

**Analytical method for mesotrione and its metabolites AMBA and MNBA in soil**

**Reports:** ECM: EPA MRID No.: MRID 50922301. Jutsum, L., and R.W. Williams. 2013. Mesotrione. Mesotrione - Analytical Method GRM007.10A for the Determination of Mesotrione and its Metabolites AMBA and MNBA in Soil. Analytical Method. Syngenta Report No.: GRM007.10A and Task No.: TK0046982. Report prepared by CEM Analytical Services Ltd. (CEMAS), Berkshire, United Kingdom, sponsored by Syngenta Ltd., Berkshire, United Kingdom, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 91 pages. Final report issued March 25, 2013.

ILV: EPA MRID No. 49458107. Bruns, G., S. Nelson, and C. Blenkinsop. 2005. Independent Laboratory Validation: Syngenta Method T001200-03: "Analytical Method 1200-03 for the Determination of Mesotrione and its Metabolites AMBA and MNBA, in Soil, Using Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry (Including Validation Data)". ETL Study No.: 04ILV07SYN and Report No.: 04SYN146.REP. Syngenta Study No.: T002655-03. Report prepared by Enviro-Test Laboratories, Alberta, Canada, and sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 41 pages. Final report issued February 2, 2005.

**Document No.:** MRIDs 50922301 & 49458107

**Guideline:** 850.6100




**Statements:** ECM: The study was not conducted in accordance with the USEPA FIFRA or OECD Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 50922301). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included. A signed and dated summary of revisions to the previous version was provided (p. 4).

ILV: The study was conducted in accordance with the USEPA GLP standards, except that stock solutions were prepared prior to study initiation under Syngenta's GLP Study No. T006450-04 (p. 3 of MRID 49458107). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5). An authenticity statement was not provided.

**Classification:** This analytical method is classified as unacceptable/upgradable for mesotrione and AMBA and unacceptable for MNBA. Since the ILV was not based specifically on the submitted ECM Syngenta Residue Method GRM007.10A, no information was available to assess the repeatability of Syngenta Residue Method GRM007.10A with regard to the LC/MS/MS analytical portion. A relevant ILV should be submitted with details to equate the LC/MS/MS analytical methods of Syngenta Residue Method GRM007.10A and discuss the preference for the simplified LC/MS/MS analytical method presented in Syngenta Residue Method GRM007.10A. The specificity of Syngenta Residue Method GRM007.10A was not

supported for MNBA based on ECM representative chromatograms. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies. In addition, the LOQ is greater than the lowest toxicological level of concern in soil for mesotrione.

Method GRM007.10A supersedes method 1200-03 which has been updated to include confirmatory LC/MS/MS transitions (Reference 1, MRID 50922301). Method 1200-03 (ECM: MRID 49458108 and ILV: MRID 49458107) was previously reviewed and deemed supplemental since the registrant failed to select the most difficult sample condition for validation analyses, the LOQ and LOD determinations were not based on scientifically acceptable procedures, and for the ECM, an insufficient number of samples were fortified at the LOQ and no fortifications were performed at 10x LOQ.

<b>PC Code:</b>	122990		
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		Date:	12/20/2019

*This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.*

## Executive Summary

The analytical methods, Syngenta Analytical Method No. 1200-03 and Syngenta Method No. GRM007.10A, is designed for the quantitative determination of mesotrione and its metabolites AMBA and MNBA in soil at the LOQ of 2.0 µg/kg using LC/MS/MS. **The LOQ is greater than the lowest toxicological level of concern in soil for mesotrione**, while the LOQ is less than the lowest toxicological level of concern in soil for MNBA, and no data are available for AMBA. Syngenta Residue Method GRM007.10A was a method which superseded Syngenta Analytical Method No. 1200-03 and updated Syngenta Analytical Method No. 1200-03 to include confirmatory LC/MS/MS transitions. ILV validated Syngenta Analytical Method No. 1200-03 in the first trial using sand soil with insignificant modifications to the analytical parameters. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and if the ILV soil matrices covered the range of soils used in

the terrestrial field dissipation studies. The submitted ECM was performed *ca.* 8 years after the ILV using loam and sandy clay loam soils. The sampling processing procedure of Syngenta Residue Method GRM007.10A matched the sampling procedure performed in the ILV; however, significant differences were noted in the LC/MS/MS analytical portion of the ILV. All ILV and ECM data was satisfactory regarding accuracy and precision for all analytes at the LOQ and 10×LOQ; the repeatability of Syngenta Residue Method GRM007.10A was acceptable based on the sampling processing procedure only. An updated ECM should be submitted with details to equate the LC/MS/MS analytical methods of Syngenta Residue Method GRM007.10A and Syngenta Analytical Method No. 1200-03, as well as to discuss the preference for the simplified LC/MS/MS analytical method presented in Syngenta Residue Method GRM007.10A. The specificity of Syngenta Residue Method GRM007.10A was not supported for MNBA based on ECM representative chromatograms.

**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						
Mesotrione	50922301 <sup>1</sup>	49458107 <sup>2</sup>		Soil	25/03/2013 <sup>3</sup>	Syngenta Crop Protection, Inc.	LC/MS/MS	0.002 mg/kg
AMBA								
MNBA								

1 In the ECM, the Gartenacker loam soil [Sample Reference CCON/033/002; pH: 7.7 (water), 7.2 (in 0.01M CaCl<sub>2</sub>); 3.5% organic matter, 2.0% organic carbon] and 18 Acres sandy clay loam soil [Sample Reference CCON/034/002; pH: 6.2 (water), 5.6 (in 0.01M CaCl<sub>2</sub>); 4.5% organic matter, 2.6% organic carbon] were obtained from Syngenta (USDA soil texture classification was not specified; Table 1, p. 27 of MRID 50922301). The soil characterization laboratory was not reported.

2 In the ILV, the sand soil (Boring ID: GP-1, GP-2, and GP-3; 0-6; Syngenta Study No. T000011-02; sand 90%, silt 5%, clay 5%; pH 5.3-5.8; 1.0-1.5% organic matter) was provided by the Sponsor and characterized by Agvise laboratories, Northwood, North Dakota (USDA soil texture classification; p. 11 of MRID 49458107). The soil characterization data was taken from Syngenta Study No. T000011-02.

3 GRM007.10A superseded Syngenta Analytical Method No. 1200-03 (p. 4 of MRID 50922301). Syngenta Analytical Method No. 1200-03 was not submitted; therefore, a method date could not be provided. The ILV was dated 02/02/2005 and validated Syngenta Analytical Method No. 1200-03.

## I. Principle of the Method

Soil samples (10 ± 0.1 g) in a 250-mL polypropylene bottle was fortified with the mixed fortification standard solution in methanol, if necessary (pp. 11, 14-15; Appendix 4, p. 91 of MRID 50922301). The samples were sequentially extracted via shaking for a minimum of 30 minutes with 0.05M NH<sub>4</sub>OH, 0.05M NH<sub>4</sub>OH:acetone (50:50, v:v), then acetone (20 mL each).

The method noted that a spatula should be used to break up the soil pellet in the second and third extraction, if necessary. After extractions, samples were centrifuged at 10000 rpm (or at a speed that visibly separates the solid sample from the supernatant) for 5 minutes, and the supernatant was decanted into another polypropylene bottle. The volume of the combined extracts was adjusted to 60 mL with 0.05M NH<sub>4</sub>OH:acetone (50:50, v:v). After centrifugation (3500 rpm for 5 minutes), a 6-mL aliquot of the supernatant (equivalent to 1 g soil) was removed. The organic solvent was removed via N-Evap unit with a bath temperature of *ca.* 40°C (*ca.* 2.5 mL volume remaining). After 2% formic acid was added to a final volume of 10 mL, the sample was sonicated and centrifuged prior to LC/MS/MS analysis.

The method contained the following precaution: bottled HPLC grade ultra-pure water should be used to prepare the LC mobile phase (p. 16 of MRID 50922301).

Samples were analyzed using an Agilent 1100 series HPLC coupled to an AB Sciex API 5000 mass spectrometer (pp. 16-21 of MRID 50922301). The LC/MS conditions consisted of a PLRP-S 100 Å column (50 x 4.6 mm, 5 µm particle size; oven temperature 35°C) with a mobile phase gradient of A) 0.1% acetic acid in HPLC-grade water and B) 0.1% acetic acid in HPLC-grade acetonitrile [percent A:B (v:v) at 0.0-0.1 min. 98:2, 4.1 min. 50:50, 6.0 min. 25:75, 7.0-8.0 min. 5:95, 8.2-10.0 min. 95:5] and Turbo Ion Spray ionization interface MS detection in negative ion mode with MRM (TEM 700°C). Injection volume was 100 µL. Two ion transitions were monitored for each analyte as follows (quantitative and confirmatory, respectively): *m/z* 338.2→291.0 and *m/z* 338.2→212.1 for mesotrione, *m/z* 213.8→170.1 and *m/z* 213.8→64.0 for AMBA, and *m/z* 244.1→200.0 and *m/z* 244.1→170.1 for MNBA. Retention times were *ca.* 5.8, 3.9, and 2.6 minutes for mesotrione, AMBA, and MNBA, respectively.

The ILV reportedly performed Syngenta Analytical Method No. 1200-03 as written, except for the use of an alternative LC/MS/MS instrument, the use of an alternative ion transition for the quantitation transition of AMBA (from *m/z* 213→171 to *m/z* 213→155) and increase of injection volume from 10 µL to 50 µL for AMBA and MNBA (pp. 11-15; Tables 1-3, pp. 16-18 of MRID 49458107). The ILV did not identify any critical steps. The submitted ECM Syngenta Residue Method GRM007.10A was a method which superseded Syngenta Analytical Method No. 1200-03 and updated Syngenta Analytical Method No. 1200-03 to include confirmatory LC/MS/MS transitions. Syngenta Analytical Method No. 1200-03 was not provided. The sampling processing procedure of Syngenta Residue Method GRM007.10A matched the sampling procedure performed in the ILV; however, significant differences were noted in the LC/MS/MS analytical portion of the ILV, including mobile phase gradient changes, the addition of a guard column, injection volume changes, monitored ion transition change for AMBA, and a different set of LC/MS/MS parameters for AMBA and MNBA. Samples were analyzed using an Applied Biosystems API-4000 Triple Quadrupole Mass Spectrometer with Turbo Ion Spray Interface. For mesotrione, the LC/MS conditions consisted of a PLRP-S 100 Å column (50 x 4.6 mm, 5 µm particle size; oven temperature not reported) and Polymer Laboratories PLRP-S (5 x 3 mm) guard column with a mobile phase gradient of A) 0.1% acetic acid in water and B) 0.1% acetic acid in acetonitrile [percent A:B (v:v) at 0.0-1.0 min. 85:15, 3.5-6.0 min. 5:95, 6.5-8.5 min. 85:15] and Turbo Ion Spray ionization interface MS detection in negative ion mode with MRM (TEM not reported). Injection volume was 20 µL. Retention time was 3.61 minutes for mesotrione. For AMBA and MNBA, the LC/MS conditions consisted of a Synergi 4µ Fusion-RP

80A column (75 x 4.6 mm, 4 µm particle size; oven temperature not reported) and Fusion-RP (4 x 3.0 mm, 5 µm) guard column with a mobile phase gradient of A) 0.1% acetic acid in water and B) 0.1% acetic acid in acetonitrile [percent A:B (v:v) at 0.0-1.0 min. 95:5, 5.0-8.0 min. 5:95, 9.5-12.5 min. 95:5] and Turbo Ion Spray ionization interface MS detection in negative ion mode with MRM (TEM not reported). Injection volume was 50 µL. Retention times were 4.20 and 6.93 for AMBA and MNBA, respectively. One ion transition was monitored for each analyte as follows:  $m/z$  338.2→291.00 for mesotrione,  $m/z$  213.80→155.10 for AMBA, and  $m/z$  243.70→199.80 for MNBA. Since the ILV was not based specifically on the submitted ECM Syngenta Residue Method GRM007.10A, no information was available to assess the repeatability of Syngenta Residue Method GRM007.10A with regard to the LC/MS/MS analytical portion. An updated ECM should be submitted with details to equate the LC/MS/MS analytical methods of Syngenta Residue Method GRM007.10A and Syngenta Analytical Method No. 1200-03, as well as to discuss the preference for the simplified LC/MS/MS analytical method presented in Syngenta Residue Method GRM007.10A.

In the ECM and ILV, Limit of Quantification (LOQ) in soil was 0.002 mg/kg for mesotrione, AMBA, and MNBA (pp. 11, 22-23 of MRID 50922301; p. 9 of MRID 49458107). In the ECM, the Limits of Detection (LODs) in soil were 0.2-0.3 pg, 1-3 pg, and 1-5 pg for mesotrione, AMBA, and MNBA, respectively, which was equivalent to 0.002-0.003 ng/mL, 0.01-0.03 ng/mL, and 0.01-0.05 ng/mL for mesotrione, AMBA, and MNBA, respectively, when using a 100 µL injection volume. The LOD was not reported in the ILV.

## II. Recovery Findings

ECM (MRID 50922301): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of mesotrione, AMBA, and MNBA in two soil matrices at fortification levels of 0.002 mg/kg (LOQ) and 0.02 mg/kg (10×LOQ; Tables 2-7, pp. 28-30). Analytes were identified and quantified using one ion transition; a confirmatory method is not usually required when LC/MS or GC/MS is the primary method to generate study data. The Gartenacker loam soil [Sample Reference CCON/033/002; pH: 7.7 (water), 7.2 (in 0.01M CaCl<sub>2</sub>); 3.5% organic matter, 2.0% organic carbon] and 18 Acres sandy clay loam soil [Sample Reference CCON/034/002; pH: 6.2 (water), 5.6 (in 0.01M CaCl<sub>2</sub>); 4.5% organic matter, 2.6% organic carbon] were obtained from Syngenta (USDA soil texture classification was not specified; Table 1, p. 27). The soil characterization laboratory was not reported.

ILV (MRID 49458107): Mean recoveries and RSDs were within guidelines for analysis of mesotrione, AMBA, and MNBA in one soil matrix at fortification levels of 0.002 mg/kg (LOQ) and 0.02 mg/kg (10×LOQ; Tables 4-6, pp. 18-20). Analytes were identified and quantified using two ion transitions. The sand soil (Boring ID: GP-1, GP-2, and GP-3; 0-6; Syngenta Study No. T000011-02; sand 90%, silt 5%, clay 5%; pH 5.3-5.8; 1.0-1.5% organic matter) was provided by the Sponsor and characterized by Agvise laboratories, Northwood, North Dakota (USDA soil texture classification; p. 11). The soil characterization data was taken from Syngenta Study No. T000011-02. The ILV validated Syngenta Analytical Method No. 1200-03 in the first trial as

written, except for the use of an alternative LC/MS/MS instrument, the use of an alternative ion transition for the quantitation transition of AMBA and increase of injection volume for AMBA and MNBA analysis (pp. 11-15; Tables 1-3, pp. 16-18). The ILV did not identify any critical steps. The only submitted ECM was Syngenta Residue Method GRM007.10A which was a method which superseded Syngenta Analytical Method No. 1200-03. The sampling processing procedure of Syngenta Residue Method GRM007.10A matched the sampling procedure performed in the ILV; however, significant differences were noted in the LC/MS/MS analytical portion of the ILV. Since the ILV was not based specifically on the submitted ECM Syngenta Residue Method GRM007.10A, no information was available to assess the repeatability of Syngenta Residue Method GRM007.10A with regard to the LC/MS/MS analytical portion. An updated ECM should be submitted with details to equate the LC/MS/MS analytical methods of Syngenta Residue Method GRM007.10A and Syngenta Analytical Method No. 1200-03, as well as to discuss the preference for the simplified LC/MS/MS analytical method presented in Syngenta Residue Method GRM007.10A.

**Table 2. Initial Validation Method Recoveries for Mesotrione and its Metabolites AMBA and MNBA in Soil<sup>1,2</sup>**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>3</sup>	Relative Standard Deviation (%)
<b>Loam Soil</b>						
Quantitation ion transition						
Mesotrione	0.002 (LOQ)	5	85-92	89	3	3.4
	0.02	5	83-92	88	4	4.3
AMBA	0.002 (LOQ)	5	76-95	89	8	8.7
	0.02	5	71-84	79	6	7.0
MNBA	0.002 (LOQ)	5	94-111	100	7	6.6
	0.02	5	91-101	98	4	4.2
Confirmation ion transition						
Mesotrione	0.002 (LOQ)	5	85-93	91	5	5.4
	0.02	5	82-100	91	6	6.9
AMBA	0.002 (LOQ)	5	80-94	88	5	5.8
	0.02	5	73-85	80	5	6.3
MNBA	0.002 (LOQ)	5	93-104	97	4	4.6
	0.02	5	90-101	98	4	4.6
<b>Sandy Clay Loam Soil</b>						
Quantitation ion transition						
Mesotrione	0.002 (LOQ)	5	78-88	82	4	4.9
	0.02	5	86-90	87	2	2.2
AMBA	0.002 (LOQ)	5	77-89	83	5	5.5
	0.02	5	70-73	71	2	2.6
MNBA	0.002 (LOQ)	5	108-115	111	3	2.4
	0.02	5	99-104	101	2	2.0
Confirmation ion transition						
Mesotrione	0.002 (LOQ)	5	76-87	82	5	5.7
	0.02	5	85-89	88	2	2.1
AMBA	0.002 (LOQ)	5	76-89	81	6	6.8
	0.02	5	70-76	72	2	3.5
MNBA	0.002 (LOQ)	5	90-101	96	4	4.7
	0.02	5	97-101	99	1	1.6

Data (uncorrected recovery results; pp. 19-21) were obtained from Tables 2-7, pp. 28-30 of MRID 50922301.

1 The Gartenacker loam soil [Sample Reference CCON/033/002; pH: 7.7 (water), 7.2 (in 0.01M CaCl<sub>2</sub>); 3.5% organic matter, 2.0% organic carbon] and 18 Acres sandy clay loam soil [Sample Reference CCON/034/002; pH: 6.2 (water), 5.6 (in 0.01M CaCl<sub>2</sub>); 4.5% organic matter, 2.6% organic carbon] were obtained from Syngenta (USDA soil texture classification was not specified; Table 1, p. 27). The soil characterization laboratory was not reported.

2 Two ion transitions were monitored for each analyte as follows (quantitative and confirmatory, respectively): *m/z* 338.2→291.0 and *m/z* 338.2→212.1 for mesotrione, *m/z* 213.8→170.1 and *m/z* 213.8→64.0 for AMBA, and *m/z* 244.1→200.0 and *m/z* 244.1→170.1 for MNBA.

3 The standard deviations were reviewer-calculated since these values were not reported in the study report. Rules of significant figures were followed.

**Table 3. Independent Validation Method Recoveries for Mesotrione and its Metabolites AMBA and MNBA in Soil<sup>1,2</sup>**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Sand Soil</b>						
Quantitation ion transition <sup>3</sup>						
Mesotrione	0.002 (LOQ)	5	84-106	95	7.9	8.3
	0.02	5	87-96	93	3.6	3.9
AMBA	0.002 (LOQ)	6	71-85	79	5.6	7.1
	0.02	5	79-83	82	1.7	2.1
MNBA	0.002 (LOQ)	6	78-96	87	8.0	9.2
	0.02	5	94-106	100	4.4	4.4

Data (uncorrected recovery results; Table 7, pp. 22-23) were obtained from Tables 4-6, pp. 18-20 of MRID 49458107.

- 1 The sand soil (Boring ID: GP-1, GP-2, and GP-3; 0-6; Syngenta Study No. T000011-02; sand 90%, silt 5%, clay 5%; pH 5.3-5.8; 1.0-1.5% organic matter) was provided by the Sponsor and characterized by Agvise laboratories, Northwood, North Dakota (USDA soil texture classification; p. 11). The soil characterization data was taken from Syngenta Study No. T000011-02.
- 2 One ion transition was monitored for each analyte as follows:  $m/z$  338.2→291.00 for mesotrione,  $m/z$  213.80→155.10 for AMBA, and  $m/z$  243.70→199.80 for MNBA. These were similar to those of the ECM, except for the use of an alternative ion transition for the quantitation transition of AMBA (from  $m/z$  213→171 to  $m/z$  213→155).
- 3 Only the quantitation ion transition was monitored for the analytes.

### III. Method Characteristics

In the ECM and ILV, LOQ in soil was 0.002 mg/kg for mesotrione, AMBA, and MNBA (pp. 11, 22-23 of MRID 50922301; p. 9 of MRID 49458107). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, i.e. which yielded a mean recovery of 70-110% and relative standard deviation of  $\leq 20\%$ . The LOQ for accurate quantitation should yield a response which is no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No justifications were reported in the ILV. In the ECM, the Limits of Detection (LODs) in soil were 0.2-0.3 pg, 1-3 pg, and 1-5 pg for mesotrione, AMBA, and MNBA, respectively, which was equivalent to 0.002-0.003 ng/mL, 0.01-0.03 ng/mL, and 0.01-0.05 ng/mL for mesotrione, AMBA, and MNBA, respectively, when using a 100  $\mu$ L injection volume. The LOD 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. An estimate of the LOD can be taken as three times the mean amplitude of the background noise. The ECM study author noted that the LOD may vary between runs and from instrument to instrument. The LOD was not reported in the ILV. No LOQ calculations were reported in ECM or ILV; no LOD calculations were reported in ECM.



**Table 4. Method Characteristics for Mesotrione and its Metabolites AMBA and MNBA in Soil**

Analyte		Mesotrione	AMBA	MNBA	
Limit of Quantitation (LOQ)		0.002 mg/kg			
Limit of Detection (LOD)	ECM	0.2-0.3 pg (0.002-0.003 ng/mL when using a 100 µL injection volume)	1-3 pg (0.01-0.03 ng/mL when using a 100 µL injection volume)	1-5 pg (0.01-0.05 ng/mL when using a 100 µL injection volume)	
	ILV	Not reported			
Linearity (calibration curve r and concentration range)	ECM <sup>1,2</sup>	Loam	r = 0.9996 (Q & C)	r = 0.9994 (Q) r = 0.9999 (C)	r = 0.9993 (Q) r = 0.9995 (C)
		Sandy Clay Loam	r = 0.9996 (Q) r = 0.9999 (C)	r = 0.9995 (Q) r = 0.9996 (C)	r = 0.9995 (Q) r = 0.9999 (C)
		Range			
	ILV <sup>2</sup>	r = 0.9988	r = 0.9957	r = 0.9946	
		0.100-5.00 ng/mL			
Repeatable	ECM (Syngenta Residue Method GRM007.10A) <sup>3</sup>	Yes at LOQ and 10×LOQ (two uncharacterized soil matrices) <sup>4</sup>			
	ILV (Syngenta Analytical Method No. 1200-03) <sup>5,6</sup>	Yes at LOQ and 10×LOQ (one characterized soil matrix) <sup>7</sup>			
Reproducible		Yes at LOQ and 10×LOQ (based on sample processing procedure only) <sup>8</sup>			
Specific	ECM	Yes, no matrix interferences were observed. Minor baseline noise interfered with LOQ analyte peak integration and attenuation.	Yes, no matrix interferences were observed. Multiple minor contaminants surrounded the LOQ analyte peak.	No, significant baseline noise was noted at the analyte RT preventing accurate integration. <sup>9</sup>	
	ILV	Yes, no matrix interferences were observed. Minor baseline noise interfered with LOQ analyte peak integration and attenuation.		Yes, no matrix interferences were observed.	

Data were obtained from pp. 12, 25 (LOQ/LOD); Tables 2-7, pp. 28-30 (recovery results); Figures 4-33, pp. 40-70 (chromatograms); Figures 34-45, pp. 71-82 (calibration coefficients) of MRID 50922301; p. 8; pp. 8-9, 22-27 (recovery results); Appendix 1, pp. 25-27 (calibration coefficients); Appendix 3, pp. 37-41 (chromatograms) of MRID 49458107; DER Attachment 2.

- 1 ECM correlation coefficient (r) values are reviewer-generated from reported coefficient of determination (r<sup>2</sup>) values (Figures 34-45, pp. 71-82 of MRID 50922301; DER Attachment 2). Matrix-matched calibration standards were used in the ECM (p. 23 of MRID 50922301).
- 2 A confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.
- 3 The submitted ECM Syngenta Residue Method GRM007.10A (dated 03/25/2013) was a method which superseded Syngenta Analytical Method No. 1200-03 and updated Syngenta Analytical Method No. 1200-03 to include confirmatory LC/MS/MS transitions. Syngenta Analytical Method No. 1200-03 was not provided.
- 4 In the ECM, the Gartenacker loam soil [Sample Reference CCON/033/002; pH: 7.7 (water), 7.2 (in 0.01M CaCl<sub>2</sub>); 3.5% organic matter, 2.0% organic carbon] and 18 Acres sandy clay loam soil [Sample Reference CCON/034/002; pH: 6.2 (water), 5.6 (in 0.01M CaCl<sub>2</sub>); 4.5% organic matter, 2.6% organic carbon] were obtained

- from Syngenta (USDA soil texture classification was not specified; Table 1, p. 27 of MRID 50922301). The soil characterization laboratory was not reported.
- 5 The ILV (dated 02/02/2005) validated Syngenta Analytical Method No. 1200-03 in the first trial as written, except for the use of an alternative LC/MS/MS instrument, the use of an alternative ion transition for the quantitation transition of AMBA (from  $m/z$  213→171 to  $m/z$  213→155) and increase of injection volume from 10  $\mu$ L to 50  $\mu$ L for AMBA and MNBA (pp. 11-15; Tables 1-3, pp. 16-18 of MRID 50922301). The ILV did not identify any critical steps.
  - 6 The sampling processing procedure of Syngenta Residue Method GRM007.10A matched the sampling procedure performed in the ILV (Syngenta Analytical Method No. 1200-03); however, significant differences were noted in the LC/MS/MS analytical portion of the ILV, including mobile phase gradient changes, the addition of a guard column, injection volume changes, monitored ion transition change for AMBA, and a different set of LC/MS/MS parameters for AMBA and MNBA.
  - 7 In the ILV, the sand soil (Boring ID: GP-1, GP-2, and GP-3; 0-6; Syngenta Study No. T000011-02; sand 90%, silt 5%, clay 5%; pH 5.3-5.8; 1.0-1.5% organic matter) was provided by the Sponsor and characterized by Agvise laboratories, Northwood, North Dakota (USDA soil texture classification; p. 11 of MRID 49458107). The soil characterization data was taken from Syngenta Study No. T000011-02.
  - 8 Since the ILV was not based specifically on the submitted ECM Syngenta Residue Method GRM007.10A, no information was available to assess the repeatability of Syngenta Residue Method GRM007.10A with regard to the LC/MS/MS analytical portion. An updated ECM should be submitted with details to equate the LC/MS/MS analytical methods of Syngenta Residue Method GRM007.10A and Syngenta Analytical Method No. 1200-03, as well as to discuss the preference for the simplified LC/MS/MS analytical method presented in Syngenta Residue Method GRM007.10A.
  - 9 Based on Figure 16, p. 53 and Figure 31, p. 68 of MRID 50922301.

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

1. This Method Validation submission was accompanied by the following note from the registrant: Please note, that MRID 49458107 says it is the ILV for Analytical Method 1200-03 but it is also the appropriate ILV for Analytical Method GRM007.10A (MRID 50922301). The two analytical methods only differ slightly. GRM007.10A has additional ion monitoring conditions that 1200-03 does not have, but the analytical procedures on which the ILV is based are not different. We believe MRID 49458107 also satisfies the requirement as the ILV for GRM007.10A.
2. The ILV (dated 02/02/2005) validated Syngenta Analytical Method No. 1200-03; however, the submitted ECM was Syngenta Residue Method GRM007.10A (dated 03/25/2013; p. 1 of MRID 50922301; pp. 1, 15 of MRID 49458107). Syngenta Residue Method GRM007.10A was a method which superseded Syngenta Analytical Method No. 1200-03 and updated Syngenta Analytical Method No. 1200-03 to include confirmatory LC/MS/MS transitions. Syngenta Analytical Method No. 1200-03 was not provided; therefore, the reviewer could not compare Syngenta Residue Method GRM007.10A to Syngenta Analytical Method No. 1200-03. The sampling processing procedure of Syngenta Residue Method GRM007.10A matched the sampling procedure performed in the ILV; however, significant differences were noted in the LC/MS/MS analytical portion of the ILV, including mobile phase gradient changes, the addition of a guard column, injection volume changes, monitored ion transition change for AMBA, and a different set of LC/MS/MS parameters for AMBA and MNBA (pp. 11-15; Tables 1-3, pp. 16-18 of MRID 49458107). Since the ILV was not based specifically on the submitted ECM Syngenta Residue Method GRM007.10A, no information was available to assess the repeatability of Syngenta Residue Method GRM007.10A with regard to the LC/MS/MS analytical portion. An updated ECM should be submitted with details to equate the LC/MS/MS analytical methods of Syngenta Residue Method GRM007.10A and Syngenta Analytical Method No. 1200-03, as well as to discuss the preference for the simplified LC/MS/MS analytical method presented in Syngenta Residue Method GRM007.10A.
3. The specificity of Syngenta Residue Method GRM007.10A was not supported for MNBA based on ECM representative chromatograms. Significant baseline noise was noted at the analyte RT preventing accurate integration (Figure 16, p. 53 and Figure 31, p. 68 of MRID 50922301). The ECM method needed another procedure to enhance specificity during LC/MS/MS analysis.
4. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method since only one characterized soil matrix was tested. OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. Even though a certain number of soil matrices is not specified in the OCSPP guidelines, more than one soil/soil matrix would need to be included in an ILV in order to cover the range of soils used in the terrestrial field dissipation studies.

The ILV soil matrix was sand soil (Boring ID: GP-1, GP-2, and GP-3; 0-6; Syngenta Study No. T000011-02; sand 90%, silt 5%, clay 5%; pH 5.3-5.8; 1.0-1.5% organic matter) which was provided by the Sponsor and characterized by Agvise laboratories, Northwood, North Dakota (USDA soil texture classification; p. 11 of MRID 49458107). The soil characterization data was taken from Syngenta Study No. T000011-02 which is a small-scale prospective ground-water monitoring study for mesotrione in NC (MRID 47418901). It could not be determined if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies.

5. The ECM soils were inadequately characterized. ECM soil matrix texture classification was not specified as USDA soil texture classification, and soil partition percentages were not reported.
6. Although there were no communication details for the ILV to the submitted ECM, the reviewer assumed that no communications occurred since the ILV (dated 02/02/2005) was performed *ca.* 8 years prior to the submitted ECM (dated 03/25/2013; p. 1 of MRID 50922301; p.1 of MRID 49458107).

It could not be determined if the ILV was performed independently of Syngenta Analytical Method T001200-03 due to lack of communication details. The ILV communications with the Sponsor (Syngenta) regarding the independent laboratory validation of Syngenta Analytical Method T001200-03 included the discussion of the ILV modifications and Sponsor approval of the ILV modifications; however, only a summary of the communications was provided (p. 15 of MRID 49458107). OCSPP 850.6100 guidelines state that the analysts, study director, equipment, instruments, and supplies of the ECM and ILV laboratories must be distinct and operated separately and without collusion. Also, the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies.

7. Matrix effects were studied in the ECM and determined to be significant (>20%) for AMBA; matrix-matched standards were recommended (p. 23; Table 8, p. 31 of MRID 50922301).
8. Storage stability of the final soil extracts and working solutions were determined to be up to 7 days and up to 87 days, respectively, when stored at 2-8°C (pp. 23-24; Table 9-14, pp. 32-34 of MRID 50922301).
9. 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, 22-23 of MRID 50922301; p. 9 of MRID 49458107). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated, i.e. which yielded a mean recovery of 70-110% and relative standard deviation of  $\leq 20\%$ . The LOQ for accurate quantitation should yield a response which is no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No justifications were reported in the ILV. In the ECM, the LOD 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. An estimate of the LOD can be taken as three times the mean amplitude of the background noise. The ECM study author noted that the LOD may vary between runs and from instrument to instrument. The LOD was not reported in the ILV. No LOQ calculations were reported in ECM or ILV; no LOD calculations were reported in ECM. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

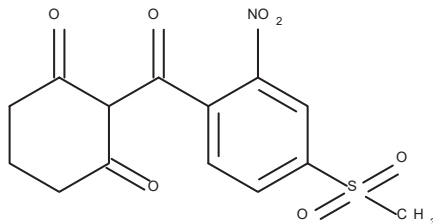
10. It was reported for the ILV that each set of 12 samples required one working day (8-person hours), with HPLC/MS/MS analysis (p. 14 of MRID 49458107).
11. The reviewer noted the following significant typographical error: the limit of quantification of the method is 0.002 mg/kg (**0.002 ppb**) in the Method Summary section, which should have been written as the limit of quantification of the method is 0.002 mg/kg (**2 ppb**; p. 11 of MRID 50922301).
12. It was determined that MRID 50922301 is a later version of MRID 50612105 dated March 14, 2013.

## V. References

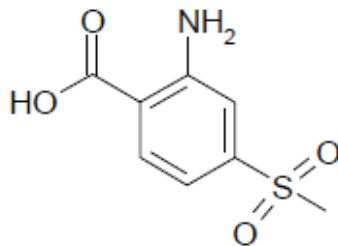
- Jutsum, Lisa (2013). Mesotrione - Validation of Draft Residue Method GRM007.10A For the Determination of Mesotrione and its Metabolites AMBA And MNBA in Soil. Method Validation Amendment 1. Performing Laboratory: CEM Analytical Services Ltd (CEMAS), Berkshire, UK. Report Number: CEMR-5657-REG Amendment 1, Sponsor(S): Syngenta Ltd, Bracknell, UK. Volume 1, pp 95. DP 448821: MRID 50612105.
- 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****Mesotrione (ZA1296; CSAA587961)**

**IUPAC Name:** 2-(4-Mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione  
**CAS Name:** 2-[4-(Methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione  
**CAS Number:** 104206-82-8  
**SMILES String:** O=C1C(C(C2=CC=C(S(=O)(C)=O)C=C2[N+](O-)=O)=O)C(=O)C1=O

**AMBA (NOA422848; CSCA041838)**

**IUPAC Name:** 2-Amino-4-methylsulfonyl benzoic acid  
**CAS Name:** Not reported  
**CAS Number:** 393085-45-5  
**SMILES String:** Not found



**MNBA (R169649; CSAA720221)****IUPAC Name:** 2-Nitro-4-methylsulfonyl benzoic acid**CAS Name:** Not reported**CAS Number:** 110964-79-9**SMILES String:** Not found