Analytical method for S-metolachlor (CGA-77102) and its metabolites CGA-51202, CGA-354743, and CGA-37735 in soil, thatch, and grass

Reports: ECM: MRID 46829512. Vargo, J.D., R. Rezaaiyan. 1999. CGA-77102: METHOD – Analytical Method For The Determination Of CGA-77102 And Degradates CGA-51202, CGA-354743, And CGA-37735 In Soil And Thatch, And CGA-77102 In Grass By High Performance Liquid Chromatography With Mass Spectrometric Detection. Novartis Study No.: 127-99. Report prepared, sponsored and submitted by Novartis Crop Protection, Inc., Greensboro, North Carolina; 76 pages. Final report issued September 2, 1999.

ILV: MRID 45848002. Evans, P.G. 2002. CGA-77102: FINAL REPORT – S-metolachor - Independent Laboratory Validation of an Analytical Method for the Determination of CGA-77102 and Degradates CGA-51202, CGA-354743, and CGA-37735 in Soil and Thatch, and CGA-77102 in Grass by High Performance Liquid Chromatography with Mass Spectrometric Detection. Novartis Study No.: 127-99. Jealott's Hill Study No.: RJ3292B. Syngenta Study No. 52-99. Report prepared by Syngenta, Jealott's Hill International Research Centre, Berkshire, United Kingdom, and sponsored and submitted by Syngenta, Berkshire, United Kingdom, and Syngenta Crop Protection, LLC, Greensboro, North Carolina; 169 pages. Final report issued June 24, 2002.

Document No.: MRIDs 46829512 & 45848002

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with the USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 46829512). Signed and dated No Data Confidentiality, Quality Assurance and GLP statements were provided (pp. 2-3, 5). An authenticity statement was not included.

ILV: The study was conducted in accordance with the OECD GLP standards (p. 3 of MRID 45848002). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A certification of authenticity was included with the Quality Assurance statement.

Classification: This analytical method is classified as **Supplemental**. The LOQ in soil (10 µg/kg) is greater than the lowest toxicological level of concern for plants (EC_{25} = 2.4 µg/kg; Monocot Ryegrass EC_{25} = 0.0048 lb a.i./A; MRID 43928932). Performance data was not satisfactory (RSD = 46%) for the 10×LOQ analysis of S-metolachlor in thatch. The ILV matrices were not characterized.

PC Code: 108800, 108801

EFED Final Reviewer: Christopher M. Koper, M.S., Chemist

Signature:

Date: June 19, 2018

CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature:	Lisa Muto
		Date:	5/10/18
	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature:	Kathleen P. Ferguson
		Date:	5/10/18

Executive Summary

The analytical method, Novartis Study No. 127-99, is designed for the quantitative determination of S-metolachlor (CGA-77102) and its metabolites CGA-51202, CGA-354743, and CGA-37735 in soil and thatch at the LOQ of 0.01 mg/kg and of S-metolachlor in grass at the LOQ of 1 mg/kg using LC/MS/MS. **The LOQ in soil (0.010 mg/kg) is greater than the lowest toxicological level of concern for plants (EC_{25} = 2.4 x 10^{-3} mg/kg; Monocot Ryegrass EC_{25} = 0.0048 lb a.i./A; MRID 43928932).** The ECM validated the method using one characterized soil, thatch, and grass matrix; the ILV validated the method using one uncharacterized soil, thatch, and grass matrix. It could not be determined if the ILV was provided with the most difficult soil matrix with which to validate the method and that the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The ILV validated the method for CGA-77102 in soil, thatch, and grass, CGA-51202 and CGA-354743 in soil and thatch and for CGA-37735 in soil at both fortification levels after the first trial, with insignificant analytical instrument and parameter modifications. The method was validated for CGA-37735 in thatch at both fortification levels after the second trial, with insignificant analytical instrument and parameter modifications, as well as a 4x dilution of the sample prior to analysis to reduce matrix effects (suppression); the first trial failed due to low recoveries. The ILV modifications did not warrant an updated ECM. All ILV data was satisfactory regarding accuracy, precision, linearity, and specificity. All ECM data was satisfactory regarding accuracy, precision, linearity, and specificity, except for the recovery data for the 10×LOQ analysis of CGA-77102 in thatch; therefore, reproducibility for the 10×LOQ analysis of CGA-77102 in thatch was not supported by the data.

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						
S-metolachlor (CGA-77102)	46829512[1]	45848002[2]		Soil and Thatch	02/09/1999	Novartis Crop Protection, Inc. Syngenta Crop Protection, Inc.	LC/MS/MS	0.01 mg/kg
CGA-51202								
CGA-354743								
CGA-37735								
S-metolachlor (CGA-77102)				Grass				1 mg/kg

1 In the ECM, Georgia sandy loam soil (0-6"; 79% sand 12% silt 9% clay, 0.7% organic matter, pH 6.8), Fresno thatch (0-3") consisting of sandy loam soil (77% sand 14% silt 9% clay, 2.6% organic matter, pH 8.3) and roots and vegetative matter from the surface, and grass (grass clipping from a control turf plot from a turf study conducted in

California, Novartis Study 280-98, inventory No. INV18146.2, sample number 236625) were used in the study. The soil characterization for the soil and thatch was provided by Novartis Crop Protection and Agvise Laboratories (Northwood, North Dakota).

2 In the ILV, the soil, thatch, and grass matrices were given the reference names SMET-SOIL, SMET-THATCH, and SMET-GRASS, respectively. The matrices were obtained from Study No. 51-99, Research Options Inc., Montezuma, Georgia. No matrix characterization data was provided.

I. Principle of the Method

Soil/Thatch

For soil and thatch, samples (10 ± 0.1 g) was measured into a plastic extraction bottle and fortified, as necessary (pp. 11, 16-18; Figure 2, p. 61 of MRID 46829512). The samples were extracted twice (2 x 75 mL) with 70% methanol/water with 1% ammonium hydroxide via shaking at room temperature for thirty minutes at high speed on an orbital shaker, then centrifugation at *ca*. 9000 rpm for 7 minutes. The combined extracts were mixed with *ca*. 10 mL of purified water, then the methanol was removed via rotary evaporation in a water bath (*ca*. 35°C). The sample was acidified by adding 0.2 mL of phosphoric acid then applied to a Varian ENV Solid Phase Extraction (SPE) cartridge (1 g; pre-conditioned with 10 mL of methanol, then 10 mL of 0.1% phosphoric acid). The sample flask was rinsed with *ca*. 5 mL of 0.1% phosphoric acid which was applied to the column. The column was rinsed sequentially with 2-3 mL of 0.1% phosphoric acid, 6 mL of 0.25% ammonium hydroxide and 6 mL of purified water. The analytes were eluted from the column using 10 mL of methanol with 0.1% ammonium hydroxide. The eluate was concentrated via rotary evaporation in a water bath (*ca*. 35°C). The residue was reconstituted in 1.0 mL of acetonitrile, and the volume was adjusted to 5 mL using purified water. The sample was further diluted, if necessary. An aliquot was transferred to an autosampler vial for analysis by LC/MS/MS.

Grass

For grass, samples (5 ± 0.1 g) was measured into a plastic extraction bottle and fortified, as necessary (only fortified with CGA-77102; pp. 11, 18-20; Figure 3, p. 62 of MRID 46829512). The samples were extracted twice (100 mL then 50 mL) with 80% acetonitrile/water via shaking at room temperature for thirty minutes at high speed on an orbital shaker, then centrifugation at *ca*. 9000 rpm for 10 minutes. An aliquot (5.0 mL) of the combined extracts was acidified by adding 5 mL of 1% formic acid then applied to a Varian ENV Solid Phase Extraction (SPE) cartridge (1 g; pre-conditioned with 5 mL of acetonitrile, then 5 mL of 40% acetonitrile/water with 0.1% phosphoric acid). The sample flask was rinsed with *ca*. 5 mL of 40% acetonitrile/water with 0.1% phosphoric acid which was applied to the column. The column was dried for *ca*. 30 seconds with vacuum. The analytes were eluted from the column using 10 mL of 80% acetonitrile/water. The eluate was reduced to dryness via rotary evaporation in a water bath (*ca*. 35-40°C). The residue was reconstituted in 10 mL of 20% acetonitrile/water. The sample was further diluted, if necessary. An aliquot was transferred to an autosampler vial for analysis by LC/MS/MS.

LC/MS/MS

Samples were analyzed using a Perkin-Elmer Series 200 LC pump coupled to a PE Sciex API-365 Triple Quadrupole MS (p. 20; Tables 2-4, pp. 31-35 of MRID 46829512). The following LC conditions were used: Zorbax SB-C8 column (3.0 mm x 150 mm, 3.5 µm, column temperature

30°C), Upchurch (#A-318) pre-column filter (2.0 µm), mobile phase of (A) 0.1% acetic acid in acetonitrile and (B) 0.1% acetic acid in purified water [percent A:B (v:v) at 0.0 min. 20:80, 10.0-13.0 min. 100:0, 14.0-21.0 min. 20:80], and injection volume of 20 µL. The following MS/MS conditions were used: positive mode (temperature 350°C) or negative mode (temperature 420°C), and multiple reaction monitoring (MRM). Analytes were identified using one ion pair transition as follows: *m/z* 284.1→252.1 for S-metolachlor (CGA-77102), *m/z* 278.2→206.0 for CGA-51202, *m/z* 328.3→121.0 for CGA-354743, and *m/z* 192.3→134.0 for CGA-37735. All analytes were monitored in negative ion mode, except S-metolachlor (CGA-77102) which was monitored in positive mode. CGA-51202 was reported to exhibit a split peak in chromatography, and the entire area should be integrated for quantitation (p. 24). Expected retention times were not reported. The method noted that trace amounts of CGA-77102 (< 0.1 ppb) are commonly seen in method blank and control samples (p. 22). Also, the method noted that metolachlor (CGA-24705) has the same retention time and mass fragmentation spectra as S-metolachlor (CGA-77102), since metolachlor consists of two rotational isomers (R and S).

ILV

The independent laboratory performed the ECM as written, except for 4x dilution of samples of CGA-37735 in thatch and insignificant modifications of analytical instrumentation and parameters (pp. 8-9, 15; Appendix 1, pp. 24-27; Appendix 14, pp. 91-167 of MRID 45848002). The 4x dilution of the CGA-37735 in thatch samples prior to analysis was performed to reduce matrix effects (suppression); this modification was decided upon after communication with the sponsor. A PE Sciex API 3000 LC/MS/MS coupled with an Agilent 1100 series LC were used. All LC and MS parameters were the same as the ECM, except that the switch from negative to positive mode was reduced to 7 minutes from 10 minutes due to the retention times of the analytes being shorter than expected. Analytes were identified using one ion pair transition as follows: *m/z* 284.02→252.07 for S-metolachlor (CGA-77102), *m/z* 278.15→206.20 for CGA-51202, *m/z* 328.18→121.03 for CGA-354743, and *m/z* 192.01→134.24 for CGA-37735. Ion transitions were similar to those used in the ECM. All analytes were monitored in negative ion mode, except S-metolachlor (CGA-77102) which was monitored in positive mode. Expected retention times were not reported. The ILV modifications did not warrant an updated ECM. The ILV reported minor improvements or clarifications for the method, including the suggestion of dilution in the case of matrix effects; these comments were ordinary laboratory improvisations for extraction methods (p. 21).

LOQ and LOD

In the ECM and ILV, Limit of Quantification (LOQ) for S-metolachlor (CGA-77102) and its metabolites CGA-51202, CGA-354743, and CGA-37735 was 0.010 mg/kg in soil and thatch; the LOQ of CGA-77102 was 1 mg/kg in grass (pp. 11, 28 of MRID 46829512; pp. 8, 16 of MRID 45848002). The Limit of Detection (LOD) for all analytes in soil, thatch, and grass was reported as 0.005 ng/µL (0.1 ng injected) in the ECM and 0.0025 mg/kg in the ILV.

II. Recovery Findings

ECM (MRID 46829512): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of S-metolachlor (CGA-77102) and its transformation products CGA-51202, CGA-354743, in CGA-37735 in one soil and thatch matrix at fortification levels of 0.01 mg/kg (LOQ), 0.10 mg/kg (10×LOQ), and 2 mg/kg (200×LOQ), except for the 10×LOQ analysis of CGA-77102 in thatch (RSD 45.5%; Tables 8-10, pp. 54-58; DER Attachment 2). The mean, standard deviation and RSD for the 10×LOQ analysis of CGA-77102 in thatch were reviewer-calculated based on the five recovery values provided in the study report since the study author omitted one of the values as an outlier and calculated statistics; rules of significant figures were followed. Mean recoveries and relative RSDs were within guideline requirements for analysis of CGA-77102 in one grass matrix at fortification levels of 1.0 mg/kg (LOQ), 10 mg/kg (10×LOQ), and 200 mg/kg (200×LOQ). Analytes were identified and quantified using one ion transition; a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate data. Georgia sandy loam soil (0-6”; 79% sand 12% silt 9% clay, 0.7% organic matter, pH 6.8), Fresno thatch (0-3”) consisting of sandy loam soil (77% sand 14% silt 9% clay, 2.6% organic matter, pH 8.3) and roots and vegetative matter from the surface, and grass (grass clipping from a control turf plot from a turf study conducted in California, Novartis Study 280-98, inventory No. INV18146.2, sample number 236625) were used in the study (p. 18; Table 1, p. 30). The soil characterization for the soil and thatch was provided by Novartis Crop Protection and Agvise Laboratories (Northwood, North Dakota).

ILV (MRID 45848002): Mean recoveries and RSDs were within guidelines for analysis of S-metolachlor (CGA-77102) and its transformation products CGA-51202, CGA-354743, in CGA-37735 in one soil and thatch matrix at fortification levels of 0.01 mg/kg (LOQ) and 0.10 mg/kg (10×LOQ); mean recoveries and relative RSDs were within guideline requirements for analysis of CGA-77102 in one grass matrix at fortification levels of 1.0 mg/kg (LOQ) and 10 mg/kg (10×LOQ; Tables 7-9, p. 17; DER Attachment 2). Analytes were identified and quantified using one ion transition; a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate data. The soil, thatch, and grass matrices were given the reference names SMET-SOIL, SMET-THATCH, and SMET-GRASS, respectively (p. 13). The matrices were obtained from Study No. 51-99, Research Options Inc., Montezuma, Georgia. No matrix characterization data was provided. The method was validated for CGA-77102 in soil, thatch, and grass, CGA-51202 and CGA-354743 in soil and thatch and for CGA-37735 in soil at both fortification levels after the first trial, with insignificant analytical instrument and parameter modifications (pp. 8-9, 15). The method was validated for CGA-37735 in thatch at both fortification levels after the second trial, with insignificant analytical instrument and parameter modifications, as well as a 4x dilution of the sample prior to analysis to reduce matrix effects (suppression); the first trial failed due to low recoveries.

Table 2. Initial Validation Method Recoveries for S-metolachlor (CGA-77102) and Its Transformation Products CGA-51202, CGA-354743, and CGA-37735 in Soil, Thatch, and Grass.[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Georgia Loamy Sand Soil					
S-Metolachlor (CGA-77102)	0.01 (LOQ)	5	68.1-95.0	83.7	11.0	13.1
	0.10	5	78.5-96.9	87.5	8.2	9.4
	2	5	94.7-103.3	98.7	3.1	3.2
CGA-51202	0.01 (LOQ)	5	81.8-90.3	85.5	3.9	4.5
	0.10	5	92.5-102.4	97.9	4.2	4.3
	2	5	96.7-100.3	98.9	1.5	1.5
CGA-354743	0.01 (LOQ)	5	83.2-90.6	86.8	3.3	3.8
	0.10	5	92.4-101.5	96.9	4.0	4.1
	2	5	95.6-106.9	100.0	4.50	4.50
CGA-37735	0.01 (LOQ)	5	83.9-92.4	88.1	3.1	3.6
	0.10	5	91.6-102.5	96.5	4.5	4.6
	2	5	100.7-104.7	102.3	2.0	2.0
	Fresno Thatch (Sandy Loam Soil with Roots and Vegetative Matter)					
S-Metolachlor (CGA-77102)	0.01 (LOQ)	5	72.8-80.0	76.0	3.1	4.0
	0.10	5[4]	82.6-203.2	112.1	51.1	**45.5**
	2	5	85.2-95.4	89.2	5.0	5.6
CGA-51202	0.01 (LOQ)	5	76.3-80.3	78.1	1.9	2.4
	0.10	5	84.1-89.9	86.0	2.3	2.6
	2	5	87.6-92.6	90.2	1.9	2.1
CGA-354743	0.01 (LOQ)	5	67.0-74.6	70.2	2.9	4.1
	0.10	5	82.6-91.6	86.8	3.3	3.8
	2	5	85.1-90.3	90.0	4.0	4.5
CGA-37735	0.01 (LOQ)	5	80.3-87.2	83.1	2.8	3.4
	0.10	5	83.3-92.4	88.8	4.4	4.9
	2	5	87.2-93.9	91.4	2.8	3.0
	Grass					
S-Metolachlor (CGA-77102)	1.0 (LOQ)	5	88.3-105.3	96.2	6.3	6.6
	10	5	90.5-100.8	96.9	4.2	4.3
	200	5	97.3-102.9	99.5	2.3	2.3

Data (recovery results were corrected when residues were quantified in the controls; pp. 25-27; Tables 5-7, pp. 36-53) were obtained from Tables 8-10, pp. 54-58 of MRID 46829512 and DER Attachment 2.

1 Georgia sandy loam soil (0-6"; 79% sand 12% silt 9% clay, 0.7% organic matter, pH 6.8), Fresno thatch (0-3") consisting of sandy loam soil (77% sand 14% silt 9% clay, 2.6% organic matter, pH 8.3) and roots and vegetative matter from the surface, and grass (grass clipping from a control turf plot from a turf study conducted in California, Novartis Study 280-98, inventory No. INV18146.2, sample number 236625) were used in the study (p. 18; Table 1, p. 30). The soil characterization for the soil and thatch was provided by Novartis Crop Protection and Agvise Laboratories (Northwood, North Dakota).

2 Analytes were identified using one ion pair transition as follows: *m/z* 284.1→252.1 for S-metolachlor (CGA-77102), *m/z* 278.2→206.0 for CGA-51202, *m/z* 328.3→121.0 for CGA-354743, and *m/z* 192.3→134.0 for CGA-37735. All analytes were monitored in negative ion mode, except S-metolachlor (CGA-77102) which was monitored in positive mode.

3 Mean, standard deviation and RSD were reviewer-calculated based on the five recovery values provided in the study report since the study author omitted one of the values as an outlier and calculated statistics for n = 4 (see DER Attachment 2). Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for S-metolachlor (CGA-77102) and Its Transformation Products CGA-51202, CGA-354743, and CGA-37735 in Soil, Thatch, and Grass.[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Soil					
S-Metolachlor (CGA-77102)	0.01 (LOQ)	5	76-90	85	6	6.8
	0.1	5	76-89	82	5	6.0
CGA-51202	0.01 (LOQ)	5	87-91	88	2	2.0
	0.1	5	82-92	88	4	4.6
CGA-354743	0.01 (LOQ)	5	84-90	87	2	2.7
	0.1	5	80-92	87	5	5.3
CGA-37735	0.01 (LOQ)	5	75-81	79	2	2.9
	0.1	5	80-90	86	4	4.3
	Thatch					
S-Metolachlor (CGA-77102)	0.01 (LOQ)	5	83-98	89	6	6.4
	0.1	5	76-87	80	6	6.9
CGA-51202	0.01 (LOQ)	5	84-89	86	2	2.3
	0.1	5	90-93	92	2	1.8
CGA-354743	0.01 (LOQ)	5	83-87	85	2	1.9
	0.1	5	88-92	90	2	2.1
CGA-37735	0.01 (LOQ)	5	83-89	87	3	3.2
	0.1	5	81-85	83	2	2.3
	Grass					
S-Metolachlor (CGA-77102)	1 (LOQ)	5	91-97	95	2	2.4
	10	5	96-102	98	3	2.7

Data (uncorrected recovery results; Appendix 12, p. 85) were obtained from Tables 7-9, p. 17 of MRID 45848002 and DER Attachment 2.

1 The soil, thatch, and grass matrices were given the reference names SMET-SOIL, SMET-THATCH, and SMET-GRASS, respectively (p. 13). The matrices were obtained from Study No. 51-99, Research Options Inc., Montezuma, Georgia. No matrix characterization data was provided.

2 Analytes were identified using one ion pair transition as follows: *m/z* 284.02→252.07 for S-metolachlor (CGA-77102), *m/z* 278.15→206.20 for CGA-51202, *m/z* 328.18→121.03 for CGA-354743, and *m/z* 192.01→134.24 for CGA-37735. Ion transitions were similar to those used in the ECM. All analytes were monitored in negative ion mode, except S-metolachlor (CGA-77102) which was monitored in positive mode.

3 Standard deviations were reviewer-calculated based on the data provided in the study report since the study author did not calculate these values (see DER Attachment 2). Rules of significant figures were followed.

III. Method Characteristics

In the ECM and ILV, LOQ for S-metolachlor (CGA-77102) and its metabolites CGA-51202, CGA-354743, and CGA-37735 was 0.010 mg/kg in soil and thatch, and 1 mg/kg in grass (pp. 11, 28 of MRID 46829512; pp. 8, 16 of MRID 45848002). No LOQ calculations or justifications were provided in the ECM or ILV. The LOD for all analytes in soil, thatch, and grass was reported as 0.005 ng/µL (0.1 ng injected) in the ECM and 0.0025 mg/kg in the ILV. In the ECM and ILV, the LOD was defined as the lowest standard used in the calibration curves. The LOD of the ECM was equivalent to that of the ILV. No LOD calculations were reported in ECM or ILV.

Table 4. Method Characteristics for S-metolachlor (CGA-77102) and Its Transformation Products CGA-51202, CGA-354743, and CGA-37735 in Soil, Thatch, and Grass.

Analyte			S-Metolachlor (CGA-77102)	CGA-51202	CGA-354743	CGA-37735
Limit of Quantitation (LOQ)	Soil/Thatch		0.01 mg/kg			
	Grass		1.0 mg/kg	**Not analyzed**		
Limit of Detection (LOD)[1]	ECM		0.005 ng/µL (0.1 ng injected)			
	ILV		0.0025 mg/kg			
Linearity (calibration curve r^2 and concentration range)	ECM[2,3]	Soil	r^2 = 0.9971-0.9978	r^2 = 0.9997-1.0000	r^2 = 0.9994-0.9998	r^2 = 0.9995-0.9997
		Thatch	r^2 = 0.9967-0.9979	r^2 = 0.9996-1.0000	r^2 = 0.9995-0.9999	r^2 = 0.9998-0.9999
		Grass	r^2 = 0.9971-0.9993	**Not analyzed**		
			0.005-1.0 ng/µL			
	ILV[4]		r^2 = 0.9987	r^2 = 0.9999	r^2 = 0.9998	r^2 = 0.9998
			0.005-0.1 ng/µL			
Repeatable	ECM[4]	Soil	Yes at LOQ, 10×LOQ and 200×LOQ			
		Thatch	Yes at LOQ and 200×LOQ **No** at 10×LOQ (RSD **45.5**%)	Yes at LOQ, 10×LOQ and 200×LOQ		
		Grass	Yes at LOQ, 10×LOQ and 200×LOQ	**Not analyzed**		
	ILV[5,6]	Soil/ Thatch	Yes at LOQ and 10×LOQ			
		Grass	Yes at LOQ and 10×LOQ	**Not analyzed**		
Reproducible	Soil		Yes at LOQ and 10×LOQ			
	Thatch		Yes at LOQ **No** at 10×LOQ	Yes at LOQ and 10×LOQ		
	Grass		Yes at LOQ and 10×LOQ	**Not analyzed**		
Specific	ECM	Soil/ Thatch	Yes, matrix interferences were *ca*. 2-10% of the LOQ (based on peak area).	Yes, no matrix interferences were observed.		
		Grass	Yes, matrix interferences were *ca*. 1% of the LOQ (based on peak area).	**Not analyzed**		
	ILV	Soil/ Thatch	Yes, matrix interferences were *ca*. 6-13% of the LOQ (based on peak area).	Yes, matrix interferences were <1% of the LOQ (based on peak area).		Yes, matrix interferences were <4% of the LOQ (based on peak area).
		Grass	Yes, matrix interferences were <1% of the LOQ (based on peak area).	**Not analyzed**		

Data were obtained from pp. 11, 28; Tables 8-10, pp. 54-58 (recovery results); Tables 5-7, pp. 36-53 (correlation coefficients); Figures 6-9, pp. 66-75 (chromatograms) of MRID 46829512; pp. 8, 15-16; Tables 7-9, p. 17 (recovery

results); Appendix 13, pp. 87-90 (calibration curves); Appendices 3-11, pp. 31-83 (chromatograms) of MRID 45848002; DER Attachment 2.

1 The LOD of the ECM was equivalent to that of the ILV, based on LOD definition.

2 ECM coefficient of determination (r^2) values are reviewer-generated from reported correlation coefficient (r) values (1/x weighting; Tables 5-7, pp. 36-53 of MRID 46829512; DER Attachment 2). The reviewer limited the calculated r^2 to 4 significant figures although 5-6 significant figures were reported in the ECM and ILV for r.

3 Values were combined fortification/sample sets.

4 In the ECM, Georgia sandy loam soil (0-6"; 79% sand 12% silt 9% clay, 0.7% organic matter, pH 6.8), Fresno thatch (0-3") consisting of sandy loam soil (77% sand 14% silt 9% clay, 2.6% organic matter, pH 8.3) and roots and vegetative matter from the surface, and grass (grass clipping from a control turf plot from a turf study conducted in California, Novartis Study 280-98, inventory No. INV18146.2, sample number 236625) were used in the study (p. 18; Table 1, p. 30 of MRID 46829512). The soil characterization for the soil and thatch was provided by Novartis Crop Protection and Agvise Laboratories (Northwood, North Dakota).

5 In the ILV, the soil, thatch, and grass matrices were given the reference names SMET-SOIL, SMET-THATCH, and SMET-GRASS, respectively (p. 13 of MRID 45848002). The matrices were obtained from Study No. 51-99, Research Options Inc., Montezuma, Georgia. No matrix characterization data was provided.

6 The ILV validated the method for CGA-77102 in soil, thatch, and grass, CGA-51202 and CGA-354743 in soil and thatch and for CGA-37735 in soil at both fortification levels after the first trial, with insignificant analytical instrument and parameter modifications (pp. 8-9, 15 of MRID 45848002). The method was validated for CGA-37735 in thatch at both fortification levels after the second trial, with insignificant analytical instrument and parameter modifications, as well as a 4x dilution of the sample prior to analysis to reduce matrix effects (suppression); the first trial failed due to low recoveries.

IV. Method Deficiencies and Reviewer's Comments

1. ECM performance data was unsatisfactory for the 10×LOQ analysis of CGA-77102 in thatch (RSD 45.5%; Tables 8-10, pp. 54-58 of MRID 46829512; DER Attachment 2). The mean, standard deviation and RSD for the 10×LOQ analysis of CGA-77102 in thatch were reviewer-calculated based on the five recovery values provided in the study report since the study author omitted one of the values as an outlier and calculated statistics; rules of significant figures were followed. OCSPP guidelines state that mean recoveries and RSDs were 70-120% and RSD ≤20%), respectively, at the LOQ and higher fortifications.

2. ILV matrices (soil and thatch) were not characterized (p. 13 of MRID 45848002). It could not be determined if the ILV was provided with the most difficult soil matrix with which to validate the method and that the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies.

3. Split peak for CGA-51202 in chromatography was observed, as expected, and the entire area should be integrated for quantitation (p. 24 of MRID 46829512).

4. The ILV study author provided a communication log between Peter Evans (ILV study author) and Kent Kabler (Appendix 15, p. 169 of MRID 45848002). These communications included trial success and discussion of problem and solution for the analysis of CGA-37735 in thatch. The reviewer noted that Kent Kabler was not identified in the study report, but was identified in the communications as the Sponsor (pp. 1-4, 21).

5. The ILV modifications did not warrant an updated ECM.

6. The estimations of the LOQ and LOD in ECM and ILV were not based on scientifically

acceptable procedures as defined in 40 CFR Part 136 (pp. 11, 28 of MRID 46829512; pp. 8, 16 of MRID 45848002). No LOQ calculations or justifications were provided in the ECM or ILV. In the ECM and ILV, the LOD was defined as the lowest standard used in the calibration curves. The LOD of the ECM was equivalent to that of the ILV. No LOD calculations were reported in ECM or ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

7. 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 one working day with LC/MS/MS analysis performed overnight (p. 24 of MRID 45848002).

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.

VI. Calculations

ECM ILV calcs

DER Attachment 1. Chemical Names and Structures.

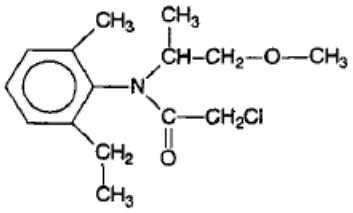
S-metolachlor (CGA-77102)

IUPAC Name: Not reported

CAS Name: (S)-2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-acetamide

CAS Number: 87392-12-9

SMILES String: Not found



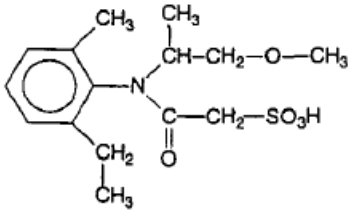
CGA-51202

IUPAC Name: Not reported

CAS Name: 2-[(2-Ethyl-6-methylphenyl)(2-methoxy-1-methylethyl)amino]-2-oxo-ethanesulfonic acid sodium salt

CAS Number: Not assigned

SMILES String: Not found



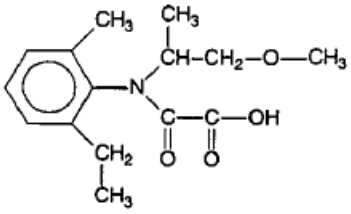
CGA-354743

IUPAC Name: Not reported

CAS Name: [(2-Ethyl-6-methylphenyl)(2-methoxy-1-methylethyl)amino]oxo-acetic acid

CAS Number: 152019-73-3

SMILES String: Not found



CGA-37735

IUPAC Name: Not reported

CAS Name: N-(2-ethyl-6-methylphenyl)-2hydroxy-acetamide

CAS Number: 97055-05-5

SMILES String: Not found