

Analytical method for trifludimoxazin (BAS 850 H) and its metabolite M850H001 in soil

Reports: ECM: EPA MRID No.: 51207301. Perez, S., and J. Adams. 2020. Validation of BASF Analytical Method R0067/01: “Method for the Determination of Residues of BAS 850 H (Reg. No. 5654329) and its Metabolite M850H001 (Reg. No. 5749359) in Soil with LOQ of 0.1 µg/kg using LC-MS/MS”. BASF Study No.: 899173. BASF Registration Document No.: 2020/2088003. ADPEN Study No.: 20C1007. Report prepared by APDEN Laboratories, Inc., Jacksonville, Florida, and sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 142 pages. Final report issued July 15, 2020.

ILV: EPA MRID No. 51207302. Rodriguez, D. 2020. Independent Laboratory Validation of BASF Method R0067/01: Method for the Determination of BAS 850 H (Reg. No. 5654329) and its Metabolite M850H001 (Reg. No. 5749359), in Soil with LOQ of 0.1 µg/kg using LC-MS/MS. BASF Study ID No.: 899174. BASF Registration Document No.: 2020/2088004. JRF America Study No.: AU-2020-11. Report prepared by JRF America, Inc., Audubon, Pennsylvania, and sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 191 pages. Final report issued July 28, 2020.

Document No.: MRIDs 51207301 & 51207302

Guideline: 850.6100

Statements: ECM: The study was not conducted in accordance with USEPA FIFRA (40 CFR Part 160) Good Laboratory Practice (GLP) standards, with the exception that three typographical/omission errors were corrected in the study protocol (p. 3 of MRID 51207301). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 51207302). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as **Acceptable**. There was extensive communication between the BASF (ECM) Study Monitor and the ILV Study Author prior to the pre-trial phase and the ILV did not include a complete report of the communications. However, BASF emailed to Manjula Unnikrishnan (RD), who emailed to EFED on August 26, 2020, a complete report of communications between the BASF Study Monitor and the ILV Study Author. These communications indicate the ILV was independent. All ILV data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for both analytes. All ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for trifludimoxazin, except for the linearity at LOQ and precision at 10×LOQ in clay loam soil. All ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for M850H001.

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

Executive Summary

This analytical method, BASF Method R0067/01, is designed for the quantitative determination of trifludimoxazin (BAS 850 H) and its metabolite M850H001 in soil at the LOQ of 0.1 µg/kg using LC/MS/MS. The LOQ is lower than the lowest toxicological level of concern in soil for trifludimoxazin (0.37 µg/kg, MRID 50406450) and its degradate M850H001 (0.17 µg/kg, MRID 50406451). Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the reported method LOQ for trifludimoxazin and its metabolite M850H001 in soil.

The ECM performed the method with two characterized soil matrices sourced from BASF terrestrial field dissipation and aquatic dissipation studies (study test materials were not trifludimoxazin). The ILV soil matrix was the ECM soil from the aquatic dissipation study - clay loam (0.50% organic carbon). It could not be determined if the ILV was conducted with the most difficult matrix with which to validate the method and that the single soil matrix could cover the range of soil matrices used in terrestrial field dissipation studies. The ILV validated BASF Method R0067/01 for trifludimoxazin and M850H001 during the first trial with insignificant modifications to the analytical instrument and parameters. It could not be determined if the ILV was conducted independently of the ECM since the ILV Study Monitor was also the ECM Study Monitor and an author of the Technical Procedure for BASF Method R0067/01.

Communications were extensive and involved some method clarification, but most of the communication was non-technical.

All ILV data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for both analytes. All ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for trifludimoxazin, except for the linearity at LOQ and precision at 10×LOQ in clay loam soil. All ECM data regarding repeatability, accuracy,

precision, linearity, and specificity were satisfactory for M850H001. The ECM calculated LODs for trifludimoxazin were $\geq 30\%$ of the LOQ.

Table 1. Analytical Method Summary – BASF Analytical Method R0067/01

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						
Trifludimoxazin (BAS 850 H; Reg. No. 5654329)	51207301 ¹	51207302 ²		Soil	15/07/2020	BASF Corporation	LC/MS/MS	0.1 µg/kg
M850H001 (Reg.No. 5749359)								

1 In the ECM, soil matrices were clay loam (Sample R1801810004; pH 7.1 (in saturated paste); 39% sand, 24% silt, 37% clay, 0.50% organic carbon (Walkley-Black), 26.2 meq/100 g cation exchange capacity) from a BASF aquatic dissipation study in California (Trial R180181; 12-18" untreated control soil; BASF Study No. 834740; BASF Reg. Doc. No. 2020/2033370) and sandy loam (Sample R1700530000; pH 6.0 (in 1:1 soil:water) and 6.1 (in saturated paste); 59% sand, 26% silt, 15% clay, 2.3% organic carbon (Walkley-Black), 14.0 meq/100 g cation exchange capacity) from a BASF terrestrial field dissipation study in Iowa (Trial R170053; 0-3" untreated control soil; BASF Study No. 780082; BASF Reg. Doc. No. 2020/2033022; pp. 13, 21; Appendix A, pp. 31-32 of MRID 51207301). The soil characterization (USDA soil texture classification) was performed by Agvise Laboratories, Northwood, North Dakota.

2 In the ILV, soil matrix was clay loam (BASF Sample R1801810004R02; JRFA Sample ID 205205; pH 7.1 (in saturated paste); 39% sand, 24% silt, 37% clay, 0.50% organic carbon (Walkley-Black), 26.2 meq/100 g cation exchange capacity) from the Sponsor (BASF; 12-18" untreated control soil; BASF Study No. 834740; p. 13; Appendix 10.2, p. 41 of MRID 51207302). The soil characterization (USDA soil texture classification) was performed by Agvise Laboratories, Northwood, North Dakota. The soil texture was verified by the reviewer using USDA-NRCS technical support tools. The soil matrix was the exact same as one of those used in the ECM.

I. Principle of the Method

Method R0067/01

Soil samples (5 ± 0.1 g each) in a 50-mL Teflon centrifuge tube were fortified with 0.05 mL of the 0.01 or 0.10 µg/mL mixed fortification solution, as necessary, and extracted by shaking (mechanical shaker at *ca.* 300 rpm for *ca.* 45 min.) twice with 25 mL of methanol:water with 0.1% formic acid (70:30, v:v; pp. 7, 15; Appendix D, pp. 46-50 of MRID 51207301). Each extraction was followed by centrifugation (*ca.* 4000 rpm for *ca.* 5 min.). The volume of the combined extracts was adjusted to 50 mL with the extraction solvent. An aliquot (25 mL) of the combined extracts was concentrated to an aqueous remainder (*ca.* 7.4 mL remaining; all methanol must be removed) and partitioned with 10 mL of cyclohexane:ethyl acetate (90:10, v:v). An aliquot (9.5 mL) of the organic phase are then evaporated to dryness under nitrogen at 50°C. The residue was reconstituted in 0.5 mL methanol with 0.1% formic acid then mixed with 2.0 mL water with 0.1% formic acid. Samples were transferred to autosampler vials via a 0.45 µm PTFE syringe and analyzed by LC/MS/MS. A method flow chart was provided (p. 55; Appendix L, p. 142).

The method noted that only Teflon or glass equipment should be used for analysis of trifludimoxazin and M850H001 since plastics have been confirmed to cause interference and

suppression on LC/MS/MS (Appendix D, p. 50 of MRID 51207301). The method also noted that all volumes must be exactly what is written, and the method should not be interrupted until ready for LC/MS/MS.

Residues of trifludimoxazin are determined by LC/MS/MS-ESI with an Agilent 1290 HPLC system coupled with an AB Sciex 6500 MS in positive ion mode (ionization temperature 200°C; Appendix D, pp. 45, 52-53 of MRID 51207301). The following parameters for analysis of trifludimoxazin (primary and confirmatory) and M850H001 (primary): Phenomenex Kinetex EVO C18 column (3.0 x 50 mm, 2.6 µm particle size; column temperature 50°C) using a mobile phase gradient of (A) water with 1.0% formic acid and (B) acetonitrile with 0.1% formic acid [time, percent A:B; 0.00-0.05 min. 80:20, 6.00 min. 50:50, 7.00-7.90 min. 1:99, 7.91-8.00 min. 80:20 (flow rate 500 µL/minute)] and injection volume of 100 µL. The following parameters for analysis of M850H001 (confirmatory): Waters Xbridge BEH C18 XP column (4.6 x 50 mm, 2.5 µm particle size; column temperature 50°C) using a mobile phase gradient of (A) water with 1.0% formic acid and (B) acetonitrile with 0.1% formic acid [time, percent A:B; 0.00-0.05 min. 80:20, 6.00 min. 50:50, 8.00-8.90 min. 1:99, 9.00-10.00 min. 80:20 (flow rate 800 µL/minute)] and injection volume of 100 µL. Trifludimoxazin was identified using two ion pair transitions (quantitation and confirmation, respectively): m/z 413→74 and m/z 413→134, and expected retention time was *ca.* 6.53 minutes. M850H001 was identified using one ion pair transition: m/z 397→114, and expected retention times were *ca.* 4.50 and 5.90 minutes in the primary and confirmatory LC methods, respectively.

ILV

The ILV performed the ECM method as written, except for insignificant modifications of analytical instrumentation and equipment (pp. 7, 14-15; Appendix 10.7, pp. 52-53 of MRID 51207302). The concentration step was performed stepwise with 5-10 mL volumes instead of the total 25 mL at once. An Agilent 1290 Infinity LC coupled to an AB Sciex QTrap 6500 MS was used. The following parameters differed from those of the ECM: column temperature 40°C, injection volume of 20 µL, and MS ionization temperature 150°C. The same ion pair transitions were used as those of the ECM. Trifludimoxazin expected retention time was *ca.* 6.87 minutes. M850H001 expected retention times were *ca.* 4.97 and 6.1 minutes in the primary and confirmatory LC methods, respectively

LOQ/LOD

In the ECM and ILV, the method Limit of Quantification (LOQ) for trifludimoxazin and M850H001 in soil is 0.1 µg/kg for each analyte (pp. 7, 17-19; Table 4, p. 25 of MRID 51207301; pp. 7, 19 of MRID 51207302). In the ECM and ILV, the method Limit of Detection (LOD) in soil was 0.026 µg/kg for each analyte. In the ECM, the LODs were calculated as 0.030-0.045 µg/kg and 0.023-0.040 µg/kg for trifludimoxazin and M850H001, respectively.

II. Recovery Findings

Method R0067/01

ECM (MRID 51207301): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of trifludimoxazin (BAS 850 H; Reg.No. 5654329) and M850H001 (Reg.No. 5749359) in two soil matrices at fortification levels of 0.1 $\mu\text{g}/\text{kg}$ (LOQ) and 1.0 $\mu\text{g}/\text{kg}$ (10 \times LOQ), except for the analysis of trifludimoxazin at 1.0 $\mu\text{g}/\text{kg}$ in clay loam soil [RSD 28% (Q) and 36% (C); p. 9; Appendix F, pp. 62-71]. The unacceptable RSDs were caused by the low recovery of one of the samples (30-38%). A replacement sample (n = 1) of trifludimoxazin at 1.0 $\mu\text{g}/\text{kg}$ in clay loam soil was re-extracted yielding acceptable recoveries (83-86%; see Reviewer's Comment #4). Analytes were identified using two ion transitions or two analytical method; performance data (recovery results) from primary and confirmatory analyses were comparable. Matrix-matched calibration standards were used (p. 19). The soil matrices were clay loam (Sample R1801810004; pH 7.1 (in saturated paste); 39% sand, 24% silt, 37% clay, 0.50% organic carbon (Walkley-Black), 26.2 meq/100 g cation exchange capacity) from a BASF aquatic dissipation study in California (Trial R180181; 12-18" untreated control soil; BASF Study No. 834740; BASF Reg. Doc. No. 2020/2033370) and sandy loam (Sample R1700530000; pH 6.0 (in 1:1 soil:water) and 6.1 (in saturated paste); 59% sand, 26% silt, 15% clay, 2.3% organic carbon (Walkley-Black), 14.0 meq/100 g cation exchange capacity) from a BASF terrestrial field dissipation study in Iowa (Trial R170053; 0-3" untreated control soil; BASF Study No. 780082; BASF Reg. Doc. No. 2020/2033022; pp. 13, 21; Appendix A, pp. 31-32). The soil characterization (USDA soil texture classification) was performed by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 51207302): Mean recoveries and RSDs were within guideline requirements for analysis of trifludimoxazin (BAS 850 H; Reg.No. 5654329) and M850H001 (Reg.No. 5749359) in one soil matrix at fortification levels of 0.1 $\mu\text{g}/\text{kg}$ (LOQ) and 1.0 $\mu\text{g}/\text{kg}$ (10 \times LOQ; Table 6, pp. 27-30). Analytes were identified using two ion transitions or two analytical method; performance data (recovery results) from primary and confirmatory analyses were comparable. Matrix-matched calibration standards were used (p. 15). The soil matrix was clay loam (BASF Sample R1801810004R02; JRFA Sample ID 205205; pH 7.1 (in saturated paste); 39% sand, 24% silt, 37% clay, 0.50% organic carbon (Walkley-Black), 26.2 meq/100 g cation exchange capacity) from the Sponsor (BASF; 12-18" untreated control soil; BASF Study No. 834740; p. 13; Appendix 10.2, p. 41). The soil characterization (USDA soil texture classification) was performed by Agvise Laboratories, Northwood, North Dakota. The soil matrix was the exact same as one of those used in the ECM. The ILV validated BASF Method R0067/01 in one soil for BAS 850 H and M850H001 during the first trial with insignificant modifications to the analytical instrument and parameters (pp. 7, 14-16; see Reviewer's Comment #6).

Table 2. Initial Validation Method Recoveries for Trifludimoxazin (BAS 850 H) and Its Metabolite M850H001 in Soil^{1,2}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Clay Loam Soil						
Quantitation ion transition or method						
Trifludimoxazin (BAS 850 H)	0.1 (LOQ)	5	79-100	90	8.8	9.8
	1.0	5 ³	38-89	70	19	28
	1.0	1 ⁴	83	--	--	--
M850H001	0.1 (LOQ)	5	80-95	89	6.2	7.0
	1.0	5	74-90	82	5.9	7.2
Confirmation ion transition or method						
Trifludimoxazin (BAS 850 H)	0.1 (LOQ)	5	86-111	97	11.9	12.3
	1.0	5 ³	30-96	77	28	36
	1.0	1 ⁴	86	--	--	--
M850H001	0.1 (LOQ)	5	74-102	87	10.7	12.3
	1.0	5	69-90	80	7.6	9.5
Sandy Loam Soil						
Quantitation ion transition or method						
Trifludimoxazin (BAS 850 H)	0.1 (LOQ)	5	79-98	88	7.9	9.0
	1.0	5	73-101	84	11.6	13.8
M850H001	0.1 (LOQ)	5	74-86	79	6.1	7.8
	1.0	5	75-93	82	8.4	10.1
Confirmation ion transition or method						
Trifludimoxazin (BAS 850 H)	0.1 (LOQ)	5	85-113	101	10.3	10.2
	1.0	5	85-120	99	14.8	14.9
M850H001	0.1 (LOQ)	5	78-93	85	6.3	7.4
	1.0	5	81-112	95	12.9	13.6

Data (uncorrected results, Appendix I, pp. 91-92; Appendix F, pp. 62-71) were obtained from p. 9; Appendix F, pp. 62-71 of MRID 51207301 (DER Attachment 2). Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

- The soil matrices were clay loam (Sample R1801810004; pH 7.1 (in saturated paste); 39% sand, 24% silt, 37% clay, 0.50% organic carbon (Walkley-Black), 26.2 meq/100 g cation exchange capacity) from a BASF aquatic dissipation study in California (Trial R180181; 12-18" untreated control soil; BASF Study No. 834740; BASF Reg. Doc. No. 2020/2033370) and sandy loam (Sample R1700530000; pH 6.0 (in 1:1 soil:water) and 6.1 (in saturated paste); 59% sand, 26% silt, 15% clay, 2.3% organic carbon (Walkley-Black), 14.0 meq/100 g cation exchange capacity) from a BASF terrestrial field dissipation study in Iowa (Trial R170053; 0-3" untreated control soil; BASF Study No. 780082; BASF Reg. Doc. No. 2020/2033022; pp. 13, 21; Appendix A, pp. 31-32). The soil characterization (USDA soil texture classification) was performed by Agvise Laboratories, Northwood, North Dakota. The soil texture was verified by the reviewer using USDA-NRCS technical support tools. Sediment matrices were not included.
- Trifludimoxazin was identified using two ion pair transitions (quantitation and confirmation, respectively): m/z 413→74 and m/z 413→134. M850H001 was identified using one ion pair transition, m/z 397→114, with quantitation and confirmation analytical methods.
- The recovery of one of the samples was re-extracted due to low recovery (30-38%; Appendix F, pp. 62-63, 66-67). The recovery results from the re-extraction (83-86%) were used for statistics in the study report. The values reported in the table above are reviewer-calculated statistics with the original recovery values from Work Order No. WO-20052010 (n = 5; DER Attachment 2). Rules of significant figures were followed.
- Recovery values from Work Order No. WO-20052010A (Appendix F, pp. 66-67).

Table 3. Independent Validation Method Recoveries for Trifludimoxazin (BAS 850 H) and Its Metabolite M850H001 in Soil^{1,2}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Clay Loam Soil						
Quantitation ion transition or method						
Trifludimoxazin (BAS 850 H)	0.1 (LOQ)	5	106-114	110	4	3
	1.0	5	102-113	108	4	4
M850H001	0.1 (LOQ)	5	102-113	109	4	4
	1.0	5	98.5-109	104	4	4
Confirmation ion transition or method						
Trifludimoxazin (BAS 850 H)	0.1 (LOQ)	5	106-120	112	6	5
	1.0	5	106-111	110	2	2
M850H001	0.1 (LOQ)	5	107-113	111	3	2
	1.0	5	107-116	111	4	3

Data (uncorrected results, Appendix 10.5, p. 46) were obtained from p. 9 of MRID 51207302. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 The soil matrix was clay loam (BASF Sample R1801810004R02; JRFA Sample ID 205205; pH 7.1 (in saturated paste); 39% sand, 24% silt, 37% clay, 0.50% organic carbon (Walkley-Black), 26.2 meq/100 g cation exchange capacity) from the Sponsor (BASF; 12-18" untreated control soil; BASF Study No. 834740; p. 13; Appendix 10.2, p. 41). The soil characterization (USDA soil texture classification) was performed by Agvise Laboratories, Northwood, North Dakota. The soil texture was verified by the reviewer using USDA-NRCS technical support tools. Sediment matrices were not included. The soil matrix was the exact same as one of those used in the ECM.

2 Trifludimoxazin was identified using two ion pair transitions (quantitation and confirmation, respectively): m/z 413→74 and m/z 413→134. M850H001 was identified using one ion pair transition, m/z 397→114, with quantitation and confirmation analytical methods.

III. Method Characteristics

The LOQ for trifludimoxazin and M850H001 in soil is 0.1 µg/kg for each analyte (pp. 7, 17-19; Table 4, p. 25 of MRID 51207301; pp. 7, 19 of MRID 51207302). The LOQ was defined as the lowest fortification level successfully tested. In the ECM and ILV, the method LOD in soil was 0.026 µg/kg for each analyte based on the signal-to-noise ratio of the M850H001 confirmatory method in the ECM. In the ECM, the LODs were calculated for each soil matrix using the following equation:

$$\text{LOD} = S \times t_{(0.99)},$$

where S is the standard deviation of the matrix-spiked sample set concentrations (n =5) and $t_{(0.99)}$ = one tailed t-statistic at 99% confidence level for n-1 (3.747). The calculated LODs were 0.030-0.045 µg/kg and 0.023-0.040 µg/kg for trifludimoxazin and M850H001, respectively (combined matrices/analyses). The calculated LODs for trifludimoxazin were $\geq 30\%$ of the LOQ; the calculated LODs for M850H001 were $< 30\%$ of the LOQ and less than the method LOD, except for the confirmation analysis in clay loam soil.

Since the reported method LOQ were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ.

Table 4. Method Characteristics - Analytical Method R0067/01

Analyte		Trifludimoxazin (BAS 850 H; Reg.No. 5654329)	M850H001 (Reg.No. 5749359)
Limit of Quantitation (LOQ)*	ECM	0.1 µg/kg	
	ILV		
Limit of Detection (LOD)	ECM (calculated)	0.033 µg/kg (Q, CL) ¹	0.023 µg/kg (Q, CL)
		0.045 µg/kg (C, CL) ¹	0.040 µg/kg (C, CL) ¹
	ECM	0.030 µg/kg (Q, SL) ²	0.023 µg/kg (Q, SL)
		0.038 µg/kg (C, SL) ¹	0.024 µg/kg (C, SL)
ILV	0.026 µg/kg (method)		
Linearity (calibration curve r and concentration range)	ECM	r = 0.9916 (Q, CL) r = 0.9978 (C, CL) r = 0.9952 (Q, CL, re-analysis) ³ r = 0.9972 (C, CL, re-analysis) ³	r = 0.9985 (Q, CL) r = 0.9960 (C, CL)
		r = 0.9960 (Q & C, SL)	r = 0.9979 (Q, SL) r = 0.9925 (C, SL) ⁴
	ILV	r = 0.99576041587 (Q, CL) r = 0.99588445526 (C, CL)	r = 0.99764530039 (Q, CL) r = 0.99753842193 (C, CL)
	Range	0.025-0.5 ng/mL	
Repeatable	ECM ⁵	Yes for LOQ, but No for 10×LOQ (n = 5; RSDs 28-36%) ⁶ (characterized clay loam soil matrix) Yes for 10×LOQ (n = 1 ; re-analysis) ³ (characterized clay loam soil matrix)	Yes for LOQ and 10×LOQ (characterized clay loam soil matrix)
		Yes for LOQ and 10×LOQ (characterized sandy loam soil matrix)	
	ILV ^{7,8}	Yes for LOQ and 10×LOQ (characterized clay loam soil matrix).	
Reproducible ⁹		Yes at 0.1 µg/kg (LLMV)* and 1.0 µg/kg	
Specific	ECM	Yes, no matrix interferences were observed. Analyte peaks were small compared to baseline noise. Some minor baseline noise near the analyte peak was noted in most LOQ and 10×LOQ representative chromatograms. ¹⁰	
		Loamy sand soil representative chromatograms contained less matrix interferences than those of clay soil.	
	ILV	Yes, matrix interferences were <5% of the LOQ (based on peak area). Minor baseline noise interfered with analyte peak attenuation and integration.	

Data were obtained from pp. 7, 17-19; Table 4, p. 25 (LOQ/LOD); p. 9 (recovery results); Appendix F, pp. 62-71 (calibration data); Appendix K, pp. 100-140 (chromatograms) of MRID 51207301; and pp. 7, 19 (LOQ/LOD); p. 9 (recovery results); Appendix 10.6, pp. 48-51 (calibration data); Appendix 10.8, pp. 64-113 (chromatograms) of MRID 51207302; DER Attachment 2. Q = Quantitation ion transition or method; C = Confirmation ion transition or method; CL = Clay Loam soil; SL = Sandy Loam soil.

* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 The calculated LOD was >30% of the LOQ and greater than the method LOD.

2 The calculated LOD was greater than the method LOD.

3 The recovery of one of the 10×LOQ samples was re-extracted due to low recovery (30-38%; Appendix F, pp. 62-63, 66-67 of MRID 51207301). The recovery results from the re-extraction (83-86%) were used for statistics in the study report (see Reviewer's Comment #4).

4 Deficiencies in the confirmation analysis do not affect the linearity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

- 5 In the ECM, soil matrices were clay loam (Sample R1801810004; pH 7.1 (in saturated paste); 39% sand, 24% silt, 37% clay, 0.50% organic carbon (Walkley-Black), 26.2 meq/100 g cation exchange capacity) from a BASF aquatic dissipation study in California (Trial R180181; 12-18" untreated control soil; BASF Study No. 834740; BASF Reg. Doc. No. 2020/2033370) and sandy loam (Sample R1700530000; pH 6.0 (in 1:1 soil:water) and 6.1 (in saturated paste); 59% sand, 26% silt, 15% clay, 2.3% organic carbon (Walkley-Black), 14.0 meq/100 g cation exchange capacity) from a BASF terrestrial field dissipation study in Iowa (Trial R170053; 0-3" untreated control soil; BASF Study No. 780082; BASF Reg. Doc. No. 2020/2033022; pp. 13, 21; Appendix A, pp. 31-32 of MRID 51207301). The soil characterization (USDA soil texture classification) was performed by Agvise Laboratories, Northwood, North Dakota. The soil texture was verified by the reviewer using USDA-NRCS technical support tools.
- 6 Reviewer-calculated statistics at 10×LOQ fortification with the original recovery values from Work Order No. WO-20052010 (n = 5; DER Attachment 2; see Reviewer's Comment #4).
- 7 In the ILV, soil matrix was clay loam (BASF Sample R1801810004R02; JRFA Sample ID 205205; pH 7.1 (in saturated paste); 39% sand, 24% silt, 37% clay, 0.50% organic carbon (Walkley-Black), 26.2 meq/100 g cation exchange capacity) from the Sponsor (BASF; 12-18" untreated control soil; BASF Study No. 834740; p. 13; Appendix 10.2, p. 41 of MRID 51207302). The soil characterization (USDA soil texture classification) was performed by Agvise Laboratories, Northwood, North Dakota. The soil texture was verified by the reviewer using USDA-NRCS technical support tools. The soil matrix was the exact same as one of those used in the ECM. The soil texture was verified by the reviewer using USDA-NRCS technical support tools.
- 8 The independent laboratory validation of BASF method (R0067/01) was successfully completed for both analytes in the first trial with insignificant modifications to the analytical instrument and parameters (pp. 7, 14-16 of MRID 51207302).
- 9 Based on overall results; not soil matrix specific.
- 10 Representative chromatograms were zoomed-in which caused analyte peaks to appear multi-peaked. Peak integration range between chromatograms was fairly uniform for each analyte/method. Linearity is satisfactory when $r \geq 0.995$ [updated DER acceptance criteria (11/2019)]; Linearity criterion is consistent with Superfund analytical methods for inorganic analytes (National Functional Guidelines for Inorganic Superfund Methods Data Review, EPA-540-R-2017-001, January 2017. https://www.epa.gov/sites/production/files/201701/documents/national_functional_guidelines_for_inorganic_superfund_methods_data_review_01302017.pdf].

IV. Method Deficiencies and Reviewer's Comments

1. The ILV report did not provide enough information with which to determine if the ILV was conducted independently of the ECM since there was extensive communication between the BASF Study Monitor/ ECM Study Monitor (Manasi G. Saha of BASF Corporation) and the ILV Study Author (David Rodriguez of JRFA) prior to the pre-trial phase and the ILV did not submit a complete report of the communications for the Study Monitor and ILV Study Author. However, BASF emailed to Manjula Unnikrishnan (RD), who emailed to EFED on August 26, 2020, a complete report of communications between the BASF Study Monitor and the ILV Study Author. These communications indicate the ILV was independent.

In the original ILV report, early June email communications appeared to be provided for only Manasi Saha and not David Rodriguez (p. 20; Appendix 10.9, pp. 114-191 of MRID 51207302). BASF emailed David Rodriguez's communications on August 26, 2020. The ILV study author briefly summarized that communications between the ILV and BASF Study Monitor (Manasi Saha) involved communication of test matrix receipt, method acceptability (see Reviewer's Comment #6), and method performance progress. Manasi Saha specifically communicated that "method detail cannot be discussed" and no phone communication would occur unless the first ILV trial failed (Appendix 10.9, pp. 114, 122). However, the reviewer noted that Technical Procedure typographical errors or changes (X-Bridge column and pipette description) occurred due to ILV communications with M. Saha prior to ILV trial (p. 3 of MRID 51207301; Appendix 10.9, pp. 119-123, 126, 132 of MRID 51207302). The Technical Procedure was provided to the ILV prior to the finalization date (Technical Procedure dated June 23, 2020; Appendix D, p. 39 of MRID 51207301). Communications between M. Saha and D. Rodriguez were extensive; however, most communication was non-technical, including many ILV report formatting/editing concerns (see specifically Appendix 10.9, pp. 168-191 of MRID 51207302). The only technical question regarding the method which was communicated was regarding the ILV using different fragments from those directed in the method (Appendix 10.9, pp. 149-150 of MRID 51207302). M. Saha advised the ILV to use the fragments directed in the method. This ILV question did not appear to be in response to an ILV method issue since it occurred prior to the ILV "Method Try-Out" (Appendix 10.9, p. 151).

2. The reviewer noted that the ILV performed a "Method Try-Out (MB, UTC, & 2 @ LOQ)" to check the LC/MS/MS system prior to a full ILV trial (Appendix 10.9, pp. 151, 153 of MRID 51207302). The results of this pre-trial was communicated to the ILV Study Monitor (Manasi G. Saha of BASF Corporation) for approval but not included in the ILV. The reviewer considered this as a pre-trial analytical system optimization and not ILV trial 1.
3. The reviewer noted the following typographical error in the ECM: "The calculated method LOD's for BAS 850 H (both transitions) and M850H001 (both methods) in clay loam soil were 0.03, **0.04**, 0.02 and 0.04 µg/kg, respectively." should have been reported

- as: “The calculated method LOD’s for BAS 850 H (both transitions) and M850H001 (both methods) in clay loam soil were 0.03, **0.05**, 0.02 and 0.04 µg/kg, respectively.” (p. 19 of MRID 51207301).
4. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ (pp. 7, 17-19; Table 4, p. 25 of MRID 51207301; pp. 7, 19 of MRID 51207302). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the reported method LOQ for trifludimoxazin and its metabolite M850H001 in soil
 5. The ECM included two soils, while the ILV only included one. The ILV should be a more rigorous test of the method, and therefore, should include at least as many test matrices as the ECM. 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 two ECM soil matrices were the untreated control samples sourced from BASF terrestrial field dissipation and aquatic dissipation studies with study test materials of BAS550 I and BAS 310 I, respectively (pp. 13, 21; Appendix A, pp. 31-32 of MRID 51207301). The ILV soil matrix was the ECM soil from the aquatic dissipation study - clay loam (BASF Sample R1801810004R02; JRFA Sample ID 205205; 12-18” untreated control soil; BASF Study No. 834740; p. 13; Appendix 10.2, p. 41 of MRID 51207302). The reviewer noted that the organic carbon content (Walkley-Black) was 0.50% for the ILV soil matrix. 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.
 6. ECM performance data was unacceptable for analysis of trifludimoxazin at 1.0 µg/kg in clay loam soil [RSD 28% (Q) and 36% (C); p. 9; Appendix F, pp. 62-71 of MRID 51207301]. The unacceptable RSDs were caused by the low recovery of one of the samples (30-38%). A replacement sample (n = 1) of trifludimoxazin at 1.0 µg/kg in clay loam soil was re-extracted yielding acceptable recoveries (83-86%). The recovery results from the re-extraction (83-86%) were used in the place of the low recoveries for statistics in the study report [means and RSDs: 79% and 10.0% (Q) and 88% and 9.0% (C); p. 9]; however, no explanation or justification for this substitution was provided. The mixing or substitution of these values was determined to be unacceptable by the reviewer since the extractions appeared to be performed on different samples and on different days, and the recoveries were calculated using different calibration curves. The analysis/extraction sample sets were reported separately in Table 2 of the DER with reviewer-calculated statistics for the recovery values from Work Order No. WO-20052010 (n = 5; DER Attachment 2).
 7. ECM linearity was not satisfactory for the LOQ (Q) analysis of trifludimoxazin in clay loam soil ($r^2 = 0.9916$; Appendix F, pp. 62-71 of MRID 51207301). Linearity is

satisfactory when $r \geq 0.995$. The linearity of the confirmation analysis calibration curve for M850H001 in sandy loam soil ($r = 0.9925$) was not satisfactory; however, deficiencies in the confirmation analysis do not affect the validity of the method since a confirmation method is not usually required when LC/MS or GC/MS is the primary method to generate study data.

The ECM was performed under the following guidelines, in addition to OCSPP 850.6100: SANCO/3029/99 rev 4 (11/07/2000) and SANCO/825/00 rev 8.1 (11/07/2000; p. 1 of MRID 51207301). Under these guidelines, the requirement for the linear correlation coefficient (r) is ≥ 0.99 . No deviation from these linearity requirements was noted.

8. BASF Method R0067/01 contained the following precautions: 1) only Teflon or glass equipment should be used for analysis of trifludimoxazin and M850H001 since plastics have been confirmed to cause interference and suppression on LC/MS/MS; 2) all volumes must be exactly what is written; and 3) the method should not be interrupted until ready for LC/MS/MS (Appendix D, p. 50 of MRID 51207301).
9. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 7, 17-19; Table 4, p. 25 of MRID 51207301; pp. 7, 19 of MRID 51207302). The LOQ was defined as the lowest fortification level successfully tested. No calculations or comparisons to baseline noise were reported for the LOQ in the ECM or ILV. In the ECM, the method LOD was estimated based on the signal-to-noise ratio of the M850H001 confirmatory method. In the ECM, the calculated LOD was based on the equation $S \times t_{(0.99)}$, where S is the standard deviation of the matrix-spiked sample set concentrations ($n = 5$) and $t_{(0.99)}$ = one tailed t-statistic at 99% confidence level for $n-1$ (3.747). Detection limit should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Since the reported method LOQ were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ.

The calculated LODs for trifludimoxazin were $\geq 30\%$ of the LOQ; the calculated LODs for M850H001 were $< 30\%$ of the LOQ and less than the method LOD, except for the confirmation analysis in clay loam soil (Table 4, p. 25 of MRID 51207301).

The calculation used to calculate LOD was similar to the MDL calculations; however, MDL is calculated as $S \times t_{(N-1, 1-\infty=, .99)}$, where S is the Standard deviation of the matrix-spiked sample set concentrations (n must be ≥ 7) and $t_{(N-1, 1-\infty=, .99)}$ = Critical t value from a student t-test table at 99% confidence (equal to 3.143 for $n = 7$).

10. The LOQ for residues of trifludimoxazin in soil was 0.1 $\mu\text{g}/\text{kg}$ for each analyte which is lower than the lowest relevant endpoint from a seedling emergence study ($\text{LC}_{50} > 1000$ mg a.i./kg dry soil; p. 7 of MRID 51207301).

11. In the ECM, matrix effects were determined to be significant ($\geq\pm 20\%$) in several analyses; therefore, matrix-matched calibration standards were used and recommended (pp. 17, 19; Table 2, p. 23 of MRID 51207301).
12. In the ECM, the storage stability of the final extracts was determined to be stable for up to 3 days at $<10^{\circ}\text{C}$ (p. 17; Table 3, p. 24 of MRID 51207301). In the ILV, it was reported that the soil extracts were analyzed within 1 day of extraction (p. 15 of MRID 51207302).
13. It was reported for the ILV that one sample set of 13 samples required *ca.* 8 hours of work, excluding LC/MS/MS and calculation of results (p. 18 of MRID 51207302).

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, and Revision 2; 1994 and 2016.
- Sindermann, A.B., J.R. Porch, H.O. Krueger, and K.H. Martin. 2013. BAS 850 H: A Toxicity Test to Determine the Effects on Seedling Emergence of Ten Species of Plants. Study performed by Wildlife International, Easton, Maryland. Laboratory Study ID: 147P-101; BASF Corporation Study ID: 411591; BASF Corporation Registration Document Number; 2013/7000405. Study sponsored by BASF Corporation, Research Triangle Park, North Carolina. Study completed on March 19, 2013. MRID 50406450.
- Dobbins, L.L., J.R. Porch, K. Keller, and K.H. Martin. 2015. BAS M850H001: A Toxicity Test to Determine the Effects on Seedling Emergence of Ten Species of Plants. Study performed by Wildlife International, Easton, Maryland. Laboratory Study ID: 147P-113; BASF Corporation Study ID: 433363; BASF Corporation Registration Document Number; 2015/7006085. Study sponsored by BASF Corporation, Research Triangle Park, North Carolina. Study completed on October 30, 2015. MRID 50406450.

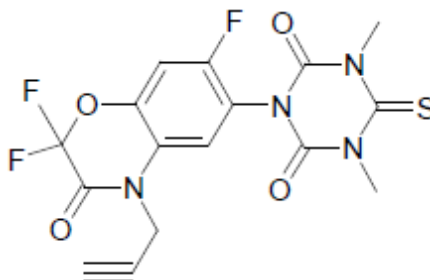
Attachment 1: Chemical Names and Structures**Trifludimoxazin (BAS 850 H; Reg.No. 5654329)**

IUPAC Name: 1,5-Dimethyl-6-thioxo-3-[2,2,7-trifluoro-3-oxo-4-(prop-2-yn-1-yl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-1,3,5-triazinane-2,4-dione

CAS Name: Not reported

CAS Number: 1258836-72-4

SMILES String: Not found

**M850H001 (Reg.No. 5749359)**

IUPAC Name: 1,3-Dimethyl-5-[2,2,7-trifluoro-3-oxo-4-(prop-2-yn-1-yl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-1,3,5-triazinane-2,4,6-trione

CAS Name: Not reported

CAS Number: None

SMILES String: Not found

