

Test Material: Triasulfuron

MRID: 49323903

Title: Triasulfuron, CGA131036 - Independent Laboratory Validation of Residue Method (AG-509) for the Determination of CGA131036 in Soil by HPLC. Final Report

EPA PC Code: 128969

OCSPP Guideline: 850.6100

**For CDM Smith**

**Primary Reviewer:** Lynne Binari

**Signature:** 

**Date:** 10/1/14

**Secondary Reviewer:** Lisa Muto

**Signature:** 

**Date:** 10/1/14

**QC/QA Manager:** Joan Gaidos

**Signature:** 

**Date:** 10/1/14

**Analytical method for triasulfuron in soil**

**Reports:** ECM: EPA MRID No.: 49323903 (Appendix 1, pp. 37-55). Edmonds, M. 1986. Syngenta Method No. AG-509 "Analytical Method for the Determination of CGA-131036 in Soil by Column Switching High Performance Liquid Chromatography" (Appendix 1, pp. 35, 37). Report prepared by Ciba-Geigy Corporation, Greensboro, North Carolina, sponsor not specified, submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 19 pages. Method Edition December 30, 1986.  
 ILV: EPA MRID No. 49323903. Yang, J. and X. Cui. 2013. Triasulfuron, CGA131036 - Independent Laboratory Validation of Residue Method (AG-509) for the Determination of CGA131036 in Soil by HPLC, Final Report. Primera Analytical Solutions Corp. (PASC) Study No.: 141-0892 and Report No.: PASC-REP-0415. Syngenta Task No.: TK0120986. Report prepared by Primera Analytical Solutions Corp., Princeton, New Jersey, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 64 pages. Final report issued December 13, 2013.

**Document No.:** MRID 49323903

**Guideline:** 850.6100  
 OECD ENV/JM/MONO(2007)17  
 EC SANCO/3029/99 rev. 4 & SANCO/825/00 rev. 8.1 (p. 18)

**Statements:** ECM: A statement pertaining to the conduct of the study in regards to Good Laboratory Practice (GLP) standards was not provided. Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were not provided.  
 ILV: The study was conducted in accordance with USEPA GLP standards (p. 3). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An Authenticity Certification statement was not provided.

**Classification:** This analytical method is classified as **Supplemental. However, the LOQ (0.01 mg/kg) is above the toxicological level of concern (LOC =  $4.24 \times 10^{-5}$  mg/kg); therefore, a more sensitive ECM is requested.** In addition, the ECM did not provide sufficient performance data at the LOQ and 10x LOQ for all soil matrices. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. Sufficient chromatographic data were not provided to support validation of the ECM. A confirmatory method was not used. Soil matrices used in the ECM were not adequately characterized, and the registrant did not specify that the soil used in the ILV was either an equivalent, or more difficult, analytical sample condition as that used in the ECM.

**PC Code:** 128969

**Reviewer:** Christopher M. Koper, M.S., Chemist **Date:** January 23, 2015

*Christopher M. Koper* 23 JAN 2015

## Executive Summary

This analytical method, Syngenta AG-509, is designed for the quantitative determination of triasulfuron in soil using HPLC. The method is quantitative for triasulfuron at the stated LOQ of 0.01 mg/kg. **The LOQ is greater than the lowest toxicological level of concern (lettuce (dicot) =  $4.24 \times 10^{-5}$  mg/kg) in soil. Therefore, a more sensitive ECM is requested.** The independent laboratory validated the method for analysis of triasulfuron in soil after one trial. Although ILV performance data were within guidelines (mean 70-110%, RSD  $\leq$ 20%), peak resolution at the LOQ indicate the ECM may not be adequate as an enforcement method for triasulfuron in soil. A confirmatory method was not employed. The registrant did not specify that the soil used in the ILV was either an equivalent, or more difficult, analytical sample condition as that used in the ECM.

**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						
Triasulfuron	49323903	49323903		Soil	30/12/1986	Syngenta	HPLC	0.01 mg/kg

## I. Principle of the Method

Samples (20 g) of soil were extracted once with 200 mL of methanol:pH 9 sodium carbonate buffer (1:1, v:v) by shaking (mechanical shaker, speed not specified) for 2 hours (Appendix 1, pp. 37-39, 50). Soil and extract were separated by filtration (glass wool>Reeve Angel 802 filter paper>Whatman 2V filter paper). An aliquot (5 g, *ca.* 51 mL, volume corrected for soil moisture content) of the filtrate was diluted with 100 mL water plus 10 mL 1.2M phosphoric acid, acidified to  $\leq$ pH 4 with 1.2M phosphoric acid, if needed, then partitioned twice with methylene chloride (2 x 25 mL; Appendix 1, p. 40). Organic phases were combined, taken to dryness (rotary evaporation, 40°C), and the resulting residues reconstituted in acetonitrile:water (75:25, v:v; 2.0 mL; Appendix 1, pp. 40, 43).

Samples were analyzed for triasulfuron (CGA131036) by HPLC using a Lichrosorb-CN (4 mm x 250 mm, 10  $\mu$ m) column coupled with a Zorbax-ODS (4.6 mm x 250 mm, 5-6  $\mu$ m) column and UV detection at 232 nm (Appendix 1, pp. 45-46, 51). Mobile phases were isocratic as follows: 0.5% tetrabutylammonium bromide in 0.001M phosphoric acid:acetonitrile (75:25, v:v) for the Lichrosorb-CN column and 0.5% tetrabutylammonium bromide in pH 7 phosphate buffer:acetonitrile (70:30, v:v) for the Zorbax-ODS column. The columns were coupled with a switching valve unit; column switch time set at 7.4-8.4 min. Injection volume was 200  $\mu$ L. Triasulfuron retention times were *ca.* 7.5 minutes for the Lichrosorb-CN column and *ca.* 20.0 minutes for both columns. A confirmatory method was not reported.

The ILV performed the method as written with the following exceptions: a Lichrosorb-CN (4.6 mm x 250 mm, 5  $\mu$ m) column was substituted for the discontinued Lichrosorb-CN (4 mm x 250 mm, 10  $\mu$ m) column, with resulting triasulfuron retention times of *ca.* 12.5 minutes after the first column and *ca.* 18 minutes after both columns and a column switch time set at 11.5-13.5 minutes (pp. 9, 13-14). In addition, final residues of 10x LOQ fortifications were reconstituted in 5.0 mL of

acetonitrile:water (75:25, v:v) diluent, rather than 2.0 mL. The instrument parameter and method modifications are not considered substantial changes to the ECM (Appendix 4, p. 60).

In the ECM and ILV, the LOQ for triasulfuron was 0.01 mg/kg (ppm; p. 8; Appendix 1, pp. 37, 44; Appendix 4, p. 60). In the ECM, the LOD was set at 2.5 ng (0.0125 ng/ $\mu$ L = ppm, based on a 200  $\mu$ L injection; Appendix 1, p. 46). Although not specified, the ILV appeared to have also set the LOD at 0.0125 ng/ $\mu$ L (Figures 3-4, pp. 23-24).

## II. Recovery Findings

ECM (Appendix 1, pp. 37-55 of 49323903): The reported recoveries appear to be selected procedural recovery samples analyzed with field trial samples, rather than results from a method validation trial (Appendix 1, pp. 48, 54-55). Triasulfuron was identified and quantified using coupled-column HPLC and UV detection. A confirmatory method was not utilized. Soil matrices were not characterized other than soil type and source (State) location.

CA sandy loam soil: Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD  $\leq$ 20%) for analysis of triasulfuron at fortification levels of 0.01 mg/kg (LOQ, n = 4), 0.02 mg/kg (n = 5), and 0.05 mg/kg (n = 3; DER Attachment 2). At fortification levels of 0.03 mg/kg and 0.04 mg/kg individual (n = 1) recoveries were 129% and 88%, respectively. Recoveries from fortification at 10x LOQ (0.1 mg/kg) were not reported.

MS silt loam soil: Mean recoveries and RSDs were within guidelines for analysis of triasulfuron at fortification levels of 0.01 mg/kg (LOQ, n = 7), 0.02 mg/kg (n = 4), 0.03 mg/kg (n = 2), 0.04 mg/kg (n = 2), and 0.05 mg/kg (n = 3; DER Attachment 2). Recoveries from fortification at 10x LOQ (0.1 mg/kg) were not reported.

KS silt loam soil: Mean recoveries and RSDs were within guidelines for analysis of triasulfuron at fortification levels of 0.01 mg/kg (LOQ, n = 8), 0.02 mg/kg (n = 5), and 0.05 mg/kg (n = 5; DER Attachment 2). Recoveries from fortification at 10x LOQ (0.1 mg/kg) were not reported.

NY silt loam soil: Mean recoveries and RSDs were within guidelines for analysis of triasulfuron at fortification levels of 0.01 mg/kg (LOQ, n = 5), 0.02 mg/kg (n = 2), 0.05 mg/kg (n = 6), and 0.1 mg/kg (10x LOQ, n = 2; DER Attachment 2). At a fortification level of 0.2 mg/kg (20x LOQ, n = 1) recovery was 95%.

ILV (MRID 49323903): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD  $\leq$ 20%) for analysis of triasulfuron in soil (uncharacterized) at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10x LOQ; p. 16; Table 1, p. 20). The method was validated for triasulfuron at both fortification levels after one trial, with minor instrument parameter and method modifications (pp. 13, 15). The soil matrix, provided by Syngenta, was characterized, but the characterization results were not provided (p. 10; Appendix 4, p. 58). Syngenta did not establish that the soil used in the ILV was either an equivalent, or more difficult, analytical sample condition as that used for the ECM.

**Table 2. Initial Validation Method Recoveries for Triasulfuron (CGA131036) in Soil<sup>1</sup>**

Matrix <sup>2</sup>	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
California (CA) Sandy loam soil	0.01 (LOQ)	4	75-103	87	13	16
	0.02	5	86-92	88	2	3
	0.03	1	129	*	*	*
	0.04	1	88	*	*	*
	0.05	3	82-83	83	1	1
Mississippi (MS) Silt loam soil	0.01 (LOQ)	7	77-100	86	8	9
	0.02	4	82-97	91	6	7
	0.03	2	79, 101	90	16	17
	0.04	2	80, 91	86	8	9
	0.05	3	85-92	89	4	4
Kansas (KS) Silt loam soil	0.01 (LOQ)	8	82-96	91	4	5
	0.02	5	87-100	93	6	6
	0.05	5	85-99	90	6	6
New York (NY) Silt loam soil	0.01 (LOQ)	5	69-99	81	12	15
	0.02	2	83, 84	84	1	1
	0.05	6	69-91	82	7	9
	0.10	2	75, 87	81	8	10
	0.20	1	95	*	*	*

Data were obtained from Appendix 1, p. 48 and DER Attachment 2 (means, standard deviations, relative standard deviations). Although not specified, example calculations indicate recovery results were uncorrected (Appendix 1, p. 43).

\* = Not applicable, n = 1.

1 Reported results were a "Summary of Recovery Data", rather than results from a method validation trial (Appendix 1, p. 48). The reported recoveries appear to be selected procedural recovery samples analyzed with field trial samples.

For example, recoveries from fortified samples shown in Figure 6 (AG-A 8099-05,06; Appendix 1, p. 54) were reported in Table III (Appendix 1, p. 48), whereas, recoveries from fortified samples shown in Figure 7 (AG-A 8099-02; Appendix 1, p. 55) were not included in Table III.

2 The four soil matrices were not characterized other than soil type and source (State) location (Appendix 1, p. 48).

**Table 3. Independent Validation Method Recoveries for Triasulfuron (CGA131036) in Soil**

Matrix <sup>1</sup>	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil (uncharacterized)	0.01 (LOQ)	5	68-84	72	6.8	9.4
	0.1	5	69-76	72	2.5	3.5

Data were obtained from Table 1, p. 20. Example calculations allow for the correction of recovery results, but the reported residue results indicate recoveries were uncorrected (Table 1, p. 20; Appendix 3, p. 57).

1 The control soil used was provided by Syngenta (p. 10). The soil was characterized by Agvise Laboratories, Northwood, North Dakota, but the characterization results were not provided (p. 10; Appendix 4, p. 58).

### III. Method Characteristics

In the ECM and ILV, the LOQ for triasulfuron in soil was 0.01 mg/kg (pp. 8-9, Appendix 1, pp. 37, 44; Appendix 4, p. 60). The LOQ was established by the lowest fortification level. In the ECM, the LOD was set at 2.5 ng (0.0125 ng/ $\mu$ L, based on a 200  $\mu$ L injection), the lowest calibration standard, with no justification (Appendix 1, pp. 46-47, 52-53). Although not specified, the ILV appeared to have also set the LOD at 0.0125 ng/ $\mu$ L (Figures 3-4, pp. 23-24).

**Table 4. Method Characteristics for Triasulfuron in Soil**

	<b>Triasulfuron</b>
Limit of Quantitation (LOQ)	0.01 mg/kg
Limit of Detection (LOD)	2.5 ng (0.0125 ng/ $\mu$ L)
Linearity (calibration curve $r^2$ and concentration range) <sup>1</sup>	ECM: $r^2 = 0.9994$ (2.5-25 ng, 0.0125-0.125 ng/ $\mu$ L) ILV: $r^2 = 0.998$ (0.0125-0.25 ng/ $\mu$ L)
Repeatable	ECM: Yes at LOQ for silt loam soils (n = 5-8), but only n = 4 for sandy loam soil. Insufficient performance data at 10x LOQ for all soils.
	ILV: Yes at LOQ and 10x LOQ
Reproducible	Undetermined. The soil matrix, provided by Syngenta, was characterized, but the characterization results were not provided, and Syngenta did not establish that the soil used in the ILV was either an equivalent, or more difficult, analytical sample condition as that used for the ECM.
Specific	Undetermined. A confirmatory method was not reported. ECM: Insufficient chromatographic data were provide to establish that are no known interferences from matrices, reagents, solvents, or equipment. <sup>2</sup> ILV: There were no significant interferences (<30% of LOD) in reagent blank and matrix control chromatograms, but the soil matrix was uncharacterized.

Data were obtained from pp. 8, 10; Table 1, p. 20; Figures 2-4, pp. 23-24; Appendix 1, pp. 37, 44, 46; Appendix 4, p. 58; and DER Attachment 2.

1 Linearity of the ECM and ILV calibration curves was verified by the reviewer;  $r^2$  values are reviewer-generated (DER Attachment 2). ECM and ILV reported r values were 0.9997 and 0.999, respectively (Figure 1, p. 22; Appendix 1, p. 47).

2 Only one chromatogram of a matrix control from a MS silt loam soil sample set (AG-A 8099-05,06) was provided (Appendix 1, p. 54). The additional chromatogram (AG-A 8099-02) provided was not of the same sample sets as the reported recovery results (Appendix 1, pp. 48, 55).

### IV. Method Deficiencies and Reviewer's Comments

1. Sufficient ECM performance data at the LOQ and 10x LOQ for all soil matrices were not provided. At the LOQ, sufficient performance data were provided for the three silt loam soils (MS, KS, NY, n = 5-8), but not for the CA sandy loam soil (n = 4; Appendix 1, p. 48). At 10x LOQ, only performance data for two spiked samples were reported for the NY silt loam soil, and no performance data were reported for the MS and KS silt loam soils and CA sandy loam soil.
2. The determination of the LOQ and LOD were not based on scientifically acceptable procedures. The LOQ (0.01 mg/kg) was reported as established by the lowest fortification level (Appendix 1, pp. 37, 44). The LOD was set at 2.5 ng (0.0125 ng/ $\mu$ L, based on a 200

µL injection), the lowest calibration standard, with no justification (Appendix 1, pp. 46-47, 52-53).

Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

3. Sufficient chromatographic data were not provided to support validation of the ECM. ECM chromatograms of reagent blanks, matrix blanks for all soil matrices, and spiked samples at LOQ and 10x LOQ for the reported recovery results were not provided. Only chromatograms of a matrix blank and spiked samples at 2x LOQ and 5x LOQ for MS silt loam sample set AG-A 8099-05,06 were provided (Appendix 1, pp. 48, 54). The additional chromatograms (sample set AG-A 8099-02) provided were not of the same sample sets as the reported recovery results (Appendix 1, pp. 48, 55).

The ECM study author reported that "All control samples from all four locations contained <0.01 ppm." (Appendix 1, pp. 44, 48), but only the one chromatogram of a matrix control from the one MS silt loam soil sample set (AG-A 8099-05,06) was provided (Appendix 1, p. 54).

ECM chromatograms of the lowest spiking levels showing that the analyte peak can be measured accurately (accounting for baseline noise) were not provided. For the ILV, a chromatogram of the lowest spiking level shows the analyte peak only minimally resolved above the baseline (Figures 4-5, p. 24; Figure 11, p. 27). Although the ILV performance data were within guidelines (mean 70-110%, RSD ≤20%; Table 1, p. 20), peak resolution at the LOQ indicate the ECM may not be adequate as an enforcement method for triasulfuron in soil (Figures 4-5, p. 24; Figure 11, p. 27).

4. Triasulfuron was identified and quantified using coupled-column HPLC with UV detection, a confirmatory method was not employed.
5. Soil matrices used in the ECM were not adequately characterized. The soil matrices used in the ECM were not characterized other than soil type and source (State) location (Appendix 1, p. 48).
6. The registrant did not specify that the soil used in the ILV was either an equivalent, or more difficult, analytical sample condition as that used in the ECM. The soil matrix used in the ILV, provided by Syngenta, was characterized, but the characterization results were not provided (p. 10; Appendix 4, p. 58).
7. The Lichrosorb-CN (4 mm x 250 mm, 10 µm) column used in the ECM has been discontinued. The ILV substituted a Lichrosorb-CN (4.6 mm x 250 mm, 5 µm) column, which Syngenta considered an acceptable replacement (p. 13; Appendix 4, p. 60). A finalized ECM with the updated LC column information should be provided.
8. The analytical purity of the triasulfuron standard used for fortifications in the ECM was not reported (Appendix 1, p. 39).

9. The reported ECM recoveries appear to be selected procedural recovery samples analyzed with field trial samples, rather than results from a method validation trial (Appendix 1, pp. 48, 54-55). However, insufficient information was provided to determine if the LOQ is less than 10% of the expected or actual peak concentration of the test compound in the field.
10. In Appendix 3 Example Calculations (p. 57), the "Amount of analyte fortified (mg/kg)" should be 0.01 mg/kg, rather than the reported 0.1 mg/kg. In addition, the reviewer could not re-calculate the reported 10xLOQ Residue (mg/kg) values (Table 1, p. 20) from the provided equations.
11. It was reported for the ILV that one analyst could complete a single set of thirteen samples in one working day with HPLC analysis performed overnight (p. 16).

## 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.



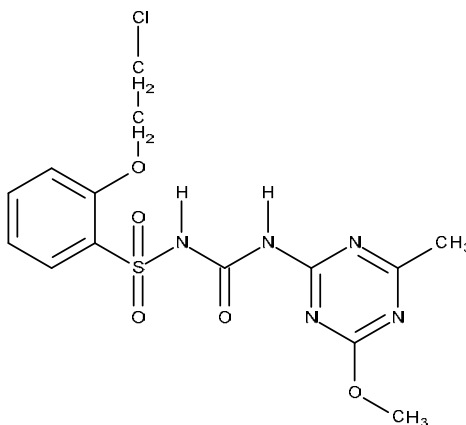
**Attachment 1: Chemical Names and Structures****Triasulfuron (CGA131036)**

**IUPAC Name:** 1-[2-(2-Chloroethoxy)phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea

**CAS Name:** 2-(2-Chloroethoxy)-N-[[4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide

**CAS Number:** 82097-50-5

**SMILES String:** ClCCOc1ccccc1S(=O)(=O)NC(=O)Nc2nc(OC)nc(C)n2

**Attachment 2: Raw Data and Calculations**

raw data and calcs