Test Material: Mesotrione

MRID: 49458108

Title: 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)

MRID: 49458107

Title: 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)"

EPA PC Code: 122990

OCSPP Guideline: 850.6100

For CDM Smith

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

Date: 3/25/15

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

Date: 3/25/15

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

Date: 3/25/15

Analytical method for mesotrione and its transformation products AMBA and MNBA in soil

Reports: ECM: EPA MRID No.: 49458108. Williams, R. 2004. 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). Syngenta Study No.: 1200-03 (p. 5). Syngenta Protocol No.: T001200-03 (p. 22). Report prepared, sponsored and submitted by Syngenta Crop Protection, Inc., Greensboro, North Carolina; 40 pages. Final report issued September 27, 2004.

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 No.: T002655-03. Report prepared by Enviro-Test Laboratories (ETL), Edmonton, Alberta, Canada, sponsored and submitted by Syngenta Crop Protection, Inc., Greensboro, North Carolina; 41 pages. Final report issued February 2, 2005.

Document No.: MRIDs 49458108 & 49458107

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49458108). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5). A signature page was provided, but an Authenticity Certification statement was not provided (p. 4).

ILV: The study was conducted in compliance with USEPA GLP standards, with the exception that the stock solutions were prepared prior to study initiation (p. 3 of MRID 49458107). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5). A signature page was provided, but an Authenticity Certification statement was not provided (p. 4).

Classification: This analytical method is classified as supplemental for analysis of mesotrione residues in sand soil. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. The ILV did not report LODs. The registrant failed to select the most difficult analytical sample condition to analyze to demonstrate how well the method performs; only sand soil (1.0-1.5% organic matter, 4-6% clay) was used in the ECM and ILV. For the ECM, an insufficient number of samples were fortified at the LOQ and no fortifications were performed at 10x LOQ.

PC Code: 122990

Reviewer: Iwona L. Maher **Signature:** IWONA MAHER Digitally signed by IWONA MAHER Date: 2019.05.21 15:00:20 -04'00'

Chemist **Date:** 05/21/2019

Executive Summary

This analytical method, Syngenta Analytical Method 1200-03, is designed for the quantitative determination of mesotrione and its transformation products AMBA and MNBA in soil using LC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 2.0 µg/kg (ppb).The independent laboratory validated the method for analysis of mesotrione, AMBA, and MNBA in sand soil after one trial. No major modifications were made by the independent laboratory. A sand soil (1.0-1.5% organic matter, 4-6% clay) was used in the ECM and ILV; therefore, the registrant failed to select the most difficult analytical sample condition to analyze to demonstrate how well the method performs.

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	49458108	49458107		Soil[1]	27/09/2004	Syngenta Crop Protection, Inc.	LC/MS/MS	2.0 µg/kg (ppb)
AMBA								
MNBA								

1 A sand soil (1.0-1.5% organic matter, 4-6% clay) was used in the ECM and ILV (p. 22; Table 5, p. 30 of MRID 49458108; p. 11 of MRID 49458107).

I. Principle of the Method

Soil (10 ± 0.1 g) was fortified with separate solutions of mesotrione, AMBA, and MNBA in methanol for procedural recoveries, and the fortified sample allowed to equilibrate for 5 minutes (pp. 13-14 of MRID 49458108). Soil samples (10 g) are sequentially extracted with 20 mL of 0.05M ammonium hydroxide (NH_4OH), followed by 20 mL of 0.05M NH_4OH:acetone (50:50, v:v), and finally 20 mL of acetone (pp. 14-15; Figure 1, p. 31). For each extraction, soil is shaken on a flat/horizontal mechanical shaker at a speed "that visibly agitates the samples" for at least 30 minutes. Soil and extract are separated by centrifugation (6,000 rpm or speed "that visibly separates the solid sample from the supernatant", *ca*. 5 minutes). Extracts are combined and centrifuged (*ca*. 6,000 rpm or speed that separates solid from supernatant, *ca*. 5 minutes). Solvent is removed from an aliquot (6-mL) of the extract under nitrogen (N-Evap, *ca*. 40°C); final volume *ca*. 2.5 mL. The remaining aqueous sample is brought to volume (10 mL) with 2% formic acid to precipitate soil acids, hand-shaken, sonicated (3-5 minutes), and centrifuged (4,500 rpm or speed that separates solid and supernatant, 5 minutes; pp. 12, 15). An aliquot of the supernatant is taken for LC/MS/MS analysis.

Samples are analyzed for mesotrione using a Waters Alliance Model 2695 LC System coupled with a Micromass Quattro Ultima MS/MS employing electrospray ionization (ESI, pp. 16-19 of MRID 49458108). Mesotrione exhibits binding and severe chromatographic peak tailing on most silica-based LC columns, therefore, polymeric columns are preferred for analysis. For AMBA and MNBA, better retention and peak shape are obtained using a Synergi^{TM} reverse-phase column, as compared to a polymeric column.

For mesotrione the following LC conditions were used: Polymer Laboratories PLRP-S column (4.6 mm x 50 mm, 100 Å, column temperature 30-35°C), with a Polymer Laboratories PLRP-S guard column (5 x 3 mm), using a mobile phase of (A) 0.1% glacial acetic acid in HPLC grade water and

(B) 0.1% glacial 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]. Injection volume was 20 µL. Expected retention time was *ca*. 4.2 minutes.

For AMBA and MNBA the following LC conditions were used: Phenomenex Synergi^{TM} 4µ Fusion-RP column (4.6 mm x 75 mm, 80 Å, column temperature 30-35°C), with a Phenomenex Fusion-RP guard column (4 x 3 mm), using a mobile phase of (A) 0.1% glacial acetic acid in HPLC grade water and (B) 0.1% glacial 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 min 95:5]. Injection volume was 10 µL, to minimize matrix effects. Expected retention times were *ca*. 4.8 and 7.6 minutes for AMBA and MNBA, respectively.

For all three analytes the following MS/MS-ESI conditions were used: negative ion mode detection and multiple reaction monitoring (MRM). Analytes are identified using one ion transition. Ion transitions monitored were as follows: *m/z* 338.2→291.0 for mesotrione, *m/z* 214.0→169.9 for AMBA, and *m/z* 244.0→199.8 for MNBA. A confirmatory method was not used.

ILV: Reference substances and soil matrix were supplied by Syngenta (pp. 10-11 of MRID 49458107). The soil matrix used in the ILV was from the same Syngenta field study (prospective groundwater monitoring test site, Study No.: T000011-02, Moore County, North Carolina, 0-6" depth) as that used in the ECM (p. 22; Table 5, p. 30 of MRID 49458108). The independent laboratory performed the extraction method as written (p. 11 of MRID 49458107). The following modifications to the LC/MS/MS conditions were made: a PE Sciex API 4000 MS/MS system was used in substitution of the Waters Alliance Model 2695 LC/Micromass Ultima MS/MS system (p. 12). Serial dilutions of 10.0, 1.00 and 0.100 ppm were performed instead of 5, 0.5 and 0.02 ppm. The AMBA ion transition monitored was adjusted to *m/z* 213.8→155.1 (Table 3, p. 18). Injection volume for AMBA and MNBA was 50 µL. Approximate retention times were 3.61, 4.20, and 6.93 minutes for mesotrione, AMBA, and MNBA, respectively (Tables 1-2, pp. 16-17).

LOQ and LOD: In the ECM and ILV, the LOQ for all analytes was 2.0 µg/kg (ng/g, ppb; p. 23 of MRID 49458108; pp. 9, 12 of MRID 49458107). In the ECM, LODs were set at 2.0 pg for mesotrione and at 1.0 pg for AMBA and MNBA, equivalent to 0.1 µg/L for all analytes (pp. 23-24 of MRID 49458108). In the ILV, LODs were not reported.

II. Recovery Findings

ECM (MRID 49458108): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of mesotrione and its transformation products AMBA and MNBA in a sand soil at fortification levels of 2.0 µg/kg (LOQ) and 50 µg/kg (25x LOQ; Tables 1-3, pp. 26-28). Fortifications at 10x LOQ were not performed. Analytes were identified and quantified using one ion transition; a confirmatory method was not used. The soil matrix was characterized (Table 5, p. 30).

ILV (MRID 49458107): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of mesotrione and its products AMBA and MNBA in a sand soil at fortification levels of 2.0 µg/kg (LOQ) and 20.0 µg/kg (10x LOQ; Tables 4-6, pp. 19-21; DER Attachment 2). The method was validated for all three analytes at both fortification levels after one trial (p. 14). The soil matrix used in the ILV was supplied by Syngenta and was from the same Syngenta field study (Study No.: T000011-02, North Carolina, 0-6" depth) as that used in the ECM (p. 11).

Table 2. Initial Validation Method Recoveries for Mesotrione and Its Transformation Products AMBA and MNBA in Sand Soil[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Mesotrione	2.0 (LOQ)	4[2]	97-100	99	1.3	1.3
	50	5	92-98	96	3.0	3.2
AMBA	2.0 (LOQ)	4[2]	88-97	93	3.9	4.2
	50	5	91-94	93	1.6	1.8
MNBA	2.0 (LOQ)	4[2]	104-108	106	1.7	1.6
	50	5	107-112	110	2.0	1.8

Data (uncorrected recovery results) were obtained from Tables 1-3, pp. 26-28 of MRID 49458108.

[1] The soil matrix was obtained from a Syngenta field study (prospective groundwater monitoring test site, Study No.: T000011-02, Moore County, North Carolina, 0-6" depth; p. 22; Table 5, p. 30). Soil from three areas of the field site was characterized (% sand, % silt and % clay, pH, % organic matter) by Agvise Laboratories, Northwood, Dakota.

[2] One sample was "unintentionally" not fortified (Table 1, p. 26).

Table 3. Independent Validation Method Recoveries for Mesotrione and Its Transformation Products AMBA and MNBA in Sand Soil[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Mesotrione	2.0 (LOQ)	5	84-106	95	7.9	8.3
	20.0	5	87-96	93	3.6	3.9
AMBA	2.0 (LOQ)	5[2]	71-85	77	5.4	7.0
	20.0	5	79-83	82	1.7	2.1
MNBA	2.0 (LOQ)	5[2]	79-96	89	7.5	8.4
	20.0	5	94-106	100	4.4	4.4

Data (uncorrected recovery results) were obtained from Tables 4-6, pp. 19-21 of MRID 49458107.

[1] The soil matrix used in the ILV was supplied by Syngenta and was from the same Syngenta field study (prospective groundwater monitoring site, Study No.: T000011-02, Moore County, North Carolina, 0-6" depth) as that used in the ECM (p. 11 of MRID 49458107; p. 22; Table 5, p. 30 of MRID 49458108).

[2] Mean, standard deviation, and relative standard deviation calculated by reviewer (DER Attachment 2). One sample was injected twice, and the reviewer used the average recovery of the two injections in statistic determinations. The study authors determined statistics as if the two injections were two separate sample recoveries (Tables 5-6, pp. 20-21 of MRID 49458107).

III. Method Characteristics

In the ECM and ILV, the LOQ for mesotrione, AMBA, and MNBA in soil was 2.0 µg/kg (ng/g, ppb; p. 23 of MRID 49458108; pp. 9, 12 of MRID 49458107). The ECM defined the LOQ as the lowest analyte concentration at which the methodology has been validated. The ECM estimated the LOD based on the smallest calibration standard used during analysis, equivalent to 0.1 pg/µL for this validation (pp. 23-24 of MRID 49458108). Therefore, LODs were estimated at 2.0 pg for mesotrione based on the 20-µL injection volume used for analysis and 1.0 pg for AMBA and MNBA based on the 10-µL injection volume (pp. 16-17, 23-24). In the ILV, LODs were not reported.

Table 4. Method Characteristics for Mesotrione and Its Transformation Products AMBA and MNBA in Soil

		Mesotrione	AMBA	MNBA
Limit of Quantitation (LOQ)		2.0 µg/kg (ng/g, ppb)		
Limit of Detection (LOD)		2.0 pg (0.1 µg/L)	1.0 pg (0.1 µg/L)	
Linearity (calibration curve r^2 and concentration range)[1]	ECM:	$r^2 = 0.9982$	$r^2 = 0.9992$	$r^2 = 0.9988$
		Range: 2-100 pg	Range: 1-50 pg	
	ILV:	$r^2 = 0.9976$	$r^2 =$ **0.9914**	$r^2 =$ **0.9892**
		Range: 0.1-5.0 ng/mL		
Repeatable	ECM:	Yes at LOQ and 25x LOQ; however, n = 4 at LOQ for all analytes. Fortifications at 10x LOQ were not performed.		
	ILV:	Yes at LOQ and 10x LOQ.		
Reproducible		Yes.		
Specific[2]	ECM:	Yes; interferences (analyte carryover) at the analyte retention times were <10% of the LOD (lowest calibration standard).		
	ILV:	Yes; no interferences detected at analyte retention times.		

Data were obtained from pp. 23-24; Tables 1-4, pp. 26-29; Figures 3-8, pp. 33-38 of MRID 49458108; Tables 4-6, pp. 19-21; Appendix 1, pp. 25-27; Appendix 3, p. 38 of MRID 49458107; DER Attachment 2.

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

1 ECM and ILV r^2 values are reviewer-generated from reported r values (1/x weighted) of 0.9991-0.9996 for the ECM and 0.9946-0.9988 for the ILV (Table 4, p. 29 of MRID 49458108; Appendix 1, pp. 25-27; Appendix 3, pp. 34-36 of MRID 49458107; DER Attachment 2).

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

IV. Method Deficiencies and Reviewer's Comments

1. The determination of the LOQ and LOD were not based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B. The ECM defined the LOQ as the lowest analyte concentration at which the methodology has been validated. The ECM estimated the LOD based on the smallest calibration standard used during analysis, equivalent to 0.1 pg/µL for this validation (pp. 23-24 of MRID 49458108). Therefore, LODs were estimated at 2.0 pg for mesotrione based on the 20-µL injection volume used for analysis and 1.0 pg for AMBA and MNBA based on the 10-µL injection volume, or 0.1 µg/L (pg/µL) for all three analytes (pp. 23-24; Figure 3, p. 33; Figure 5, p. 35; Figure 7, p. 37). In the ILV, LODs were not reported.

   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.

2. A sand soil (1.0-1.5% organic matter, 4-6% clay) was used in the ECM and ILV; therefore, the registrant failed to select the most difficult analytical sample condition to analyze to demonstrate how well the method performs (p. 22; Table 5, p. 30 of MRID 49458108; p. 11 of MRID 49458107).

3. For the ECM, an insufficient number of samples were fortified (n = 4) at the LOQ and no fortifications were performed at 10x LOQ (Tables 1-3, pp. 26-28 of MRID 49458108).

4. For the ILV, chromatograms were not provided for reagent blanks. For the calibration standards, only chromatograms of the 0.100 and 5.00 ppb standards were provided (calibration standard range 0.100-5.00 ppb), and the 0.100 ppb standard did not chromatograph as an attenuated peak (Appendix 3, pp. 37-41 of MRID 49458107). Linearity (r^2) of the AMBA and MNBA calibration curves was not ≥ 0.995.

5. For AMBA and MNBA data at the LOQ in the ILV, the reviewer recalculated mean, standard deviation, and relative standard deviation values (DER Attachment 2). One sample was injected twice, and the reviewer used the average recovery of the two injections in statistic determinations. The study authors determined statistics as if the two injections were two separate sample recoveries (Tables 5-6, pp. 20-21 of MRID 49458107).

6. A confirmatory method was not employed; however, typically, a confirmatory method is not required where GC/MS and LC/MS methods are used as the primary method(s) to generate study data.

7. The independent laboratory made the following modifications to the LC/MS/MS conditions of the ECM: a PE Sciex API 4000 MS/MS system was used in substitution of the Waters Alliance Model 2695 LC/Micromass Ultima MS/MS system (pp. 11-12 of MRID 49458107). Serial dilutions of 10.0, 1.00 and 0.100 ppm were performed instead of 5, 0.5 and 0.02 ppm. The AMBA ion transition monitored was adjusted to *m/z* 213.8→155.1 (Table 3, p. 18). Injection volume for AMBA and MNBA was increased to 50 µL. None of the modifications are considered a substantial change to the ECM.

8. It was reported for the ILV that 8 person-hours are required to extract and complete one set of twelve samples, including instrument data analysis (p. 14 of MRID 49458107).

V. References

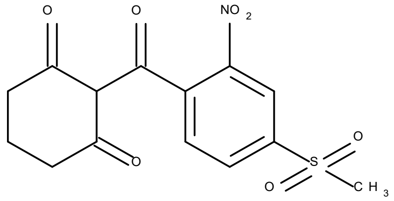
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

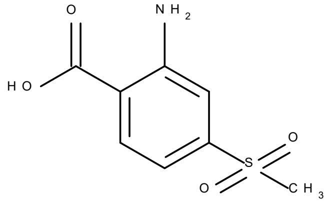
Mesotrione (ZA1296)

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: CS(=O)(=O)c1ccc(c(c1)[N+](=O)[O-])C(=O)C2C(=O)CCCC2=O



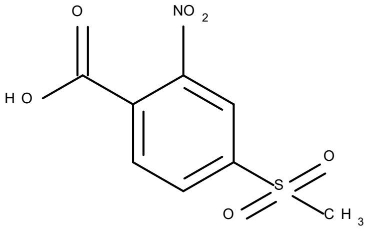
AMBA (NOA-422848; CSCA041838)

IUPAC Name: 2-Amino-4-methylsulfonyl-benzoic acid
CAS Name: Benzoic acid, 2-amino-4-(methylsulfonyl)-
CAS Number: 393085-45-5
SMILES String: CS(=O)(=O)c1ccc(c(c1)N)C(=O)



MNBA (NOA-437130; CSAA720221; R169649; NMSBA)

IUPAC Name: 4-Methylsulfonyl-2-nitro-benzoic acid
CAS Name: Benzoic acid, 4-(methylsulfonyl)-2-nitro-
CAS Number: 110964-79-9
SMILES String: CS(=O)(=O)c1ccc(c(c1)[N+](=O)[O-])C(=O)O



Section III. Environmental Chemistry Method Report Review Considerations

This list of considerations is provided to help reviewers assess the acceptability of environmental chemistry method reports and their associated independent laboratory validation reports. This list may be used as a screen or a checklist but is not meant to be attached to the method report reviews. Listed considerations carry unequal weight. Evaluate them using best professional judgment. Consider all information from the method reports and from reports for similar methods to determine whether any deficiencies affect the method report classification.

ECM Report (MRID 49458108)

» The required instrumentation, glassware, and chemicals were identified in the report and are commercially available.

Appendices 1-2 (pp. 39-40) list recommended equipment, labware, reagents and analytical standards along with recommended suppliers. Section **4.0 Final Determination by LC/MS/MS** (pp. 16-19) describes recommended instrumentation.

» The matrix/matrices was/were well characterized. (For example, for soil, pH and percentages of organic carbon, moisture, sand, silt, and clay, *etc.* were reported.)

Yes; pH and percentages of organic matter, sand, silt, and clay were reported (Table 5, p. 30). The soil matrix was obtained from a Syngenta field study (prospective groundwater monitoring test site, Study No.: T000011-02, Moore County, North Carolina, 0-6" depth; p. 22; Table 5, p. 30). A sand soil (1.0-1.5% organic matter, 4-6% clay) was used in the ECM and ILV; therefore, the registrant failed to select the most difficult analytical sample condition to analyze to demonstrate how well the method performs.

» All steps in the ECM are scientifically sound. Mass spectrometry or another technique was used to confirm the identity of the analyte(s).

The steps are scientifically sound. HPLC/MS/MS was utilized (pp. 16-19). Analytes were identified using one ion transition; a confirmatory method was not used. Typically, a confirmatory method is not required where GC/MS and LC/MS methods are used as the primary method.

An insufficient number of samples were fortified (n = 4) at the LOQ and no fortifications were performed at 10x LOQ (Tables 1-3, pp. 26-28).

» Any encountered interferences, problem areas, or critical steps were described and/or explained.

Mesotrione exhibits binding and severe chromatographic peak tailing on most silica-based LC columns, therefore, polymeric columns are preferred for analysis (p. 16). For AMBA and MNBA, better retention and peak shape are obtained using a Synergi^{TM} reverse-phase column, as compared to a polymeric column.

To minimize ion source contamination, it is recommended that a timed event controlled switching valve be used to divert the LC stream to waste during periods of no data collection (*e.g.*, from injection to 3.2 minutes and from 5.1 minutes to completion; p. 17).

» The matrix blank was free of interference(s).

Yes; interferences (analyte carryover) at the analyte retention times were <10% of the LOD (lowest calibration standard; pp. 21, 23; Figures 3-8, pp. 33-38).

» Representative chromatograms were provided for reagent blanks, matrix blanks, standard curves, and spiked samples at the LOQ and 10× LOQ for all analytes in each matrix.

ECM (Figures 3-8, pp. 33-38 of MRID 49458108): Yes; except no samples were fortified at 10x LOQ. Standard curves for all analytes were provided, with the individual calibration standard data (Table 4, p. 29). MS/MS product ion spectra were also provided (Figure 2, p. 32).

ILV (Appendix 3, pp. 34-41 of MRID 49458107): No. Chromatograms were not provided for reagent blanks. For the calibration standards, only chromatograms of the 0.100 and 5.00 ppb standards were provided (calibration standard range 0.100-5.00 ppb), and the 0.100 ppb mesotrione standard did not chromatograph as an attenuated peak (Appendix 3, pp. 37, 41). Standard curves for all analytes were provided, with the individual calibration standard data (Appendix 1, pp. 25-27; Appendix 3, pp. 34-36). Linearity (r^2) of the AMBA and MNBA calibration curves was not ≥ 0.995.

» The chromatograms of the lowest spiking level are attenuated to where one can measure the peak accurately (accounting for the noise on the baseline).

ECM (MRID 49458108): Yes (Figure 4, p. 34; Figure 6, p. 36; Figure 8, p. 38).

ILV (MRID 49458107): Yes (Appendix 3, p. 39).

» There are explanations of how the LOD and LOQ were calculated. The procedures are scientifically acceptable. A best effort was demonstrated to achieve a low LOQ. (LOD and LOQ are often calculated as the mean matrix blank value plus 3 times the standard deviation and 10 times the standard deviation, respectively. 40 CFR Part 136, Appendix B lists some scientifically accepted procedures for estimating detection limits. Actual detection limits are not based on the arbitrarily selected lowest concentration in the spiked samples.)

The determination of the LOQ and LOD were not based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B. The ECM defined the LOQ as the lowest analyte concentration at which the methodology has been validated (pp. 23-24). The ECM estimated the LOD based on the smallest calibration standard used during analysis, equivalent to 0.1 pg/µL for this validation. Therefore, LODs were estimated at 2.0 pg for mesotrione based on the 20-µL injection volume used for analysis and 1.0 pg for AMBA and MNBA based on the 10-µL injection volume, or 0.1 µg/L (pg/µL) for all three analytes (pp. 23-24; Figure 3, p. 33; Figure 5, p. 35; Figure 7, p. 37). In the ILV, LODs were not reported.

» The LOQ(s) is/are less than toxicological levels of concern. (Concentrations in soil with units of mass/area (*e.g.*, lbs/acre) are converted to units of mass/mass (*e.g.*, mg/kg) using a soil depth of six inches and the soil density. The 6-inch soil depth is a default to use unless there is a reason to use an alternative depth.)

No toxicological levels of concern were reported for soil.

» For ECMs used in submitted field studies, the LOQ(s) is/are less than 10% of the expected or actual peak concentration of the test compound in the field.

Not applicable to this review.

ILV Report (MRID 49458107)

» An ILV was performed and documented in a report separate from the ECM report.

Yes.

» The ILV was independent. (If the laboratory that conducted the validation belonged to the same organization as the originating laboratory, the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion. 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.)

Yes. The laboratory that conducted the ILV, Enviro-Test Laboratories, was independent of the originating laboratory Syngenta Crop Protection, Inc.

» All communication prior to running the samples between the independent laboratory and the developers or previous users of the ECM was documented.

Communications between the independent laboratory and study sponsor were only briefly summarized (p. 15). The independent laboratory requested two changes with the ECM; increasing the injection volume for AMBA and MNBA to 50 µL to improve sensitivity and changing the Q1 to Q3 transition for AMBA to *m/z* 213.8→155.1 (p. 15; Table 3, p. 18). Both changes were approved by Syngenta.

» A maximum of three sample sets were used to validate the ECM (*i.e.*, produce recoveries with acceptable precision and accuracy). A minimally complete sample set includes a reagent blank, two matrix blanks, five samples spiked at the LOQ, and five samples spiked at 10× LOQ for each matrix.

Yes. The method was validated for mesotrione, AMBA, and MNBA at both fortification levels after one trial (p. 14).

A validation set consisted of one reagent blank, two matrix blank control samples, five samples spiked at the LOQ, and five samples spiked at 10x LOQ (p. 12). The soil matrix used in the ILV was from the same Syngenta field study (prospective groundwater monitoring test site, Study No.: T000011-02, Moore County, North Carolina, 0-6" depth) as that used in the ECM (p. 11 of MRID 49458107; p. 22; Table 5, p. 30 of MRID 49458108).

» Interferences with peak areas were less than 50% at the LOD.

Yes, based on 0.100 ppb calibration standard (Appendix 3, pp. 37-38).

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» Sample recoveries were not corrected for reagent blanks, matrix blanks, or other recoveries.

No, not corrected (pp. 19-20; Tables 1-3, pp. 26-28; Figure 4, p. 34; Figure 6, p. 36; Figure 8, p. 38 of MRID 49458108; Table 7, pp. 22-23; Appendix 3, pp. 31-33 of MRID 49458107).

» A minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte. (For the initial method validation, the number of spiked samples analyzed at each concentration is at least equal to that of the independent method validation and is preferably seven.)

ECM (MRID 49458108): No at the LOQ (n = 4), and no fortifications were performed at 10x LOQ (Tables 1-3, pp. 26-28).

ILV (MRID 49458107): Yes (Tables 4-6, pp. 19-21).

» The method recoveries met OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) ≤20%) at the stated LOQ and at higher concentrations.

ECM (MRID 49458108): Yes (Tables 1-3, pp. 26-28).

ILV (MRID 49458107): Yes (Tables 4-6, pp. 19-21). For AMBA and MNBA at the LOQ, the reviewer recalculated mean, standard deviation, and relative standard deviation values (DER Attachment 2). One sample was injected twice, and the reviewer used the average recovery of the two injections in statistic determinations. The study authors determined statistics as if the two injections were two separate sample recoveries (Tables 5-6, pp. 20-21).

» Two sets of performance data were submitted, one for the initial or other internal validation and one for the ILV, with the following exception. If the initial validation was performed by a governmental agency, a reference to the agency's documentation of the ECM will serve as the ECM report. In this case, the applicant submitted an ILV report and documentation of the agency's ECM if not the full initial validation report for the ECM.

Yes.

» Any modifications to the method recommended by the independent laboratory were implemented in the ECM report. If substantial changes to the ECM were recommended, an internal validation was conducted for the updated ECM report.

The independent laboratory performed the extraction method as written (p. 11 of MRID 49458107). The following modifications to the LC/MS/MS conditions were made: a PE Sciex API 4000 MS/MS system was used in substitution of the Waters Alliance Model 2695 LC/Micromass Ultima MS/MS system (p. 12). Serial dilutions of 10.0, 1.00 and 0.100 ppm were performed instead of 5, 0.5 and 0.02 ppm. The AMBA ion transition monitored was adjusted to *m/z* 213.8→155.1 (Table 3, p. 18). Injection volume for AMBA and MNBA was increased to 50 µL. None of the modifications are considered a substantial change to the ECM.