Test Material:	Mesotrione
MRID:	49458109
Title:	Analytical Method 6179-04 for the Determination of Mesotrione and its Degradates AMBA and MNBA in Water by Direct Injection High Performance Liquid Chromatography with Mass Spectrometric Detection
MRID:	49458106
Title:	Independent Laboratory Validation: Syngenta Method No. T006179-04 "Analytical Method 6179-04 for the Determination of Mesotrione and its Degradates AMBA and MNBA in Water by Direct Injection High Performance Liquid Chromatography with Mass Spectrometric Detection"
EPA PC Code:	122990
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** 

Date: 3/25/15

Secondary Reviewer: Lisa Muto **Signature:** 

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 water

Reports:	ECM: EPA MRID No.: 49458109. Chamkasem, N. 2004. Analytical Method 6179-04 for the Determination of Mesotrione and its Degradates AMBA and MNBA in Water by Direct Injection High Performance Liquid Chromatography with Mass Spectrometric Detection. Syngenta Protocol and Study No.: T006179-04 (pp. 5, 19). Report prepared, sponsored and submitted by Syngenta Crop Protection, Inc., Greensboro, North Carolina; 50 pages. Final report issued May 6, 2004. ILV: EPA MRID No. 49458106. McLean, N. 2005. Independent Laboratory Validation: Syngenta Method No. T006179-04 "Analytical Method 6179-04 for the Determination of Mesotrione and its Degradates AMBA and MNBA in Water by Direct Injection High Performance Liquid Chromatography with Mass Spectrometric Detection. ETL Study No.: 04ILV08SYN and Report No.: 04SYN145.REP. Syngenta No.: T006450-04. Report prepared by Enviro-Test Laboratories (ETL), Edmonton, Alberta, Canada, sponsored and submitted by Syngenta Crop Protection, Inc., Greensboro, North Carolina; 67 pages. Final report issued August 3, 2005.
Document No.:	MRIDs 49458109 & 49458106
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49458109). 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 (p. 3 of MRID 49458106). 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. An updated ECM report implementing a significant ILV modification to the method was not provided (modification to fortification solution preparation; Comment 1). The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. The ILV did not report LODs. In the ILV, interferences with AMBA peak areas were *ca*. 60% of the LOQ in the surface water matrix control. Characterizations for the surface and ground water matrices used in the ECM validation were not provided.
PC Code:	122990
Reviewer:	Iwona L. Maher Chemist
Signature:	IWONA MAHER Digitally signed by IWONA MAHER Date: 2019.05.21 14:31:59 -04'00'
Date:	05/21/2019

Executive Summary

This analytical method, Syngenta Analytical Method 6179-04, is designed for the quantitative determination of mesotrione and its transformation products AMBA and MNBA in water using LC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 0.05 µg/L (ppb). The independent laboratory validated the method for analysis of mesotrione, AMBA, and MNBA at both fortification levels in surface water after one trial and in ground water after two trials. In the ILV, interferences with AMBA peak areas were *ca*. 60% of the LOQ (based on peak height) in the surface water matrix control. An updated ECM report implementing a significant ILV modification to fortification solution preparation was not provided.

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	49458109	49458106		Surface and ground water	06/05/2004	Syngenta Crop Protection, Inc.	LC/MS/MS	0.05 µg/L (ppb)
AMBA								
MNBA								

I. Principle of the Method

Water samples are allowed to warm to ambient temperature prior to processing (p. 14 of MRID 49458109). Water (5 mL) is acidified with 1 drop of acetic acid and analyzed directly by LC/MS/MS. For procedural recoveries, fortifications are done following acidification of the water using a mixed standard of mesotrione, AMBA, and MNBA in 5% acetonitrile/water + 0.1% acetic acid (pp. 13-14, 17-18).

Samples are analyzed using a Waters Model 2690 LC System coupled with a Micromass Quattro Ultima MS/MS employing an Ion-Spray atmospheric pressure ionization (API) interface (pp. 12, 17; Tables 1-2, pp. 22-24 of MRID 49458109). Mesotrione may chelate with metal ion impurities in silica-based HPLC columns and cause chromatographic peak tailing, therefore, a polymer column is preferred for analysis. The following LC conditions were used: Polymer Laboratories PLRP-S column (4.6 mm x 50 mm, 5 µm, 100 Å, column temperature 35°C), with a Phenomenex Polymerx RP-1 guard column (dimensions not reported) plus an Upchurch (A-318) pre-column filter (0.5 µm), using a mobile phase of (A) 0.1% acetic acid in HPLC grade water and (B) acetonitrile [percent A:B (v:v) at 0.0-0.5 min. 98:2, 7.0-10 min. 5:95, 15 min 98:2]. Injection volume was 50 µL. The following MS/MS conditions were used: electrospray ionization in 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. Expected retention times were *ca*. 4.35, 5.48, and 7.24 minutes for MNBA, AMBA, and mesotrione, respectively. A confirmatory method was not used.

ILV: Reference substances were supplied by Syngenta (pp. 10-11 of MRID 49458106). Ground water was obtained from a well and surface water from Gull Lake, with both collection sites located in a rural area of Rimbey, Alberta, Canada (p. 11). The water matrices were characterized by the independent laboratory under GLP (pp. 11-12). The following modifications to the ECM were made: a PE Sciex API 4000 MS/MS system was used in substitution of the Waters Model 2690

LC/Micromass Ultima MS/MS system, mixed analyte fortification solutions were prepared in methanol instead of 5% acetonitrile/water + 0.1% acetic acid, and injection volume was increased to 100 µL (pp. 12-13). Approximate retention times were 3.38, 4.12, and 5.20 minutes for MNBA, AMBA, and mesotrione, respectively (Table 1, p. 21).

LOQ and LOD: In the ECM and ILV, the LOQ for mesotrione, AMBA, and MNBA was 0.05 µg/L (ppb; pp. 12, 19 of MRID 49458109; pp. 9, 16 of MRID 49458106). In the ECM, the LOD was set at 1.25 pg for all three analytes, equivalent to 0.025 µg/L based on an injection volume of 50 µL (pp. 12, 15 of MRID 49458109). In the ILV, LODs were not reported.

II. Recovery Findings

ECM (MRID 49458109): 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 surface water (irrigation pond, creek) and ground water (well) at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10x LOQ; DER Attachment 2). Analytes were identified and quantified using one ion transition; a confirmatory method was not used. The water matrices were not characterized.

ILV (MRID 49458106): 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 ground water (well) and surface water (lake) at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10x LOQ; Tables 6-11, pp. 26-31). The method was validated for all three analytes at both fortification levels in ground water after two trials and in surface water after one trial (pp. 18-19). Both water matrices were characterized (pp. 11-12; Appendix 1, pp. 42-44). During Trial 1/ground water, the independent laboratory believed poor recoveries (7.1-8.8%) of MNBA at the 10x LOQ fortification were probably due to poor solubility of MNBA in the 5% acetonitrile/water + 0.1% acetic acid solution used to prepare fortification solutions (p. 17; Table 4, p. 24). For Trial 2/ground water and Trial 1/surface water, mixed analyte fortification solutions were prepared using methanol (pp. 18-19).

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

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface (Irrigation Pond) Water					
Mesotrione	0.05 (LOQ)	5	108-114	112	2.68	2.39
	0.5	5	101-105	103	1.58	1.54
AMBA	0.05 (LOQ)	5	94-104	100	3.70	3.71
	0.5	5	93-96	95	1.14	1.21
MNBA	0.05 (LOQ)	5	93-106	99	4.76	4.82
	0.5	5	101-107	104	2.24	2.15
	Surface (Buffalo Creek) Water					
Mesotrione	0.05 (LOQ)	5	106-111	109	1.92	1.76
	0.5	5	101-105	103	1.52	1.48
AMBA	0.05 (LOQ)	5	92-102	96	3.77	3.93
	0.5	5	96-102	98	2.35	2.39
MNBA	0.05 (LOQ)	5	97-110	103	5.86	5.66
	0.5	5	104-107	105	1.14	1.08
	Ground (Well) Water					
Mesotrione	0.05 (LOQ)	5	100-107	105	2.95	2.80
	0.5	5	100-105	102	1.79	1.75
AMBA	0.05 (LOQ)	5	97-106	102	3.83	3.75
	0.5	5	98-101	100	1.52	1.52
MNBA	0.05 (LOQ)	5	98-106	102	3.27	3.20
	0.5	5	100-107	104	2.68	2.58

Means, standard deviations, and relative standard deviations for each fortification level and matrix were determined by the reviewer using uncorrected recovery results, because the study author only provided summary results (Tables 3-6, pp. 25-36 of MRID 49458109; DER Attachment 2).

1 Water matrices were not characterized. Surface and well water matrices were obtained from Irrigation Pond AW-3 IRG BRR-1 and Well AW-3 P4BRR-4, respectively, located in Eagle Springs, North Carolina (Syngenta Study No.: T000011-02), with a second surface water obtained from Buffalo Creek, Greensboro, North Carolina (pp. 8-9).

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

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Ground (Well) Water					
Mesotrione	0.05 (LOQ)	5	95.2-103	99	3.7	3.7
	0.5	5	96.8-104	100	2.8	2.8
AMBA	0.05 (LOQ)	5	106-117	110	4.3	3.9
	0.5	5	105-109	107	1.4	1.3
MNBA	0.05 (LOQ)	5	110-115	113	1.9	1.7
	0.5	5	92.6-102	96	4.0	4.2
	Surface (Lake) Water					
Mesotrione	0.05 (LOQ)	5	98-119	110	8.0	7.3
	0.5	5	107-120	113	5.2	4.6
AMBA	0.05 (LOQ)	5	83-86	85	1.1	1.3
	0.5	5	83-91	86	3.5	4.1
MNBA	0.05 (LOQ)	5	75-118	94	17	18
	0.5	5	70-79	73	3.7	5.1

Data (uncorrected recovery results) were obtained from Tables 6-11, pp. 26-31 of MRID 49458106.

1 Water matrices were characterized (pp. 11-12). Ground water was obtained from a well and surface water from Gull Lake, with both collection sites located in a rural area of Rimbey, Alberta, Canada.

III. Method Characteristics

In the ECM and ILV, the LOQ for mesotrione, AMBA, and MNBA in water was 0.05 µg/L (ng/mL, ppb; pp. 12, 19 of MRID 49458109; p. 9 of MRID 49458106). The ECM defined the LOQ as the lowest fortification specified by the method which yields adequate recovery data as specified by USEPA guidelines. The ECM estimated the LOD based on the smallest calibration standard used during analysis, equivalent to 0.025 pg/µL for this validation (p. 12; Tables 3-5, pp. 25-33 of MRID 49458109). Therefore, LODs were estimated at 1.25 pg for all three analytes based on the 50-µL injection volume used for analysis (p. 12; Table 1, p. 22 of MRID 49458109). In the ILV, LODs were not reported.

Table 4. Method Characteristics for Mesotrione and Its Transformation Products AMBA and MNBA in Surface and Ground Water

			Mesotrione	AMBA	MNBA
Limit of Quantitation (LOQ)			0.05 µg/L (ng/mL, ppb)		
Limit of Detection (LOD)			1.25 pg (0.025 µg/L)		
Linearity (calibration curve r^2 and concentration range)[1]	ECM:		r^2 = 0.9995-0.9998	r^2 = 0.9997-0.9999	r^2 = 0.9998-0.9999
	ILV:[2]	Surface:	r^2 = 0.9958	r^2 = 0.9960	r^2 = **0.9926**
		Ground:	r^2 = 0.9998	r^2 = 0.9994	r^2 = 0.9996
	Range:		0.025-1.00 ng/mL (pg/µL)		
Repeatable	ECM:		Yes at LOQ and 10x LOQ.		
	ILV:		Yes at LOQ and 10x LOQ.		
Reproducible			Yes.		
Specific[3]	ECM:		Yes; interferences at the analyte retention times were <20% of the LOD (lowest calibration standard; Tables 3-5, pp. 25-33).		
	ILV:		Yes for all three analytes in ground water and for mesotrione and MNBA in surface water. No for AMBA in surface water; interference of *ca*. 60% of LOQ (based on peak height) detected at analyte retention time.		

Data were obtained from p. 12; Tables 3-5, pp. 25-33; Figures 5-6, pp. 43-44; Figure 7, p. 46; Figure 8, p. 48 of MRID 49458109; Tables 6-11, pp. 26-31; Figures 1-6, pp. 36-41; Appendix 2, pp. 49-51, 53, 60-62, 64-65 of MRID 49458106; DER Attachment 2.

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

1 ECM r^2 values (no weighting) are reviewer-generated from study results (peak area counts); it appears the study author did not correctly report calibration curve data, because the slope, Y-intercept, and corr. coeff. for mesotrione were identical for the pond and creek water sample sets and likewise for AMBA and MNBA (Tables 3-5, pp. 25-33 of MRID 49458109; DER Attachment 2). ILV r^2 values are reviewer-generated from reported r values (no weighting) of 0.9963-0.9999 (Figures 1-6, pp. 36-41; Appendix 2, pp. 49-51, 60-62 of MRID 49458106; DER Attachment 2).

2 Results presented by sample set (analyte/matrix), but calibration standards were solvent prepared, not matrix-matched.

3 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 following modifications to the ECM were made: a PE Sciex API 4000 MS/MS system was used in substitution of the Waters Model 2690 LC/Micromass Ultima MS/MS system, mixed analyte fortification solutions were prepared in methanol instead of 5% acetonitrile/water + 0.1% acetic acid, and injection volume was increased to 100 µL (pp. 12-13 of MRID 49458106). The equipment substitution and modification to the injection volume are not considered substantial changes to the ECM.

   However, during Trial 1/ground water, the independent laboratory believed poor recoveries (7.1-8.8%) of MNBA at the 10x LOQ fortification were probably due to poor solubility of MNBA in the 5% acetonitrile/water + 0.1% acetic acid solution used to prepare the fortification solutions (p. 17; Table 4, p. 24 of MRID 49458106). The 50.0 ppb fortification stock solution was analyzed and the concentration of MNBA was "similar" to the concentration in the 5.00 ppb stock solution. For Trial 2/ground water and Trial 1/surface water, mixed analyte fortification solutions were prepared using methanol (pp. 18-19). This modification was approved by the study monitor. An updated ECM report implementing the ILV modification to fortification solution preparation was not provided.

2. 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 fortification specified by the method which yields adequate recovery data as specified by USEPA guidelines (p. 12 of MRID 49458109). The ECM estimated the LOD based on the smallest calibration standard used during analysis, equivalent to 0.025 pg/µL for this validation (p. 12; Tables 3-5, pp. 25-33). Therefore, LODs were estimated at 1.25 pg for all three analytes based on the 50-µL injection volume used for analysis (p. 12; Table 1, p. 22). 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 water was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

3. In the ILV, interferences with AMBA peak areas were *ca*. 60% of the LOQ (based on peak height) in the surface water matrix control (Appendix 2, pp. 64-65 of MRID 49458106).

4. Characterizations of the surface (irrigation pond, creek) and ground (well) water matrices used in the ECM validation were not provided.

5. For the ECM, standard curve plots were not provided. Individual calibration standard data with regression curve (slope, Y-intercept, corr. coeff.) analysis were provided; however, it appears the study author did not correctly report regression curve results, because the slope, Y-intercept, and corr. coeff. for mesotrione were identical for the pond and creek water sample sets and likewise for AMBA and MNBA with the pond and creek water sample sets (Tables 3-5, pp. 25-33 of MRID 49458109). Standard curve plots and coefficients of determination (r^2) were generated by the reviewer using the provided calibration standard data (DER Attachment 2).

6. For the ILV, chromatograms were not provided for reagent blanks. For the calibration standards, only chromatograms of the 0.025 and 1.00 ppb standards were provided (calibration standard range 0.025-1.00 ppb), and the 0.025 ppb standard did not chromatograph as an attenuated peak (Appendix 2, pp. 52, 56, 63, 67 of MRID 49458106). Linearity (r^2) of the MNBA calibration curve (non-matrix matched) for the surface water sample set was not ≥ 0.995 (DER Attachment 2).

7. 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.

8. For the ECM, means, standard deviations, and relative standard deviations for each fortification level and matrix were determined by the reviewer, because the study author only provided summary results (Tables 3-6, pp. 25-36; DER Attachment 2).

9. It was reported for the ILV that 2 person-hours are required to fortify and complete one set of thirteen samples per matrix and instrument analysis requires approximately 15 minutes per run; therefore, a sample set can be analyzed in *ca*. 6 hours (p. 19 of MRID 49458106).

V. References

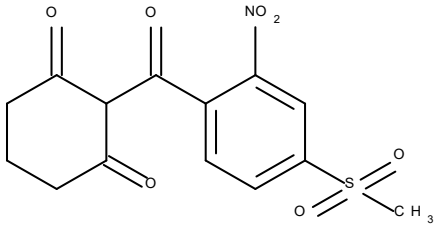
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

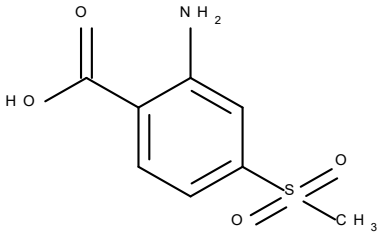
Mesotrione (ZA 1296)

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



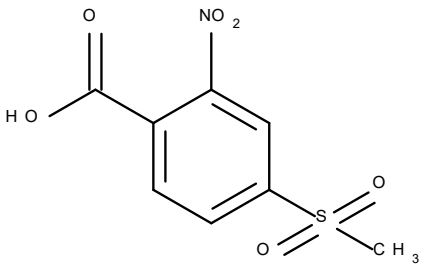
AMBA (NOA-422848)

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)

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 49458109)

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

Sections **2.1 Apparatus** and **2.2 Reagents and Analytical Standards** (pp. 12-14) list recommended equipment, labware, reagents and analytical standards along with recommended suppliers. **Tables 1-2** (pp. 22-24) describe 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.)

No; source locations for the three water matrices were provided, but characterizations were not provided (pp. 8-9). Surface and well water matrices were obtained from Irrigation Pond AW-3 IRG BRR-1 and Well AW-3 P4BRR-4, respectively, located in Eagle Springs, North Carolina (Syngenta Study No.: T000011-02), with a second surface water obtained from Buffalo Creek, Greensboro, North Carolina.

» 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 (p. 12; Tables 1-2, pp. 22-24). Analytes were identified using one ion transitions; 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.

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

Yes (pp. 16-17); potential problems of note were:

Mesotrione may chelate with metal ion impurities in silica-based HPLC columns and cause chromatographic peak tailing, therefore, a polymer column is preferred for analysis. An alternative column was recommended: Luna Phenyl-Hexyl 3 x 50 mm, 3 µm (Phenomenex Cat# 00B-4256-Y0).

Long-term optimization of the LC/MS signal by infusion of a test mixture of analytes into the system will result in lingering high backgrounds for the molecular ions and may be severe enough to affect the ability to achieve desired signal to noise ratios for the lowest standards. It is recommended that optimizing/calibration with analytical standards be done using dilute solutions, optimizing/calibration time be minimized, and the Ion-Spray interface orifice plate be routinely cleaned.

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

Yes; interferences at the analyte retention times were <20% of the LOD (lowest calibration standard; Tables 3-5, pp. 25-33; Figures 5-6, pp. 43-44; Figure 7, p. 46; Figure 8, p. 48).

» 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 4-8, pp. 40-49 of MRID 49458109): Representative chromatograms were provided for calibration standards, reagent blanks, matrix blanks, and spiked samples at the LOQ and 10× LOQ for all analytes in each matrix. Standard curve plots were not provided. Individual calibration standard data with regression curve (slope, Y-intercept, corr. coeff.) analysis were provided; however, it appears the study author did not correctly report regression curve analyses, because the slope, Y-intercept, and corr. coeff. for mesotrione were identical for the pond and creek water sample sets and likewise for AMBA and MNBA with the pond and creek water sample sets (Tables 3-5, pp. 25-33). Standard curve plots and coefficients of determination (r^2) were generated by the reviewer (DER Attachment 2). MS/MS product ion spectra were provided (Figure 3, p. 39).

ILV (Appendix 2, pp. 49-67 of MRID 49458106): No. Chromatograms were not provided for reagent blanks. For the calibration standards, only chromatograms of the 0.025 and 1.00 ppb standards were provided (calibration standard range 0.025-1.00 ppb), and the 0.025 ppb standard did not chromatograph as an attenuated peak (Appendix 2, pp. 52, 56, 63, 67). Standard curves for all analytes were provided, with the individual calibration standard data (Figures 1-6, pp. 36-41; Appendix 2, pp. 49-51). Linearity (r^2) of the MNBA calibration curve (non-matrix matched) for the surface water sample set was not ≥ 0.995 (DER Attachment 2).

» 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 49458109): Yes (Figure 6, p. 45; Figure 7, p. 47; Figure 8, p. 49).

ILV (MRID 49458106): Yes (Appendix 2, pp. 54, 65).

» 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 fortification specified by the method which yields adequate recovery data as specified by USEPA guidelines (p. 12 of MRID 49458109). The ECM estimated the LOD based on the smallest calibration standard used during analysis, equivalent to 0.025 pg/µL for this validation (p. 12; Tables 3-5, pp. 25-33). Therefore, LODs were estimated at 1.25 pg for all three analytes based on the 50-µL injection volume used for analysis (p. 12; Table 1, p. 22). 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 water.

» 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 49458106)

» 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.

Yes (Table 12, p. 32).

» 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 all three analytes at both fortification levels in ground water after two trials and in surface water after one trial (pp. 18-19). During Trial 1/ground water, the independent laboratory believed poor recoveries (7.1-8.8%) of MNBA at the 10x LOQ fortification were probably due to poor solubility of MNBA in the 5% acetonitrile/water + 0.1% acetic acid solution (p. 17; Table 4, p. 24). For Trial 2/ground water and Trial 1/surface water, mixed analyte fortification solutions were prepared using methanol (pp. 18-19).

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 (Tables 6-11, pp. 26-31). Ground water was obtained from a well and surface water from Gull Lake, with both collection sites located in a rural area of Rimbey, Alberta, Canada (p. 11). The water matrices were characterized by the independent laboratory under GLP (pp. 11-12; Appendix 1, pp. 42-44).

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

Yes for all three analytes in ground water and for mesotrione and MNBA in surface water (Appendix 2, pp. 52-54, 63-65).
No for AMBA in surface water; interference of *ca*. 60% of the LOQ (based on peak height) detected at analyte retention time (Appendix 2, pp. 64-65).

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

No, recoveries were not corrected (p. 19; Tables 3-5, pp. 25-33 of MRID 49458109; Tables 6-11, pp. 26-31; Table 13, pp. 33-34; Figures 1-6, pp. 36-41 of MRID 49458106).

» 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 49458109): Yes (Tables 3-5, pp. 25-33).

ILV (MRID 49458106): Yes (Tables 6-11, pp. 26-31).

» 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 49458109): Yes (DER Attachment 2). Means, standard deviations, and relative standard deviations for each fortification level and matrix were determined by the reviewer, because the study author only provided summary results (Tables 3-6, pp. 25-36).

ILV (MRID 49458106): Yes (Tables 6-11, pp. 26-31).

» 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 following modifications to the ECM were made: a PE Sciex API 4000 MS/MS system was used in substitution of the Waters Model 2690 LC/Micromass Ultima MS/MS system, mixed analyte fortification solutions were prepared in methanol instead of 5% acetonitrile/water + 0.1% acetic acid, and injection volume was increased to 100 µL (pp. 12-13 of MRID 49458106). The equipment substitution and modification to the injection volume are not considered substantial changes to the ECM.

However, during Trial 1/ground water, the independent laboratory believed poor recoveries (7.1-8.8%) of MNBA at the 10x LOQ fortification were probably due to poor solubility of MNBA in the 5% acetonitrile/water + 0.1% acetic acid solution used to prepare fortification solutions (p. 17; Table 4, p. 24). The 50.0 ppb fortification stock solution was analyzed and the concentration of MNBA was "similar" to the concentration in the 5.00 ppb stock solution. For Trial 2/ground water and Trial 1/surface water, mixed analyte fortification solutions were prepared using methanol (pp. 18-19). This modification was approved by the study monitor. An updated ECM report implementing the ILV modification to fortification solution preparation was not provided.