mL (0.005 µg/L) for each of the analytes in the water matrices tested (p. 12 of MRID 51228148).

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

		Isocycloseram (SYN547407)	SYN549107	SYN549431	SYN550455	SYN551485
Lowest Levels of Method Validated (LLMV)*	ECM	0.05 µg/L (ppb) [SW and GW]				
	ILV					
Limit of Detection (LOD)	ECM	0.005 µg/L (ppb) (3x background noise) [SW and GW]				
	ILV					
Linearity (calibration curve r and concentration range)	ECM[3]	r = 0.9980 (Q); r = 0.9999 (C)	r = 0.9999 (Q); r = 0.9999 (C)	r = 0.9993 (Q); r = 0.9998 (C)	r = 0.9999 (Q); r = 0.9999 (C)	r = 0.9999 (Q); r = 0.9998 (C)
		Calibration Range: 0.005 to 5 µg/L (0.15 pg to 150 pg injected on column using a 30 µL injection volume)				
	ILV[4]	r = 0.9996 (Q)	r = 0.9991 (Q)	r = 0.9978 (Q)	r = 0.9984 (Q)	r = 0.9998 (Q)
		Calibration Range: 0.005 to 5 µg/L				
Repeatable	ECM	Yes for 0.05 µg/L (LLMV) and 0.5 µg/L (10x LLMV) in one characterized surface water matrix and one characterized ground water matrix where n = 5.				
	ILV[5]	Yes for 0.05 µg/L (LLMV) and 0.5 µg/L (10x LLMV) in one characterized surface water matrix and one characterized ground water matrix where n = 5.				
Reproducible		Yes for 0.05 µg/L (LLMV) and 0.5 µg/L (10x LLMV) in tested water matrices.				
Specific	ECM	Yes, matrix interferences were <2% of the LOQ (based on peak areas). Some minor baseline noise was noted at the LOQ which interfered with analyte peak integration.	Yes, matrix interferences were <12% of the LOQ (based on peak areas). Some minor baseline noise was noted at the LOQ which interfered with analyte peak integration.	Yes, matrix interferences were <4% of the LOQ (based on peak areas). Some minor baseline noise was noted at the LOQ which interfered with analyte peak integration.	**No**, analyte peak was split into two peaks.[6] Matrix interferences were <7% of the LOQ (based on peak areas). Some baseline noise was noted at the LOQ which interfered with analyte peak integration.	Yes, matrix interferences were <2% of the LOQ (based on peak areas). Some minor baseline noise was noted at the LOQ which interfered with analyte peak integration.
	ILV	Yes, no matrix interferences were observed.	Yes, matrix interferences were <3% of the LOQ (based on peak areas).	Yes, matrix interferences were <2% of the LOQ (based on peak areas). Minor contaminants were observed near the LOQ analyte peak.	Yes, matrix interferences were *ca*. 5% of the LOQ (based on peak areas).	Yes, no matrix interferences were observed. Insignificant baseline noise was noted.

Data obtained from pp. 14 and 18 (LOQ/LOD); Tables 2-5, pp. 29-32 (recovery data); p. 24, and Figures 18-19, p. 47; Figures 32-33, p. 54; Figures 46-47, p. 61; Figures 60-61, p. 68; and Figures 74-75, p. 75 (calibration curves); Figures 6-105, pp. 51-90 (chromatograms) of MRID 51228142; pp. 8 and 12 (LOQ/LOD); pp. 8, 14-15, Tables 4-5, pp. 21-30 (recovery data); p. 14, and Appendix 6, pp. 100-102 (calibration curves); Appendices 1-5, pp. 50-99 (chromatograms) of MRID 51228148.

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 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→354 and *m/z* 434→238 for SYN549107, *m/z* 435→392 and *m/z* 435→160 for SYN549431, *m/z* 553→277 and *m/z* 553→247 for SYN550455 and *m/z* 503→390 and *m/z* 503→431 for SYN551485 (pp. 20-21 of MRID 51228142, and Figure 7, p. 46 of MRID 51228148).

2 In the ECM, the specific source of the surface water was reported as a local lake. The source of the ground water was reported as residential (Table 1, p. 28 of MRID 51228142). The surface water matrix had the following characteristics: pH: 7.5; conductivity: 0.13 mmhos/cm; hardness: 33 mg; SAR 0.51. The ground water matrix had the following characteristics: pH: 7.3; conductivity: 0.1 mmhos/cm; hardness: 31 mg; SAR 0.53 (Table 1, p. 28 of MRID 51228142). The water characterization report was not provided in the MRID. In the ILV, the surface and ground water matrices were provided by the study sponsor (pp. 8 and 11 of MRID 51228148). The source of the samples was not reported. The surface water matrix had the following characteristics: pH: 6.3; conductivity: 0.24 mmhos/cm; hardness: 73 mg as $CaCO_3$/L; SAR 0.26; Turbidity 20.3 NTU, Alkalinity 64 mg $CaCO_3$/L (Appendix 9, p. 107 of MRID 51228148). 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 did not report calibration chromatograms for the confirmatory ion pair transition.

5 The ILV validated the method, GRM072.17A, in the first trial as written with minor modifications to the equipment and mobile phase used in the LC (pp. 9 and 17 of MRID 51228148). An updated ECM is not required.

6 Two peaks were observed for SYN550455 in ECM representative chromatograms (Figures 48-59, pp. 62-67; Figures 96-99, pp. 86-87 of MRID 51228142). The ECM study report noted that baseline separation occurs under reverse phase (RP) conditions (Phenomenex Kinetex C18 column used; pp. 14, 19; Appendix 1, p. 95). However, the ILV used the same RP LC column, and only a single analyte peak was observed in representative chromatograms (Figure 7, p. 45; Appendix 4, pp. 80-89 of MRID 51228148). The Certificate of Analysis of the ECM test material of SYN550455 was not provided for review to assess the purity of the test material.

IV. Method Deficiencies and Reviewer's Comments

1. Since the reported method LOQ of the ECM and ILV were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ of the ECM and ILV are the lowest level of method validation (LLMV) rather than LOQs (pp. 14 and 18 of MRID 51228142; pp. 8 and 12 of MRID 51228148). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

2. The LLMV is not sensitive enough to detect down to the lowest toxicological level of concern in water for all analytes. For aquatic invertebrates, the most sensitive toxicity endpoint available was determined to the be the No Observed Adverse Effect Concentration (NOAEC) for mysid (*Americamysis bahia*). The value of this endpoint is 0.00098 µg active ingredient/liter (a.i./L) (Marini 2019; MRID 51229455).

3. The specificity of the method for SYN550455 was not supported by ECM representative chromatograms since two peaks were observed for SYN550455 (Figures 48-59, pp. 62-67; Figures 96-99, pp. 86-87 of MRID 51228142). The ECM study report noted that baseline separation occurs under reverse phase (RP) conditions (Phenomenex Kinetex C18 column used; pp. 14, 19; Appendix 1, p. 95). However, the ILV used the same RP LC column, and only a single analyte peak was observed in representative chromatograms (Figure 7, p. 45; Appendix 4, pp. 80-89 of MRID 51228148). The differing LC conditions could have led to the elution of SYN550455 as a single peak; however, in this case, the ILV modifications of the method would require an updated ECM since the formation of a single peak for each analyte in a non-enantiomer-specific method is expected. The Certificate of Analysis of the ECM test material of SYN550455 was not provided for review to assess the purity of the test material.

4. The ILV made the following modifications to the ECM (Syngenta Report No: GRM072.17A): 1) The equipment used differed from the equipment in the ECM. A Shimadzu LC-30AD UHPLC LC system was used coupled with an AB MDS Sciex API 6500 triple quad MS LC-MS/MS (pp. 11, 12, and 45 of MRID 51228148). 2) The mobile phase and gradient differed from the ECM. Mobile phase A was 0.1% formic acid in water. Mobile phase B was 0.1% formic acid in acetonitrile. The mobile phase gradient was A:B (v:v) at 0-0.5 min. 100:0, 1.2-3.0 min. 20:80, 3.0-3.5 min. 0:100, 4.0 min 100:0. The Pump B concentration was changed from 10% to 0%. The column specifications, the column temperature, the injection volume, and flow rate were the same as used in the ECM. The ILV lists a strong wash solution as the autosampler rinse solution. The ECM did not report the rinse solution. 3) Dwell times of the transitions of SYN547407, SYN549431 and SYN551485 were changed from 25 msec to 50 msec.

   The analytical modifications included the following: 1) changing the LC instrumentation; 2) mobile phase and gradient change; and dwell time change for three of the analytes. Overall, the ILV method (PASC Report No.: PASC-REP-3882) was a suitable version of the ECM. The ECM allows for use of different instrumentation and optimization of parameters other than those specified as long as separation and sensitivity requirements are met (p. 18 of MRID 51228142).

5. In the ECM, the final samples fortified at the LLMV and stored in acetonitrile:ultra-pure water (50:50; v:v) extracts in the ground water and surface water matrices were determined to be stable for up to 7 days when refrigerated at 4ºC (p. 25; Tables 7-8, p. 34 of MRID 51228142). The extracts should be allowed to equilibrate to room temperature prior to use. Stock and standard solutions should be refrigerated or frozen to prevent decomposition; the temperature and stability were not reported in the ECM (p. 17 of MRID 51228142). An expiration date of 3 months for SYN547407 and metabolites in acetonitrile:ultra-pure water (50:50; v:v) was recommended unless additional data are generated to support a longer expiration date. The ILV reported solution stability evaluation results demonstrated that the control sample solution, LLMV, and 10× LLMV recovery solutions have up to 8 days of solution stability when stored in refrigeration at 5°C (working temperature range, 2-8°C) for each sample type (p. 16, Tables 6-7, pp. 31-40 of MRID 51228148).

6. The ILV reported that there were no communications between the performing laboratory (PASC) and sponsor (Syngenta) regarding the ILV procedure or progress, until the ILV was completed (p. 17 of MRID 51228148).

7. In the ECM, no significant matrix effects (<20%) were observed (p. 33 of MRID 51228142). There were no significant matrix effects observed in the ILV (pp. 11 and 16of MRID 51228148). Solvent-based calibrations were used in the ECM and ILV (p. 24 of MRID 51228142; and p. 14 of MRID 51228148).

8. The ECM reported that analysis of a batch, considered to be 30 samples, could be completed in one working day (8 hours; p. 18 of MRID 51228142). The time requirement for the method was not reported in the ILV. 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. 18 of MRID 51228142). The ECM reported that samples should be stored refrigerated in sealed containers when analysis cannot be completed in a single day.

9. This ECM does not resolve the diastereomers or the R and S enantiomers of each diastereomeric pair and will detect and quantify all enantiomers of each compound as a single chromatographic peak with the exception of SYN550455 where baseline separation occurs under reverse phase conditions (p. 14 of MRID 51228142).

V. References

Marini, J. 2019. Isocycloseram: SYN547407 - Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*): Final Report. Project Number: 1781/7164, TK0081829, MRID 51229455. Unpublished Study Prepared by Smithers Viscient Laboratories.

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.

U.S. Environmental Protection Agency. 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

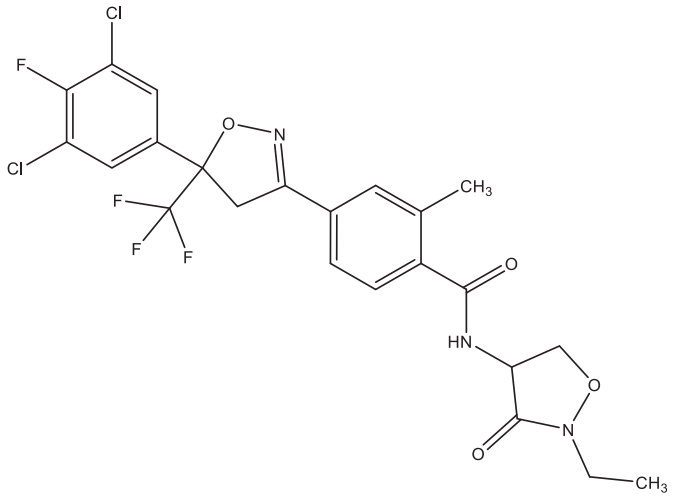
Isocycloseram (SYN547407)

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

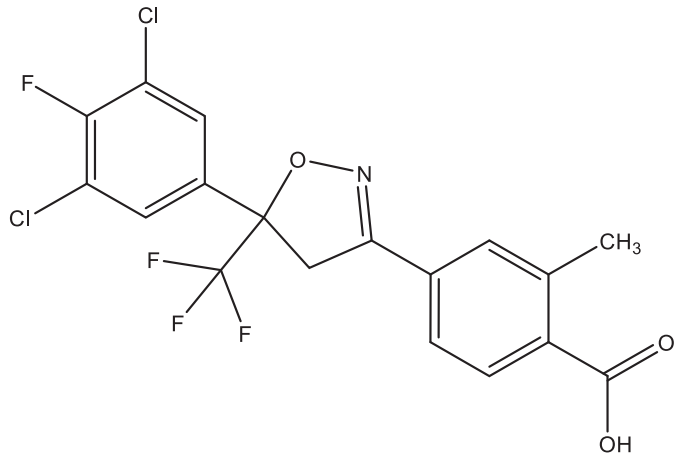
CAS Number: 2061933-85-3

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



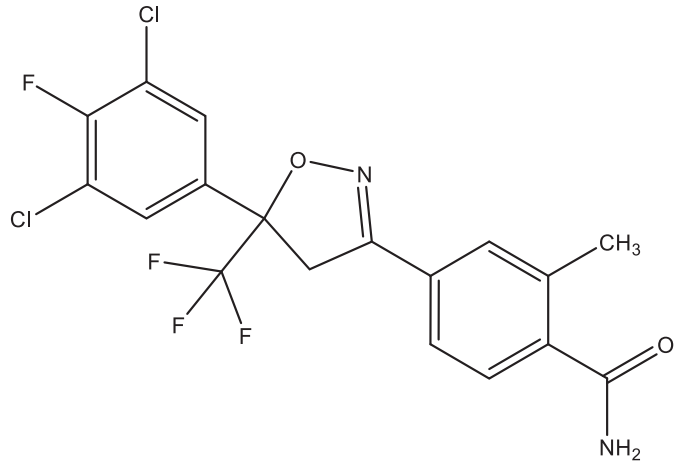
SYN549107

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



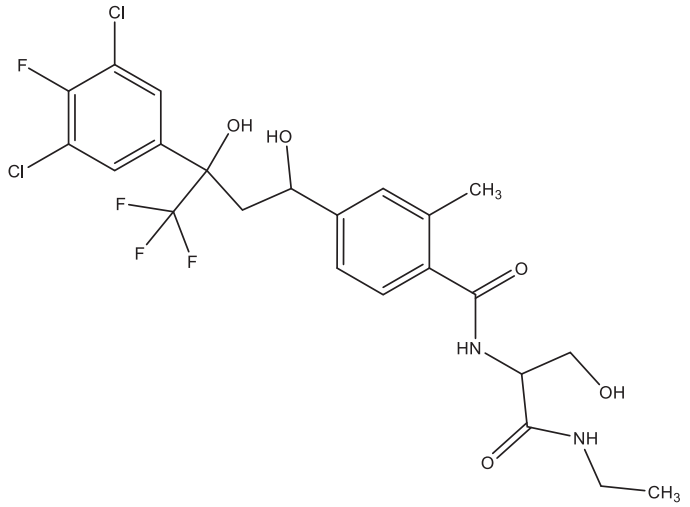
SYN550455

IUPAC Name: 4-(3-(3,5-Dichloro-4-fluorophenyl)-4,4,4-trifluoro-1,3-dihydroxybutyl)-N-(1-(ethylamino)-3-hydroxy-1-oxopropan-2-yl)-2-methylbenzamide

CAS Name: Not reported

CAS Number: Not reported

SMILES String: ClC1=C(F)C(Cl)=CC(C(O)(C(F)(F)F)CC(C2=CC=C(C(NC(CO)C(NCC)=O)=O)C(C)=C2)O)=C1



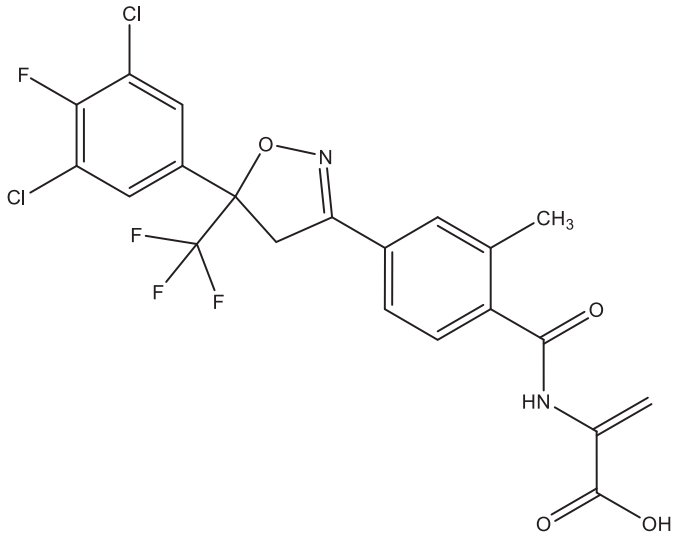
SYN551485

IUPAC Name: 2-(4-(5-(3,5-Dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5- dihydro-1,2-oxazol-3-yl)-2-methylbenzamido)acrylic 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(NC(C(O)=O)=C)=O)C(C)=C3)=NO2)=C1



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