Test Material: Primisulfuron-methyl

MRID: 49447004

Title: Analytical Method (GRM070.01A) for Determination of CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 in Water by LC-MS/MS

MRID: 49447003

Title: Primisulfuron-methyl - Independent Laboratory Validation of Residue Method (CIGPSM1) For the Determination of Primisulfuron-Methyl (CGA136872), CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913 in Water by LC-MS/MS

EPA PC Code: 128973

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** Lynne Binari

Date: 4/22/15

Secondary Reviewer: Lisa Muto **Signature:** Lisa Muto

Date: 4/22/15

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

Date: 4/22/15

Analytical method for primisulfuron-methyl (CGA136872) and its transformation products CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913 in water

Reports: ECM: EPA MRID No.: 49447004. Lin, K. 2014. Analytical Method (GRM070.01A) for Determination of CGA136872, CGA191429, CGA120844, CGA27913, CGA171683 and CGA177288 in Water by LC-MS/MS. Report prepared, sponsored, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 87 pages. Syngenta Report No.: GRM070.01A and Task No.: TK0165283. Final report issued July 11, 2014.

ILV: EPA MRID No. 49447003. Perez, R., S. Perez, and D. Patel. 2014. Primisulfuron-methyl - Independent Laboratory Validation of Residue Method (CIGPSM1) For the Determination of Primisulfuron-methyl (CGA136872), CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913 in Water by LC-MS/MS. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 176 pages. Study No.: 2K13-TK0165283. Report and Task No.: TK0165283. Final report issued June 24, 2014.

Document No.: MRIDs 49447004 & 49447003

Guideline: 850.6100

Statements: ECM: There was no claim of compliance for the study with USEPA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49447004). Signed and dated Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity Certification statements were not provided.

ILV: The study was conducted in compliance with USEPA GLP standards (p. 3 of MRID 49447003). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). The certification of the authenticity of the study report is included in the Quality Assurance Statement (p. 4).

Classification: This analytical method is classified as unacceptable. For the ECM validation, only a single procedural recovery (n = 1) per fortification level and ion pair transition (quantitation, confirmation) was reported for each analyte. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. For the ILV, selected recovery results were corrected for the matrix control. The ECM validation water matrix was not characterized.

PC Code: 128973

Reviewer:

Andrew Shelby
Physical Scientist

Signature:

Date: 5/22/2015

Executive Summary

The analytical method, Syngenta Analytical Method GRM070.1A, is designed for the quantitative determination of primisulfuron-methyl (CGA136872) and its transformation products CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913 in ground water using LC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 0.05 ppb (µg/L). The LOQ is greater than the lowest toxicological level of concern in water. The independent laboratory validated the method for analysis of primisulfuron-methyl, CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913 at the LOQ and 10x LOQ in ground water after one trial. However, ILV chromatograms for quantitation ion analyses of CGA177288 and CGA27913 showed significant baseline noise although interferences were <30% of the LOQ, and for CGA191429 interferences were 25-75% of the LOQ with significant baseline noise. No major modifications were made by the independent laboratory.

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						
Primisulfuron-methyl (CGA136872)	49447004	49447003		Ground Water	11/07/2014	Syngenta Crop Protection, LLC	LC/MS/MS	0.05 ppb (µg/L)
CGA191429								
CGA120844								
CGA177288								
CGA171683								
CGA27913								

I. Principle of the Method

Water (20 mL) was fortified with a mixed standard solution of primisulfuron-methyl (CGA136872), CGA191429, CGA120844, CGA27913, CGA171683, and CGA177288 in methanol for procedural recoveries (pp. 11-12, 14 of MRID 49447004). Ground water was used for validation; source not reported (p. 10; Figures 9-14, pp. 59-76). Water samples should be stored in darkness in plastic containers, rather than glass, to prevent losses of primisulfuron-methyl (CGA136872), CGA191429, CGA120844, CGA27913, CGA171683, and CGA177288 due to absorption or photodegradation (p. 14). Water samples (20 mL) are acidified to pH 4 ± 1 with 20 µL of concentrated acetic acid, hand shaken to mix, then analyzed directly using LC/MS/MS (p. 14; Appendix 3, p. 80). If particles are visible, centrifuge sample prior to analysis.

Samples are analyzed using a Waters Acquity UPLC® system (I Class) and a AB Sciex API 4000 triple quadrupole MS with TurboIonSpray interface (pp. 15-18; Appendix 4, pp. 81-82 of MRID 49447004). The following LC conditions were used: Atlantis® T3 column (3.0 mm x 100 mm, 5 µm, column temperature 40°C), mobile phase of (A) 0.1mM ammonium acetate in ultrapure water and (B) methanol [percent A:B (v:v) at 0-1 min. 85:15, 5 min. 50:50, 8-11 min 5:95, 11.1-14 min. 85:15], and injection volume of 100 µL. The following MS/MS conditions were used: negative ion mode and multiple reaction monitoring (MRM). Analytes are identified using two ion pair transitions; one for quantitation (Q, "primary") and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 467.2→225.8 (Q) and *m/z* 467.2→176.0 (C) for primisulfuron-methyl (CGA136872), *m/z* 453.0→156.0 (Q) and *m/z* 453.0→92.1 (C) for CGA191429, *m/z* 213.9→182.0 (Q) and *m/z* 213.9→106.0 (C) for CGA120844, *m/z* 200.0→92.0 (Q) and *m/z* 200.0→155.8 (C) for CGA177288, *m/z* 226.0→175.8 (Q) and *m/z* 226.0→125.9 (C) for CGA171683, and *m/z* 181.9→106.0 (Q) and *m/z* 181.9→42.0 (C) for CGA27913 (Appendix 4, p. 81). Expected retention times are *ca*. 7.5, 7.2, 6.4, 4.6, 3.0, and 2.1 minutes for CGA171683, primisulfuron-methyl (CGA136872), CGA191429, CGA120844, CGA27913, and CGA177288, respectively.

ILV: Test compounds and ground water were supplied by Syngenta (pp. 13-15 of MRID 49447003). ADPEN Laboratories was initially contracted to independently validate Syngenta Analytical Method CIGPSM1, *Determination of Primisulfuron-Methyl (CGA-136872) and its Metabolites in Water and Soil by High Performance Liquid Chromatography Thermospray Mass Spectrometry (TSP-LC/MS)*, EPA MRID No. 42040102, for primisulfuron-methyl (CGA136872) and its transformation products CGA191429, CGA120844, CGA177288, CAG171683, and CGA27913 in water (p. 11; 22; Appendix 2, pp. 82-105). The first trial of Method CIGPSM1 was unsuccessful for all analytes, potentially due to losses during four extraction and four evaporation steps (p. 19; Appendix 8, p. 176). Syngenta then modified the method by reducing the water sample volume to 20 mL, reducing volume of the acidifying agent to 20 µL, eliminating concentration steps, and using LC/MS/MS for the final determination (p. 17; Appendix 3, pp. 107-142). The detailed procedures and analysis conditions for the modified method are described above.

The independent laboratory performed the modified ECM as written with the following changes: Agilent 1200 SL HPLC system and Agilent 6490 Series triple quadrupole MS with Agilent Jet Stream electrospray ionization, Atlantis® T3 column (3.0 mm x 150 mm, 3 µm), mobile phase of (A) 0.1mM ammonium acetate in HPLC water and (B) methanol [percent A:B (v:v) at 0 min. 97:3, 5.2 min. 45:55, 5.6-8.5 min 0:100, 8.6-11 min. 97:3], and injection volume of 80 µL (p. 18; Appendix 7, pp. 154-175 of MRID 49447003). Ion transitions monitored were as follows: *m/z* 466.99→226 (Q) and *m/z* 466.99→175.9 (C) for primisulfuron-methyl (CGA136872), *m/z* 453.01→175.9 (Q) and *m/z* 453.01→155.9 (C) for CGA191429, *m/z* 214.02→181.9 (Q) and *m/z* 214.02→41.8 (C) for CGA120844, *m/z* 199.99→155.7 (Q) and *m/z* 199.99→92 (C) for CGA177288, *m/z* 226.02→175.9 (Q) and *m/z* 226.02→125.9 (C) for CGA171683, and *m/z* 181.89→105.9 (Q) CGA27913; no C ion pair for CGA27913. Approximate retention times were 7.8-8.0, 7.7, 7.6, 6.3, 4.6-4.7, and 3.6 minutes for primisulfuron-methyl (CGA136872), CGA171683, CGA191429, CGA120844, CGA27913, and CGA177288, respectively (Figures 7-40, pp. 43-76).

LOQ and LOD: In the ECM and ILV, the LOQ and LOD for all analytes were 0.05 µg/L (ppb, ng/mL, pg/µL) and 0.01 µg/L, respectively (p. 22 of MRID 49447004; p. 20 of MRID 49447003).

II. Recovery Findings

ECM (MRID 49447004): Recovery results presented in Tables 1-11 (pp. 26-36) of the study report are data generated by ADPEN Laboratories for the ILV (Tables 2-12, pp. 25-35 of MRID 49447003) and are discussed in the following paragraph. Recovery results generated by Syngenta were obtained from the provided chromatograms (Figure 9, pp. 60-61; Figure 10, pp. 63-64; Figure 11, pp. 66-67, 69-70; Figure 13, pp. 72-73, Figure 14, pp. 75-76). Only a single procedural recovery per fortification level and ion pair transition (quantitation, confirmation) was reported for each analyte to support validation of the ECM. Therefore, statistics (mean, standard deviation, RSD) for the recovery results per fortification level and ion pair transition could not be generated. All individual recovery results were within 70-120% at fortification levels of 0.05 µg/L (ppb, LOQ) and 0.5 µg/L (10x LOQ). Analytes were identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The water matrix was not characterized.

ILV (MRID 49447003): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of primisulfuron-methyl (CGA136872) and its transformation products CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913 in ground water at fortification levels of 0.05 ppb (LOQ) and 0.5 ppb (10x LOQ; Tables 2-12, pp. 25-35). Except for CGA27913, analytes were identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. CGA27913 was identified and quantified using one ion pair transition. The modified method was validated for all analytes at both fortification levels in ground water after one trial, with alternate MS/MS parameters and MRM transitions (pp. 11, 19-20). The water matrix was characterized by Agvise Laboratories, Northwood, North Dakota (Appendix 5, p. 151).

Table 2. Initial Validation Method Recoveries for Primisulfuron-methyl (CGA136872) and Its Transformation Products CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913 in Ground Water

Analyte	Fortification Level (ppb)[1]	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Quantitation Ion					
Primisulfuron-methyl (CGA136872)	0.05 (LOQ)	1	109	--[2]	--	--
	0.5	1	107	--	--	--
CGA191429	0.05 (LOQ)	1	98	--	--	--
	0.5	1	100	--	--	--
CGA120844	0.05 (LOQ)	1	98	--	--	--
	0.5	1	99	--	--	--
CGA177288	0.05 (LOQ)	1	103	--	--	--
	0.5	1	111	--	--	--
CGA171683	0.05 (LOQ)	1	100	--	--	--
	0.5	1	105	--	--	--
CGA27913	0.05 (LOQ)	1	98	--	--	--
	0.5	1	91	--	--	--
	Confirmation Ion					
Primisulfuron-methyl (CGA136872)	0.05 (LOQ)	1	116	--	--	--
	0.5	1	111	--	--	--
CGA191429	0.05 (LOQ)	1	90	--	--	--
	0.5	1	95	--	--	--
CGA120844	0.05 (LOQ)	1	95	--	--	--
	0.5	1	97	--	--	--
CGA177288	0.05 (LOQ)	1	86	--	--	--
	0.5	1	100	--	--	--
CGA171683	0.05 (LOQ)	1	99	--	--	--
	0.5	1	103	--	--	--
CGA27913	0.05 (LOQ)	1	95	--	--	--
	0.5	1	97	--	--	--

Data (recovery results) were obtained from Figure 9, pp. 60-61; Figure 10, pp. 63-64; Figure 11, pp. 66-67, 69-70; Figure 13, pp. 72-73; Figure 14, pp. 75-76 of MRID 49447004. Data presented in Tables 1-11 (pp. 26-36) are recovery results generated by the ILV (Tables 2-12, pp. 25-35 of MRID 49447003).

1 Ppb analyte = µg/L, ng/mL, or pg/µL.

2 Not applicable, n = 1.

Table 3. Independent Validation Method Recoveries for Primisulfuron-methyl (CGA136872) and Its Transformation Products CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913 in Ground Water

Analyte	Fortification Level (ppb)[1]	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Quantitation Ion					
Primisulfuron-methyl (CGA136872)	0.05 (LOQ)	5	105-115	108	4.3	4.0
	0.5	5	113-119	117	2.3	2.0
CGA191429	0.05 (LOQ)	5	108-120	113	4.6	4.1
	0.5	5	84-120	105	15.0	14.3
CGA120844	0.05 (LOQ)	5	88-94	90	2.6	2.9
	0.5	5	91-96	94	1.8	1.9
CGA177288	0.05 (LOQ)	5	91-118	104	9.6	9.2
	0.5	5	115-118	117	1.4	1.2
CGA171683	0.05 (LOQ)	5	68-72	69	2.0	2.9
	0.5	5	82-89	85	3.0	3.6
CGA27913	0.05 (LOQ)	5	85-110	101	10.1	10.1
	0.5	5	105-119	114	5.8	5.1
	Confirmation Ion					
Primisulfuron-methyl (CGA136872)	0.05 (LOQ)	5	84-99	90	5.9	6.5
	0.5	5	108-117	114	3.4	3.0
CGA191429	0.05 (LOQ)	5	90-108	102	7.4	7.3
	0.5	5	109-121	116	4.5	3.9
CGA120844	0.05 (LOQ)	5	92-109	99	7.3	7.4
	0.5	5	88-94	91	2.5	2.7
CGA177288	0.05 (LOQ)	5	103-119	112	5.8	5.2
	0.5	5	116-120	118	2.0	1.7
CGA171683	0.05 (LOQ)	5	68-75	70	2.9	4.1
	0.5	5	80-93	86	4.9	5.6
CGA27913	0.05 (LOQ)	5	Not reported.			
	0.5	5				

Data (recovery results, corrected when residues found in matrix controls) were obtained from Tables 2-12, pp. 25-35 of MRID 49447003.

1 Ppb analyte = µg/L, ng/mL, or pg/µL.

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD for primisulfuron-methyl (CGA136872) and its transformation products CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913 in ground water were 0.05 and 0.01 ppb (ng/mL, pg/µL), respectively (p. 22 of MRID 49447004; p. 20 of MRID 49447003). The updated ECM defined the LOQ as the lowest analyte concentration at which the methodology has been validated and a mean recovery of 70-110% and RSD of ≤20% has been obtained. The updated ECM defined the LOD as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated matrix control sample at the corresponding retention time and an estimate of the LOD can be taken as three times the background noise.

Table 4. Method Characteristics for Primisulfuron-methyl (CGA136872) and Its Transformation Products CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913 in Ground Water

			CGA136872	**CGA191429**	**CGA120844**	**CGA177288**	**CGA171683**	**CGA27913**
Limit of Quantitation (LOQ)			0.05 ppb (µg/L, ng/mL, pg/µL)					
Limit of Detection (LOD)			0.01 ppb					
Linearity (calibration curve r^2 and concentration range)[1]	ECM:[2]	Q ion:	$r^2 = 0.9990$	$r^2 = 0.9982$	$r^2 = 0.9994$	$r^2 = 0.9990$	$r^2 = 0.9984$	$r^2 = 0.9970$
		C ion:	$r^2 = 0.9992$	$r^2 = 0.9980$	$r^2 = 0.9980$	$r^2 = 0.9988$	$r^2 = 0.9992$	$r^2 = 0.9952$
		Range:	0.01-1.0 ng/mL					
	ILV:	Q ion:	$r^2 = 0.99973575$	$r^2 = 0.99937945$	$r^2 = 0.99976533$	$r^2 = 0.99968404$	$r^2 = 0.99893232$	0.99980353
		C ion:	$r^2 = 0.99995448$	$r^2 = 0.99980037$	$r^2 = 0.99970301$	$r^2 = 0.99975897$	$r^2 = 0.99908533$	Not reported.
		Range:	0.01-10 ng/mL (0.0008-0.8 ng/0.08 mL injected)					
Repeatable	ECM:		Undetermined, n = 1 at LOQ and 10x LOQ.					
	ILV:		Yes at LOQ and 10x LOQ.					
Reproducible			Yes.					
Specific	ECM:	Q ion:	Interferences were <10% of the LOQ (based on peak area).					
		C ion:	Interferences were <25% of the LOQ (based on peak area).					
	ILV:	Q ion:	Interferences were <30% of the LOQ (based on peak area).	Interferences were 25-75% of the LOQ (based on peak area), with significant baseline noise.	Interferences were <30% of the LOQ (based on peak area), but there was significant baseline noise for CGA177288 and CGA27913.			
		C ion:	Interferences were 4-51% of the LOQ (based on peak area).					Not reported.[3]

Data were obtained from pp. 12, 22; Figure 8, pp. 53-58; Figures 9-14, pp. 59-76 of MRID 49447004; p. 20; Tables 2-12, pp. 25-35; Figures 1-18, pp. 37-54; Figure 19-21, pp. 55-57; Figures 23-25, pp. 59-61; Figures 27-29, pp. 63-65; Figures 31-33, pp. 67-69; Figures 35-37, pp. 71-73; Figures 39-41, pp. 75-77; Appendix 7, pp. 154-175 of MRID 49447003; DER Attachment 2.

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

1 1/x weighting (Figure 8, pp. 53-58 of MRID 49447004; Appendix 7, pp. 155, 157, 159, 161, 163, 165, 167, 169171, 173, 175 of MRID 49447003).

2 ECM coefficient of determination (r^2) values are reviewer-generated from reported correlation coefficient (r) values (Figure 8, pp. 53-58 of MRID 49447004; DER Attachment 2).

3 A confirmatory method was not used for CGA27913; however, a confirmatory method is typically not required where GC/MS and/or LC/MS methods are used as the primary method.

IV. Method Deficiencies and Reviewer's Comments

1. ADPEN Laboratories was initially contracted to independently validate Syngenta Analytical Method CIGPSM1, *Determination of Primisulfuron-Methyl (CGA-136872) and its Metabolites in Water and Soil by High Performance Liquid Chromatography Thermospray Mass Spectrometry (TSP-LC/MS)*, EPA MRID No. 42040102, for primisulfuron-methyl (CGA136872) and its transformation products CGA191429, CGA120844, CGA177288, CAG171683, and CGA27913 in water (p. 11; 22; Appendix 2, pp. 82-105). The first trial of Method CIGPSM1 was unsuccessful for all analytes, potentially due to losses during four extraction and four evaporation steps (p. 19; Appendix 8, p. 176). Syngenta then modified the method by reducing the water sample volume to 20 mL, reducing volume of acidifying agent to 20 µL, eliminating concentration steps, and using LC/MS/MS for the final determination (p. 17; Appendix 3, pp. 107-142). Soil analysis is not included in the modified method. ADPEN Laboratories successfully validated the LC/MS/MS method for all analytes in ground water, with minor modifications. Syngenta submitted the updated method as Analytical Method GRM070.01A (MRID 49447004); however, <u>all of the recovery results presented in the tables</u> (Tables 1-11, pp. 26-36) are the data generated by ADPEN Laboratories as part of the ILV of the modified method (Tables 2-12, pp. 25-35 of MRID 49447003). ECM validation data generated by Syngenta could only be obtained from the provided chromatograms (Figure 9, pp. 60-61; Figure 10, pp. 63-64; Figure 11, pp. 66-67, 69-70; Figure 13, pp. 72-73; Figure 14, pp. 75-76 of MRID 49447004). The author, Kaijun Lin, of the updated ECM report was the study monitor for the ILV (Appendix 8, p. 176 of MRID 49447003).

2. For the ECM validation, only a single procedural recovery (n = 1) per fortification level and ion pair transition (quantitation, confirmation) was reported for each analyte (Figure 9, pp. 60-61; Figure 10, pp. 63-64; Figure 11, pp. 66-67, 69-70; Figure 13, pp. 72-73; Figure 14, pp. 74-75 of MRID 49447004). Consequently, the ECM validation did not establish precision and accuracy of the method.

3. The determination of the LOQ and LOD were not based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B. The updated ECM defined the LOQ as the lowest analyte concentration at which the methodology has been validated and a mean recovery of 70-110% and RSD of ≤20% has been obtained (p. 22 of MRID 49447004). The updated ECM defined the LOD as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated matrix control sample at the corresponding retention time and an estimate of the LOD can be taken as three times the background noise. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

4. For the ILV, CGA191429 C ion, CGA120844 C ion, CGA177288 Q ion, and CGA27913 Q ion recovery results were corrected for residues detected in the matrix control samples (Tables 2-12, pp. 25-35 of MRID 49447003).

5. The ground water matrix used in the ECM validation was not characterized. The purities of the test compounds used were not specified, only described as "analytical standards" (Appendix 2, p. 79 of MRID 49447004).

6. For the ILV calibration standards, only chromatograms of 0.01 and 10 ng/mL standards were provided; calibration standard ranges were 0.01-10 ng/mL (0.008-0.8 ng; Figures 7-18, pp. 43-54; Appendix 7, pp. 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175 of MRID 49447003).

   Only chromatograms of primisulfuron-methyl (CGA136872) Q and C ions, CGA120844 Q ion, and CGA177288 C ion at the LOQ showed attenuation to where one can measure the peak accurately (Figure 21, p. 57; Figure 29, p. 65; Figure 33, p. 69). At the LOQ, CGA191429 Q and C ions had an interfering peak and/or significant baseline noise, CGA120844 C ion showed poor attenuation, CGA177288 Q ion had significant baseline noise, CGA171683 Q and C ions had an interfering peak on the tailing edge, and CGA27913 Q ion had significant baseline noise (Figure 25, p. 61; Figure 29, p. 65; Figure 33, p. 69; Figure 37, p. 73; Figure 41, p. 77).

   The ILV study authors reported that prior to analysis of validation samples, the matrix control was determined to contain no detectable residues of primisulfuron-methyl (CGA136872) and its products CGA191429, CGA120844, CGA177288, CGA171683, and CGA27913 and had no peaks which might interfere with targeted analyte responses (p. 19). However, the chromatograms showed interferences with peak areas were not less than 50% at the LOD (based on peak area) for primisulfuron-methyl (CGA136872) C ion, CGA191429 Q and C ions, CGA120844 C ion, CGA177288 Q ion, and CGA27913 Q ion (Figures 7-12, pp. 43-48; Figures 19-20, pp. 55-56; Figures 23-24, pp. 59-60; Figures 27-28, pp. 63-64; Figures 31-21, pp. 67-68; Figures 39-40, pp. 75-76; Appendix 7, pp. 157-175).

7. For the ECM, chromatograms for reagent blank samples were not provided. For the calibration standards, only chromatograms of 0.01 and 1 ng/mL standards were provided; calibration standard ranges were 0.01-1 ng/mL (p. 12; Figures 2-7, pp. 41-52 of MRID 49447004). Standard curve plots with regression curve analyses were provided, but individual calibration standard data were not provided (Figure 8, pp. 53-58). Chromatograms of the analytes at the LOQ showed attenuation to where one can measure the peak accurately, except for CGA177288 C ion which barely peaked above baseline (Figure 9, p. 60; Figure 10, p. 63; Figure 11, pp. 66, 69; Figure 13, p. 72; Figure 14, p. 75).

8. All communication prior to running the samples between the independent laboratory and the developers or previous users of the ECM were not provided. The independent laboratory provided "all pertinent communications" (p. 21; Appendix 8, p. 176 of MRID 49447003).

9. For the ILV, a confirmatory ion pair transition was not used for CGA27913; however, typically, a confirmatory method is not required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data.

10. The equipment substitutions and modifications to optimize LC and MS/MS conditions implemented by the independent laboratory (see section **I. Principle of the Method**, ILV: above for details) are not considered substantial changes to the ECM. The alternate procedures used during the ILV are included in the updated ECM (p. 19; Appendix 6, p. 86 of MRID 49447004).

11. The IUPAC name and chemical structure for CGA27913 which were reported in Attachment 1 of this DER were based on data provided on pp. 14, 149 of MRID 49447003 and p. 39 of MRID 49447004; however, the reviewer noted several discrepancies/errors in the provided information. The chemical structure should contain $–NH_2$, instead of –NH, and $–SO_2H$, instead of $–SO_2$. As shown, the chemical structure for CGA27913 looked like Saccharin (CGA147087) without the N-S bond. Also, based on the IUPAC chemical name for Saccharin (1,1-dioxo-**1,2**-benzothiazol-3-one), the reviewer believed that the provided chemical name for CGA27913 "**1,3**-Benz-isothiazol-3(2H)-one, 1,1-dioxide" should have been "**1,2**-Benz-isothiazol-3(2H)-one, 1,1-dioxide". Additionally, the reviewer noted that the molecular formulas were inconsistent between the two MRIDs. In MRID 49447003 (p. 14), the molecular formula was reported as "$C_6H_7N_1O_3S$", where the numbers of carbons and hydrogen were incorrect for the chemical structure <u>as illustrated</u>. In MRID 49447004 (p. 39), the molecular formula was reported as "$C_7H_5NO_3S$", where the numbers of carbons and hydrogen were correct for the chemical structure <u>as illustrated</u>. The reviewer did not have any previous information regarding CGA27913 in order to provide more accurate chemical information.

12. It was reported for the ILV that a single analyst can complete a set of thirteen samples (one reagent blank, two matrix controls, and ten fortified samples) in less than three hours, with LC/MS/MS analysis performed overnight (p. 21 of MRID 49447003).

13. For the ECM (MRID 49447004), the ion transitions *m/z* 200.0→92.0 (Q) and *m/z* 200.0→155.8 (C) for CGA177288 in Appendix 4 (p. 81) and chromatograms (Figure 14, pp. 74-76) are reversed in the study report text (p. 18). Figure 4-2b description "0.02 ng/mL" should read 0.01 ng/mL, and "Figure 11" (pp. 69-70) should read Figure 12.

    The analyte retention times reported in Appendix 6 (p. 86) are the retention times for the gradient mobile phase used for the ECM validation (p. 16), not the ILV validation. The ILV did not reported expected retention times and a different HPLC system and gradient mobile phase were used. Approximate retention times for the ILV LC/MS/MS conditions were obtained from the provided chromatograms (Figures 7-40, pp. 43-76 of MRID 49447003).

V. References

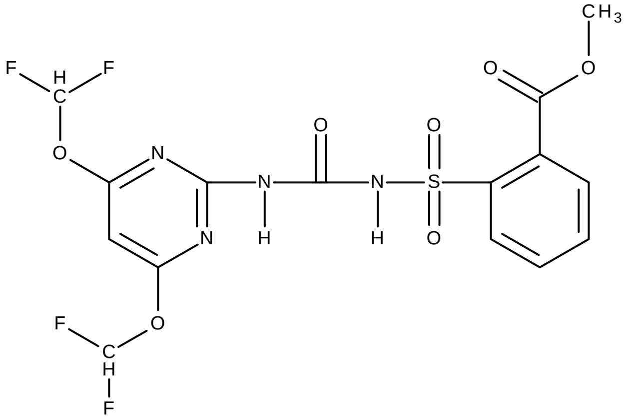
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Attachment 1: Chemical Names and Structures

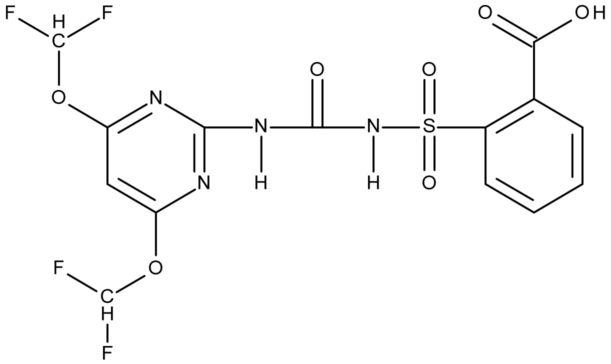
Primisulfuron-methyl (CGA136872)

IUPAC Name:	Methyl 2-[4,6-bis(difluoromethoxy)pyrimidin-2-ylcarbamoylsulfamoyl]benzoate. 2-{3-[4,6-Bis(difluoromethoxy)-pyrimidin-2-yl]-ureidosulfonyl}benzoic acid methyl ester.
CAS Name:	Methyl 2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoate. Benzoic acid, 2-[[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]-carbonyl]amino]sulfonyl-, methyl ester.
CAS Number:	86209-51-0
SMILES String:	COC(=O)c1ccccc1S(=O)(=O)NC(=O)Nc2nc(OC(F)F)cc(OC(F)F)n2



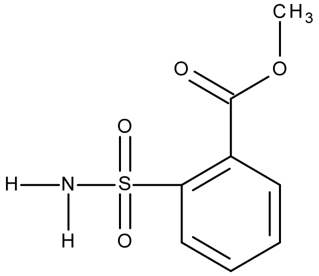
CGA191429 (Metabolite G)

IUPAC Name:	2-(3-[4,6-Bis(difluoromethoxy)-phenyl)-2-yl]-ureidosulfonyl)benzoic acid 2-[4,6-Bis(difluoromethoxy)-pyrimidin-2-yl]-aminocarbonyl-aminosulfonylbenzoic acid
CAS Name:	Not found.
CAS Number:	Not registered.
SMILES String:	[H]N(c1nc(cc(n1)OC(F)F)OC(F)F)C(=O)N([H])S(=O)(=O)c2ccccc2C(=O)O



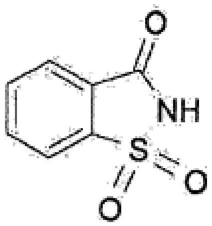
CGA120844

IUPAC Name: 2-Aminosulfonyl benzoic acid methylester
Methyl-2-[aminosulfonyl]benzoate
CAS Name: Not found.
CAS Number: Not registered.
SMILES String: [H]N([H])S(=O)(=O)c1ccccc1C(=O)OC



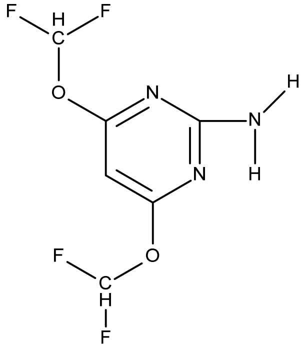
CGA27913 (IUPAC name and chemical structure provided by Registrant in MRIDs 49447003 and 49447004; see Reviewer Comment #11)

IUPAC Name: 1,3-Benz-isothiazol-3(2H)-one, 1,1-dioxide
CAS Name: Not found.
CAS Number: Not registered.
SMILES String: Not found.



CGA171683 (Metabolite B)

IUPAC Name: 2-Amino-4,6-bis(difluoromethoxy)pyrimidine
4,6-Bis[difluoromethoxy]-2-aminopyrimidine
CAS Name: Not found.
CAS Number: Not registered.
SMILES String: [H]N([H])c1nc(cc(n1)OC(F)F)OC(F)F



CGA177288

IUPAC Name: 2-Aminosulfonyl benzoic acid
2-[Aminosulfonyl]benzoic acid

CAS Name: Not found.

CAS Number: Not registered.

SMILES String: [H]N([H])S(=O)(=O)c1ccccc1C(=O)O