

Two equivalent analytical methods for mefentrifluconazole (BAS 750 F; Reg.No. 5834378) and its metabolites M750F003 (Reg.No. 5924326) and 1,2,4-Triazole (Reg.No. 87084) in soil

Reports: ECM: EPA MRID No.: 49762549. Saha, M. 2017. Methods of Analysis of BAS 750 F and its Relevant Metabolites in Soil with Limit of Determination (LOD) Calculation. BASF Study Nos.: 784705, 430689, 835161. BASF Registration Document No.: 2017/7008101. Report prepared, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 480 pages. Final report issued May 17, 2017.

ILV: EPA MRID No. 49762443. Perez, R., A. Ratliff. 2016. Independent Laboratory Validation of the following methods entitled: BASF Analytical Method D1513/01: "Method for the Determination of Residues of BAS 750 F (Reg. No. 5834378) and its Metabolites, M750F003 (Reg. No. 5924326) and 1,2,4-Triazole (Reg. No. 87084) in Soil by LC-MS/MS using Micro-Extraction Procedure" And BASF Analytical Method L0214/01: "Validation of Analytical Method L0214/01 for the Determination of BAS No. 750 F (Reg. No. 5834378) and Metabolites of Reg. No. 5924326 and 1,2,4-Triazole (Reg. No. 87084) in soil by LC-MS/MS". BASF Study ID No.: 703438_1. BASF Registration Document No.: 2016/7006411. ADPEN Study No.: 16G0204. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 188 pages. Final report issued October 4, 2016.

Document No.: MRIDs 49762549 & 49762443

Guideline: 850.6100

Statements: ECM: The study was not conducted in accordance with Good Laboratory Practice (GLP) standards since it was not a study (p. 3 of MRID 49762549). Signed and dated No Data Confidentiality and GLP statements were provided; Quality Assurance and Authenticity statements were not provided (pp. 2-3). The ECM was a compilation of the study reports for two equivalent methods and LOD determination (p. 5). These three study reports (BASF Study # 784705; Study ID 430689; BASF Study # 835161) were conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (Appendix A, p. 10; Appendix B, p. 209; Appendix C, p. 412). Study ID 430689 was also conducted in accordance with OECD and German GLP (Appendix B, pp. 209, 211). Signed and dated No Data Confidentiality, GLP, Quality Assurance statements were provided (Appendix A, pp. 9-11; Appendix B, pp. 208-211; Appendix C, pp. 411-413). Authenticity statements were provided by BASF Study #s 784705 and 835161, but not Study ID 430689 (Appendix A, p. 12; Appendix C, p. 414).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (p. 3 of MRID 49762443). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

Classification: The analytical methods are classified as supplemental. **For Both Methods**, the ILV did not validate the methods using a range of soils representative of the test soils used in the terrestrial field dissipation studies. Based on the general unsatisfactory linearity and specificity data for 1,2,4-triazole over the full range tested, an updated ECM should be provided to include the ILV recommendation of alternative caps. **For BASF Method L0214/01**, ECM data was not entirely satisfactory for linearity and specificity of 1,2,4-triazole and for linearity of M750M003. **For BASF Method D1513/01**, ILV data was not entirely satisfactory for linearity and specificity of 1,2,4-triazole and for linearity of M750M003. Additionally, the ECM data was not entirely satisfactory for linearity of 1,2,4-triazole.

PC Code: 122000

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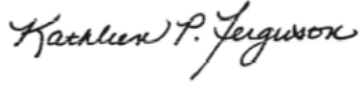
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Executive Summary

The two analytical methods, BASF Methods L0214/01 and D1513/01, are designed for the quantitative determination of mefentrifluconazole (BAS 750 F; Reg.No. 5834378), M750F003 (Reg.No. 5924326), and 1,2,4-triazole (Reg.No. 87084) in soil at the LOQ of 0.002 mg/kg (or ppb) using LC/MS/MS. The LOQ for these methods should be less than the lowest toxicological level of concern in soil for mefentrifluconazole and any metabolites included in the Residues of Concern for drinking water exposure to humans or for ecological exposure. This cannot be assessed at this time since no risk assessment has yet been completed for this new chemical petition for registration. BASF Methods L0214/01 and D1513/01 were equivalent methods, except that Method D1513/01 was a micro-extraction version. Both methods were validated by the ILV in the first trial with insignificant modifications to the analytical instrumentation, as well as the ILV recommendation that alternative caps should be used in the place of the Thermo Scientific SeptraSeal caps in order to reduce residual background of 1,2,4-triazole. Based on the general unsatisfactory linearity and specificity data for 1,2,4-triazole, an updated ECM should be provided to include the ILV recommendation of alternative caps. For each method, the ECM used two characterized soil matrices, while the ILV used one soil matrix per method. The ECM methods used two test soils each that corresponded to some of the test soils in environmental fate studies, with soils samples from two of the field dissipation study sites used for Method 1513/01 (Table 5a.) The ILV did not validate the methods using a range of soils representative of the test soils used in the terrestrial field dissipation studies since the terrestrial field dissipation studies contained soils from six test sites. For BASF Method L0214/01, all ILV data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for all three analytes. In the ECM, all data regarding repeatability,

accuracy, and precision were satisfactory for all three analytes; linearity of the method was not supported for M750F003 and 1,2,4-triazole. Additionally, it could not be determined if specificity of the method was supported for 1,2,4-triazole based on the ECM representative chromatograms. For BASF Method D1513/01, all ILV data regarding repeatability, accuracy, and precision were satisfactory for all three analytes; specificity and linearity of the method were not supported for 1,2,4-triazole. Additionally, linearity of the method was not supported for M750F003. In the ECM, all data regarding repeatability, accuracy, and precision were satisfactory for all three analytes; linearity of the method was not supported for 1,2,4-triazole. ECM representative chromatograms of 1,2,4-triazole contained very small analyte peaks.

In a separate study, the method LOD of BASF Analytical Method No. D1513/01 (micro-extraction procedure) was validated in accordance with the methodology set forth in 40 CFR Ch. 1 Part 136 Appendix B and determined to be 0.0004 mg/kg for each analyte in soil. The LOD for method L0214/01 was not conducted, as both method L0214/01 and D1513/01 are equivalent.

Table 1. Analytical Method Summary – BASF Analytical Methods D1513/01 and L0214/01

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						
Mefentrifluconazole (BAS 750 F)	49762549 ¹	49762443		Soil ^{2,3}	17/05/2017 ⁴	BASF Corporation	LC/MS/MS	0.002 mg/kg
M750F003 (Reg.No. 5924326)								
1,2,4-Triazole (Reg.No. 87084)								

1 A compilation of BASF Study # 784705; Study ID 430689; BASF Study # 835161.

2 Analytical Method D1513/01: In the ECM, sandy loam soil (TRT-BARESOIL SCS-19 0-3"; 74% sand 18% silt 8% clay; pH 6.9 in saturated paste; 0.39% organic carbon) and sand soil (SCS1 0-3"; 91% sand 8% silt 1% clay; pH 7.1 in saturated paste; 0.41% organic carbon) were used. Soils were characterized by Agvise Laboratories, Northwood, North Dakota; soil sources were not further specified. The test soils were soils from the top 3 inches of soil from BASF study 715267: trial R140864 (sandy loam) and trial R140865 (sand). In the ILV, loamy sand soil (TRT-BARESOIL SCS-23 18-24"; 76% sand 20% silt 4% clay; pH 8.1 in saturated paste; 0.05% organic carbon) was used. Soil was provided by the sponsor and characterized by Agvise Laboratories, Northwood, North Dakota.

3 Analytical Method L0214/01: In the ECM, loamy fine sand soil (LUFA 2.2; 87.4% sand 7.7% silt 5.0% clay; pH (water) 6.4; 1.72% organic carbon) and sandy loam soil (LUFA 2.3; 64.1% sand 28.3% silt 7.6% clay; pH (water) 6.1; 0.67% organic carbon) were used. Soils were characterized by BioChem agrar; soil sources were not further specified. In the ILV, silty clay loam soil (PA.IL.T.CHAR 30-36.A.; 7% sand 54% silt 39% clay; pH 7.0 in saturated paste; 0.32% organic carbon) was used. Soil was provided by the sponsor and characterized by Agvise Laboratories, Northwood, North Dakota.

4 Method date was September 30, 2016 for BASF Study # 784705; method date was July 18, 2016 for Study ID 430689; method date was May 16, 2017 for BASF Study # 835161.

All referenced page numbers for MRID 49762549 refer to those reported on the right-handed margin of the document pages.

I. Principle of the Method

Method L0214/01

Briefly, residues of BAS 750 F in soil samples (5 g each) are extracted twice by mechanical shaking with acetonitrile/water (70/30, v/v), centrifuged, and residues in an aliquot of the combined extracts are analyzed for parent BAS 750 F and M750F003 using LC-MS/MS. A separate aliquot from the combined extracts is concentrated to an aqueous remainder and diluted with water for the determination of 1,2,4-triazole using LC-MS/MS.

The method uses MS/MS detection monitoring ion transitions m/z 398→182 and 398→133 for parent BAS 750 F; m/z 288→159 and 288→103 for M750F003; and m/z 70→43 for 1,2,4-triazole. In lieu of a secondary (alternate) ion transition for 1,2,4-triazole, confirmatory analysis is performed using a different LC-MS/MS column (Hypercarb™ for the primary analysis and Synergi™ Hydro-RP for confirmation). The results are calculated by direct comparison of the sample peak responses to those of external standards.

For validation, soil samples were fortified with each analyte and analyzed according to the established method validation guidelines. The analytical sets for each soil type (LUFA 2.2 and LUFA 2.3) consisted of two controls, five replicates fortified with analyte at the method limit of quantitation, 0.002 mg/kg, and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation, 0.02 mg/kg. The final determination of residues of BAS 750 F was performed by LC-MS/MS monitoring the ion transitions listed in the table shown below (Table 2).

Table 2: Summary Parameters for the Analytical Method L0214/01 Used for the Quantitation of BAS 750 F and Metabolite Residues in Soil

Method ID	BASF method L0214/01	
Analyte(s)	Residues of BAS 750 F, M750F003, and 1,2,4-triazole in soil	
Extraction Solvent/technique	Residues of BAS 750 F in soil samples (5 g each) are extracted twice by mechanical shaking with acetonitrile/water (70/30, v/v)	
Cleanup Strategies	Centrifugation and decantation	
Instrument	<p>Analyses for BAS 750 F and M750F003 are performed on an LC system using an Aquasil™ C18 column (150 x 3 mm, 3 µm particle size) and mobile phase gradient of water:acetonitrile each acidified with 1% formic acid (95:5 to 1:99, v/v, over ~5 minutes, flow rate 800 µL/minute).</p> <p>Analyses of 1,2,4-triazole is performed on an LC system using either a Hypercarb™ (100 x 4.6 mm, 5 µm) or a Synergi™ Hydro-RP column (150 x 4.6 mm, 4 µm, for confirmation) and mobile phase gradients consisting of water:acetonitrile each acidified with 1% formic acid (95:5 to 90:10, v/v, over 2.5 minutes, or 99:1 to 95:5, v/v, over 3.0 minutes, flow rates 1 mL/minute).</p>	
Detector	AB Sciex API 5500 Mass Spectrometer	
	Quantitation (<i>m/z</i>)	Confirmation (<i>m/z</i>)
Analyte		
BAS 750 F	398→182	398→133
M750F003	288→159	288→103
1,2,4-Triazole	70→43	70→43*
Ionization Mode	Positive	Positive
Standardization Method	Linear Regression, equal and 1/x weighting were used	
Stability of Std Solutions	The available storage stability data indicate that stock and fortification solutions of each analyte prepared in acetonitrile or acetonitrile:water (70:30, v/v), and water for 1,2,4-triazole, are stable held under refrigeration for at least 3 months, and that calibration standards prepared in acetonitrile:water (70:30, v/v) (or water for 1,2,4-triazole) are stable held under refrigeration for at least 1 month.	
Expected Retention times (approximate minutes)	BAS 750 F, 4.5; M750F003, 3.5; 1,2,4-triazole, 1.80 (Hypercarb) and 2.25 (Synergi).	

*In lieu of a secondary (alternate) ion transition for 1,2,4-triazole, confirmatory analysis is performed using a different LC-MS/MS column (Hypercarb™ for the primary analysis and Synergi™ Hydro-RP column for confirmation).

Instrument/Detector for Confirmatory Method: For parent BAS 750 F and M750F003, accurate quantitation is possible using one chromatographic method and two different mass transitions; therefore, no additional confirmatory techniques are required for these analytes. In lieu of secondary (alternate) ion transitions for 1,2,4-triazole, a confirmatory technique is available using a different LC-MS/MS column (Synergi™ Hydro-RP column).

A Method Flow Chart was provided (Appendix B, Figure 18, p. 294 of MRID 49762549).

Method D1513/01

Briefly, residues of BAS 750 F in soil samples (0.1 g each) are extracted twice by mechanical shaking with acetonitrile/water (70/30, v/v), centrifuged, and residues in an aliquot of the combined extracts are diluted with acetonitrile:water (10:90, v/v) analyzed for parent BAS 750 F and M750F003 using LC-MS/MS. A separate aliquot from the combined extracts is concentrated to an aqueous remainder and diluted with water for the determination of 1,2,4-triazole using LC-MS/MS. The ion transitions monitored for BAS 750F are at *m/z* 398→70 and *m/z* 400→70 for primary and confirmation quantitation. For M750F003 and 1,2,4-triazole *m/z* 288→70 and *m/z* 70→43, respectively, are monitored for primary quantitation, and secondary chromatographic methods

monitoring the same ion transitions are used for confirmation. All transitions are monitored in positive ionization mode for primary and confirmation quantification. The results are calculated by direct comparison of the sample peak responses to those of external standards.

For validation, soil samples were fortified with each analyte and analyzed according to the established method validation guidelines. The analytical sets for each soil type (sandy loam and sand) consisted of a reagent blank, two controls, five replicates fortified with analyte at the method limit of quantitation, 0.002 mg/kg, and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation, 0.02 mg/kg. The final determination of residues of BAS 750 F was performed by LC-MS/MS monitoring the ion transitions listed in the table shown below (Table 3).

Table 3: Summary Parameters for the Analytical Method D1513/01 Used for the Quantitation of BAS 750 F and Metabolite Residues in Soil

Method ID	BASF method D1513/01	
Analyte(s)	Residues of BAS 750 F, M750F003, and 1,2,4-triazole in soil	
Extraction Solvent/technique	Residues of BAS 750 F in soil samples (0.1 g each) are extracted twice by mechanical shaking with acetonitrile/water (70/30, v/v)	
Cleanup Strategies	Centrifugation and decantation	
Instrument	<p>Analyses for BAS 750 F and M750F003 are performed on an LC system using either an Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.7 µm) and mobile phase gradient of acidified water:acetonitrile (95:5 to 1:99, v/v, over ~3.5 minutes, flow rate 600 uL/minute) or for confirmatory purposes for M750F003, an Xbridge phenyl column (2.1 x 100 mm, 2.5 µm) and mobile phase gradient of acidified water:acetonitrile (85:15 to 1:99, v/v, over ~2.5 minutes, flow rate 600 uL/minute).</p> <p>Analyses of 1,2,4-triazole is performed on an LC system using either a Hypercarb column (100 x 4.6 mm, 3 µm) with a mobile phase gradient consisting of acidified water:acetonitrile (99:1 to 50:50, v/v, over 3.5 minutes, flow rate 800 uL/minute), or for confirmation, a Unison UK-C18 column (75 x 3 mm, 3 µm) coupled to a Thermo Hypercarb (50 x 4.6, 3 µm) and mobile phase gradient of acidified water:acetonitrile (99:1, v/v, over 3.0 minutes, flow rate 1 mL/minute).</p>	
Detector	AB Sciex API 5500 Mass Spectrometer	
Analyte	Quantitation (m/z)	Confirmation (m/z)
BAS 750 F	398→70	400→70
M750F003	288→70	288→70*
1,2,4-Triazole	70→43	70→43*
Ionization Mode	Positive	Positive
Standardization Method	Linear Regression, equal weighting	
Stability of Std Solutions	Stock and intermediate (fortification) standard solutions of BAS 750 F and M750F003 were prepared in acetonitrile and were shown to be stable up to 3 months (92 days) in acetonitrile when held under refrigeration. The calibration solutions of BAS 750 F or M750F003 were prepared by serial dilution of the fortification standards with acetonitrile:water (20:80, v/v) and were shown to be stable for up to 1 month (31 days) under refrigeration. Stock, intermediate and calibration standards solutions of 1,2,4 triazole were prepared in water and exhibited stability under refrigeration for up to 3 months (92 days) in water.	
Expected Retention times (approximate minutes)	BAS 750 F, 2.5 (both primary and confirmatory); M750F003, 1.6 (primary); 2.1 (confirmatory); 1,2,4-triazole, 2.9 (primary); 2.8 (confirmatory).	

*In lieu of a secondary ion transition for M750F003 and 1,2,4-triazole, confirmatory analysis was performed using a different LC-MS/MS column. The report indicated that, due to instrument sensitivity, the secondary transition available for M750F003 (mz 288→103) was not strong enough for quantitation; therefore the alternative chromatographic conditions for M750F003 were used.

Instrument/Detector for Confirmatory Method: For parent BAS 750 F, accurate quantitation is possible using one chromatographic method and two different mass transitions; therefore, no additional confirmatory techniques are required for these analytes. No matrix suppression or enhancement was found to affect the analyte. For M750F003 and 1,2,4-Triazole, secondary mass transitions were not reliable for quantitation, therefore two independent chromatographic method using the primary quantitation transition were used.

ILV

The ILV performed the ECM methods as written with insignificant modifications of the analytical method. For Analytical Methods D1513/01 and L0214/01, the ILV recommended that alternative caps should be used in the place of the Thermo Scientific SeptraSeal caps in order to reduce residual background of 1,2,4-triazole (pp. 25-26; Appendix A, p. 128 of MRID 49762443; pp. 20-21 of MRID 49762549). Also, a typographical error was identified. The ILV parameters are reported in Tables 4 and 5 below.

Table 4: Summary Parameters for the Analytical Method D1513/01 Used in the ILV for the Quantitation of BAS 750 F and Metabolite Residues in Soil

Method ID	BASF method D1513/01		
Analyte(s)	Residues of BAS 750 F, M750F003, and 1,2,4-triazole in soil		
Extraction Solvent/technique	Residues of BAS 750 F in soil samples (0.1 g each) are extracted twice by mechanical shaking with acetonitrile:water (70:30, v/v), centrifuged and residues in an aliquot of the combined extracts are diluted with acetonitrile:water (10:90, v/v) and analyzed by LC-MS/MS. A separate aliquot from the combined extracts is concentrated to an aqueous remainder and diluted with water for the determination of 1,2,4-triazole using LC-MS/MS.		
Cleanup Strategies	Centrifugation and decantation		
Instrument	<p>Analyses for BAS 750 F and M750F003 were performed on an LC system using either an Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.7 µm) and mobile phase gradient of acidified water:acetonitrile (95:5 to 1:99, v/v, over ~3.5 minutes, flow rate 600 uL/minute) or for confirmatory purposes for M750F003, an Xbridge BEH phenyl column (2.1 x 100 mm, 1.7 µm) and mobile phase gradient of acidified water:acetonitrile (85:15 to 1:99, v/v, over ~2.5 minutes, flow rate 600 uL/minute).</p> <p>Analyses of 1,2,4-triazole was performed on an LC system using either a Hypercarb column (100 x 4.6 mm, 3 µm) with a mobile phase gradient consisting of acidified water:acetonitrile (95:5 to 90:10, v/v, over 2.5 minutes, flow rate 800 uL/minute), or for confirmation, a Unison UK-C18 column (75 x 3 mm, 3 µm) coupled to a Thermo Hypercarb (50 x 4.6, 3 µm) and mobile phase gradient of acidified water:acetonitrile (99:1, v/v, over 3.0 minutes, flow rate 1 mL/minute).</p>		
Detector	AB Sciex API 5500 Mass Spectrometer		
	Analyte	Quantitation (m/z)	Confirmation (m/z)
	BAS 750 F	398→70	400→70
	M750F003	288→70	288→70*
	1,2,4-Triazole	70→43	70→43*
	Ionization Mode	Positive	Positive
	Standardization Method	Linear Regression, equal weighting	
	Stability of Std Solutions	The available storage stability data indicate that stock and intermediate (fortification) standard solutions of BAS 750 F and M750F003 prepared in acetonitrile are stable up to 3 months when held under refrigeration and that calibration solutions of BAS 750 F and M750F003 prepared by serial dilution of intermediate standards with acetonitrile:water (20:80, v/v) are	

Table 4: Summary Parameters for the Analytical Method D1513/01 Used in the ILV for the Quantitation of BAS 750 F and Metabolite Residues in Soil

Method ID	BASF method D1513/01
	stable for at least 1 month under refrigeration. The storage stability data also indicate that standard solutions of 1,2,4 triazole in water are stable for up to 3 months held under refrigeration.
Retention times (minutes)	BAS 750 F, 3.0 (both primary and confirmatory); M750F003, 2.1 (primary); 2.0 (confirmatory); 1,2,4-triazole, 1.6 (primary); 1.4 (confirmatory).

*In lieu of a secondary ion transition for M750F003 and 1,2,4-triazole, confirmatory analysis was performed using a different LC-MS/MS column.

Table 5: Summary Parameters for the Analytical Method L0214/01 Used in the ILV for the Quantitation of BAS 750 F and Metabolite Residues in Soil

Method ID	BASF method L0214/01	
Analyte(s)	Residues of BAS 750 F, M750F003, and 1,2,4-triazole in soil	
Extraction Solvent/technique	Residues of BAS 750 F in soil samples (5 g each) are extracted twice by mechanical shaking with acetonitrile:water (70:30, v/v), centrifuged, and residues in an aliquot of the combined extracts are then analyzed for BAS 750 F and M750F003 using LC-MS/MS. A separate aliquot from the combined extracts is concentrated to an aqueous remainder and diluted with water for the determination of 1,2,4-triazole using LC-MS/MS.	
Cleanup Strategies	Centrifugation and decantation	
Instrument	Analyses for BAS 750 F and M750F003 were performed on an LC system using an Aquasil™ C18 column (150 x 3 mm, 3 µm particle size) and mobile phase gradient of water:acetonitrile each acidified with 0.1% formic acid (95:5 to 1:99, v/v, over ~5 minutes, flow rate 800 µL/minute). Analyses of 1,2,4-triazole was performed on an LC system using either a Hypercarb™ (100 x 4.6 mm, 3 µm) and mobile phase gradient consisting of water:acetonitrile each acidified with 0.1% formic acid (95:5 to 90:10, v/v, over 2.5 minutes, flow rate 1 mL/minute), or a Synergi™ Hydro-RP column (150 x 3.9 mm, 4 µm) and a mobile phase gradient consisting of water:acetonitrile each acidified with 0.1% formic acid (99:1 to 95:5, v/v, over 3.0 minutes, flow rate 1 mL/minute).	
Detector	AB Sciex API 5500 Mass Spectrometer	
	Analyte	Quantitation (m/z)
	BAS 750 F	398→182
	M750F003	288→159
	1,2,4-Triazole	70→43
		Confirmation (m/z)
		398→133
		288→103
		70→43*
Ionization Mode	Positive	
Standardization Method	Linear Regression, 1/x weighting	
Stability of Std Solutions	The available storage stability data indicate that stock and intermediate (fortification) standard solutions of BAS 750 F and M750F003 prepared in acetonitrile are stable up to 3 months when held under refrigeration and that calibration solutions of BAS 750 F and M750F003 prepared by serial dilution of intermediate standards with acetonitrile:water (70:30, v/v) are stable for at least 1 month under refrigeration. The storage stability data also indicate that standard solutions of 1,2,4 triazole in water are stable for up to 3 months held under refrigeration.	
Retention times (minutes)	BAS 750 F, 5.3; M750F003, 4.2; 1,2,4-triazole, 1.8 (Hypercarb) and 1.7 (Synergi).	

*In lieu of a secondary (alternate) ion transition for 1,2,4-triazole, confirmatory analysis is performed using a different LC-MS/MS column (Hypercarb™ and Synergi™ Hydro-RP columns are available)

For method L0214/01, all samples were analyzed using matrix-matched standards. For method D1513/01, all samples were analyzed using matrix-matched standards, except for the analyses of M750F003 and 1,2,4-triazole under secondary chromatographic conditions which used solvent-based standards.

Methodology to Evaluate MDL and LOD

Evaluation of LOD of BASF Analytical Method No. D1513/01 required the experimental determination of MDL as defined by 40 CFR Ch.1 Part 136 appendix B. Within the scope of the method to determine the residues of BAS 750 F, two independent injections were used for analysis. An MDL determination was conducted on both of two independent injections that are required by the method. A summary of the test soils used to develop and evaluate the methods is provided in Table 5a. These test soils did correspond in location and characteristics to some of the soils used in specific environmental fate studies, including two soils taken from the location of two field dissipation studies for Method D1513/01 (see Table 5a). Different soils (not related to any of those used in field dissipation or other environmental fate studies) were used in the ILV. Analysis of the physical-chemical properties of these soils was provided but no location or taxonomic information is provided for the soil samples used for the ILV. A brief description of the methodology to determine MDL is as follows:

1. Standards containing all analytes were injected using the LC-MS/MS parameters from Method D1513/01. All transitions were monitored according to the method, including those set forth for the secondary chromatographic techniques. The least sensitive transition of the least sensitive analyte for each independent injection of the method was determined qualitatively through visual inspection of factors such as peak height, relative background level, area count, etc. Once the appropriate analytes and transitions were selected, an estimation was made to what level a sample in matrix would produce a S/N of 2-10.
2. Using BASF Analytical Method No. D1513/01, seven control samples (0.1 g each) were extracted by shaking twice with a mixture of acetonitrile-water (70:30, v/v), using 0.8 mL for each extraction. For analysis of BAS 750 F and M750F003, an aliquot (0.1 mL) from the combined extract was diluted with a calibration standard (acetonitrile-water, 20:80, v/v, 0.2 mL) and water (0.2 mL) to an appropriate spiking level for analysis by LC-MS/MS. For analysis of 1,2,4-triazole, a 0.2 mL aliquot was concentrated to ~0.025 mL under nitrogen at 20°C brought to 0.5 mL in a calibration standard to an appropriate spiking level for the LC-MS/MS analysis. These seven matrix spiked samples were injected on the LC-MS/MS with bracketing neat standards for quantitation.

3. Using the standard curve to calculate the concentrations of the seven matrix-spiked samples, the results are put into the equation: $MDL = S \times t_{(N-1, 1-\alpha=0.99)}$, where

MDL = Method detection limit

S = Standard deviation of the matrix-spiked sample set concentrations

$t_{(N-1, 1-\alpha=0.99)}$ = Critical t value from a student t-test table at 99% confidence

The acceptance criteria for the MDL calculation was:

- a. The calculated MDL must be able to be seen on the instrument with S/N of ≥ 2 .
- b. The concentration of the matrix-spiked samples must be no greater than 10X the calculated MDL.

If either of the above to criteria were not met, the experiment had to be repeated at a higher or lower spiking concentration, respectively, until all criteria are met. Reinjection of the samples with lower or higher injection volumes could be done in place of preparing new matrix-spiked samples as it changes the amount (pg) injected on the column the same.

4. A matrix-spiked sample at the calculated MDL was injected on the LC-MSMS (no standard curve is required) to verify the MDL can be seen at ≥ 2 S/N.

Based on an evaluation of the susceptibility of the analyte of interest to instrument variability, LC-MS/MS drift, unexpected contamination, and untested matrix effects, the MDL was raised to an appropriate value that will mitigate the anticipated issues. This new value is the LOD.

LOQ/LOD

The LOQ was defined by the lowest fortification level successfully tested. The validated LOQ for residues of BAS 750 F and its metabolites in soil is 0.002 mg/kg, for each analyte. The limit of determination is set to be 0.0004 mg/kg for each analyte in soil.

II. Recovery Findings

Method L0214/01

ECM (MRID 49762549): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD $\leq 20\%$) for analysis of mefentrifluconazole (BAS 750 F; Reg.No. 5834378), M750F003 (Reg.No. 5924326), and 1,2,4-triazole (Reg.No. 87084) in two soil matrices at fortification levels of 0.002 mg/kg (LOQ) and 0.02 mg/kg (10 \times LOQ; Appendix B, pp. 213). Mefentrifluconazole, M750F003, and 1,2,4-triazole were identified using two ion transitions or techniques; performance data (recovery results) from primary and confirmatory analyses were comparable. The loamy fine sand soil (LUF 2.2; 87.4% sand 7.7% silt 5.0% clay; pH (water) 6.4; 1.72% organic carbon) and sandy loam soil (LUF 2.3; 64.1% sand 28.3% silt 7.6% clay; pH (water) 6.1; 0.67% organic carbon) were used (USDA soil textural classification; Appendix B, p. 228; Appendix B, Figures 70-71, pp. 392-397). Soils were characterized by BioChem agrar; soil sources were not further specified.

Table 5a: Summary Characteristics of Soils Used to Develop and Evaluate Mefentrifluconazole Residue Analytical Methods.

Methods and soils for ECM Original and Independent Evaluations	Summary of Soil Characteristics	Relationship to Environmental Fate Test Soils
<u>Method L0214/01</u> ECM (MRID 49762549)	<p>1. loamy fine sand soil (LUFA 2.2; 87.4% sand 7.7% silt 5.0% clay; pH (water) 6.4; 1.72% organic carbon).</p> <p>2. sandy loam soil (LUFA 2.3; 64.1% sand 28.3% silt 7.6% clay; pH (water) 6.1; 0.67% organic carbon).</p> <p>(USDA soil textural classification; Appendix B, p. 228; Appendix B, Figures 70-71, pp. 392-397).</p>	<p>Identical in soil taxonomy and from the same general location as the LUFA 2.1 soil used for the soil adsorption / desorption study (MRID 49762346).</p> <p>Similar to LUFA 2.1 and 2.2.</p>
<u>ILV (MRID 49762443)</u>	<p>1. silty clay loam soil (PA.IL.T.CHAR 30-36.A.; 7% sand 54% silt 39% clay; pH 7.0 in saturated paste; 0.32% organic carbon)</p> <p>(USDA soil textural classification; p. 17; Appendix F, p. 183)</p>	<p>“The control soil sample was homogenized and provided by BASF”. No soil taxonomic information or location information was provided.</p>
<u>Method D1513/01</u> ECM (MRID 49762549):	<p>1. sandy loam soil (TRT-BARESOIL SCS-19 0-3”; 74% sand 18% silt 8% clay; pH 6.9 in saturated paste; 0.39% organic carbon)</p> <p>2. sand soil (SCS1 0-3”; 91% sand 8% silt 1% clay; pH 7.1 in saturated paste; 0.41% organic carbon)</p> <p>(USDA soil textural classification; Appendix A, p. 21-22; Appendix A, Appendix A, pp. 43-44)</p>	<p>Soils #1 and #2 are stated to correspond to the test soils used for two field dissipation studies in MRID 49762441: “Terrestrial field dissipation of the fungicide BAS 750 F following application of a suspension concentrate formulation to bare soil and turf plots in California and Georgia”</p>
<u>ILV (MRID 49762443)</u>	<p>1. loamy sand soil (TRT-BARESOIL SCS-23 18-24”; 76% sand 20% silt 4% clay;</p>	<p>“The control soil sample was homogenized and provided by BASF”. No soil taxonomic</p>

	pH 8.1 in saturated paste; 0.05% organic carbon) (USDA soil textural classification; p. 17; Appendix F, p. 184).	information or location information was provided.
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ILV (MRID 49762443): Mean recoveries and RSDs were within guideline requirements for analysis of mefentrifluconazole (BAS 750 F; Reg.No. 5834378), M750F003 (Reg.No. 5924326), and 1,2,4-triazole (Reg.No. 87084) in one soil matrix at fortification levels of 0.002 mg/kg (LOQ) and 0.02 mg/kg (10×LOQ; p. 22). Mefentrifluconazole, M750F003, and 1,2,4-triazole were identified using two ion transitions or techniques; performance data (recovery results) from primary and confirmatory analyses were comparable. The silty clay loam soil (PA.IL.T.CHAR 30-36.A.; 7% sand 54% silt 39% clay; pH 7.0 in saturated paste; 0.32% organic carbon) was used (USDA soil textural classification; p. 17; Appendix F, p. 183). Soil was provided by the sponsor and characterized by Agvise Laboratories, Northwood, North Dakota. The method was validated in the first trial with insignificant modifications to the analytical instrumentation (pp. 25-26; Appendix A, p. 128). For both methods, the ILV recommended that alternative caps should be used in the place of the Thermo Scientific SeptraSeal caps in order to reduce residual background of 1,2,4-triazole.

Method D1513/01

ECM (MRID 49762549): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of mefentrifluconazole (BAS 750 F; Reg.No. 5834378), M750F003 (Reg.No. 5924326), and 1,2,4-triazole (Reg.No. 87084) in two soil matrices at fortification levels of 0.002 mg/kg (LOQ) and 0.02 mg/kg (10×LOQ; Appendix A, Table 1, pp. 32-33). Mefentrifluconazole, M750F003, and 1,2,4-triazole were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. The sandy loam soil (TRT-BARESOIL SCS-19 0-3"; 74% sand 18% silt 8% clay; pH 6.9 in saturated paste; 0.39% organic carbon) and sand soil (SCS1 0-3"; 91% sand 8% silt 1% clay; pH 7.1 in saturated paste; 0.41% organic carbon) were used (USDA soil textural classification; Appendix A, p. 21-22; Appendix A, Appendix A, pp. 43-44). Soils were characterized by Agvise Laboratories, Northwood, North Dakota; soil sources were not further specified. The test soils were soils from the top 3 inches of soil from BASF study 715267: trial R140864 (sandy loam) and trial R140865 (sand).

ILV (MRID 49762443): Mean recoveries and RSDs were within guideline requirements for analysis of mefentrifluconazole (BAS 750 F; Reg.No. 5834378), M750F003 (Reg.No. 5924326), and 1,2,4-triazole (Reg.No. 87084) in one soil matrix at fortification levels of 0.002 mg/kg (LOQ) and 0.02 mg/kg (10×LOQ; p. 21). Mefentrifluconazole, M750F003, and 1,2,4-triazole were identified using two ion transitions or techniques; performance data (recovery results) from primary and confirmatory analyses were comparable. The loamy sand soil (TRT-BARESOIL SCS-23 18-24"; 76% sand 20% silt 4% clay; pH 8.1 in saturated paste; 0.05% organic carbon) was used (USDA soil textural classification; p. 17; Appendix F, p. 184). Soil was provided by the sponsor and characterized by Agvise Laboratories, Northwood, North Dakota. The method was validated in

the first trial with insignificant modifications to the analytical instrumentation (pp. 25-26; Appendix A, p. 128). For both methods, the ILV recommended that alternative caps should be used in the place of the Thermo Scientific SepraSeal caps in order to reduce residual background of 1,2,4-triazole.

Table 6a. Initial Validation Method Recoveries for Mefentrifluconazole (BAS 750 F; Reg.No. 5834378), M750F003 (Reg.No. 5924326), and 1,2,4-Triazole (Reg.No. 87084) in Soil¹ Using BASF Analytical Method L0214/01

Analyte	Matrix	Fortification Levels (mg/kg)	n	Recovery (%)	Average Rec. (%)	SD ²	%RSD
BAS 750 F	LUFA 2.2	Primary Quantitation (m/z 398→182)					
		0.002	5	101, 102, 104, 100, 101	102	--	1.6
		0.02	5	102, 105, 102, 102, 102	102	--	1.4
		Overall	10	Range, 100 - 105	102	--	1.4
		Confirmatory Quantitation (m/z 398→133)					
		0.002	5	97, 101, 97, 99, 96	98	--	2.1
		0.02	5	102, 104, 102, 102, 102	102	--	1.0
		Overall	10	Range, 96 - 104	100	--	2.8
BAS 750 F	LUFA 2.3	Primary Quantitation (m/z 398→182)					
		0.002	5	108, 109, 112, 105, 110	109	--	2.4
		0.02	5	108, 105, 82, 99, 85	96	--	12.1
		Overall	10	Range, 82 - 112	102	--	10.2
		Confirmatory Quantitation (m/z 398→133)					
		0.002	5	106, 102, 110, 110, 107	107	--	3.2
		0.02	5	107, 110, 81, 104, 81	96	--	15.0
		Overall	10	Range, 81 - 110	102	--	11.2
M750F003	LUFA 2.2	Primary Quantitation (m/z 288→159)					
		0.002	5	103, 103, 100, 101, 98	101	--	2.3
		0.02	5	100, 102, 101, 104, 103	102	--	1.6
		Overall	10	Range, 98 - 104	101	--	2.0
		Confirmatory Quantitation (m/z 288→103)					
		0.002	5	102, 95, 102, 98, 94	98	--	3.7
		0.02	5	99, 101, 104, 105, 106	103	--	2.7
		Overall	10	Range, 94 - 106	100	--	4.0
M750F003	LUFA 2.3	Primary Quantitation (m/z 288→159)					
		0.002	5	102, 80, 106, 105, 105	99	--	11.2
		0.02	5	84, 79, 82, 80, 84	82	--	2.7
		Overall	10	Range, 79 - 106	91	--	13.3
		Confirmatory Quantitation (m/z 288→103)					
		0.002	5	104, 88, 97, 102, 106	99	--	7.3
		0.02	5	82, 78, 84, 86, 80	82	--	4.1
		Overall	10	Range, 78 - 106	91	--	11.6
1,2,4-Triazole	LUFA 2.2	Primary Quantitation (m/z 70→43) – Hypercarb column					
		0.002	5	100, 103, 106, 104, 98	102	--	3.1
		0.02	5	99, 82, 86, 86, 88	88	--	7.5
		Overall	10	Range, 82 - 106	95	--	9.3
		Confirmatory Technique (m/z 70→43) – Synergi column					
		0.002	5	93, 94, 93, 94, 93	93	--	0.5
0.02	5	99, 98, 98, 93, 97	97	--	2.5		

Analyte	Matrix	Fortification Levels (mg/kg)	n	Recovery (%)	Average Rec. (%)	SD ²	%RSD
		Overall	10	Range, 94 - 106	95	--	2.5
1,2,4-Triazole	LUFA 2.3	Primary Quantitation (m/z 70→43) – Hypercarb column					
		0.002	5	94, 76, 89, 92, 92	88	--	8.4
		0.02	5	85, 86, 88, 84, 80	85	--	3.7
		Overall	10	Range, 76 - 94	87	--	6.7
		Confirmatory Technique (m/z 70→43) – Synergi column					
		0.002	5	95, 86, 99, 99, 96	95	--	5.6
		0.02	5	100, 100, 87, 97, 96	96	--	5.6
		Overall	10	Range, 86 - 100	96	--	5.3

Data (uncorrected recovery results, Appendix B, pp. 236-237 and Tables 10-21, pp. 246-251) were obtained from Appendix B, pp. 213 of MRID 49762549.

1 The loamy fine sand soil (LUFA 2.2; 87.4% sand 7.7% silt 5.0% clay; pH (water) 6.4; 1.72% organic carbon) and sandy loam soil (LUFA 2.3; 64.1% sand 28.3% silt 7.6% clay; pH (water) 6.1; 0.67% organic carbon) were used (USDA soil textural classification; Appendix B, p. 228; Appendix B, Figures 70-71, pp. 392-397). Soils were characterized by BioChem agrar; soil sources were not further specified.

2 SD = Standard Deviation; the SD was not reported.

Table 6b. Independent Validation Method Recoveries for Mefentrifluconazole (BAS 750 F; Reg.No. 5834378), M750F003 (Reg.No. 5924326), and 1,2,4-Triazole (Reg.No. 87084) in Soil¹ Using BASF Analytical Method L0214/01

Matrix	Fortification Levels (ppm)	n	Recovery (%)	Average Recovery (%)	Standard Deviation	%RSD ²
Silty Clay Loam Soil (Sample R1405960007 from BASF study no. 433578)	BAS 750 F					
	Primary Quantitation (m/z 398 → 182)					
	0.002	5	104, 107, 110, 90, 86	99	10.7	10.8
	0.02	5	111, 99, 99, 95, 88	98	8.4	8.6
	Overall	10	Range: 86–111	99	9.1	9.2
	Secondary Quantitation (m/z 398 → 133)					
	0.002	5	108, 97, 109, 78, 87	96	13.4	14.0
	0.02	5	109, 88, 97, 94, 85	95	9.4	9.9
	Overall	10	Range: 78–109	95	10.9	11.5
	M750F003					
	Primary Quantitation (m/z 288 → 159)					
	0.002	5	109, 98, 104, 100, 106	103	4.4	4.2
	0.02	5	99, 95, 102, 104, 104	101	3.9	3.8
	Overall	10	Range: 95–109	102	4.1	4.0
	Secondary Quantitation (m/z 288 → 103)					
	0.002	5	103, 110, 113, 105, 104	107	4.4	4.1
	0.02	5	91, 94, 99, 102, 92	96	4.8	5.1
	Overall	10	Range: 91–113	101	7.4	7.4
	1,2,4-Triazole					
	Primary Quantitation (m/z 70 → 43)					
	0.002	5	98, 99, 99, 97, 99	98	0.9	1.0
0.02	5	106, 99, 117, 108, 114	109	7.1	6.6	
Overall	10	Range: 97–117	104	7.2	6.9	
Secondary Chromatographic Condition for Quantitation (m/z 70 → 43) ³						
0.002	5	94, 100, 96, 96, 97	97	2.2	2.3	
0.02	5	99, 97, 102, 96, 94	98	3.3	3.4	
Overall	10	Range: 94–102	97	2.7	2.8	

Data (uncorrected recovery results) were obtained from p. 22 of MRID 49762443

¹ Silty clay loam soil (PA.IL.T.CHAR 30-36.A.; 7% sand 54% silt 39% clay; pH 7.0 in saturated paste; 0.32% organic carbon) was used (USDA soil textural classification; p. 17; Appendix F, p. 183). Soil was provided by the sponsor and characterized by Agvise Laboratories, Northwood, North Dakota.

² Relative Standard Deviation = (Standard Deviation ÷ Average Recovery) × 100

³ Confirmatory analysis was conducted using the secondary chromatographic conditions, which uses the Synergi Hydro RP column (4 µm, 4.6 × 150 mm).

Table 7a. Initial Validation Method Recoveries for Mefentrifluconazole (BAS 750 F; Reg.No. 5834378), M750F003 (Reg.No. 5924326), and 1,2,4-Triazole (Reg.No. 87084) in Soil¹ Using BASF Analytical Method D1513/01

Matrix	Fortification Levels (mg/kg)	n	Recovery (%)	Average Recovery (%)	Standard Deviation	%RSD ^a
BAS 750 F from Soil (Sandy Loam)	BAS 750 F: Primary Transition (<i>m/z</i> 398 → 70)					
	0.002	5	102, 106, 97.7, 90.9, 99.4	99.2	5.60	5.65
	0.02	8	97.4, 97.4, 126, 100, 93.8, 91.0, 99.8, 90.2	99.5	11.5	11.53
	Overall	13	Range: 90.2–126	99.4	9.30	9.40
	BAS 750 F: Secondary Transition (<i>m/z</i> 400 → 70)					
	0.002	5	98.0, 103, 90.3, 96.4, 89.9	95.5	5.50	5.76
	0.02 ^b	8	98.0, 105, 119, 91.4, 98.9, 87.5, 97.7, 84.4	97.7	10.9	11.13
Overall	13	Range: 84.4–119	96.9	9.00	9.26	
BAS 750 F from Soil (Sand)	BAS 750 F: Primary Transition (<i>m/z</i> 398 → 70)					
	0.002	5	99.3, 104, 102, 95.6, 92.1	98.6	4.80	4.88
	0.02	5	107, 89.7, 90.4, 94.9, 120	100	12.9	12.89
	Overall	10	Range: 89.7 - 120.1	99.5	9.30	9.30
	BAS 750 F: Secondary Transition (<i>m/z</i> 400 → 70)					
	0.002	5	107, 105, 93.3, 98.5, 97.7,	100	5.60	5.58
	0.02	5	107, 87.2, 84.2, 95.9, 107	96.3	10.7	11.12
Overall	10	Range: 84.2–107.2	98.3	8.30	8.48	
M750F003 from Soil (Sandy Loam)	M750F003: Primary Transition (<i>m/z</i> 288 → 70)					
	0.002	5	96.7, 96.9, 89.8, 95.2, 98.7	95.5	3.40	3.57
	0.02	5	105, 101, 93.2, 96.0, 89.2	96.9	6.20	6.44
	Overall	10	Range: 89.2–105	96.2	4.80	4.99
	M750F003: Confirmatory method; Transition (<i>m/z</i> 288 → 70) ^b					
	0.002	5	94.2, 96.9, 107, 88.8, 88.3	95.0	7.60	8.00
	0.02	5	103, 107, 91.8, 85.4, 73.4	92.1	13.6	14.71
Overall	10	Range: 73.4–107	93.5	10.5	11.19	
M750F003 from Soil (Sand)	M750F003: Primary Transition (<i>m/z</i> 288 → 70)					
	0.002	5	105, 91.3, 95.3, 96.7, 91.1	95.9	5.70	5.90
	0.02	5	99.0, 104, 95.5, 99.8, 99.0	99.4	3.00	3.06
	Overall	10	Range: 91.1–105	97.7	4.70	4.79
	M750F003: Confirmatory method; Transition (<i>m/z</i> 288 → 70) ^b					
	0.002	5	79.7, 66.0, 94.9, 92.4, 96.7	85.9	13.0	15.13
	0.02	5	71.9, 81.6, 77.3, 92.4, 94.4	83.5	9.70	11.61
Overall	10	Range: 66.0–96.7	84.7	10.9	12.85	
1,2,4-Triazole from Soil (Sandy Loam)	1,2,4-Triazole: Primary Transition (<i>m/z</i> 70 → 43)					
	0.002	5	93.6, 83.5, 80.1, 85.7, 80.0	84.6	5.60	6.62
	0.02	5	87.0, 86.6, 73.8, 83.5, 69.4	80.1	8.00	10.00
	Overall	10	Range: 69.4–93.6	82.3	6.90	8.42
	1,2,4-Triazole: Confirmatory method; Transition (<i>m/z</i> 70 → 43) ^b					
0.002	5	80.7, 83.0, 82.6, 88.1, 90.7	85.0	4.20	4.95	

Matrix	Fortification Levels (mg/kg)	n	Recovery (%)	Average Recovery (%)	Standard Deviation	%RSD ^a
	0.02	5	96.0, 96.5, 81.6, 99.4, 81.2	90.9	8.80	9.70
	Overall	10	Range: 80.7–99.4	88.0	7.20	8.21
1,2,4-Triazole from Soil (Sand)	1,2,4-Triazole: Primary Transition (<i>m/z</i> 70 → 43)					
	0.002	5	77.2, 84.0, 89.2, 85.5, 80.0	83.2	4.70	5.65
	0.02	5	78.0, 80.2, 89.0, 83.9, 92.6	84.7	6.10	7.15
	Overall	10	Range: 77.2–92.6	84.0	5.20	6.17
	1,2,4-Triazole: Confirmation Transition (<i>m/z</i> 70 → 43) ^b					
	0.002	5	79.6, 86.6, 110, 81.7, 84.1	88.4	12.4	13.99
	0.02	5	81.8, 80.9, 84.6, 85.9, 88.8	84.4	3.20	3.79
	Overall	10	Range: 79.6–110	86.4	8.80	10.15

Data (uncorrected recovery results, Appendix A, Appendices D-E, pp. 71-92) were obtained from Appendix A, Table 1, pp. 32-33 of MRID 49762549.

¹ The sandy loam soil (TRT-BARESOIL SCS-19 0-3”); 74% sand 18% silt 8% clay; pH 6.9 in saturated paste; 0.39% organic carbon) and sand soil (SCS1 0-3”); 91% sand 8% silt 1% clay; pH 7.1 in saturated paste; 0.41% organic carbon) were used (USDA soil textural classification; Appendix A, p. 21-22; Appendix A, Appendix A, pp. 43-44). Soils were characterized by Agvise Laboratories, Northwood, North Dakota; soil sources were not further specified. The test soils were soils from the top 3 inches of soil from BASF study 715267: trial R140864 (sandy loam) and trial R140865 (sand).

^a Relative Standard Deviation = (Standard Deviation ÷ Average Recovery) × 100

^b Secondary Chromatographic method used for confirmation

Table 7b. Independent Validation Method Recoveries for Mefentrifluconazole (BAS 750 F; Reg.No. 5834378), M750F003 (Reg.No. 5924326), and 1,2,4-Triazole (Reg.No. 87084) in Soil¹ Using BASF Analytical Method D1513/01

Matrix	Fortification Levels (ppm)	n	Recovery (%)	Average Recovery (%)	Standard Deviation	%RSD ²
Loamy Sand Soil (Sample R1408640049 from BASF study no. 715267)	BAS 750 F					
	Primary Quantitation (m/z 398 → 70)					
	0.002	5	98, 118, 103, 105, 103	105	7.5	7.1
	0.02	5	87, 102, 92, 93, 96	94	5.5	5.8
	Overall	10	Range: 87–118	100	8.7	8.7
	Secondary Quantitation (m/z 400 → 70)					
	0.002	5	99, 125, 107, 94, 106	106	11.8	11.1
	0.02	5	83, 87, 94, 98, 90	90	5.8	6.4
	Overall	10	Range: 83–125	98	12.1	12.4
	M750F003					
	Primary Quantitation (m/z 288 → 70)					
	0.002	5	89, 112, 106, 105, 103	103	8.4	8.1
	0.02	5	83, 87, 92, 90, 90	88	3.4	3.8
	Overall	10	Range: 83–112	96	9.8	10.3
	Secondary Chromatographic Conditions for Quantitation (m/z 288 → 70) ³					
	0.002	5	119, 113, 108, 114, 114	113	3.8	3.4
	0.02	5	114, 116, 113, 116, 106	113	4.3	3.8
	Overall	10	Range: 106–119	113	3.8	3.4
	1,2,4-Triazole					
	Primary Quantitation (m/z 70 → 43)					
0.002	5	109, 82, 104, 90, 92	95	11.1	11.6	
0.02	5	119, 114, 112, 117, 96	112	9.1	8.1	
Overall	10	Range: 82–119	104	12.8	12.4	
Secondary Chromatographic Conditions for Quantitation (m/z 70 → 43) ⁴						
0.002	5	79, 75, 79, 74, 98	81	9.9	12.2	
0.02	5	77, 106, 92, 80, 94	90	11.8	13.2	
Overall	10	Range: 74–106	86	11.3	13.2	

Data (uncorrected recovery results) were obtained from p. 21 of MRID 49762443.

¹ Loamy sand soil (TRT-BARESOIL SCS-23 18-24²; 76% sand 20% silt 4% clay; pH 8.1 in saturated paste; 0.05% organic carbon) was used (USDA soil textural classification; p. 17; Appendix F, p. 184). Soil was provided by the sponsor and characterized by Agvise Laboratories, Northwood, North Dakota.

² Relative Standard Deviation = (Standard Deviation ÷ Average Recovery) × 100

³ Confirmatory analysis was conducted using the alternative chromatographic method, which uses the XBridge BEH Phenyl column (1.7 µm, 2.1 × 100 mm).

⁴ Confirmatory analysis was conducted using the alternative chromatographic method, which uses the Unison UK-C18 column (3 µm, 3 × 75 mm) coupled to a Thermo Hypercarb column; (3 µm, 4.6 x 50 mm).

III. Method Characteristics

The LOQ was defined by the lowest fortification level successfully tested. The validated LOQ for residues of BAS 750 F and its metabolites in soil is 0.002 mg/kg, for each analyte, corresponding to a concentration of 0.125 ng/mL for BAS 750 F and M750F003, and 0.625 ng/mL for 1,2,4-triazole in the soil extract. The reported LOD for each analyte in soil was 20% of the LOQ, equivalent to 0.0004 mg/kg.

Evaluation of the LOD. In a separate study (BASF Study No. 835161), the method limit of detection (LOD) of BASF Analytical Method No. D1513/01 (micro-extraction procedure) was tested and evaluated in accordance with the methodology set forth in 40 CFR Ch. 1 Part 136 Appendix B and determined to be 0.0004 mg/kg for each analyte in soil.

The LOD for method L0214/01 was not conducted, as both method L0214/01 and D1513/01 are equivalent.

The results for Injection 1 (for analysis of BAS 750 F and M750F003) are summarized below.

Table 8: Calculation of MDL for M750F003 (m/z 288→70) for Method D1513/01, using the Methodology Set Forth in 40 CFR Ch 1 Part 136 Appendix B

0.002 ng/mL Matrix-Spike Replicate	Calculated Concentration (ng/mL)
1	0.00172
2	0.00167
3	0.00271
4	0.00201
5	0.00171
6	0.00169
7	0.00180
Standard Deviation (S) =	0.000375
N-1 =	6
Critical t value (t) =	3.143
Injection Volume (µL)	20
MDL (ng/mL) =	0.00118
MDL (ng on-column) =	0.0000236
MDL (pg on-column) =	0.024
LOD (pg on-column) =	0.1
LOD Equivalent to incurred sample at =	0.0004 ppm

The results for Injection 2 (for analysis of 1,2,4-Triazole) are summarized below.

Table 9: Calculation of MDL for 1,2,4-Triazole (m/z 70→43) for Method D1513/01, using the Methodology Set Forth in 40 CFR Ch 1 Part 136 Appendix B

0.0095 ng/mL Matrix-Spike Replicate	Calculated Concentration (ng/mL)
1	0.00725
2	0.00839
3	0.01110
4	0.00759
5	0.01000
6	0.00671
7	0.00855
Standard Deviation (S) =	0.001561
N-1 =	6
Critical t value (t) =	3.143
Injection Volume (µL)	50
MDL (ng/mL) =	0.00491
MDL (ng on-column) =	0.0002453
MDL (pg on-column) =	0.25
LOD (pg on-column) =	1

In summary, the MDL was successfully determined to be 0.024 pg on-column for the first injection (BAS 750 F and M750F003) of method D1513/01 and 0.25 pg on-column for the second injection (1,2,4-Triazole) of the method. After an evaluation of the analytes of interest taking into consideration instrument variability, LC-MS/MS drift, unexpected contamination, and untested matrix effects, the LOD was determined to be 0.1 pg on-column for the first injection of the method and 1 pg on-column for the second injection of the method.

Table 10a. Method Characteristics - Analytical Method L0214/01¹

Analyte		Mefentrifluconazole (BAS 750 F; Reg.No. 5834378)	M750F003 (Reg.No. 5924326)	1,2,4-Triazole (Reg.No. 87084)	
Limit of Quantitation (LOQ)	ECM	0.002 mg/kg			
	ILV				
Limit of Detection (LOD)	ECM	0.0004 mg/kg			
	ILV				
Linearity (calibration curve r^2 and concentration range) ²	ECM	Solvent	$r^2 = 0.9984$ (Q) $r^2 = 0.9966$ (C)	$r^2 = \mathbf{0.9944}$ (Q) $r^2 = 0.9970$ (C)	$r^2 = \mathbf{0.9944}$ (Q) $r^2 = 1.0000$ (C)
		Lufa 2.2	$r^2 = 0.9996$ (Q) $r^2 = 0.9994$ (C)	$r^2 = 0.9998$ (Q) $r^2 = 0.9996$ (C)	NA
		Lufa 2.3	$r^2 = 0.9986$ (Q) $r^2 = 0.9988$ (C)	NA	NA
	ILV	$r^2 = 0.9970$ (Q) $r^2 = 0.9966$ (C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9992$ (C)	$r^2 = 0.9968$ (Q) $r^2 = 0.9990$ (C)	
	Concentration Range	0.025-3 ng/mL		0.125-15 ng/mL	
Repeatable	ECM	Yes at LOQ and 10×LOQ (two characterized soil matrices)			
	ILV ³	Yes at LOQ and 10×LOQ (one characterized soil matrix)			
Reproducible		Yes at LOQ and 10×LOQ			
Specific	ECM ⁴	No 10×LOQ chromatograms were provided.			
	ILV ⁶	Yes, no matrix interferences were observed.	Could not be fully determined. No matrix interferences were observed; however, the LOQ peak in one soil (Lufa 2.2) was not resolved from the baseline noise (Q-Hypercarb). ⁵		
		Yes, no matrix interferences were observed.	Yes, matrix interferences were <14% of the LOQ (based on peak area).		

Data were obtained from Appendix B of MRID 49762549; MRID 49762443. Q = Quantitation ion transition or primary method; C = Confirmation ion transition or method. NA = Not applicable.

1 Analytical Method L0214/01: In the ECM, loamy fine sand soil (LUFA 2.2; 87.4% sand 7.7% silt 5.0% clay; pH (water) 6.4; 1.72% organic carbon) and sandy loam soil (LUFA 2.3; 64.1% sand 28.3% silt 7.6% clay; pH (water) 6.1; 0.67% organic carbon) were used (USDA soil textural classification; Appendix B, p. 228; Appendix B, Figures 70-71, pp. 392-397 of MRID 49762549). Soils were characterized by BioChem agrar; soil sources were not further specified. In the ILV, silty clay loam soil (PA.IL.T.CHAR 30-36.A.; 7% sand 54% silt 39% clay; pH 7.0 in saturated paste; 0.32% organic carbon) was used (USDA soil textural classification; p. 17; Appendix F, p. 183 of MRID 49762443). Soil was provided by the sponsor and characterized by Agvise Laboratories, Northwood, North Dakota.

2 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report. Solvent-based calibration standards were used in the ILV. Correlation values below 0.995 are in bold red text.

3 The method was validated by the ILV in the first trial with insignificant modifications to the analytical instrumentation (pp. 11, 18-19, 21-22, Table 19, p. 43 of MRID 49762443). For both methods, the ILV recommended that alternative caps should be used in the place of the Thermo Scientific SeptraSeal caps in order to reduce residual background of 1,2,4-triazole.

4 Based on Appendix B, Figures 19-66, pp. 296-319 of MRID 49762549.

5 Based on Appendix B, Figures 53-54, p. 313 (Lufa 2.2) and Figures 61-62, p. 317 (Lufa 2.3) of MRID 49762549.

Specificity was satisfactory using the Synergi column (C) in both test soils.

6 Based on Figures 21-28, pp. 114-125 of MRID 49762443.

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

Table 10b. Method Characteristics - Analytical Method D1513/01¹

Analyte		Mefentrifluconazole (BAS 750 F; Reg.No. 5834378)	M750F003 (Reg.No. 5924326)	1,2,4-Triazole (Reg.No. 87084)
Limit of Quantitation (LOQ)	ECM	0.002 mg/kg		
	ILV			
Limit of Detection (LOD)	ECM	0.0004 mg/kg		
	ILV			
Linearity (calibration curve r^2 and concentration range) ²	ECM	$r^2 = 0.9982$ (Q) $r^2 = 0.9990$ (C)	$r^2 = 0.9958$ (Q & C)	$r^2 = \mathbf{0.9884}$ (Q) $r^2 = 0.9988$ (C)
	ILV	$r^2 = 0.9986$ (Q & C)	$r^2 = \mathbf{0.9892}$ (Q) $r^2 = 0.9994$ (C)	$r^2 = \mathbf{0.9896}$ (Q) $r^2 = 0.9952$ (C)
	Concentration Range	0.005-0.25 ng/mL		0.01-0.50 ng/mL (Q) 0.01-1.0 ng/mL (C)
Repeatable	ECM	Yes at LOQ and 10×LOQ (two characterized soil matrices)		
	ILV ³	Yes at LOQ and 10×LOQ (one characterized soil matrix)		
Reproducible	Yes at LOQ and 10×LOQ			
Specific	ECM ⁴	Yes, matrix interferences were <5% of the LOQ (based on peak area).	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed; however, the LOQ peak was very small compared to baseline noise.
	ILV ⁵	Yes, matrix interferences were <10% of the LOQ (based on peak area).	Yes, no matrix interferences were observed; however, the LOQ peak was very small.	No , matrix interferences were <i>ca.</i> ≤45% (Q) and ≤95% (C) of the LOQ (based on peak area) ⁶ .

Data were obtained from Appendix A of MRID 49762549; MRID 49762443. Q = Quantitation ion transition or primary method; C = Confirmation ion transition or method.

1 Analytical Method D1513/01: In the ECM, sandy loam soil (TRT-BARESOIL SCS-19 0-3"; 74% sand 18% silt 8% clay; pH 6.9 in saturated paste; 0.39% organic carbon) and sand soil (SCS1 0-3"; 91% sand 8% silt 1% clay; pH 7.1 in saturated paste; 0.41% organic carbon) were used (USDA soil textural classification; Appendix A, p. 21-22; Appendix A, Appendix A, pp. 43-44 of MRID 49762549). Soils were characterized by Agvise Laboratories, Northwood, North Dakota; soil sources were not further specified. The test soils were soils from the top 3 inches of soil from BASF study 715267: trial R140864 (sandy loam) and trial R140865 (sand). In the ILV, loamy sand soil (TRT-BARESOIL SCS-23 18-24"; 76% sand 20% silt 4% clay; pH 8.1 in saturated paste; 0.05% organic carbon) was used (USDA soil textural classification; p. 17; Appendix F, p. 184 of MRID 49762443). Soil was provided by the sponsor and characterized by Agvise Laboratories, Northwood, North Dakota.

2 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report. Matrix-based calibration standards were used in the ILV (p. 23 of MRID 49762443). Correlation values below 0.995 are in bold red text.

3 The method was validated by the ILV in the first trial with insignificant modifications to the analytical instrumentation (pp. 11, 18-19, 21-22, Table 19, p. 43 of MRID 49762443). For both methods, the ILV recommended

that alternative caps should be used in the place of the Thermo Scientific SeptraSeal caps in order to reduce residual background of 1,2,4-triazole.

4 Based on Appendix A, Appendix J, Figures J.37-J.78, pp. 160-201 of MRID 49762549.

5 Based on Figures 7-14, pp. 73-84 of MRID 49762443.

6 Based on Figures 7, 8, and 13, pp. 75, 78, 83 of MRID 49762443 (reagent blank and matrix blank control interferences considered).

A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

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

IV. Method Deficiencies and Reviewer's Comments

1. For BASF Analytical Method L0214/01, the following deficiencies were noted in the ECM:

It could not be determined if specificity of the method was supported for 1,2,4-triazole based on the ECM representative chromatograms because the LOQ peak in the Lufa 2.2 soil was not resolved from the baseline noise using the Hypercarb column (primary method; Appendix B, Figures 53-54, p. 313 (Lufa 2.2) and Figures 61-62, p. 317 (Lufa 2.3) of MRID 49762549). The LOQ peak in the Lufa 2.3 soil was well-resolved. Specificity for 1,2,4-triazole was satisfactory using the Synergi column (C) in both test soils.

Linearity was slightly below the 0.995 correlation coefficient standard for the primary methods of M750F003 ($r^2 = 0.9944$) and 1,2,4-triazole ($r^2 = 0.9944$; solvent calibration standards).

No 10×LOQ chromatograms were provided. Representative chromatograms of all fortifications/matrices/ions should be provided for review.

2. For BASF Analytical Method D1513/01, the following deficiencies were noted:

The specificity of the method was not supported for 1,2,4-triazole based on the ILV representative chromatograms because matrix interferences were *ca.* ≤45% (Q) and ≤95% (C) of the LOQ (based on peak area; reagent blank and matrix blank control interferences considered; Figures 7, 8, and 13, pp. 75, 78, 83 of MRID 49762443). Additionally, in the ECM representative chromatograms, the LOQ peak was very small compared to baseline noise (Figures 7-14, pp. 73-84 of MRID 49762443).

Linearity was not slightly below the 0.995 correlation coefficient standard for the primary methods of M750F003 (ILV $r^2 = 0.9892$) and 1,2,4-triazole (ILV $r^2 = 0.9884$; ECM $r^2 = 0.9896$; solvent calibration standards. Linearity is satisfactory when $r^2 \geq 0.995$.

3. Based on the residual background level of 1,2,4-triazole observed adversely affecting method linearity and specificity, an updated ECM should be provided to include the ILV recommendation of alternative caps for samples to be analyzed. For both methods, the ILV recommended that alternative caps should be used in the place of the Thermo Scientific SeptraSeal caps in order to reduce residual background of 1,2,4-triazole (pp. 11, 18-19, 21-22, Table 19, p. 43 of MRID 49762443).
4. The ILV only used one soil matrix to validate each method; the ILV did not validate the

methods using a range of soils representative of the test soils used in the referenced terrestrial field dissipation studies. The terrestrial field dissipation studies contained soils from six test sites: New York (silt loam, 0-48"), North Dakota (clay, 0-24" and 30-48"; sandy clay loam, 24-30"), Washington (loamy sand, 0-6" and 18-30"; sand 6-18"; sandy loam 30-48"), California (loamy sand 0-48"), Oklahoma (sandy loam, 0-18"; loam, 18-42"; sandy clay loam, 42-48"), and Illinois (silty clay loam, 0-18" and 24-42"; silty clay, 18-24"; clay loam, 42-48"; soil classification taken from Tier II Summary for Field Studies). The reviewer noted that the silty clay loam soil (PA.IL.T.CHAR 30-36.A.; 7% sand 54% silt 39% clay; pH 7.0 in saturated paste; 0.32% organic carbon) used to validate the BASF Analytical Method L0214/01 had a high clay content (p. 17; Appendix F, p. 183 of MRID 49762443).

5. In MRID 49762343 (*Investigation into the extractability of BAS 750 F in samples from 14C soil degradation studies*), BASF Method No. L0214/01 was compared to the soil extraction method used in soil metabolism/degradation studies, in which soils were extracted twice with acetonitrile, acetonitrile:water (80:20, v:v), and acetonitrile:water (50:50, v:v) by shaking. The extractability of the total residues and of individual compounds was similar between methods.
6. In the ECM, stock and intermediate (fortification) standard solutions of BAS 750 F and M750F003 were prepared in acetonitrile and were shown to be stable up to 3 months (92 days) in acetonitrile when held under refrigeration. The calibration solutions of BAS 750 F or M750F003 were prepared by serial dilution of the fortification standards with acetonitrile:water (20:80, v/v) and were shown to be stable for up to 1 month (31 days) under refrigeration. Stock, intermediate and calibration standards solutions of 1,2,4-triazole were prepared in water and exhibited stability under refrigeration for up to 3 months (92 days) in water. During the course of the study, the test substance solutions were stored in a refrigerator and all solutions were used within the demonstrated time period of stability (Tier II Summary for MRID 49762549). Also, recoveries from stored solutions generated during extract stability experiments performed in conjunction with this study (which included tests on initial extracts and extracts at the final volume stage stored at room temperature), indicated that residues of BAS 750 F are stable in extracts for at least the time period tested (11-12 days), sufficient to support the storage intervals and conditions incurred by sample extracts in the study.
7. The ILV study author only reported that communications between the ILV and ECM or study sponsor involved notifying the Study Monitor of the successful completion of the ILV first trial (p. 26 of MRID 49762443). The documentation of the communication was noted but not provided for review.
8. It was reported for the ILV that each set of 16 samples required approximately 12 hours to process, including calculation of results (p. 25 of MRID 49762443).

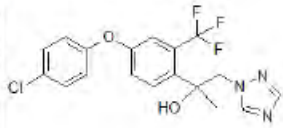
V. References

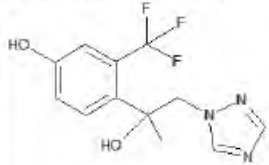
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory

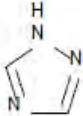
Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Code Name	BAS 750 F	<p>Chemical structure:</p> 
BASF Reg. No.	5834378	
CAS No.	1417782-03-6	
Molecular Formula	C ₁₈ H ₁₅ ClF ₃ N ₃ O ₂	
Molecular Weight	397.8 g/mol	
IUPAC Name	(2RS) 2-[4-(4-Chlorophenoxy)-2-(Trifluoromethyl)Phenyl]-1-(1H-1,2,4-Triazol-1-yl)Propan-2-ol	
Lot Number	L85-124	
Purity	99.7%	
Storage	Keep at Room Temperature or Cooler	
Expiration Date	July 01, 2017	

Code Name	M750F003	<p>Chemical structure:</p> 
BASF Reg. No.	5924326	
CAS No.	NA	
Molecular Formula	C ₁₂ H ₁₂ F ₃ N ₃ O ₂	
Molecular Weight	287.2	
IUPAC Name	4-[2-Hydroxy-1-(1H-1,2,4-Triazol-1-yl)Propan-2-yl]-3-(Trifluoromethyl)Phenol	
Lot Number	L84-250	
Purity	99.6%	
Storage	Keep at Room Temperature or Cooler	
Expiration Date	May 01, 2017	

Code Name	480M52 (old code BF 480-16)	<p>Chemical structure:</p> 
BASF Reg. No.	87084	
CAS No.	288-88-0	
Molecular Formula	C ₂ H ₃ N ₃	
Molecular Weight	69.0667	
IUPAC Name	1,2,4-(1H)- Triazole	
Lot Number	AC10194-134	
Purity	99.0%	
Storage	Keep in refrigerator	
Expiration Date	April 1, 2022	