

**Analytical method for pyraclostrobin metabolite BF 500-3 (Reg. No. 340266) in water**

**Reports:** ECM: EPA MRID No.: 50088701. Saha, M. 2016. Validation of BASF Method D1508/01: Analytical Method for the Determination of residues of Pyraclostrobin metabolite, BF 500-3 (Reg.No. 340266) in Surface and Drinking Water by LC-MS/MS. BASF Study No.: 762712. BASF Registration Document No.: 2016/7010883. Report prepared by BASF Crop Protection, Research Triangle Park, North Carolina; and sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 99 pages. Final report issued July 27, 2016; amended report issued October 24, 2016.

ILV: EPA MRID No. 50088706. Sharp, S. 2015. Independent Lab Validation of BASF Method D1508/01: “Analytical Method for the Determination of residues of Pyraclostrobin metabolite, BF 500-3 (Reg. No. 340266) in Surface and Drinking Water by LC-MS/MS”. BASF Study No.: 762713. BASF Registration Document No.: 2015/7005730. EPL Study ID No.: 137G1077. Report prepared by EPL Bio Analytical Services (EPL), Niantic, Illinois, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 70 pages. Final report issued September 17, 2015.

**Document No.:** MRIDs 50088701 & 50088706

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR Part 160) Good Laboratory Practices (GLP) standards (p. 3 of MRID 50088701). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 50088706). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).

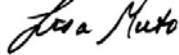
**Classification:** This analytical method is classified as **Acceptable**. However, ILV linearity was not satisfactory for BF 500-3.

**PC Code:** 099100

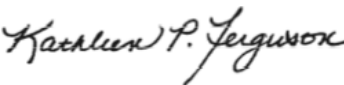
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Date: 04/16/18

**CDM/CSS-Dynamac JV Reviewers:** Lisa Muto,  
Environmental Scientist

Signature:   
Date: 9/12/17

**Reviewers:** Kathleen Ferguson, Ph.D.,  
Environmental Scientist

Signature:   
Date: 9/12/17

*This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.*

## Executive Summary

The analytical method, BASF Method D1508/01, is designed for the quantitative determination of pyraclostrobin metabolite BF 500-3 (Reg. No. 340266) in water at the LOQ of 0.03 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern of BF 500-3 in water (estuarine/marine invertebrate chronic toxicity NOAEC of 0.5 µg/L; DP 417743).

Characterized surface and drinking water matrices were used in the ECM. The ILV validated the method in the first trial with insignificant modifications to the analytical instrumentation using characterized surface and drinking water matrices. All ILV and ECM data regarding repeatability, accuracy, precision, and specificity were satisfactory in both matrices. However, ILV linearity was not satisfactory for BF 500-3 in either matrix.

**Table 1. Analytical Method Summary**

| Analyte(s) by Pesticide <sup>1</sup> | MRID                           |                                   | EPA Review | Matrix               | Method Date (dd/mm/yyyy)                                 | Registrant       | Analysis | Limit of Quantitation (LOQ) |
|--------------------------------------|--------------------------------|-----------------------------------|------------|----------------------|--|------------------|----------|-----------------------------|
|                                      | Environmental Chemistry Method | Independent Laboratory Validation |            |                      |  |                  |          |                             |
| BF 500-3 (Reg. No. 340266)           | 50088701                       | 50088706                          |            | Water <sup>2,3</sup> | 27/07/2016 (Final report)<br>24/10/2016 (Amended report) | BASF Corporation | LC/MS/MS | 0.03 µg/L                   |

1 Pyraclostrobin metabolite BF 500-3 (Reg. No. 340266) = Methyl N-(2-([1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl)phenyl)carbamate.

2 In the ECM, surface water (ID No. 201441104; JL; pH 6.9, 32 mg equiv. CaCO<sub>3</sub>/L hardness, 6.1 ppm total organic carbon) and drinking (tap/drinking well) water (ID No. 201441105; pH 7.4, 25 mg equiv. CaCO<sub>3</sub>/L hardness, 0.7 ppm total organic carbon) was used in the study (p. 13; Appendix A, pp. 25-26 of MRID 50088701). The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota; the water sources were not further specified.

3 In the ILV, surface water (BASF Study 437860; RCN R130034; PW; pH 6.9, 41 mg equiv. CaCO<sub>3</sub>/L hardness, 11.0 ppm total organic carbon) and drinking (tap) water (BASF Reference No. 22014; TW; pH 7.3, 22 mg equiv. CaCO<sub>3</sub>/L hardness, 2.4 ppm total organic carbon) was used in the study (p. 13; Appendix F, pp. 64-65 of MRID 50088706). The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota; the water sources were not further specified.

## I. Principle of the Method

Water samples (10 mL) in 10-mL volumetric flasks were fortified with 0.03 mL of 0.01 µg/mL or 0.1 µg/mL BF 500-3 fortification solution in acetonitrile (pp. 13-14; Appendix C, pp. 40, 45 of MRID 50088701). The solutions were mixed thoroughly via vortex, transferred to LC vials, and analyzed by LC/MS/MS. The method noted that BF 500-3 is known to adsorb to plastics, so only volumetric glassware should be used.

Water samples were analyzed for pyraclostrobin metabolite BF 500-3 (Reg. No. 340266) using Waters Acquity UPLC (Xbridge BEH C18 column, 2.1 mm x 50 mm, 2.5  $\mu$ m column; column temperature 50°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; 0.0-0.25 min. 85:15, 3.75-4.45 min. 1:99, 4.50-5.00 min. 85:15] coupled with a Sciex 5500 mass spectrometer using positive Turbo Ion Spray with Multiple Reaction Monitoring (MRM) mode (Appendix C, p. 43 of MRID 50088701). Injection volume was 10  $\mu$ L. Two ion transitions were monitored for BF 500-3 (quantitation and confirmatory, respectively) as follows:  $m/z$  358 $\rightarrow$ 164 and  $m/z$  358 $\rightarrow$ 132. Expected retention time was *ca.* 3.5 minutes.

In the ILV, the ECM was performed as written, except for the use of a different LC/MS/MS system (pp. 14-18; Appendix H, p. 69 of MRID 50088706). Agilent 1290 HPLC coupled to an AB Sciex 6500 Q-trap with Turbo Ion Spray was used. All LC/MS conditions were the same. Two ion transitions were monitored for BF 500-3 (quantitation and confirmatory, respectively) as follows:  $m/z$  357.9 $\rightarrow$ 164.1 and  $m/z$  357.9 $\rightarrow$ 132.0. Expected retention time was *ca.* 3.3 minutes. No other modifications to the ECM were reported.

The Limit of Quantification (LOQ) and Limit of Detection (LOD) for pyraclostrobin metabolite BF 500-3 (Reg. No. 340266) in water were 0.03  $\mu$ g/L and 0.006  $\mu$ g/L, respectively, in the ECM and ILV (p. 7; Appendix C, pp. 33, 46 of MRID 50088701; pp. 7, 21 of MRID 50088706).

## II. Recovery Findings

ECM (MRID 50088701): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of pyraclostrobin metabolite BF 500-3 (Reg. No. 340266) in two water matrices at fortification levels of 0.03  $\mu$ g/L (LOQ) and 0.3  $\mu$ g/L (10 $\times$ LOQ; p. 9). BF 500-3 was identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. The surface water (ID No. 201441104; JL; pH 6.9, 32 mg equiv. CaCO<sub>3</sub>/L hardness, 6.1 ppm total organic carbon) and drinking (tap/drinking well) water (ID No. 201441105; pH 7.4, 25 mg equiv. CaCO<sub>3</sub>/L hardness, 0.7 ppm total organic carbon) was used in the study (p. 13; Appendix A, pp. 25-26). The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota; the water sources were not further specified.

ILV (MRID 50088706): Mean recoveries and RSDs were within guideline requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of BF 500-3 in two water matrices at fortification levels of 0.03  $\mu$ g/L (LOQ) and 0.3  $\mu$ g/L (10 $\times$ LOQ; p. 8). BF 500-3 was identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. The surface water (BASF Study 437860; RCN R130034; PW; pH 6.9, 41 mg equiv. CaCO<sub>3</sub>/L hardness, 11.0 ppm total organic carbon) and drinking (tap) water (BASF Reference No. 22014; TW; pH 7.3, 22 mg equiv. CaCO<sub>3</sub>/L hardness, 2.4 ppm total organic carbon) was used in the study (p. 13; Appendix F, pp. 64-65). The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota; the water sources were not further specified. The method was validated in the first trial in surface and drinking water matrices with insignificant modifications to the analytical instrumentation (pp. 8, 23).

**Table 2. Initial Validation Method Recoveries for Pyraclostrobin Metabolite BF 500-3 (Reg. No. 340266) in Water**

| Analyte <sup>1</sup>                      | Fortification Level (µg/L) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| <b>Surface Water<sup>2,3</sup></b>        |                            |                 |                    |                   |                        |                                 |
| Quantitation ion                          |                            |                 |                    |                   |                        |                                 |
| BF 500-3<br>(Reg. No. 340266)             | 0.03 (LOQ)                 | 5               | 81.7-89.7          | 86.0              | 3.31                   | 3.85                            |
|   | 0.3                        | 5               | 94.3-105           | 99.9              | 4.19                   | 4.20                            |
| Confirmatory ion                          |                            |                 |                    |                   |                        |                                 |
| BF 500-3<br>(Reg. No. 340266)             | 0.03 (LOQ)                 | 5               | 83.4-87.3          | 85.1              | 1.43                   | 1.68                            |
|   | 0.3                        | 5               | 95.1-106           | 101               | 4.88                   | 4.85                            |
| <b>Drinking (Tap) Water<sup>2,3</sup></b> |                            |                 |                    |                   |                        |                                 |
| Quantitation ion                          |                            |                 |                    |                   |                        |                                 |
| BF 500-3<br>(Reg. No. 340266)             | 0.03 (LOQ)                 | 5               | 98.8-111           | 104               | 4.38                   | 4.21                            |
|   | 0.3                        | 5               | 95.6-113           | 104               | 6.47                   | 6.25                            |
| Confirmatory ion                          |                            |                 |                    |                   |                        |                                 |
| BF 500-3<br>(Reg. No. 340266)             | 0.03 (LOQ)                 | 5               | 99.6-111           | 105               | 4.93                   | 4.69                            |
|   | 0.3                        | 5               | 98.2-109           | 105               | 4.12                   | 3.94                            |

Data (uncorrected recovery results, Appendices D-E, pp. 49-54) were obtained from p. 9 of MRID 50088701.

1 Pyraclostrobin metabolite BF 500-3 (Reg. No. 340266) = Methyl N-(2-[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl]phenyl)carbamate.

2 The surface water (ID No. 201441104; JL; pH 6.9, 32 mg equiv. CaCO<sub>3</sub>/L hardness, 6.1 ppm total organic carbon) and drinking (tap/drinking well) water (ID No. 201441105; pH 7.4, 25 mg equiv. CaCO<sub>3</sub>/L hardness, 0.7 ppm total organic carbon) was used in the study (p. 13; Appendix A, pp. 25-26). The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota; the water sources were not further specified.

3 Two ion transitions were monitored for BF 500-3 (quantitation and confirmatory, respectively) as follows: *m/z* 358→164 and *m/z* 358→132.

**Table 3. Independent Validation Method Recoveries for Pyraclostrobin Metabolite BF 500-3 (Reg. No. 340266) in Water**

| Analyte <sup>1</sup>                      | Fortification Level (µg/L) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| <b>Surface Water<sup>2,3</sup></b>        |                            |                 |                    |                   |                        |                                 |
| Quantitation ion                          |                            |                 |                    |                   |                        |                                 |
| BF 500-3<br>(Reg. No. 340266)             | 0.03 (LOQ)                 | 5               | 75-97              | 87                | 8.71                   | 9.98                            |
|   | 0.3                        | 5               | 75-112             | 96                | 11.2                   | 11.6                            |
| Confirmatory ion                          |                            |                 |                    |                   |                        |                                 |
| BF 500-3<br>(Reg. No. 340266)             | 0.03 (LOQ)                 | 5               | 75-95              | 85                | 7.49                   | 8.79                            |
|   | 0.3                        | 5               | 81-113             | 96                | 12.0                   | 12.5                            |
| <b>Drinking (Tap) Water<sup>2,3</sup></b> |                            |                 |                    |                   |                        |                                 |
| Quantitation ion                          |                            |                 |                    |                   |                        |                                 |
| BF 500-3<br>(Reg. No. 340266)             | 0.03 (LOQ)                 | 5               | 83-92              | 87                | 3.41                   | 3.92                            |
|   | 0.3                        | 5               | 69-96              | 82                | 10.2                   | 12.4                            |
| Confirmatory ion                          |                            |                 |                    |                   |                        |                                 |
| BF 500-3<br>(Reg. No. 340266)             | 0.03 (LOQ)                 | 5               | 82-93              | 88                | 4.40                   | 5.02                            |
|   | 0.3                        | 5               | 71-100             | 84                | 10.7                   | 12.7                            |

Data (uncorrected recovery results, p. 19; Appendix D, pp. 56-59) were obtained from p. 8 of MRID 50088706.

1 Pyraclostrobin metabolite BF 500-3 (Reg. No. 340266) = Methyl N-(2-([1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl)phenyl)carbamate.

2 The surface water (BASF Study 437860; RCN R130034; PW; pH 6.9, 41 mg equiv. CaCO<sub>3</sub>/L hardness, 11.0 ppm total organic carbon) and drinking (tap) water (BASF Reference No. 22014; TW; pH 7.3, 22 mg equiv. CaCO<sub>3</sub>/L hardness, 2.4 ppm total organic carbon) was used in the study (p. 13; Appendix F, pp. 64-65). The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota; the water sources were not further specified.

3 Two ion transitions were monitored for BF 500-3 (quantitation and confirmatory, respectively) as follows: *m/z* 357.9→164.1 and *m/z* 357.9→132.0.

### III. Method Characteristics

The LOQ and LOD for pyraclostrobin metabolite BF 500-3 in water were 0.03 µg/L and 0.006 µg/L, respectively, in the ECM and ILV (pp. 7, 18; Appendix C, pp. 33, 46 of MRID 50088701; pp. 7, 21 of MRID 50088706). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested, and the LOD was set to 20% of the LOQ. In the ECM, the LOD was also defined as a level which was at least 3-5 times the baseline noise (minimum signal-to-noise ratio of 3:1). No calculations were reported to justify the LOQ and LOD for the method in the ECM and ILV.

**Table 4. Method Characteristics**

| Analyte <sup>1</sup>   |                    | Pyraclostrobin metabolite BF 500-3 (Reg. No. 340266)   |  |
|--|--------------------|--|--|
| Limit of Quantitation (LOQ)  |                    | 0.03 µg/L  |  |
| Limit of Detection (LOD)   |                    | 0.006 µg/L   |  |
| Linearity<br>(calibration curve $r^2$<br>and concentration<br>range) | ECM <sup>2</sup>   | Surface<br>Water   | $r^2 = 0.9994$ (Q)<br>$r^2 = 0.9996$ (C)                   |
|  |                    | Drinking<br>Water  | $r^2 = 0.9952$ (Q)<br>$r^2 = 0.9964$ (C)                   |
|  | ILV                | Surface<br>Water   | $r^2 = \mathbf{0.9946}$ (Q)<br>$r^2 = 0.9953$ (C)          |
|  |                    | Drinking<br>Water  | $r^2 = \mathbf{0.9948}$ (Q)<br>$r^2 = \mathbf{0.9942}$ (C) |
|  | Concentration      |  | 0.006-0.15 ng/mL   |
| Repeatable   | ECM <sup>3</sup>   | Yes at LOQ and 10×LOQ<br>(characterized surface and drinking water)  |  |
|  | ILV <sup>4,5</sup> | Yes at LOQ and 10×LOQ<br>(characterized surface and drinking water)  |  |
| Reproducible   |                    | Yes at LOQ and 10×LOQ  |  |
| Specific   | ECM                | Yes, no matrix interferences were observed.<br>Minor baseline interference around the analyte peak interfered with peak integration. |  |
|  | ILV                | Yes, no matrix interferences were observed.  |  |

Data were obtained from pp. 7, 18; Appendix C, pp. 33, 46; p 9 (recovery data); Appendix E, pp. 51-54 (correlation coefficients); Appendix I, pp. 68-93 (calibration curves & chromatograms) of MRID 50088701; pp. 7, 21; p. 8 (recovery data); Appendices A-B, pp. 25-52 (calibration curves & chromatograms); Appendix D, pp. 56-59 (correlation coefficients) of MRID 50088706. Q = Quantitation ion transition; C = Confirmatory ion transition.

1 Pyraclostrobin metabolite BF 500-3 (Reg. No. 340266) = Methyl N-(2-([1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl)phenyl)carbamate.

2 Correlation coefficients ( $r^2$ ) values were reviewer-calculated from r values provided in the study report (Appendix E, pp. 51-54 of MRID 50088701; DER Attachment 2). Calibration curves were matrix-based.

3 In the ECM, surface water (ID No. 201441104; JL; pH 6.9, 32 mg equiv. CaCO<sub>3</sub>/L hardness, 6.1 ppm total organic carbon) and drinking (tap/drinking well) water (ID No. 201441105; pH 7.4, 25 mg equiv. CaCO<sub>3</sub>/L hardness, 0.7 ppm total organic carbon) was used in the study (p. 13; Appendix A, pp. 25-26 of MRID 50088701). The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota; the water sources were not further specified.

4 In the ILV, surface water (BASF Study 437860; RCN R130034; PW; pH 6.9, 41 mg equiv. CaCO<sub>3</sub>/L hardness, 11.0 ppm total organic carbon) and drinking (tap) water (BASF Reference No. 22014; TW; pH 7.3, 22 mg equiv. CaCO<sub>3</sub>/L hardness, 2.4 ppm total organic carbon) was used in the study (p. 13; Appendix F, pp. 64-65 of MRID 50088706). The water matrices were characterized by Agvise Laboratories, Northwood, North Dakota; the water sources were not further specified.

5 In the ILV, the method was validated in the first trial in surface and drinking water matrices with insignificant modifications to the analytical instrumentation (pp. 8, 23 of MRID 50088706).

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

#### IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, linearity was not satisfactory for BF 500-3 in either water matrix [surface water  $r^2 = 0.9946$  (Q), drinking water  $r^2 = 0.9948$  (Q) and  $r^2 = 0.9942$  (C); Appendix D, pp. 56-59 of MRID 50088706]. Linearity is satisfactory when  $r^2 \geq 0.995$ .
2. In ECM representative chromatograms, minor baseline interference around the analyte peak interfered with peak integration (Appendix I, pp. 68-93 of MRID 50088701).
3. 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. 7, 18; Appendix C, pp. 33, 46 of MRID 50088701; pp. 7, 21 of MRID 50088706). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested, and the LOD was set to 20% of the LOQ. In the ECM, the LOD was also defined as a level which was at least 3-5 times the baseline noise (minimum signal-to-noise ratio of 3:1). No calculations were reported to justify the LOQ and LOD for the method in the ECM and ILV.
4. In the ECM, matrix effects were found to be insignificant (<20%) for surface and drinking water matrices (p. 16; Tables 3-4, pp. 21-22 of MRID 50088701).
5. In the ECM, the stock and fortification solutions (in acetonitrile) and calibration standards (in water) were reported to be stable for up to 42 days and 30 days, respectively, when stored refrigerated in the dark in amber glass bottles (temperature not specified; stock and fortification solution data from a previous study; p. 16; Tables 5-6, p. 23 of MRID 50088701). The extracts of the water samples were found to be stable (>70% recovery) after one week under refrigeration in the dark in amber glass bottles (temperature not specified).
6. In the ECM, the following Protocol Amendments and Deviations were noted: 1) the data from the first analysis set was not used due to large interference peak generated using plastic cap in culture tube (Amendment); and 2) product ion spectra was not generated within the study (Deviation; Appendix L, p. 98 of MRID 50088701).

In the ECM, the Changes to the Amended Report were listed in Appendix M (Appendix M, p. 99 of MRID 50088701).

The ECM reported that no method findings/recommendation were noted by the ILV after successful trial completion (p. 17 of MRID 50088701).

7. The ILV study author documented all communications between the ILV and Study Monitor (p. 23; Appendix E, pp. 61-62 of MRID 50088706). Communications consisted of trial initiation and completion and insignificant protocol changes. No technical communications between the ILV and ECM occurred; no communications between the ILV and ECM occurred during the ILV trial.

8. It was reported for the ILV that analysis of 13 samples required *ca.* 6-8 hours of work including the calculation of the results (p. 22 of MRID 50088706).

## 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****BF 500-3 (Reg. No. 340266)**

**IUPAC Name:** Methyl N-(2-[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl]phenyl)carbamate

**CAS Name:** Methyl N-[[[1-(4-chlorophenyl)pyrazol-3-yl]oxy]-tolyl]-N-carbamate

**CAS Number:** 512165-96-7

**SMILES String:** COC(=O)Nc1ccccc1COc2cn(n2)c3ccc(cc3)Cl

