

Analytical method for Boscalid (BAS 510 F) and its metabolites, M510F47 (Reg.No. 107371) and M510F49 (Reg.No. 391572), in water

Reports: ECM: EPA MRID No.: 49937102. Penning, H. 2015. Validation of Analytical Method L0127/1 for the Determination of BAS 510 F (Boscalid) Residues in Surface Water and Groundwater (Including Amendment No. 1). BASF Study No.: 357249. BASF Registration Document No.: 2015/7007586. Report prepared by BASF SE, BASF Agricultural Center Limburgerhof, Limburgerhof, Germany; and sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 56 pages. Final report issued January 30, 2009. Amendment No. 1 issued August 18, 2015.

ILV: EPA MRID No. 49937103. Göcer, M. 2016. Report Amendment No. 1/ Revision No. 1: Independent Laboratory Validation (ILV) of the BASF Method L0127 for the Determination of Boscalid (BAS 510 F) and two of its metabolites M510F47 (Reg.No. 107371) and M510F49 (Reg.No. 391572) in surface water and groundwater. BASF Study ID No.: 776664. BASF Registration Document No.: 2016/1112645. Report prepared by Eurofins Agroscience Services EcoChem GmbH, Niefem-Öschelbronn, Germany, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 155 pages. Final report issued November 18, 2015. Amendment No. 1/Revision No. 1 issued April 1, 2016.

Document No.: MRIDs 49937102 & 49937103

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with OECD and German Good Laboratory Practice (GLP) standards, which are fairly compatible with USEPA FIFRA and TSCA standards (p. 3 of MRID 49937102). 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 OECD and German Good Laboratory Practice (GLP) standards, which are fairly compatible with USEPA FIFRA and TSCA standards (pp. 3, 5 of MRID 49937103). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-5). An authenticity statement was included with the QA statement.

Classification: This analytical method is classified as Supplemental. Two sets of performance data were not included for BASF Method L0127/02 for the determination of residues of boscalid metabolites M510F47 and M510F49. No 10×LOQ chromatograms were provided in the ECM.

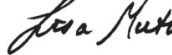
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EFED Final Reviewer: Taimei Harris, Ph.D.,
Fate Scientist

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Date: Taimei

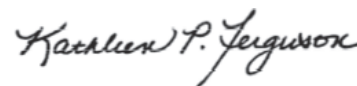
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Executive Summary

The analytical method, BASF Method L0127/1 (BASF Method L0127/01), is designed for the quantitative determination of boscalid (BAS 510 F) in water at the LOQ of 0.03 ppb (0.03 µg/kg; 0.03 µg/L) using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water. The analytical method, BASF Method L0127/02, is designed for the quantitative determination of boscalid metabolites M510F47 (Reg.No. 107371) and M510F49 (Reg.No. 391572) in water at the LOQ of 0.03 ppb (0.03 µg/kg; 0.03 µg/L) using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water. The ECM performed BASF Method L0127/1 using characterized surface and ground water, but no 10×LOQ chromatograms were provided in the ECM. The ILV performed BASF Method L0127/1 and BASF Method L0127/02 using characterized surface and ground water. In the ILV, the number of trials was not specified in the study report. Methods and results for the metabolites, M510F47 and M510F49, were not included in the ECM; therefore, two sets of validation data were not included for BASF Method L0127/02. In the ECM and ILV, matrix interferences at the retention time of boscalid were approximately equal to the LOD in some chromatograms.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Boscalid (BAS 510 F)	49937102 ¹	49937103		Water ^{3,4}	30/01/2009 (Final Report) 18/08/2015 (Amendment 1)	BASF Corporation	LC/MS/MS	0.03 ppb (0.03 µg/kg; 0.03 µg/L)
M510F47 (Reg.No. 107371)	None submitted ²							
M510F49 (Reg.No. 391572)								

1 BASF Method L0127/1 (BASF Method L0127/01).

2 BASF Method L0127/02.

3 In the ECM, ground (tap) water (pH 7.64-7.81; 4.7-14.2 °dH total hardness; 1.1-2.2 mg/L total organic carbon) was collected from the test facility which was sourced from four wells (Well Böhl, Well Schifferstadt, Well Mutterstadt and Well Waldsee) and surface water (pH 8.7; 1.25 mmol/L carbonate hardness; 14 mg/L total organic carbon) was collected from Kelmetschweiher.

4 In the ILV, surface water (pH 8.02 at 23.8°C; 10.4 °dH total hardness; 7.0 mg/L total organic carbon) was collected from Pfalz (characterized in a separate study S14-04800; Klaumann 2015) and ground water (pH 6.9; 3.2 mmol/L hardness; 2.5 mg/L total organic carbon) was collected from Grundwasser Malsch (Karlsruhe).

Page numbers cited for MRID 49937102 refer to those appearing on the bottom, right-hand corner of the document.

I. Principle of the Method

Boscalid (MRID 49937102): Water samples (10 mL) were fortified in 50 mL glass beakers (Appendix 6.4, pp. 37, 39-40, 44 of MRID 49937102). A C₁₈-solid phase extraction (SPE) cartridge (3 cc/500 mg) was pre-conditioned with 2 x 2.5 mL each of cyclohexane:ethyl acetate (1:1, v:v), methanol and pure water. The cartridge was not allowed to dry, and 2 mL of pure water added to the column. The water sample was applied to the cartridge via pipet then the beaker was rinsed with 2 mL of pure water. The beaker rinse was applied to the column. A vacuum was applied to the column to achieve a slow flow (*ca.* 3-4 mL/min.). After all of the solution passed through the cartridge, the column was mounted on to the column drier and dried with a stream of nitrogen at a temperature of 30°C for 45 minutes. The dried columns were washed with 2.5 mL of cyclohexane. The analyte was eluted with 2 x 2.5 mL of cyclohexane:ethyl acetate (1:1, v:v). The solvent of the eluate was completely evaporated via rotary evaporator at 40°C (water bath). The residue was reconstituted in methanol:water (80:20, v:v) prior to analysis by LC/MS/MS.

Water samples were analyzed for boscalid using Agilent 1100 LC Binary Pump system (Betasil C18 column, 2.1 mm x 100 mm, 5 µm column; column temperature 25°C) using a gradient mobile phase of (A) water:formic acid (1000:1, v:v) and (B) methanol:formic acid (1000:1, v:v) [time ratio A:B; 0.0 min. 66:34, 2.0 min. 26:74, 4.0-6.0 min. 10:90, 6.1-9.0 min. 66:34] coupled with a AB Sciex API 4000 triple stage quadrupole mass spectrometer using positive electrospray ionization in Multiple Reaction Monitoring (MRM) mode (pp. 40-41 of MRID 49937102). Injection volume was 50 µL. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 343.0→271.0 and m/z 343.0→307.0 for boscalid. Retention time was *ca.* 3.3 minutes for boscalid.

In the ILV, the ECM was performed as written for boscalid, except for the use of a different LC/MS/MS system (pp. 30-31, 33; Appendix 7.3, p. 86 of MRID 49937103). Sciex API 5500 LC/MS/MS system was used. All LC/MS conditions were the same, except that the column temperature was 30°C and the injection volume was 10 µL. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 342.9→271 and m/z 342.9→307 for boscalid. Retention time was *ca.* 2.8 minutes for boscalid. No other modifications to the ECM were reported.

In the ILV, procedures for M510F47 and M510F49 were reported, but they were not reported in the ECM MRID 49937102.

M510F47 (MRID 49937103): Water samples (50 mL) were acidified (<pH 2) and fortified (pp. 24, 30-31; Appendix 7.3, p. 87 of MRID 49937103). An OASIS HLB SPE cartridge (6 cc/500 mg) was pre-conditioned with 2 x 6 mL each of methanol and pure water. The water sample was applied to the cartridge without vacuum (*ca.* 1 drop per second). After all of the solution passed through the cartridge, the column was rinsed with 4.5 mL of pure water. The column was dried under vacuum at room temperature for 5 minutes. The analyte was eluted with 2 x 4.5 mL of methanol without vacuum, but vacuum was used at the end to ensure quantitative elution. The solvent of the eluate

was completely evaporated via a stream of nitrogen at 40°C. The residue was reconstituted in 1 mL of pure water via ultrasonication prior to analysis by LC/MS/MS.

Water samples were analyzed for M510F47 using Sciex API 5500 LC/MS/MS system (Waters XSelect HSS T3 column, 2.1 mm x 100 mm, 2.5 µm column; column temperature 30°C) using a gradient mobile phase of (A) water:formic acid (1000:1, v:v) and (B) methanol:formic acid (1000:1, v:v) [time ratio A:B; 0.0-0.5 min. 90:10, 1.0 min. 70:30, 4.1 min. 40:60, 4.2-6.0 min. 0.1:99.9, 6.1-7.0 min. 90:10] using positive electrospray ionization (Turbo Ion Spray) in Multiple Reaction Monitoring (MRM) mode (p. 34 of MRID 49937103). Injection volume was 10 µL. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 157.9→122 and m/z 157.9→94 for M510F47. Retention time was *ca.* 2.9 minutes for M510F47.

M510F49 (MRID 49937103): Water samples (10 mL) were fortified in a glass beaker (pp. 24, 30-31; Appendix 7.3, p. 88 of MRID 49937103). An Octadecyl C₁₈-SPE cartridge (3 cc/500 mg; Bakerbond) was pre-conditioned with 2 x 2.5 mL each of methanol and pure water. The water sample was applied to the cartridge without vacuum (*ca.* 1 drop per second). After all of the solution passed through the cartridge, the beaker was rinsed with 2 mL water which was afterward applied to the column. The column was dried under vacuum at room temperature for 2-3 minutes. The analyte was eluted with 2 x 2.5 mL of methanol. The solvent of the eluate was completely evaporated via a stream of nitrogen at 40°C. The residue was reconstituted in 2 mL of methanol:water (80:20, v:v) via ultrasonication prior to analysis by LC/MS/MS.

Water samples were analyzed for M510F49 using Sciex API 5500 LC/MS/MS (Betasil C18 column, 2.1 mm x 100 mm, 5 µm column; column temperature 30°C) using a gradient mobile phase of (A) water:formic acid (1000:1, v:v) and (B) methanol:formic acid (1000:1, v:v) [time ratio A:B; 0.0 min. 66:34, 2.0 min. 26:74, 4.0-6.0 min. 10:90, 6.1-9.0 min. 66:34] using positive electrospray ionization (Turbo Ion Spray) in Multiple Reaction Monitoring (MRM) mode (p. 35 of MRID 49937103). Injection volume was 10 µL. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 322.9→202 and m/z 322.9→94 for M510F49. Retention time was *ca.* 3.1 minutes for M510F49.

The Limit of Quantification (LOQ) for boscalid in water was 0.03 ppb (0.03 µg/kg; 0.03 µg/L) in the ECM and ILV (pp. 6, 19; Appendix 6.4, p. 44 of MRID 49937102; pp. 20, 59-61 of MRID 49937103). In the ILV, the LOQ for boscalid corresponded to 0.15 ng/mL in the extract. The Limit of Detection (LOD) for boscalid in water was reported as 0.005 ppb (0.005 µg/kg; 0.005 µg/L; 17% of the LOQ) in the ECM and ILV; the LOD corresponded to 0.00125 ng in the ECM and 0.025 ng/mL in the ILV. The boscalid metabolites, M510F47 and M510F49, were not included in the ECM, but they were included in the ILV. The LOQ and LOD for M510F47 and M410F49 were 0.03 ppb (0.03 µg/kg; 0.03 µg/L) and 0.009 ppb (0.009 µg/L), respectively, in the ILV. For M510F47, the LOQ and LOD corresponded to 1.5 ng/mL and 0.45 ng/mL, respectively, in the extract. For M510F49, the LOQ and LOD corresponded to 0.15 ng/mL and 0.045 ng/mL, respectively, in the extract

II. Recovery Findings

ECM (MRID 49937102): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of boscalid in water matrices at

fortification levels of 0.03 ppb (0.03 µg/kg; 0.03 µg/L; LOQ) and 0.3 ppb (0.3 µg/kg; 0.3 µg/L; 10×LOQ; Tables 1-4, pp. 15-18). Boscalid was identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. The metabolites, M510F47 and M510F49, were not included in the ECM. The ground (tap) water (pH 7.64-7.81; 4.7-14.2 °dH total hardness; 1.1-2.2 mg/L total organic carbon) was collected from the test facility which was sourced from four wells (Well Böhl, Well Schifferstadt, Well Mutterstadt and Well Waldsee) and surface water (pH 8.7; 1.25 mmol/L carbonate hardness; 14 mg/L total organic carbon) was collected from Kelmetschweiher (pp. 10-11; Appendices 6.1-6.2, pp. 21-24).

ILV (MRID 49937103): Mean recoveries and RSDs were within guideline requirements for analysis of boscalid and its metabolites, M510F47 and M510F49, in water matrices at fortification levels of 0.03 ppb (0.03 µg/kg; 0.03 µg/L; LOQ) and 0.3 ppb (0.3 µg/kg; 0.3 µg/L; 10×LOQ; Tables 15-26, pp. 48-56). All analytes were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. The surface water (pH 8.02 at 23.8°C; 10.4 °dH total hardness; 7.0 mg/L total organic carbon) was collected from Pfalz (characterized in a separate study S14-04800; Klaumann 2015) and ground water (pH 6.9; 3.2 mmol/L hardness; 2.5 mg/L total organic carbon) was collected from Grundwasser Malsch (Karlsruhe; p. 22; Appendix 7.7, Figures 54-55, pp. 151-152). The number of trials was not specified in the study report, but the reviewer assumed that the methods for boscalid and its metabolites were validated in the first trial with insignificant modifications to the analytical instrumentation, based on the fact that no modifications of the method and no communication with the sponsor was required by the ILV to validate the methods (pp. 10-11, 63). Methods and results for the metabolites, M510F47 and M510F49, were not included in the ECM; therefore, two sets of validation data were not included for M510F47 and M510F49.

Table 2. Initial Validation Method Recoveries for Boscalid (BAS 510 F) in Water^{1,2}

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water						
Quantitation ion						
Boscalid (BAS 510 F)	0.03 (LOQ)	5	84.7-94.0	89.9	3.4	3.8
	0.3	5	80.7-99.3	87.6	7.3	8.4
Confirmatory ion						
Boscalid (BAS 510 F)	0.03 (LOQ)	5	85.3-98.0	90.8	5.1	5.6
	0.3	5	80.0-97.3	89.6	6.2	6.9
Surface Water						
Quantitation ion						
Boscalid (BAS 510 F)	0.03 (LOQ)	5	85.3-95.3	90.1	4.1	4.5
	0.3	5	90.0-100.0	95.2	4.3	4.5
Confirmatory ion						
Boscalid (BAS 510 F)	0.03 (LOQ)	5	82.7-89.3	86.9	2.8	3.2
	0.3	5	85.3-102.7	95.1	6.6	6.9

Data (uncorrected recovery results, Tables 1-4, pp. 15-18) were obtained from Tables 1-4, pp. 15-18 of MRID 49937102. The metabolites, M510F47 and M510F49, were not included in the ECM.

¹ The ground (tap) water (pH 7.64-7.81; 4.7-14.2 °dH total hardness; 1.1-2.2 mg/L total organic carbon) was collected from the test facility which was sourced from four wells (Well Böhl, Well Schifferstadt, Well Mutterstadt and Well Waldsee) and surface water (pH 8.7; 1.25 mmol/L carbonate hardness; 14 mg/L total organic carbon) was collected from Kelmetschweiher (pp. 10-11; Appendices 6.1-6.2, pp. 21-24).

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 343.0→271.0 and m/z 343.0→307.0 for boscalid (pp. 40-41).

Table 3. Independent Validation Method Recoveries for Boscalid (BAS 510 F) and its Metabolites, M510F47 and M510F49, in Water^{1,2,3}

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water						
Quantitation ion						
Boscalid (BAS 510 F)	0.03 (LOQ)	5	97-115	109	8	7
	0.3	5	85-96	90	4	5
M510F47	0.03 (LOQ)	5	101-104	103	1	1
	0.3	5	96-108	103	5	5
M510F49	0.03 (LOQ)	5	85-92	89	3	3
	0.3	5	88-103	94	6	6
Confirmatory ion						
Boscalid (BAS 510 F)	0.03 (LOQ)	5	95-115	107	8	8
	0.3	5	86-95	90	3	4
M510F47	0.03 (LOQ)	5	101-103	102	1	1
	0.3	5	95-103	100	3	3
M510F49	0.03 (LOQ)	5	84-92	89	3	3
	0.3	5	89-100	93	4	4
Ground Water						
Quantitation ion						
Boscalid (BAS 510 F)	0.03 (LOQ)	5	104-113	108	4	4
	0.3	5	87-93	89	3	3
M510F47	0.03 (LOQ)	5	99-102	101	1	1
	0.3	5	91-100	96	4	4
M510F49	0.03 (LOQ)	5	83-97	89	6	6
	0.3	5	87-95	91	3	4
Confirmatory ion						
Boscalid (BAS 510 F)	0.03 (LOQ)	5	102-112	108	4	4
	0.3	5	86-108	89	4	4
M510F47	0.03 (LOQ)	5	99-101	100	1	1
	0.3	5	91-102	96	5	5
M510F49	0.03 (LOQ)	5	83-102	92	7	8
	0.3	5	89-95	93	3	3

Data (uncorrected recovery results, pp. 36-38) were obtained from Tables 15-26, pp. 48-56 of MRID 49937103.

1 Standard deviations were reviewer-calculated using the data in the study report because they were not calculated by the study author (DER Attachment 2).

2 The surface water (pH 8.02 at 23.8°C; 10.4 °dH total hardness; 7.0 mg/L total organic carbon) was collected from Pfalz (characterized in a separate study S14-04800; Klaumann 2015) and ground water (pH 6.9; 3.2 mmol/L hardness; 2.5 mg/L total organic carbon) was collected from Grundwasser Malsch (Karlsruhe; p. 22; Appendix 7.7, Figures 54-55, pp. 151-152).

3 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 342.9→271 and m/z 342.9→307 for boscalid; m/z 157.9→122 and m/z 157.9→94 for M510F47; and m/z 322.9→202 and m/z 322.9→94 for M510F49 (pp. 33-35).

III. Method Characteristics

The LOQ for boscalid in water was 0.03 ppb (0.03 µg/kg; 0.03 µg/L) in the ECM and ILV (pp. 6, 19; Appendix 6.4, p. 44 of MRID 49937102; pp. 20, 59-61 of MRID 49937103). In the ILV, the LOQ for boscalid corresponded to 0.15 ng/mL in the extract. In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested. The LOD for boscalid in water was reported as 0.005 ppb (0.005 µg/kg; 0.005 µg/L; 17% of the LOQ) in the ECM and ILV; the LOD corresponded to 0.00125 ng in the ECM and 0.025 ng/mL in the ILV. In the ECM, the LOD was defined as the absolute amount of analyte injected into the LC/MS/MS instrument using the lowest standard of the calibration curve. The boscalid metabolites, M510F47 and M510F49, were not included in the ECM, but they were included in the ILV. The LOQ and LOD for M510F47 and M410F49 were 0.03 ppb (0.03 µg/kg; 0.03 µg/L) and 0.009 ppb (0.009 µg/L), respectively, in the ILV. For M510F47, the LOQ and LOD corresponded to 1.5 ng/mL and 0.45 ng/mL, respectively, in the extract. For M510F49, the LOQ and LOD corresponded to 0.15 ng/mL and 0.045 ng/mL, respectively, in the extract. No calculations or comparisons to background levels were reported to justify the LOQ and LOD for the method in the ECM. No justifications of the LOQ or LOD were reported in the ILV.

Table 4. Method Characteristics

Analyte		Boscalid (BAS 510 F)	M510F47	M510F49
Limit of Quantitation (LOQ)	ECM	0.03 ppb (0.03 µg/kg; 0.03 µg/L)	Not performed	
	ILV		0.03 ppb (0.03 µg/kg; 0.03 µg/L)	
Limit of Detection (LOD)	ECM	0.005 ppb (0.005 µg/kg; 0.005 µg/L)	Not performed	
	ILV		0.009 ppb (0.009 µg/L)	
Linearity (calibration curve r^2 and concentration range) ¹	ECM	$r^2 = 0.9982$ (Q) $r^2 = 0.9990$ (C)	Not performed	
	ILV	$r^2 = 0.9982$ (Q) $r^2 = 0.9988$ (C)	$r^2 = 0.9996$ (Q & C; surface and ground water matrices)	$r^2 = 0.9990$ (Q) $r^2 = 0.9994$ (C)
	Concentration Range	(0.025-0.50 ng/mL)	(0.35-10 ng/mL)	(0.010-1.0 ng/mL)
Repeatable	ECM ²	Yes at LOQ and 10×LOQ	Not performed	
	ILV ^{3,4}	Yes at LOQ and 10×LOQ		
Reproducible		Yes at LOQ and 10×LOQ	Could not be determined	
Specific	ECM	Yes, matrix interferences were <8% of the LOQ in ground water and <i>ca.</i> 16% of the LOQ in surface water. Minor baseline interference surrounded the analyte peak in most LOQ chromatograms. 10×LOQ chromatograms were not presented.	Not performed	

Analyte		Boscalid (BAS 510 F)	M510F47	M510F49
	ILV	Yes, matrix interferences were <8% of the LOQ in ground water and <17% (Q) and <18% (C) of the LOQ in surface water. Minor baseline interference surrounded the analyte peak in C chromatograms.	Yes, matrix interferences were <6% of the LOQ in ground water and <2% of the LOQ in surface water. Minor baseline interference surrounded the analyte peak in surface water chromatograms.	Yes, matrix interferences were <3% of the LOQ in ground and surface water.

Data were obtained from pp. 6, 19; Appendix 6.4, pp. 38, 44; Tables 1-4, pp. 15-18 (recovery data); Appendix 6.3, pp. 26-29 (chromatograms); Appendix 6.3, p. 30 (calibration curves) of MRID 49937102; pp. 20, 59-61; Tables 15-26, pp. 48-56 (recovery data); Appendix 7.1, Figures 1-8, pp. 66-73 (calibration curves); Appendix 7.4, Figures 23-50, pp. 92-119 (chromatograms & reagent blank chromatograms) of MRID 49937103. Q = Quantitation ion transition; C = Confirmatory ion transition.

- 1 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Appendix 6.3, p. 30 of MRID 49937102; Figures 1-8, pp. 66-73 of MRID 49937103; DER Attachment 2). For the ECM and ILV, calibration curves were solvent-based, except for M510F47 which were matrix-based.
- 2 In the ECM, ground (tap) water (pH 7.64-7.81; 4.7-14.2 °dH total hardness; 1.1-2.2 mg/L total organic carbon) was collected from the test facility which was sourced from four wells (Well Böhl, Well Schifferstadt, Well Mutterstadt and Well Waldsee) and surface water (pH 8.7; 1.25 mmol/L carbonate hardness; 14 mg/L total organic carbon) was collected from Kelmetschweiher (pp. 10-11; Appendices 6.1-6.2, pp. 21-24 of MRID 49937102).
- 3 In the ILV, surface water (pH 8.02 at 23.8°C; 10.4 °dH total hardness; 7.0 mg/L total organic carbon) was collected from Pfalz (characterized in a separate study S14-04800; Klaumann 2015) and ground water (pH 6.9; 3.2 mmol/L hardness; 2.5 mg/L total organic carbon) was collected from Grundwasser Malsch (Karlsruhe; p. 22; Appendix 7.7, Figures 54-55, pp. 151-152 of MRID 49937103).
- 4 In the ILV, the number of trials was not specified in the study report, but the reviewer assumed that the methods for boscalid and its metabolites were validated in the first trial with insignificant modifications to the analytical instrumentation, based on the fact that no modifications of the method and no communication with the sponsor was required by the ILV to validate the methods (pp. 10-11, 63 of MRID 49937103). Methods and results for the metabolites, M510F47 and M510F49, were not included in the ECM; therefore, two sets of performance data were not included for M510F47 and M510F49.

IV. Method Deficiencies and Reviewer's Comments

1. The ILV MRID 49937103 was performed in support of BASF Method L0127/1 (also referred to as BASF Method L0127/01) for the determination of boscalid residues and BASF Method L0127/02 for the determination of residues of boscalid metabolites M510F47 and M510F49 (pp. 9-10 of MRID 49937103). The submitted ECM MRID 49937102 only included BASF Method L0127/1; therefore, only BASF Method L0127/1 was determined to be reproducible. Two sets of performance data were not included for BASF Method L0127/02; only the ILV was submitted. OCSPP guidelines state that two sets of performance data should be submitted, one for the initial or other internal validation and one for the ILV.
2. The ECM performed BASF Method L0127/1 using characterized surface and ground water, but no 10×LOQ chromatograms were provided in the ECM. OCSPP guidelines state that representative chromatograms should be provided for reagent blanks, matrix blanks, standard curves, and spiked samples at the LOQ and 10×LOQ for all analytes in each matrix.

3. In ILV representative chromatograms, minor baseline interference surrounded the analyte peak in several chromatograms (Appendix 7.4, Figures 23-50, pp. 92-119 of MRID 49937103). Additionally, the reviewer noted that matrix interferences in representative boscalid chromatograms were <17% (Q) and <18% (C) of the LOQ in surface water at the retention time of boscalid; therefore, matrix interferences were approximately equal to the LOD in the quantitative ion chromatogram and greater than the LOD in the confirmatory ion chromatogram. The reviewer noted that a confirmatory method is not always required when LC/MS/MS or GC/MS/MS is the primary identification method.

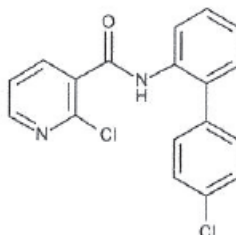
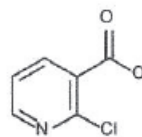
In ECM representative chromatograms, minor baseline interference surrounded the analyte peak in most LOQ chromatograms (Appendix 6.3, pp. 26-29 of MRID 49937102). Additionally, the reviewer noted that matrix interferences at the retention time of boscalid were <8% of the LOQ in ground water and *ca.* 16% of the LOQ in surface water; therefore, matrix interferences were approximately equal to the LOD in the surface water chromatograms. The reviewer noted that a confirmatory method is not always required when LC/MS/MS or GC/MS/MS is the primary identification method.
4. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 6, 19; Appendix 6.4, p. 44 of MRID 49937102; pp. 20, 59-61 of MRID 49937103). No calculations or comparisons to background levels were reported to justify the LOQ and LOD for the method. The LOQ and LOD for M510F47 and M410F49 were only reported in the ILV since they were not included as test materials in the ECM.
5. In the ECM, the Technical Procedure (non-GLP) of Method L0127/1 (Appendix 6.4, pp. 31-46 of MRID 49937102) and Report Amendment No. 1 to the Final Report of Method L0127/1 (pp. 47-56 of MRID 49937102) were included. The Technical Procedure supplied additional details about the method. The Amendment did not change any existing information; additional information was provided to demonstrate the influence of matrix load on the analysis using quality control samples (p. 48).
6. The matrix effects were found to be insignificant (<20%) for boscalid in the ECM and ILV (p. 50 of MRID 49937102; p. 60 of MRID 49937103). Matrix effects were also found to be insignificant for M510F49; however, significant matrix effects were observed for M510F47 in surface water (mean >20%) and ground water (mean *ca.* 20%). Matrix-matched standards were used for both matrices with M510F47.
7. The ILV study author reported that no communications between the ILV and ECM or study sponsor occurred (p. 10 of MRID 49937103). No justifications of the LOQ or LOD were reported in the ILV.
8. In the ILV, the number of trials was not specified in the study report, but the reviewer assumed that the methods for boscalid and its metabolites were validated in the first trial with insignificant modifications to the analytical instrumentation.

9. It was reported for the ILV that one sample set (e.g. total 15 samples with matrix-matched standards) required one working day with LC/MS/MS performed unattended overnight (p. 60 of MRID 49937103). Evaluation of the LC/MS/MS results was completed within a few hours, so a set of 15 samples was completely processed and evaluated in 24 hours. The reported time requirement was 1.5 working days for a set of 20 samples in the ECM (p. 19 of MRID 49937102).

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**Boscalid (BAS 510 F; Reg. No. 300355)****IUPAC Name:** 2-Chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide**CAS Name:** Not reported**CAS Number:** 188425-85-6**SMILES String:** Not found**M510F47 (Reg. No. 107371)****IUPAC Name:** 2-Chloronicotinic acid**CAS Name:** Not reported**CAS Number:** 2942-59-8**SMILES String:** Not found**M510F49 (Reg. No. 391572)****IUPAC Name:** N-(4'-Chlorobiphenyl-2-yl)-2-hydroxynicotinamide**CAS Name:** Not reported**CAS Number:** Not reported**SMILES String:** Not found