

**Analytical method for Fluoxapiprolin (BCS-CS55621) and seven degradates BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 in water**

**Reports:** ECM 1: MRID 52029302. Li, Y. 2021. An Analytical Method for the Determination of Residues of BCS-CS55621, and Its Metabolites BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 in Water Using LC/MS/MS. Bayer Method No: TY-004-W21-01. Activity ID: RATYN063. Report prepared by Bayer Crop Science, Chesterfield, Missouri, and sponsored and submitted by Bayer Crop Science, St. Louis, Missouri; 34 pages. Final report issued April 5, 2021.

ECM 2: MRID 51820517. Li, Y. 2021. In House Laboratory Validation of Analytical Method for the Determination of Residues of BCS-CS55621, and Its Metabolites BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 in Water Using LC/MS/MS. Study and Activity ID: RATYN063. Report prepared by Bayer Crop Science, Chesterfield, Missouri, and sponsored and submitted by Bayer Crop Science, St. Louis, Missouri; 136 pages. Final report issued June 3, 2021.

ILV: MRID 51820518. Swaim, L. 2022. Independent Laboratory Validation of "An Analytical Method for the Determination of Residues of BCS-CS55621, and Its Metabolites BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 in Water Using LC/MS/MS" with Stability Data. Bayer CropScience Study No.: RATY0249. Eurofins Study No.: 90890. eSM Study No.: S21-05737. Report prepared by Eurofins EAG Agrosience, LLC, Columbia, Missouri, and sponsored and submitted by Bayer CropScience, Chesterfield, Missouri; 355 pages. Final report issued January 24, 2022.

**Document No.:** MRIDs 52029302, 51820517, & 51820518

**Guideline:** 850.6100

**Statements:** ECM 1: No claim of compliance with USEPA FIFRA Good Laboratory Practices (GLP) are made for the document since it was not a study as defined by 40 CFR Part 160 or current OECD GLP (p. 3 of MRID 52029302). Signed and dated No Data Confidentiality, GLP, and Authenticity statements were provided (pp. 2-4). A Quality Assurance statement was not included.

ECM 2: The study was conducted in accordance with USEPA FIFRA GLP standards (40 CFR Part 160; 1989; p. 3 of MRID 51820517). 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 (40 CFR Part 160 (1989), which is compatible with OECD GLP [ENV/MC/CHEM (98) 17; p. 3 of MRID 51820518]. Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Certification statements were provided (pp. 2-6).

**Classification:** This analytical method is classified as **unacceptable, but upgradeable**. ECM 1 MRID 52029302 contained the full method written in a step-wise manner, but no performance data; while ECM 2 MRID 51820517 contained performance data, but only a method summary. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ. The water matrices used by the internal validation (ECM 2) were not characterized or described; the water sources were not reported accurately. An updated Bayer Method No. TY-004-W21-01 should be submitted to correct typographical errors related to the tabulated ion pair transitions for BCS-CY96288-IS and BCS-CZ38260. Communication between the ILV study personnel and the Sponsor Monitor was documented and included discussion of the independent laboratory validation, as well as the storage stability experiment; no collusion occurred. ECM 2 recovery results were corrected for residues quantified in the controls.

**PC Code:** 110304

**EFED Final  
Reviewer:** Richard Shamblen,  
Biologist

RICHARD

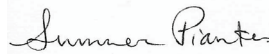
Signature: SHAMBLEN  
Date: December 17, 2024

Digitally signed by  
RICHARD SHAMBLEN  
Date: 2024.12.17 11:38:53  
-05'00'

**PB&A/CSS JV  
Reviewers:**

Summer Piantes, M.S.,  
Environmental Scientist

Signature:



Date: 04/01/2023

Lisa Muto, M.S.,  
Environmental Scientist

Signature:



Date: 04/07/2023

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

## Executive Summary

This analytical method, Bayer Method TY-004-W21-01, is designed for the quantitative determination of fluoxapiprolin (BCS-CS55621) and its metabolites BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585

at the LOQ of 0.5 ng/mL in water using LC/MS/MS. The LOQ is **less than** the lowest toxicological level of concern in water for all analytes. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ of 0.5 ng/mL for fluoxapiprolin (BCS-CS55621) and its metabolites BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 in water.

The ILV validated the method, Bayer Method TY-004-W21-01, with the first trial as written, except for minor modifications to optimize the instrumentation used in the ILV as allowed by the ECM. These modifications included: 1) changing the LC instrumentation and adding a weak needle wash. An updated ECM is not required for these modifications; however, the ECM method (Bayer Method TY-004-W21-01) should be updated to correct the following typographical errors: 1) the BCS-CY96288-IS was erroneously reported as BCS-CS55621-IS in ECM 1 and ECM 2; 2) the confirmation ion transition pair reported in the ECM 2 recovery table for BCS-CZ38260 was the quantitative ion pair for the BCS-CZ38260-(<sup>15</sup>N<sub>2</sub>) internal standard; and 3) the range of calculated LODs in the text was inconsistent with those reported in the ECM 2 tables.

The step-wise analytical method (Bayer Method TY-004-W21-01) was described in ECM 1 (MRID 52029302). No performance data was submitted in this study. Internal verification of Bayer Method TY-004-W21-01 was described in ECM 2 (MRID 21820517). The ECM 2 test matrices were uncharacterized bulk untreated water samples from two unrelated water studies in Minnesota and Wisconsin. The ILV test matrix was a characterized drinking water matrix.

Although the ILV was conducted independently of the internal validation (ECM 1 and ECM 2) since the laboratory personnel and equipment differed between the ILV laboratory and ECM 1 and ECM 2 laboratory, there was extensive communication between the ILV study personnel and the Sponsor Monitor.

All ILV and ECM 2 data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 at 0.5 ng/mL and 5.0 ng/mL in water matrices. Additionally, ILV calculated LOQs exceeded the method LOQ (0.5 ng/mL) for fluoxapiprolin (BCS-CS55621; 0.522 ng/mL), BCS-CZ38260 (0.623 ng/mL; confirmation ion pair transition), BCS-CY96288 (0.526 ng/mL) and BCS-BP32808 (0.561 ng/mL); ILV calculated LODs exceeded 30% of the method LOQ (0.15 ng/mL) for fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CZ38260 (confirmation ion pair transition), and BCS-BP32808.

**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 |            |        |                                          |                    |          |                             |
| Fluoxapiprolin (BCS-CS55621) | 52029302 <sup>1</sup> & 51820517 <sup>2</sup> | 51820518 <sup>3</sup>             |            | Water  | 05/04/2021 (ECM 1)<br>03/06/2021 (ECM 2) | Bayer Crop Science | LC/MS/MS | 0.5 ng/mL                   |
| BCS-CY96288                  |                                               |                                   |            |        |                                          |                    |          |                             |
| BCS-CU97237                  |                                               |                                   |            |        |                                          |                    |          |                             |
| BCS-DA63612                  |                                               |                                   |            |        |                                          |                    |          |                             |
| BCS-CC26101                  |                                               |                                   |            |        |                                          |                    |          |                             |
| BCS-CZ38260                  |                                               |                                   |            |        |                                          |                    |          |                             |
| BCS-BP32808                  |                                               |                                   |            |        |                                          |                    |          |                             |
| BCS-DH17585                  |                                               |                                   |            |        |                                          |                    |          |                             |

1 ECM 1 MRID 52029302 contained the full method written in a step-wise manner, but no performance data; while ECM 2 MRID 51820517 contained performance data, but only a method summary.

2 In the ECM 2 MRID 51820517, two bulk untreated water samples were included in the internal validation (pp. 9 and 20 of MRID 51820517). The source of Water 1 was untreated water from Minnesota (Sample ID: IFTMN-GW15-A001) from study FTISN003 (Minnesota; pp. 9 and 20 of MRID 51820517). The source of Water 2 was untreated water from Wisconsin (Sample ID: IFTWI-GW25-A036) from study FTISN004 (Wisconsin, pp. 9 and 20 of MRID 51820517). Water characteristics including pH, hardness, and conductivity were not reported. The ECM 2 indicated that the source of the water was associated with terrestrial field dissipation studies; however, the references provided in the ECM 2 are not consistent with that description (p. 30 of MRID 51820517). MRID numbers for the referenced studies Study FTISN003 and Study FTISN004 were not reported in ECM 1 MRID 52029302 or ECM 2 MRID 51820517 and could not be determined by the reviewer.

3 In the ILV, the water matrix was drinking water obtained from the cafeteria in the Eurofins Testing facility (p. 27 of MRID 51820518) and had the following characteristics: pH: 8.2; Conductivity: 0.50 mmhos/cm; Hardness: 278 mg equivalent CaCO<sub>3</sub>/L; Sodium Adsorption Ratio (SAR): 0.53; TDS: 350 ppm; Turbidity: 0.16 NTU; Alkalinity 293 mg CaCO<sub>3</sub>/L; Potassium: 5.1 ppm; Calcium: 64 ppm; Magnesium: 28 ppm; Sodium: 20 ppm; Carbonates 0.00 meq/L; Bicarbonates: 5.57 meq/L; Sulfate-Sulfur: 6.4 ppm; Chloride 6.1 ppm; Nitrogen (Nitrate): <0.1 ppm. Water characterization was performed by Agvise Laboratories in Northwood, North Dakota (p. 27; Appendix 3, p. 270).

## I. Principle of the Method

### Bayer Method TY-004-W21-01

Water matrix samples were diluted by adding native and isotopic internal standards (IS) diluted and analyzed by LC/MS/MS. The step-wise analytical method (TY-004-W21-01) was described in ECM 1 (MRID 52029302). Internal verification of Method TY-004-W21-01 was described in ECM 2 (MRID 51820517).

Primary native standard solutions of each analyte fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and

BCS-DH17585 were prepared by dissolving *ca.* 5 mg of the reference item in 50 mL of either acetonitrile or acetonitrile:water (4:1; v:v) to a final concentration of *ca.* 100 µg/mL (p. 8 of MRID 52029302). Fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CC26101, BCS-CZ38260, and BCS-BP32808 were dissolved in acetonitrile. BCS-CU97237, BCS-DA63612, and BCS-DH17585 were dissolved in acetonitrile:water (4:1; v:v).

Primary isotopic internal standard solutions for each analyte were prepared by dissolving *ca.* 2.5 mg of isotopic standard material in 25 mL of solvent to a final concentration of *ca.* 100 µg/mL. The solvent for each isotopic internal was either acetonitrile or acetonitrile:water (4:1; v:v) consistent with the preparation of the primary native standard solutions (p. 8 of MRID 52029302).

Primary solutions should be corrected for the purity of the standard during the initial preparation of native and internal standard solutions (p. 8 of MRID 52029302). The purity of the starting reference and isotopic internal standards was not provided in ECM 1.

A secondary mixed reference standard solution was prepared by mixing an aliquot of *ca.* 1 mL of each primary native standard in acetonitrile:water (4:1; v:v) to a final volume of 100 mL and final concentration of 1,000 ng/mL. Additional mixed native spiking solutions were prepared by serial dilution to final concentrations of 100 ng/mL and 10 ng/mL by diluting aliquots of the mixed native standard solution in acetonitrile:water (1:1; v:v; p. 9 of MRID 52029302).

Secondary mixed internal standard solutions were prepared by mixing a *ca.* 1 mL aliquot each of the primary isotopic internal standards and diluting the mixture to a final volume of 100 mL and a final concentration of 1.0 µg/mL in acetonitrile:water (4:1; v:v; p. 10 of MRID 52029302). Mixed isotopic internal standard solutions were prepared by serial dilution of the mixed internal standard solution to final concentrations of 20 ng/mL and 4 ng/mL in acetonitrile:water (1:1; v:v) and acetonitrile:water (1:9; v:v), respectively (p. 10 of MRID 52029302).

Primary standard solutions should be stored in a freezer when not in use. Secondary native and internal standards should be stored in a refrigerator when not in use (p. 9 of MRID 52029302).

Calibration standards were prepared in water:acetonitrile (90:10, v:v) in the range of 0.1 ng/mL to 10 ng/mL with extra calibration standards at 16 ng/mL and 20 ng/mL for the analysis of high residue samples (p. 11 of MRID 52029302). Internal standard (IS) was added to each calibration standard (4 ng/mL concentration). The ratio of the analyte to the IS was used to generate calibration curves for quantifying analytes in test samples (p. 14 of MRID 52029302; Appendix 3, pp. 115-130 of MRID 51820517). The use of isotopically-labeled internal standards in quantification methods is a method which is used to negate any matrix effects which may be present.

Water samples (1.0 mL) were fortified using combined fortification solutions to LOQ concentrations of 0.5 ng/mL and 5 ng/mL of fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 (p. 10 of MRID 51820517). Untreated control samples of Water 1 and Water 2 were also analyzed (p. 24; Appendix 2, pp. 91-98 of MRID 51820517).



Samples were analyzed Shimadzu LC-20ADXR HPLC binary solvent delivery pump coupled to a triple-quadrupole tandem API 6000+ mass spectrometer (MS/MS) equipped with an ESI Turbo IonSpray interface operated in multiple reaction monitoring (MRM) mode with unit mass resolution. (p. 13 of MRID 52029302; Appendix 4, pp. 131-136 of MRID 51820517). The following LC conditions were reported in ECM 1 and ECM 2 (p. 12 of MRID 52029302; p. 21; Appendix 4, pp. 131-136 of MRID 51820517): Phenomenex Kinetex Luna Omega C18 (100 mm x 2.1 mm column; 1.6 µm particle size; column temperature ambient/40°C; injection volume 20 µL), mobile phase of (A) 0.5% acetic acid water, (B) 0.5% acetic acid in acetonitrile (binary flow rate of 0.30 mL/min). The following LC gradient and flow rate were reported in ECM 1 (p. 12 of MRID 52029302) and ECM 2 (p. 21 of MRID 51820517).

| Time (min.) | Module     | Events      | Parameters |
|-------------|------------|-------------|------------|
| 0.0         | Pumps      | Pump B Conc | 5          |
| 0.3         | Pumps      | Pump B Conc | 5          |
| 2.5         | Pumps      | Pump B Conc | 80         |
| 4.0         | Pumps      | Total Flow  | 0.3        |
| 4.1         | Pumps      | Total Flow  | 0.6        |
| 5.8         | Pumps      | Pump B Conc | 100        |
| 5.9         | Pumps      | Pump B Conc | 5          |
| 6.8         | Pumps      | Total Flow  | 0.6        |
| 6.9         | Pumps      | Total Flow  | 0.3        |
| 7.0         | Controller | Stop        |            |

The following MS/MS conditions were reported in the ECM 1 and ECM 2: 5500V positive (+) ESI mode, -4500 negative (-) ESI mode, source temperature 600°C, Turbo Ion Spray interface, and MRM (pp. 13-14 of MRID 52029302 and pp. 21-22 of MRID 51820517). Two ion pair transitions were monitored for fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-BP32808, and BCS-DH17585; three ion pair transitions were monitored for BCS-CZ38260, but only two ion transitions were quantified in the internal validation. The following MS parameters were reported in the ECM 1 and ECM 2.

| Analyte                                                                         | Primary              | Confirmatory          | Mode     | Expected Retention Times (minutes) |
|---------------------------------------------------------------------------------|----------------------|-----------------------|----------|------------------------------------|
| Fluoxapiprolin (BCS-CS55621)                                                    | <i>m/z</i> 650→610.2 | <i>m/z</i> 650→378    | Positive | <i>ca.</i> 3.93                    |
| BCS-CS55621-( <sup>13</sup> C <sub>3</sub> , <sup>15</sup> N <sub>3</sub> )-IS  | <i>m/z</i> 656→616.2 | --                    | Positive | <i>ca.</i> 3.93                    |
| BCS-CY96288                                                                     | <i>m/z</i> 666→626.1 | <i>m/z</i> 666→191    | Positive | <i>ca.</i> 3.76                    |
| BCS-CY96288-( <sup>13</sup> C <sub>3</sub> , <sup>15</sup> N <sub>3</sub> )-IS* | <i>m/z</i> 672→634.1 | --                    | Positive | <i>ca.</i> 3.73                    |
| BCS-CU97237                                                                     | <i>m/z</i> 442.2→332 | <i>m/z</i> 442.2→208  | Positive | <i>ca.</i> 2.92                    |
| BCS-CU97237-( <sup>13</sup> C <sub>6</sub> )-IS                                 | <i>m/z</i> 448→338   | --                    | Positive | <i>ca.</i> 2.92                    |
| BCS-DA63612                                                                     | <i>m/z</i> 456→345.2 | <i>m/z</i> 456→222.1  | Positive | <i>ca.</i> 2.34                    |
| BCS-DA63612-( <sup>13</sup> C <sub>6</sub> )-IS                                 | <i>m/z</i> 462→351   | --                    | Positive | <i>ca.</i> 2.34                    |
| BCS-CC26101                                                                     | <i>m/z</i> 225→161   | <i>m/z</i> 225→141    | Negative | <i>ca.</i> 3.20                    |
| BCS-CC26101-( <sup>13</sup> C <sub>2</sub> )-IS                                 | <i>m/z</i> 227→162   | --                    | Negative | <i>ca.</i> 3.20                    |
| BCS-CZ38260                                                                     | <i>m/z</i> 161→76    | --                    | Negative | <i>ca.</i> 2.74                    |
|                                                                                 | --                   | <i>m/z</i> 163.1→125  | Positive | <i>ca.</i> 2.74                    |
|                                                                                 | --                   | <i>m/z</i> 163.1→97** | Positive | <i>ca.</i> 2.74                    |
| BCS-CZ38260-( <sup>15</sup> N <sub>2</sub> )-IS                                 | <i>m/z</i> 163→77    | --                    | Negative | <i>ca.</i> 2.74                    |

|                                                 |               |               |          |          |
|-------------------------------------------------|---------------|---------------|----------|----------|
|                                                 | --            | $m/z$ 165→127 | Positive |          |
| BCS-BP32808                                     | $m/z$ 169→101 | $m/z$ 169→149 | Positive | ca. 3.31 |
| BCS-BP32808-( <sup>15</sup> N <sub>2</sub> )-IS | $m/z$ 171→101 | --            | Positive | ca. 3.31 |
| BCS-DH17585                                     | $m/z$ 403→169 | $m/z$ 403→157 | Positive | ca. 3.39 |
| BCS-DH17585-( <sup>13</sup> C <sub>6</sub> )-IS | $m/z$ 409→169 | --            | Positive | ca. 3.39 |

\* Note: BCS-CY96288-IS was erroneously reported as BCS-CS55621-IS in ECM 1 and ECM 2. The reviewer made the assignment of BCS-CY96288-IS based on the molecular weight/primary ion transition, as well as supporting chromatograms and calibration plots (Table 8, pp. 13-14 of MRID 52029302; Appendix 2, pp. 78-114; Appendix 3, pp. 115-130 of MRID 51820517). The ILV MRID 51820518 also noted this typographical error (p. 37 of MRID 51820518).

\*\* Not used in validation, based on ECM 2 representative chromatograms and linear plot (Appendix 2, pp. 78-114 and Appendix 3, p. 124 of MRID 51820517).

## ILV

The ILV was based on MRID 52089302 (Bayer Method TY-004-W21-01). The independent laboratory performed the ECM as written with the following modifications. The LC equipment used in the ILV differed from the ECM. Samples were analyzed Applied Biosystems/Sciex System with Degasser, Solvent Valve Autosampler, Column Oven, and System Controller HPLC coupled to an Applied Biosystems/Sciex API 6500+ Q-trap with Ion Chromatograph SelexION+ mass spectrometer (MS/MS) equipped with a Turbo IonSpray interface operated in multiple MRM mode with unit mass resolution (p. 32 of MRID 51820518). The LC settings including column specifications, the column temperature, the injection volume, and flow rate were the same as used in the ECM. The ILV listed a weak needle wash of acetonitrile:methanol:water (1:1:2; v:v:v) was used (p. 31 of MRID 51820518).

The MS parameters of the ILV were the same as those of the ECM (p. 32 of MRID 51820518). The ILV monitored ion transitions were the same as those of the ECM for all analytes, except that both method confirmation ion transitions for BCS-CZ38260 were quantified in the ILV. The ILV and reviewer noted ECM 1 MRID 52029302 and ECM 2 MRID 51820517 contained the incorrectly referenced listing of BCS-CY96288-IS in the mass transition tables (p. 13 of MRID 51820515; p. 22 of MRID 51820517).

## LOD/LOQ

The Limit of Quantification (LOQ) in water was reported as 0.5 ng/mL for fluoxapiprolin (BCS-CS55621) and its metabolites BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 in the ECM 1, the ECM 2, and the ILV (p. 15 of MRID 52029302; pp. 9-10, 25 of MRID 51820517; p. 36 of MRID 51820518). In the ILV, the LOQ was defined as the lowest fortification level at which acceptable recovery data was obtained. The LOQ was 0.5 ng/mL for fluoxapiprolin (BCS-CS55621) and its metabolites in drinking water (p. 36 of MRID 51820518).

The Limit of Detection (LOD) was not reported in ECM 1. The LOD for fluoxapiprolin (BCS-CS55621) and its metabolites BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 in the ECM 2 Water 1 and Water 2 matrices was calculated for each analyte based on the standard deviation at the 0.5 ng/L fortification level and was reported as 0.06-0.09 ng/mL for all analytes in water (p. 28 of MRID 51820517). In the

ILV, the LOD for each analyte in drinking water was defined as the lowest calibration standard in the matrix, 0.100 ng/mL. The LOD was confirmed in the ILV by multiplying the standard deviation of the analyte concentration at the LOQ by a one-tailed Student's t statistic (p. 28 of MRID 51820517). In the ILV drinking water matrix, the LOD for fluoxapiprolin (BCS-CS55621) and its metabolites BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 ranged from 0.0256 to 0.187 ng/mL for the primary ion pair transition (Table 5, pp. 61-63 of MRID 51820518). The LOD calculated in the ECM and the ILV varied depending on the matrix and transition ion pair for each analyte and are listed on **Table 4a**.

Since the LOQs were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQs are the lowest levels of method validation (LLMVs) rather than LOQs.

## II. Recovery Findings

ECM 1 (MRID 52029302): MRID 51820515 was a method only study report. No performance data was submitted in this study.

ECM 2 (MRID 51820517): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD  $\leq$ 20%) for the Minnesota and Wisconsin water matrix samples fortified with fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 in at the LOQ (0.5 ng/mL; n = 7) and 10 $\times$ LOQ (5.0 ng/mL; n = 5; pp. 10, 25; Tables 1-16, pp. 31-34; Appendix 1, pp. 38-77 of MRID 51820517). Performance data for the quantification and confirmation ion pair transitions were evaluated and comparable. Recovery results were corrected for residues quantified in the controls (p. 15 of MRID 52029302; pp. 23-24; Appendix 1, pp. 38-77 of MRID 51820517). Residues of fluoxapiprolin (BCS-CS55621) and metabolites BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 were below the LOD (0.06-0.09 ng/mL) for the respective analyte in the two control samples for each water matrix (p. 10; Appendix 2; pp. 91-92 of MRID 51820517).

In ECM 2 MRID 51820517, the source of Water 1 was bulk untreated water from Minnesota (Sample ID: IFTMN-GW15-A001) from study FTISN003 (Minnesota; pp. 9 and 20 of MRID 51820517). The source of Water 2 was bulk untreated water from Wisconsin (Sample ID: IFTWI-GW25-A036) from study FTISN004 (Wisconsin, pp. 9 and 20 of MRID 51820517). Water characteristics including pH, hardness, and conductivity were not reported. The ECM 2 indicated that the source of the water was associated with terrestrial field dissipation studies; however, the references provided in the ECM 2 are not consistent with that description (p. 30 of 51820517).

ILV (MRID 51820518): Mean recoveries and RSDs met requirements for drinking water matrix samples fortified with fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 at the LOQ (0.500 ng/mL; n = 5) and 10 $\times$  LOQ (5.00 ng/mL; n = 5; Tables 1, pp. 43-51 of MRID 51820518). One reagent blank and two control samples were also analyzed. The analytes were quantified one ion pair transition and confirmatory ion pair transition. The quantitation and confirmatory ion pair transitions are listed in Section I; both confirmation ion transitions for



BCS-CZ38260 were quantified in the ILV. Performance data for the quantification and confirmation ion pair transitions were comparable. Fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 were <LOD in the control samples and reagent blanks.

The ILV drinking water matrix was obtained from the cafeteria in the Eurofins Testing facility (p. 27 of MRID 51820518) and had the following characteristics: pH: 8.2; Conductivity: 0.50 mmhos/cm; Hardness: 278 mg equivalent  $\text{CaCO}_3/\text{L}$ ; Sodium Adsorption Ratio (SAR): 0.53; TDS: 350 ppm; Turbidity: 0.16 NTU; Alkalinity 293 mg  $\text{CaCO}_3/\text{L}$ ; Potassium: 5.1 ppm; Calcium: 64 ppm; Magnesium: 28 ppm; Sodium: 20 ppm; Carbonates 0.00 meq/L; Bicarbonates: 5.57 meq/L; Sulfate-Sulfur: 6.4 ppm; Chloride 6.1 ppm; Nitrogen (Nitrate): <0.1 ppm. Water characterization was performed by Agvise Laboratories in Northwood, North Dakota (p. 27; Appendix 3, p. 270).

The method (Bayer Method TY-004-W21-01) was validated in one trial by the ILV in the drinking water matrix with modifications to the analytical method (see Reviewer's Comment #8). In the ILV, no matrix suppression or enhancement was observed (pp. 36, 39; Table 1, pp. 43-51, Table 4, p. 60; Figures 12-17, pp. 167-218 of MRID 51820518). The ILV method was based on the ECM method; however, the ILV was modified to optimize the LC-MS/MS equipment in the ILV. An updated ECM is not required based on ILV modifications.

**Table 2. Initial Validation Method Recoveries for Fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 in Water<sup>1,2,3</sup>**

| Analyte                      | Fortification Level (ng/mL) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|------------------------------|-----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| <b>Water 1 - Minnesota</b>   |                             |                 |                    |                   |                        |                                 |
| Quantitation ion transition  |                             |                 |                    |                   |                        |                                 |
| Fluoxapiprolin (BCS-CS55621) | 0.5 (LOQ)                   | 7               | 90-108             | 101               | 7.3                    | 7.2                             |
|                              | 5.0                         | 5               | 88-104             | 94                | 6.4                    | 6.8                             |
| BCS-CY96288                  | 0.5 (LOQ)                   | 7               | 92-114             | 100               | 8.0                    | 8.0                             |
|                              | 5.0                         | 5               | 95-102             | 100               | 3.0                    | 3.0                             |
| BCS-CU97237                  | 0.5 (LOQ)                   | 7               | 92-108             | 97                | 6.3                    | 6.5                             |
|                              | 5.0                         | 5               | 94-103             | 97                | 3.7                    | 3.8                             |
| BCS-DA63612                  | 0.5 (LOQ)                   | 7               | 94-102             | 97                | 3.2                    | 3.3                             |
|                              | 5.0                         | 5               | 93-111             | 101               | 7.6                    | 7.5                             |
| BCS-CC26101                  | 0.5 (LOQ)                   | 7               | 90-106             | 101               | 5.5                    | 5.5                             |
|                              | 5.0                         | 5               | 94-108             | 101               | 5.4                    | 5.4                             |
| BCS-CZ38260                  | 0.5 (LOQ)                   | 7               | 96-106             | 102               | 3.9                    | 3.8                             |
|                              | 5.0                         | 5               | 95-111             | 102               | 5.6                    | 5.5                             |
| BCS-BP32808                  | 0.5 (LOQ)                   | 7               | 98-112             | 103               | 4.9                    | 4.7                             |
|                              | 5.0                         | 5               | 100-110            | 104               | 4.1                    | 4.0                             |
| BCS-DH17585                  | 0.5 (LOQ)                   | 7               | 92-108             | 101               | 5.0                    | 5.0                             |
|                              | 5.0                         | 5               | 98-111             | 102               | 5.0                    | 4.9                             |
| Confirmation ion transition  |                             |                 |                    |                   |                        |                                 |
| Fluoxapiprolin (BCS-CS55621) | 0.5 (LOQ)                   | 7               | 88-108             | 101               | 6.9                    | 6.8                             |
|                              | 5.0                         | 5               | 92-103             | 98                | 5.4                    | 5.5                             |
| BCS-CY96288                  | 0.5 (LOQ)                   | 7               | 86-124             | 102               | 12.9                   | 12.7                            |
|                              | 5.0                         | 5               | 91-101             | 96                | 4.8                    | 5.1                             |
| BCS-CU97237                  | 0.5 (LOQ)                   | 7               | 94-112             | 101               | 6.9                    | 6.8                             |
|                              | 5.0                         | 5               | 89-106             | 99                | 6.5                    | 6.6                             |
| BCS-DA63612                  | 0.5 (LOQ)                   | 7               | 94-104             | 99                | 3.4                    | 3.5                             |
|                              | 5.0                         | 5               | 89-111             | 99                | 7.9                    | 8.0                             |
| BCS-CC26101                  | 0.5 (LOQ)                   | 7               | 94-110             | 99                | 5.6                    | 5.7                             |
|                              | 5.0                         | 5               | 86-103             | 96                | 6.8                    | 7.1                             |
| BCS-CZ38260                  | 0.5 (LOQ)                   | 7               | 88-104             | 97                | 5.5                    | 5.7                             |
|                              | 5.0                         | 5               | 92-108             | 100               | 7.5                    | 7.5                             |
| BCS-BP32808                  | 0.5 (LOQ)                   | 7               | 93-109             | 103               | 8.0                    | 7.7                             |
|                              | 5.0                         | 5               | 92-106             | 97                | 5.2                    | 5.3                             |
| BCS-DH17585                  | 0.5 (LOQ)                   | 7               | 86-108             | 99                | 8.1                    | 8.2                             |
|                              | 5.0                         | 5               | 93-106             | 97                | 5.3                    | 5.4                             |
| <b>Water 2 - Wisconsin</b>   |                             |                 |                    |                   |                        |                                 |
| Quantitation ion transition  |                             |                 |                    |                   |                        |                                 |
| Fluoxapiprolin (BCS-CS55621) | 0.5 (LOQ)                   | 7               | 92-110             | 101               | 6.7                    | 6.6                             |
|                              | 5.0                         | 5               | 96-110             | 100               | 5.6                    | 5.6                             |
| BCS-CY96288                  | 0.5 (LOQ)                   | 7               | 94-108             | 102               | 6.0                    | 5.9                             |
|                              | 5.0                         | 5               | 94-105             | 99                | 4.0                    | 4.0                             |
| BCS-CU97237                  | 0.5 (LOQ)                   | 7               | 84-108             | 98                | 8.2                    | 8.4                             |

| Analyte                      | Fortification Level (ng/mL) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|------------------------------|-----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
|                              | 5.0                         | 5               | 93-114             | 102               | 7.6                    | 7.5                             |
| BCS-DA63612                  | 0.5 (LOQ)                   | 7               | 94-108             | 101               | 5.5                    | 5.5                             |
|                              | 5.0                         | 5               | 90-108             | 99                | 7.4                    | 7.4                             |
| BCS-CC26101                  | 0.5 (LOQ)                   | 7               | 90-100             | 95                | 4.9                    | 5.1                             |
|                              | 5.0                         | 5               | 94-103             | 100               | 2.0                    | 2.0                             |
| BCS-CZ38260                  | 0.5 (LOQ)                   | 7               | 89-107             | 97                | 7.5                    | 7.7                             |
|                              | 5.0                         | 5               | 93-100             | 96                | 2.5                    | 2.6                             |
| BCS-BP32808                  | 0.5 (LOQ)                   | 7               | 96-112             | 103               | 5.5                    | 5.4                             |
|                              | 5.0                         | 5               | 101-112            | 106               | 4.0                    | 3.8                             |
| BCS-DH17585                  | 0.5 (LOQ)                   | 7               | 94-106             | 98                | 5.1                    | 5.2                             |
|                              | 5.0                         | 5               | 94-111             | 99                | 6.9                    | 7.0                             |
| Confirmation ion transition  |                             |                 |                    |                   |                        |                                 |
| Fluoxapiprolin (BCS-CS55621) | 0.5 (LOQ)                   | 7               | 92-110             | 98                | 6.6                    | 6.7                             |
|                              | 5.0                         | 5               | 98-113             | 104               | 5.7                    | 5.5                             |
| BCS-CY96288                  | 0.5 (LOQ)                   | 7               | 84-110             | 101               | 8.7                    | 8.6                             |
|                              | 5.0                         | 5               | 92-119             | 102               | 10.5                   | 10.3                            |
| BCS-CU97237                  | 0.5 (LOQ)                   | 7               | 88-106             | 97                | 7.0                    | 7.2                             |
|                              | 5.0                         | 5               | 85-103             | 96                | 6.7                    | 7.0                             |
| BCS-DA63612                  | 0.5 (LOQ)                   | 7               | 94-104             | 100               | 4.6                    | 4.6                             |
|                              | 5.0                         | 5               | 92-110             | 101               | 6.4                    | 6.3                             |
| BCS-CC26101                  | 0.5 (LOQ)                   | 7               | 90-106             | 97                | 5.0                    | 5.2                             |
|                              | 5.0                         | 5               | 96-109             | 103               | 6.8                    | 6.6                             |
| BCS-CZ38260                  | 0.5 (LOQ)                   | 7               | 92-102             | 99                | 4.0                    | 4.0                             |
|                              | 5.0                         | 5               | 97-108             | 104               | 4.3                    | 4.2                             |
| BCS-BP32808                  | 0.5 (LOQ)                   | 7               | 95-107             | 102               | 4.1                    | 4.0                             |
|                              | 5.0                         | 5               | 95-108             | 102               | 4.6                    | 4.5                             |
| BCS-DH17585                  | 0.5 (LOQ)                   | 7               | 90-102             | 97                | 4.5                    | 4.6                             |
|                              | 5.0                         | 5               | 92-109             | 97                | 7.0                    | 7.2                             |

Data (recovery results were corrected for residues quantified in the controls, p. 15 of MRID 52029302; pp. 23-24; Appendix 1, pp. 38-77 of MRID 51820517) were obtained from Tables 1, 3, 5, 7, 9, 11, 13, and 15 (quantitation ion pair transition) and Tables 2, 4, 6, 8, 10, 12, 14, and 16 (confirmatory ion pair transition); pp. 31-34 of MRID 51820517). Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

- 1 1 ECM 1 MRID 52029302 contained the full method written in a step-wise manner, but no performance data; while ECM 2 MRID 51820517 contained performance data, but only a method summary.
- 2 In the ECM 2 MRID 51820517, two bulk untreated water samples were included in the internal validation (pp. 9 and 20 of MRID 51820517). The source of Water 1 was untreated water from Minnesota (Sample ID: IFTMN-GW15-A001) from study FTISN003 (Minnesota; pp. 9 and 20 of MRID 51820517). The source of Water 2 was untreated water from Wisconsin (Sample ID: IFTWI-GW25-A036) from study FTISN004 (Wisconsin, pp. 9 and 20 of MRID 51820517). Water characteristics including pH, hardness, and conductivity were not reported. The ECM 2 indicated that the source of the water was associated with terrestrial field dissipation studies; however, the references provided in the ECM 2 are not consistent with that description (p. 30 of 51820517). MRID numbers for the referenced studies Study FTISN003 and Study FTISN004 were not reported in ECM 1 MRID 52029302 or ECM 2 MRID 51820517 and could not be determined by the reviewer.
- 3 Two ion pair transitions were monitored (primary and confirmation, respectively):  $m/z$  650→610.2 and  $m/z$  650→378 for fluoxapiprolin (BCS-CS55621),  $m/z$  666→626.1 and  $m/z$  666→191 for BCS-CY96288,  $m/z$  442.2→332 and  $m/z$  442.2→208 for BCS-CU97237,  $m/z$  456→345.2 and  $m/z$  456→222.1 for BCS-DA63612,  $m/z$  225→161 and  $m/z$  225→141 for BCS-CC26101,  $m/z$  161→76 and  $m/z$  163.1→125 for BCS-CZ38260 (see

Reviewer's Comments),  $m/z$  169→101 and  $m/z$  169→149 for BCS-BP32808, and  $m/z$  403→169 and  $m/z$  403→157 for BCS-DH17585.

**Table 3. Independent Validation Method Recoveries for Fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 in Water<sup>1,2</sup>**

| Analyte                       | Fortification Level (ng/mL) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|-------------------------------|-----------------------------|-----------------|--------------------|-------------------|------------------------|---------------------------------|
| <b>Drinking Water</b>         |                             |                 |                    |                   |                        |                                 |
| Quantitation ion transition   |                             |                 |                    |                   |                        |                                 |
| Fluoxapiprolin (BCS-CS55621)  | 0.5 (LOQ)                   | 5               | 84-109             | 98                | 9.4                    | 9.6                             |
|                               | 5.0                         | 5               | 84-104             | 98                | 8.0                    | 8.2                             |
| BCS-CY96288                   | 0.5 (LOQ)                   | 5               | 86-101             | 90                | 9.3                    | 10.4                            |
|                               | 5.0                         | 5               | 82-107             | 97                | 9.6                    | 9.8                             |
| BCS-CU97237                   | 0.5 (LOQ)                   | 5               | 96-104             | 99                | 5.5                    | 5.6                             |
|                               | 5.0                         | 5               | 84-102             | 97                | 7.4                    | 7.6                             |
| BCS-DA63612                   | 0.5 (LOQ)                   | 5               | 94-117             | 104               | 8.2                    | 7.9                             |
|                               | 5.0                         | 5               | 85-109             | 101               | 9.3                    | 9.3                             |
| BCS-CC26101                   | 0.5 (LOQ)                   | 5               | 103-111            | 108               | 3.0                    | 2.8                             |
|                               | 5.0                         | 5               | 88-103             | 99                | 6.2                    | 6.3                             |
| BCS-CZ38260                   | 0.5 (LOQ)                   | 5               | 96-99              | 98                | 1.4                    | 1.4                             |
|                               | 5.0                         | 5               | 85-101             | 97                | 6.6                    | 6.8                             |
| BCS-BP32808                   | 0.5 (LOQ)                   | 5               | 90-111             | 102               | 9.8                    | 9.7                             |
|                               | 5.0                         | 5               | 78-99              | 89                | 7.8                    | 8.7                             |
| BCS-DH17585                   | 0.5 (LOQ)                   | 5               | 92-102             | 96                | 4.4                    | 4.6                             |
|                               | 5.0                         | 5               | 82-107             | 99                | 9.8                    | 9.9                             |
| Confirmation ion transition   |                             |                 |                    |                   |                        |                                 |
| Fluoxapiprolin (BCS-CS55621)  | 0.5 (LOQ)                   | 5               | 92-106             | 99                | 6.0                    | 6.1                             |
|                               | 5.0                         | 5               | 83-106             | 97                | 8.7                    | 9.0                             |
| BCS-CY96288                   | 0.5 (LOQ)                   | 5               | 89-107             | 97                | 7.7                    | 7.9                             |
|                               | 5.0                         | 5               | 86-104             | 96                | 9.1                    | 9.5                             |
| BCS-CU97237                   | 0.5 (LOQ)                   | 5               | 99-102             | 100               | 1.1                    | 1.1                             |
|                               | 5.0                         | 5               | 83-103             | 96                | 7.6                    | 7.9                             |
| BCS-DA63612                   | 0.5 (LOQ)                   | 5               | 92-110             | 100               | 7.7                    | 7.7                             |
|                               | 5.0                         | 5               | 82-103             | 97                | 8.8                    | 9.0                             |
| BCS-CC26101                   | 0.5 (LOQ)                   | 5               | 101-108            | 105               | 2.7                    | 2.6                             |
|                               | 5.0                         | 5               | 87-105             | 99                | 7.1                    | 7.1                             |
| BCS-CZ38260 <sup>3</sup>      | 0.5 (LOQ)                   | 5               | 89-101             | 94                | 4.5                    | 4.8                             |
|                               | 5.0                         | 5               | 87-106             | 99                | 7.3                    | 7.3                             |
| BCS-BP32808                   | 0.5 (LOQ)                   | 5               | 85-90              | 87                | 2.1                    | 2.4                             |
|                               | 5.0                         | 5               | 80-98              | 90                | 6.6                    | 7.3                             |
| BCS-DH17585                   | 0.5 (LOQ)                   | 5               | 87-100             | 93                | 5.7                    | 6.1                             |
|                               | 5.0                         | 5               | 82-107             | 99                | 9.7                    | 9.8                             |
| Confirmation ion transition 2 |                             |                 |                    |                   |                        |                                 |
| BCS- CZ38260 <sup>4</sup>     | 0.5 (LOQ)                   | 5               | 94-124             | 107               | 11.2                   | 10.4                            |
|                               | 5.0                         | 5               | 86-107             | 98                | 7.9                    | 8.1                             |

Data (uncorrected recovery results; p. 33) were obtained from Table 1, pp. 43-51 (quantitation ion pair transition and confirmatory ion pair transition) of MRID 51820518. Since the LOQ was not based on scientifically acceptable

procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

- 1 Two ion pair transitions were monitored (primary and confirmatory, respectively):  $m/z$  650→610.2 and  $m/z$  650→378 for fluoxapiprolin (BCS-CS55621),  $m/z$  666→626.1 and  $m/z$  666→191 for BCS-CY96288,  $m/z$  442.2→332 and  $m/z$  442.2→208 for BCS-CU97237,  $m/z$  456→345.2 and  $m/z$  456→222.1 for BCS-DA63612,  $m/z$  225→161 and  $m/z$  225→141 for BCS-CC26101,  $m/z$  161→76,  $m/z$  161→125, and  $m/z$  163.1→97.0 for BCS-CZ38260,  $m/z$  169→101 and  $m/z$  169→149 for BCS-BP32808, and  $m/z$  403→169 and  $m/z$  403→157 for BCS-DH17585. The ILV monitored ion transitions were the same as those of the ECM for all analytes except for BCS-CZ38260 which had two confirmation ion pair transition monitored:  $m/z$  163.1→125.0 and  $m/z$  163.1→97.0.
- 2 In the ILV, the water matrix was drinking water obtained from the cafeteria in the Eurofins Testing facility and had the following characteristics: pH: 8.2; Conductivity: 0.50 mmhos/cm; Hardness: 278 mg equivalent  $\text{CaCO}_3/\text{L}$ ; Sodium Adsorption Ratio (SAR): 0.53; Total Dissolved Solids: 350 ppm; Turbidity: 0.16 NTU; Alkalinity 293 mg  $\text{CaCO}_3/\text{L}$ ; Potassium: 5.1 ppm; Calcium: 64 ppm; Magnesium: 28 ppm; Sodium: 20 ppm; Carbonates 0.00 meq/L; Bicarbonates: 5.57 meq/L; Sulfate-Sulfur: 6.4 ppm; Chloride 6.1 ppm; Nitrogen (Nitrate): <0.1 ppm. Water characterization was performed by Agvise Laboratories in Northwood, North Dakota (p. 27; Appendix 3, p. 270).
- 3 Confirmation ion pair transition  $m/z$  163.1→125.0 for BCS-CZ38260.
- 4 Confirmation ion pair transition  $m/z$  163.1→97.0 for BCS-CZ38260. This ion transition was not included in the ECM1 or ECM 2 internal validation.



### III. Method Characteristics

The LOQ in water was reported as 0.5 ng/mL for fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 in the ECM 1, ECM 2, and ILV (p. 15 of MRID 52029302; pp. 9-10, 25 of MRID 51820517; p. 36 of MRID 51820518). No justification or calculation for LOQ was reported in ECM 1. In ECM 2, the LOQ was defined as the lowest fortification level in which the method was validated where mean recovery was 70-110% and relative standard deviation was  $\leq 20\%$ , as well as blank values were below 30% at this level.

The LOD was not reported in ECM 1. In ECM 2, the LOD was calculated for each analyte the standard deviation at the 0.5 ng/mL fortification ( $n = 14$ ) using the following equation (p. 28):

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

Where,  $t_{0.99}$  is the one-tailed t statistic for  $n = 14$  (2.650) and SD is the standard deviation of the analyte recovery measurements at the target LOQ.

In ECM 2, the LODs were calculated as 0.06-0.09 ng/mL for all analytes in water (p. 28; Table 17, p. 35 of MRID 51820517). No further justification for LOD was reported in ECM 2. The reviewer noted that the text reported a range of 0.04-0.11 ng/mL for the calculated LODs (p. 28 of MRID 51820517). This range does not match the range of values reported in Table 17, p. 35 of MRID 51820517.

The ILV reported the nominal LOQ of 0.5 ng/mL for fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, BCS-CZ38260, BCS-BP32808, and BCS-DH17585 in water (pp. 13, 36 of MRID 51820518) based on the lowest fortification level at which acceptable recovery data were obtained. In the ILV, the LOQ and LOD were confirmed for each analyte in water based on the standard deviation at the 0.5 ng/mL fortification ( $n = 5$ ; quantitation ion transition only) using the following equations (Table 5, pp. 61-63 of MRID 51820518):

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

Where,  $t_{0.99}$  is the one-tailed t statistic for  $n = 5$  (3.747) and SD is the standard deviation of the analyte recovery measurements at the target LOQ.

$$\text{LOQ} = 3 \times (\text{Calculated LOD})$$

In the ILV, the LOQs were calculated as range from 0.0769 to 0.561 ng/mL for all analytes (Table 5, pp. 61-63 of MRID 51820518). The LODs were calculated as 0.0256 to 0.187 ng/mL for all analytes. No justifications for the LOQ or LOD were reported in the ILV.

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

**Table 4a. Method Characteristics –Water<sup>1,2</sup>**

|                                                                     |                                         |            | Fluoxapiprolin<br>(BCS-CS55621)                                                                                                                                               | BCS-CY96288                                     | BCS-CU97237                              | BCS-DA63612                                     |
|---------------------------------------------------------------------|-----------------------------------------|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|------------------------------------------|-------------------------------------------------|
| Limit of Quantitation<br>(LOQ)*                                     | ECM 1 <sup>1</sup>                      |            | 0.5 ng/mL (noted that it can be adjusted, as required)                                                                                                                        |                                                 |                                          |                                                 |
|                                                                     | ECM 2 <sup>2</sup>                      |            | 0.5 ng/mL                                                                                                                                                                     |                                                 |                                          |                                                 |
|                                                                     | ILV                                     | Nominal    | 0.5 ng/mL                                                                                                                                                                     |                                                 |                                          |                                                 |
|                                                                     |                                         | Calculated | 0.522 ng/mL <sup>3</sup> (Q)<br>0.339 ng/mL (C)                                                                                                                               | 0.526 ng/mL <sup>3</sup> (Q)<br>0.426 ng/mL (C) | 0.304 ng/mL (Q)<br>0.0742 ng/mL (C)      | 0.466 ng/mL (Q)<br>0.437 ng/mL (C)              |
| Limit of Detection<br>(LOD)                                         | ECM 1                                   |            | Not reported                                                                                                                                                                  |                                                 |                                          |                                                 |
|                                                                     | ECM 2 (calculated)                      |            | 0.089 ng/mL                                                                                                                                                                   | 0.091 ng/mL                                     | 0.093 ng/mL                              | 0.062 ng/mL                                     |
|                                                                     | ILV (calculated)                        |            | 0.174 ng/mL <sup>4</sup> (Q)<br>0.113 ng/mL (C)                                                                                                                               | 0.175 ng/mL <sup>4</sup> (Q)<br>0.142 ng/mL (C) | 0.101 ng/mL (Q)<br>0.0247 ng/mL (C)      | 0.155 ng/mL <sup>4</sup> (Q)<br>0.146 ng/mL (C) |
| Linearity<br>(calibration curve<br>r and<br>concentration<br>range) | ECM 1                                   |            | Not Reported                                                                                                                                                                  |                                                 |                                          |                                                 |
|                                                                     |                                         |            | 0.1-20 µg/L                                                                                                                                                                   |                                                 |                                          |                                                 |
|                                                                     | ECM 2                                   |            | r = 0.9990 (Q)<br>r = 0.9982 (C)                                                                                                                                              | r = 0.9990 (Q)<br>r = 0.9977 (C)                | r = 0.9980 (Q)<br>r = 0.9991 (C)         | r = 0.9987 (Q)<br>r = 0.9985 (C)                |
|                                                                     |                                         |            | 0.1-20 µg/L                                                                                                                                                                   |                                                 |                                          |                                                 |
|                                                                     | ILV                                     |            | r = 0.99902674 (Q)<br>r = 0.99850095 (C)                                                                                                                                      | r = 0.99647978 (Q)<br>r = 0.99845013 (C)        | r = 0.99919176 (Q)<br>r = 0.99978678 (C) | r = 0.99915032 (Q)<br>r = 0.99936680 (C)        |
|                                                                     |                                         |            | 0.100-10.0 ng/mL                                                                                                                                                              |                                                 |                                          |                                                 |
| Repeatable                                                          | ECM 1                                   |            | No performance data submitted                                                                                                                                                 |                                                 |                                          |                                                 |
|                                                                     | ECM 2 <sup>5</sup>                      |            | Yes for LOQ (0.5 ng/mL) and 10×LOQ (5.0 ng/mL) in two uncharacterized and undescribed bulk water matrices.                                                                    |                                                 |                                          |                                                 |
|                                                                     | ILV <sup>6,7</sup>                      |            | Yes for LOQ (0.5 ng/mL) and 10×LOQ (5.0 ng/mL) in one characterized drinking water matrix.                                                                                    |                                                 |                                          |                                                 |
| Reproducible                                                        |                                         |            | Yes for 0.5 ng/mL (LLMV)* and 5.0 ng/mL in water.                                                                                                                             |                                                 |                                          |                                                 |
| Specific                                                            | ECM 1 - Water 1<br>(unspecified matrix) |            | Only representative LOQ chromatograms were provided. No untreated control representative chromatograms were provided to assess matrix interferences at the RT of the analyte. |                                                 |                                          |                                                 |

|  |             |         | Fluoxapiprolin<br>(BCS-CS55621)                                                                                                         | BCS-CY96288                                                                                                                                 | BCS-CU97237                                                                                                                                 | BCS-DA63612                                                                                                                                 |
|--|-------------|---------|-----------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
|  | ECM 2       | Water 1 | Yes, no matrix interferences were observed (based on quantified residues). Minor baseline noise was observed near the analyte (C only). | Yes, no matrix interferences were observed (based on quantified residues), but notable baseline noise surrounding LOQ analyte peak (Q & C). | Yes, no matrix interferences were observed (based on quantified residues), but notable baseline noise surrounding LOQ analyte peak (Q & C). | Yes, matrix interferences were <1% of the LOQ (based on quantified residues). Minor baseline noise was observed near the analyte.           |
|  |             | Water 2 |                                                                                                                                         |                                                                                                                                             |                                                                                                                                             | Yes, no matrix interferences were observed (based on quantified residues), but notable baseline noise surrounding LOQ analyte peak (Q & C). |
|  | ILV - Water |         | Yes, no matrix interferences were observed.                                                                                             | Yes, no matrix interferences were observed.                                                                                                 | Yes, no matrix interferences were observed.                                                                                                 | Yes, no matrix interferences were observed.                                                                                                 |

Data were obtained from pp. 6, 15 (LOQ/LOD); Appendix 2, pp. 24-25 (representative chromatograms); Appendix 3, pp. 26-33 (representative MS spectra) of MRID 52029302; pp. 25, 28; Table 17, p. 35 (LOQ/LOD); pp. 20-21; Tables 1-16, pp. 31-34; Appendix 1, pp. 37-77 (recovery data); Appendix 1, pp. 37-77; Appendix 3, pp. 115-130 (linearity coefficients & calibration curves); Appendix 2, pp. 78-114 (chromatograms) of MRID 51820517; p. 36, Table 5, pp. 61-63 (LOQ/LOD); Table 1, pp. 43-51; Appendix 5, pp. 277-355 (recovery data); p. 36, Figures 2-3, pp. 83-99 (linearity coefficients & calibration curves), Figures 4-9, pp. 100-150; Figures 10-17, pp. 151-218 (chromatograms) of MRID 51820518. Q = quantitative ion transition; C = confirmatory ion transition.

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

1 ECM 1 MRID 52029302 contained the full method written in a step-wise manner, but no performance data.

2 ECM 2 MRID 51820517 (Internal validation) contained the method summary and performance data.

3 Calculated LOQ > method LOQ (0.5 ng/mL).

4 Calculated LOD >30% of method LOQ (0.15 ng/mL).

5 In the ECM 2 MRID 51820517, two bulk untreated water samples were included in the internal validation (pp. 9 and 20 of MRID 51820517). The source of Water 1 was untreated water from Minnesota (Sample ID: IFTMN-GW15-A001) from study FTISN003 (Minnesota; pp. 9 and 20 of MRID 51820517). The source of Water 2 was untreated water from Wisconsin (Sample ID: IFTWI-GW25-A036) from study FTISN004 (Wisconsin, pp. 9 and 20 of MRID 51820517). Water characteristics including pH, hardness, and conductivity were not reported. The ECM 2 indicated that the source of the water was associated with terrestrial field dissipation studies; however, the references provided in the ECM 2 are not consistent with that description (p. 30 of 51820517). MRID numbers for the referenced studies Study FTISN003 and Study FTISN004 were not reported in ECM 1 MRID 52029302 or ECM 2 MRID 51820517 and could not be determined by the reviewer.

6 In the ILV, the water matrix was drinking water obtained from the cafeteria in the Eurofins Testing facility (p. 27 of MRID 51820518) and had the following characteristics: pH: 8.2; Conductivity: 0.50 mmhos/cm; Hardness: 278 mg equivalent CaCO<sub>3</sub>/L; Sodium Adsorption Ratio (SAR): 0.53; TDS: 350 ppm; Turbidity: 0.16 NTU; Alkalinity 293 mg CaCO<sub>3</sub>/L; Potassium: 5.1 ppm; Calcium: 64 ppm; Magnesium: 28 ppm; Sodium: 20 ppm; Carbonates 0.00 meq/L; Bicarbonates: 5.57 meq/L; Sulfate-Sulfur: 6.4 ppm; Chloride 6.1 ppm; Nitrogen (Nitrate): <0.1 ppm. Water characterization was performed by Agvise Laboratories in Northwood, North Dakota (p. 27; Appendix 3, p. 270 of MRID 51820518).

7 The method (Bayer Method TY-004-W21-01) was validated in one trial by the ILV in the drinking water matrix with modifications to the analytical method (see Reviewer's Comment #8). The ILV validated the method in one trial and no matrix suppression or enhancement was observed (pp. 36, 39; Table 1, pp. 43-51, Table 4, p. 60; Figures 12-17, pp. 167-218 of MRID 51820518). The ILV method was based on the ECM method; however, the ILV was modified to optimize the LC-MS/MS equipment in the ILV. An updated ECM is not required based on ILV modifications.

**Table 4b. Method Characteristics (con't)**

|                                                         |                            | BCS-CC26101                                                                                                | BCS-CZ38260                                                                                   | BCS-BP32808                                            | BCS-DH17585                              |
|---------------------------------------------------------|----------------------------|------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|--------------------------------------------------------|------------------------------------------|
| Limit of Quantitation (LOQ)*                            | ECM 1 <sup>1</sup>         | 0.5 ng/mL (noted that it can be adjusted, as required)                                                     |                                                                                               |                                                        |                                          |
|                                                         | ECM 2 <sup>2</sup>         | 0.5 ng/mL                                                                                                  |                                                                                               |                                                        |                                          |
|                                                         | ILV                        | 0.5 ng/mL                                                                                                  |                                                                                               |                                                        |                                          |
|                                                         | Calculated                 | 0.178 ng/mL (Q)<br>0.147 ng/mL (C)                                                                         | 0.0769 ng/mL (Q)<br>0.249 ng/mL (C1)<br><b>0.623 ng/mL<sup>3</sup> (C2)</b>                   | <b>0.561 ng/mL<sup>3</sup> (Q)</b><br>0.130 ng/mL (C)  | 0.262 ng/mL (Q)<br>0.307 ng/mL (C)       |
| Limit of Detection (LOD)                                | ECM 1                      | <b>Not reported</b>                                                                                        |                                                                                               |                                                        |                                          |
|                                                         | ECM 2 (calculated)         | 0.079 ng/mL                                                                                                | 0.081 ng/mL                                                                                   | 0.066 ng/mL                                            | 0.067 ng/mL                              |
|                                                         | ILV (calculated)           | 0.0593 ng/mL (Q)<br>0.0490 ng/mL (C)                                                                       | 0.0256 ng/mL (Q)<br>0.0829 ng/mL (C1)<br><b>0.208 ng/mL<sup>4</sup> (C2, m/z 163.1-97.0)</b>  | <b>0.187 ng/mL<sup>4</sup> (Q)</b><br>0.0434 ng/mL (C) | 0.0872 ng/mL (Q)<br>0.102 ng/mL (C)      |
| Linearity (calibration curve r and concentration range) | ECM 1 (unspecified matrix) | <b>Not Reported</b>                                                                                        |                                                                                               |                                                        |                                          |
|                                                         |                            | 0.1-20 µg/L                                                                                                |                                                                                               |                                                        |                                          |
|                                                         | ECM 2 <sup>5</sup>         | r = 0.9989 (Q)<br>r = 0.9986 (C)                                                                           | r = 0.9987 (Q)<br>r = 0.9988 (C1, m/z 163.1-125.0)                                            | r = 0.980 (Q)<br>r = 0.9979 (C)                        | r = 0.9980 (Q)<br>r = 0.9979 (C)         |
|                                                         |                            | 0.1-20 µg/L                                                                                                |                                                                                               |                                                        |                                          |
|                                                         | ILV <sup>6</sup>           | r = 0.99872862 (Q)<br>r = 0.99890258 (C)                                                                   | r = 0.99958045 (Q)<br>r = 0.9988 (C1, m/z 163.1-125.0)<br>r = 0.99936300 (C2, m/z 163.1-97.0) | r = 0.99951645 (Q)<br>r = 0.99982879 (C)               | r = 0.99960469 (Q)<br>r = 0.99971692 (C) |
| Repeatable                                              | ECM 1                      | <b>No performance data submitted</b>                                                                       |                                                                                               |                                                        |                                          |
|                                                         | ECM 2 <sup>5</sup>         | Yes for LOQ (0.5 ng/mL) and 10×LOQ (5.0 ng/mL) in two uncharacterized and undescribed bulk water matrices. |                                                                                               |                                                        |                                          |
|                                                         | ILV <sup>6,7</sup>         | Yes for LOQ (0.5 ng/mL) and 10×LOQ (5.0 ng/mL) in one characterized drinking water matrix.                 |                                                                                               |                                                        |                                          |
| Reproducible                                            |                            | Yes for 0.5 ng/mL (LLMV)* and 5.0 ng/mL in water.                                                          |                                                                                               |                                                        |                                          |

|          |                                      | BCS-CC26101                                                                                                                                                                                                                    | BCS-CZ38260                                                                                                                                                                                                                    | BCS-BP32808                                                                                                                                                                                                                             | BCS-DH17585                                                                                                                                                                                                                    |
|----------|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Specific | ECM 1 - Water 1 (unspecified matrix) | Only representative LOQ chromatograms were provided. No untreated control representative chromatograms were provided to assess matrix interferences at the RT of the analyte.                                                  |                                                                                                                                                                                                                                |                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                |
|          | ECM 2      Water 1 & Water 2         | Yes, no matrix interferences were observed (based on quantified residues). Minor baseline noise was observed near the analyte in the Untreated control sample; these interferences were not observed in fortification samples. | Yes, no matrix interferences were observed (based on quantified residues). Minor baseline noise was observed near the analyte in the Untreated control sample; these interferences were not observed in fortification samples. | Yes, matrix interferences were $\leq 2\%$ of the LOQ (based on quantified residue). Minor baseline noise was observed near the analyte in the Untreated control sample; these interferences were not observed in fortification samples. | Yes, no matrix interferences were observed (based on quantified residues). Minor baseline noise was observed near the analyte in the Untreated control sample; these interferences were not observed in fortification samples. |
|          | ILV - Water 1                        | Yes, no matrix interferences were observed (based on quantified residues). Elevated baseline noise was observed near the analyte.                                                                                              | Yes, matrix interferences were $< 4\%$ of the LOQ (based on quantified residues).                                                                                                                                              | Yes, no matrix interferences were observed (based on quantified residues). Elevated baseline noise was observed near the analyte.                                                                                                       | Yes, no matrix interferences were observed (based on quantified residues). Elevated baseline noise was observed near the analyte.                                                                                              |

Data were obtained from pp. 6, 15 (LOQ/LOD); Appendix 2, pp. 24-25 (representative chromatograms); Appendix 3, pp. 26-33 (representative MS spectra) of MRID 52029302; pp. 25, 28; Table 17, p. 35 (LOQ/LOD); pp. 20-21; Tables 1-16, pp. 31-34; Appendix 1, pp. 37-77 (recovery data); Appendix 1, pp. 37-77; Appendix 3, pp. 115-130 (linearity coefficients & calibration curves); Appendix 2, pp. 78-114 (chromatograms) of MRID 51820517; p. 36, Table 5, pp. 61-63 (LOQ/LOD); Table 1, pp. 43-51; Appendix 5, pp. 277-355 (recovery data); p. 36, Figures 2-3, pp. 83-99 (linearity coefficients & calibration curves), Figures 4-9, pp. 100-150; Figures 10-17, pp. 151-218 (chromatograms) of MRID 51820518. Q = quantitative ion transition; C = confirmatory ion transition.

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

1 ECM 1 MRID 52029302 contained the full method written in a step-wise manner, but no performance data.

2 ECM 2 MRID 51820517 (Internal validation) contained the method summary and performance data.

3 Calculated LOQ > method LOQ (0.5 ng/mL).

4 Calculated LOD >30% of method LOQ (0.15 ng/mL).

5 In the ECM 2 MRID 51820517, two bulk untreated water samples were included in the internal validation (pp. 9 and 20 of MRID 51820517). The source of Water 1 was untreated water from Minnesota (Sample ID: IFTMN-GW15-A001) from study FTISN003 (Minnesota; pp. 9 and 20 of MRID 51820517). The source of Water 2 was untreated water from Wisconsin (Sample ID: IFTWI-GW25-A036) from study FTISN004 (Wisconsin, pp. 9 and 20 of MRID 51820517). Water characteristics including pH, hardness, and conductivity were not reported. The ECM 2 indicated that the source of the water was associated with terrestrial field dissipation studies; however, the references provided in the ECM 2 are not consistent with that description (p. 30 of 51820517). MRID numbers for the referenced studies Study FTISN003 and Study FTISN004 were not reported in ECM 1 MRID 52029302 or ECM 2 MRID 51820517 and could not be determined by the reviewer.

6 In the ILV, the water matrix was drinking water obtained from the cafeteria in the Eurofins Testing facility (p. 27 of MRID 51820518) and had the following characteristics: pH: 8.2; Conductivity: 0.50 mmhos/cm; Hardness: 278 mg equivalent  $\text{CaCO}_3/\text{L}$ ; Sodium Adsorption Ratio (SAR): 0.53; TDS: 350 ppm; Turbidity: 0.16 NTU; Alkalinity 293 mg  $\text{CaCO}_3/\text{L}$ ; Potassium: 5.1 ppm; Calcium: 64 ppm; Magnesium: 28 ppm; Sodium: 20 ppm; Carbonates 0.00 meq/L;



Bicarbonates: 5.57 meq/L; Sulfate-Sulfur: 6.4 ppm; Chloride 6.1 ppm; Nitrogen (Nitrate): <0.1 ppm. Water characterization was performed by Agvise Laboratories in Northwood, North Dakota (p. 27; Appendix 3, p. 270).

7 The method (Bayer Method TY-004-W21-01) was validated in one trial by the ILV in the drinking water matrix with modifications to the analytical method (see Reviewer's Comment #8). The ILV validated the method in one trial and no matrix suppression or enhancement was observed (pp. 36, 39; Table 1, pp. 43-51, Table 4, p. 60; Figures 12-17, pp. 168-218 of MRID 51820518). The ILV method was based on the ECM method; however, the ILV was modified to optimize the LC-MS/MS equipment in the ILV. An updated ECM is not required based on ILV modifications.

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

1. This DER was prepared with two MRIDs as the internal laboratory validations (ECMs): MRID 52029302 & MRID 51820517. ECM 1 = MRID 52029302 (Bayer Method TY-004-W21-01) ECM 2 = MRID 51820517 (Internal validation). ECM 2 MRID 51820517 contained only a method summary, while ECM 1 MRID 52029302 contained the full method written in a step-wise manner (pp. 11-12 of MRID 52029302; pp. 20-21 of MRID 51820517). In OCSPP guideline 850.6100 states that "The ECM should be clearly written with complete analytical methods that describe the exact procedure, materials, and equipment in sufficient detail to be used by laboratory chemists to review the ECM and its associated ILV results".

OCSPP guideline 850.6100 could not have been satisfied using only ECM 2 MRID 51820517 since the ECM method (Bayer Method No. TY-004-W21-01) was not completely reproduced in ECM 2 MRID 51820517. Method details such as volume stock solutions, volume of mixed internal standard solution to add to samples, and calibration standard preparation were not included in ECM 2 MRID 51820517. These details were found in ECM 1 MRID 52029302.

2. Since the reported method LOQ of the ECM and ILV were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ of the ECM and ILV are the lowest level of method validation (LLMV) rather than LOQs (pp. 25, 28, Table 17, p. 35 of MRID 51820517; p. 36, Table 5, pp. 61-63 of MRID 51820518). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

Based on the performance data submitted by the ILV and ECM 2, the LLMV was equivalent to the ECM 2 and ILV reported method LOQ for all analytes in water (0.5 ng/mL).

3. The sample collection details such as time, date, and location of the bulk water samples and water characteristics including pH, hardness, and conductivity for the ECM 2 test matrices (Water 1 from Minnesota and Water 2 from Wisconsin) were not reported (p. 16 of MRID 51820517) as required by OCSPP guideline 850.6100. In addition, the ECM 2 indicated that the source of the water was associated with terrestrial field dissipation studies (p. 9 of MRID 51820517); however, the references provided in the ECM 2 are not consistent with that description (isoxaflutole water analysis studies; p. 30 of 51820517). MRID numbers for the referenced studies Study FTISN003 and Study FTISN004 were not reported in ECM 1 MRID 52029302 or ECM 2 MRID 51820517 and could not be determined by the reviewer.
4. Although the ILV was conducted independently of the internal validation (ECM 1 and ECM 2) since the laboratory personnel and equipment differed between the ILV laboratory and ECM 1 and ECM 2 laboratory, there was extensive communication between the ILV study personnel and the Sponsor Monitor (Leah Riter, Bayer Crop Science) which included discussion of the independent laboratory validation, as well as

- the storage stability experiment. The ILV included a list of correspondence between the ILV and the Study Monitor (Sponsor; Appendix 4, pp. 271-276 of MRID 51820518) which referenced 31 email correspondences between October 8, 2021 and January 5, 2022. OCSPP guidelines specify that the ILV must be conducted independently of the internal validation without collusion between the laboratory personnel or equipment. The communications between the ILV Study Director/Author (Lisa Swaim, Eurofins EAG Agroscience, LLC), ILV personnel (Emily Vogel and Erin Martin, Eurofins EAG Agroscience, LLC), Study Sponsor (Leah Riter, Bayer Crop Science), and other unspecified personnel (Suman Shrestha) was listed in detail. Two emails requesting information and clarification of the method protocol were sent prior to the ILV trial. Specifically, Eurofins requested clarification on the stability of standard solutions, verification of timing continuing calibration verification, units for the calibration curves, LC flow rate and solvent gradient, and MS mode for positive and negative monitoring parameters. Other email communications involved protocol and report template issue, ILV trial results, and guideline discussion, specifically about LOD/LOQ determination. There were several email exchanges between Leah Riter and Lisa Swaim regarding the storage stability portion of the study. These correspondences did not impact the integrity of the ILV.
5. Based on the method (Bayer Method TY-004-W21-01), ECM 2 recovery results were corrected for residues quantified in the controls (p. 15 of MRID 52029302; pp. 23-24; Appendix 1, pp. 38-77 of MRID 51820517). OCSPP 850.6100 guidance states that “for an acceptable ECM, reported sample values should not be corrected for recoveries”.
  6. The ILV performed the analytical method in ECM 1 (Bayer Method TY-004-W21-01) except for the use of different equipment. Samples were analyzed Applied Biosystems/Sciex System with Degasser, Solvent Valve Autosampler, Column Oven, and System Controller HPLC coupled to an Applied Biosystems/Sciex API 6500+ Q-trap with Ion Chromatograph SelexION+ mass spectrometer (MS/MS) equipped with a Turbo IonSpray interface operated in multiple MRM mode with unit mass resolution. (p. 32 of MRID 51820518). The LC settings including column specifications, the column temperature, the injection volume, and flow rate were the same as used in the ECM. The ILV listed a weak needle wash of acetonitrile:methanol:water (1:1:2; v:v:v) was used. Overall, the ILV method (Eurofins Study No.: 90890) was a suitable version of the ECM. The ECM allows for use of different instrumentation and optimization of parameters other than those specified as long as separation and sensitivity requirements are met (pp. 12-13 of MRID 51820517).
  7. The determinations of the LOD and LOQ in the ECM 1, ECM 2, and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 6 of MRID 52029302; pp. 25, 28; Table 17, p. 35 of MRID 51820517; pp. 36; Table 5, pp. 61-63 of MRID 51820518). No justification or calculation for LOQ was reported in ECM 1. In ECM 2, the LOQ was defined as the lowest fortification level in which the method was validated where mean recovery was 70-110% and relative standard deviation was  $\leq 20\%$ , as well as blank values were below 30% at this level. The LOD was not reported in ECM 1. In ECM 2, the LOD was calculated for each analyte the standard deviation at the 0.5

ng/mL fortification ( $n = 14$ ) using the following equation:  $LOD = (t_{0.99} \times SD)$ , where,  $t_{0.99}$  is the one-tailed  $t$  statistic for  $n = 14$  (2.650) and  $SD$  is the standard deviation of the analyte recovery measurements at the target LOQ. In the ILV, the LOQ and LOD were calculated for each analyte in water based on the standard deviation at the 0.5 ng/mL fortification ( $n = 5$ ) using the following equations:  $LOD = (t_{0.99} \times SD)$  and  $LOQ = 3 \times (\text{Calculated LOD})$ , where,  $t_{0.99}$  is the one-tailed  $t$  statistic for  $n = 5$  (3.747) and  $SD$  is the standard deviation of the analyte recovery measurements at the target LOQ. Calculated LOQs exceeded the method LOQ for fluoxapiprolin (BCS-CS55621; 0.522 ng/mL), BCS-CZ38260 (0.623 ng/mL; confirmation ion pair transition), BCS-CY96288 (0.526 ng/mL) and BCS-BP32808 (0.561 ng/mL). ILV calculated LODs exceeded 30% of the method LOQ (0.15 ng/mL) for fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CZ38260 (confirmation ion pair transition), and BCS-BP32808 (Table 5, pp. 61-63 of MRID 51820518).

Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

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

The reviewer noted that the LOD calculation provided in ECM 1 and ECM 2 corresponded to the MDL calculation detailed in USEPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2; 2016.

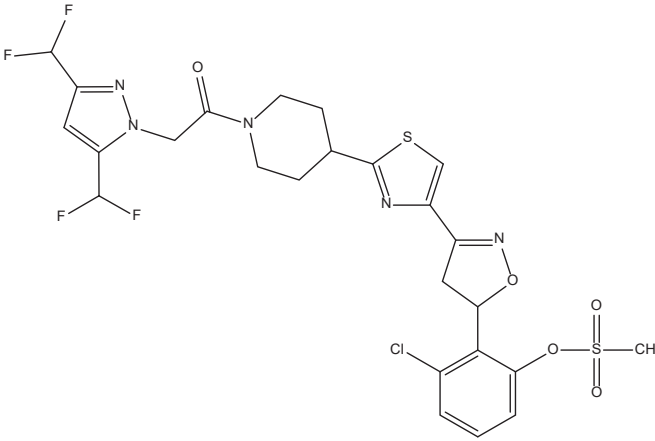
8. In ECM 2, the stability of the final sample extract was determined to be up to 9 days under refrigerated (mean temperature 4°C) storage (stability defined as  $\pm 15\%$ ; p. 28; Table 18, p. 36 of MRID 51820517).
9. The time requirement for the method was reported in the ILV as 8 hours (one workday) for a set of 13 samples, if standards, solutions, and reagents are prepared in advance, with LC/MS/MS analysis performed overnight (p. 37 of MRID 51820518). Standard preparation required two analysts *ca.* 22 hours (two working days); preparation of solutions and reagents required *ca.* 1.5 hours. In the ECM 2, the time requirement for the method was reported as 1 hour for a batch of 30 samples, with HPLC/MS/MS analysis performed overnight (p. 28 of MRID 51820517). No time requirement was reported in ECM 1.
10. A refrigerated storage stability study was conducted in the ILV. The ILV reported that, fluoxapiprolin (BCS-CS55621), BCS-CY96288, BCS-CU97237, BCS-DA63612, BCS-CC26101, and BCS-DH17585 are stable ( $\leq 30\%$  loss during storage) for at least 30 days of refrigerated storage. BCS-CZ38260 and BCS-BP32808 are stable ( $\leq 30\%$  loss during storage) for *ca.* three days and one day of refrigerated storage, respectively (p. 39 of MRID 51820518).

11. The reviewer noted several typographical errors in the ECM 2. The BCS-CY96288-IS was erroneously reported as BCS-CS55621-IS in ECM 1 and ECM 2. The reviewer made the assignment of BCS-CY96288-IS based on the molecular weight/primary ion transition, as well as supporting chromatograms and calibration plots (Table 8, pp. 13-14 of MRID 52029302; Appendix 2, pp. 78-114; Appendix 3, pp. 115-130 of MRID 51820517). The ILV MRID 51820518 also noted this typographical error (p. 37 of MRID 51820518). The reviewer noted that the ion transition pair reported in ECM 2 Table 11 12, p. 33 of MRID 51820517, was the quantitative ion pair for BCS-CZ38260-(<sup>15</sup>N<sub>2</sub>) internal standard instead of the confirmation ion pair for BCS-CZ38260. Representative chromatograms showing the primary ion transition pair and confirmatory ion transition pairs were provided in Appendix 2, pp. 78-114 of MRID 51820517. The ECM 1 and ECM 2 should be updated to correct these errors. In ECM 2, the LODs were calculated as 0.06-0.09 ng/mL for all analytes in water (p. 28; Table 17, p. 35 of MRID 51820517). No further justification for LOD was reported in ECM 2. The reviewer noted that the text reported a range of 0.04-0.11 ng/mL for the calculated LODs (p. 28 of MRID 51820517). This range does not match the range of values reported in Table 17, p. 35 of MRID 51820517.

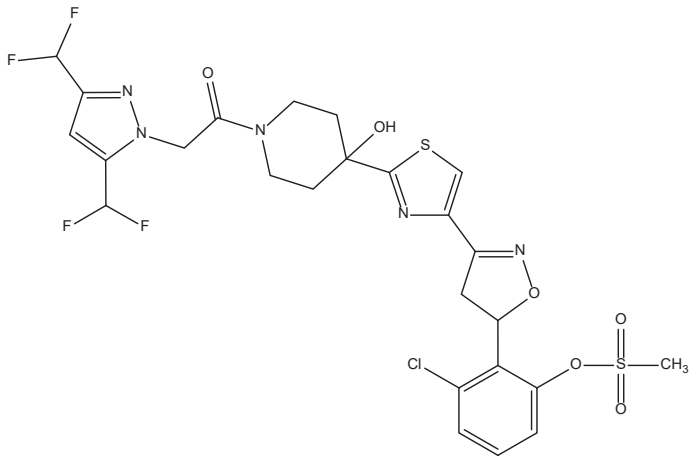
## V. References

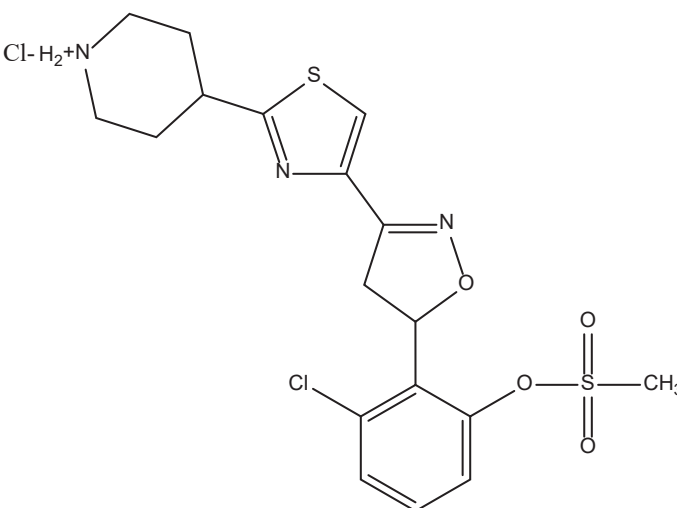
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides>.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347, and Revision 2; 2015 and 2016.

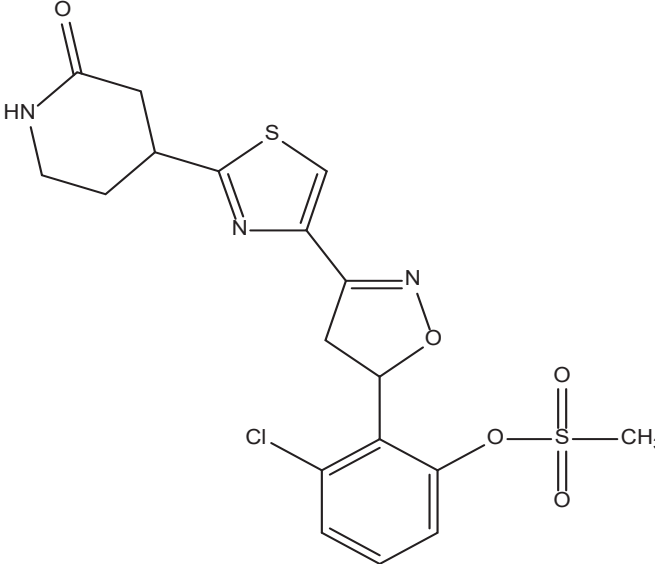
DER ATTACHMENT 1. Fluoxapiprolin and Its Environmental Transformation Products. <sup>A</sup>

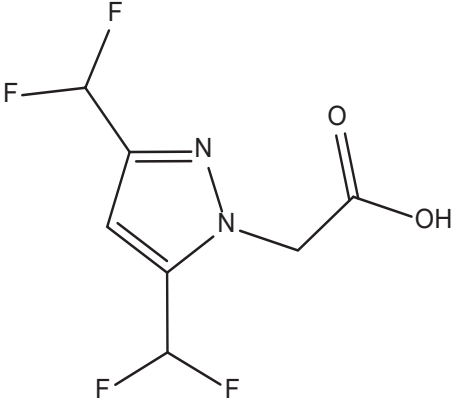
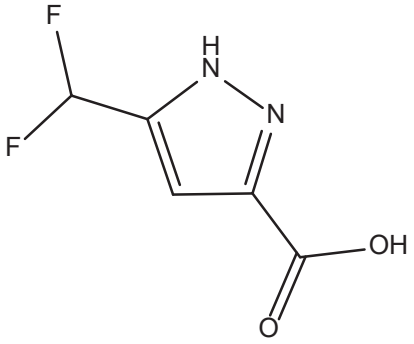
| Code Name/<br>Synonym                   | Chemical Name                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Chemical Structure                                                                  | Study<br>Type         | MRID                 | Maximum<br>%AR<br>(day) | Final<br>%AR<br>(study<br>length) |
|-----------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-----------------------|----------------------|-------------------------|-----------------------------------|
| <b>PARENT</b>                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                     |                       |                      |                         |                                   |
| <b>Fluoxapiprolin<br/>(BCS-CS55621)</b> | <b>IUPAC:</b> 2-[(5RS)-3-[2-(1-{[3,5-bis(difluoromethyl)-1H-pyrazol-1-yl]acetyl}-4-piperidyl)thiazol-4-yl]-4,5-dihydroisoxazol-5-yl]-3-chlorophenyl methanesulfonate<br><br><b>CAS:</b> 2-[3,5-bis(difluoromethyl)-1H-pyrazol-1-yl]-1-[4-[4-[5-[2-chloro-6-[(methylsulfonyl)oxy]phenyl]-4,5-dihydro-3-isoxazolyl]-2-thiazolyl]-1-piperidinyl]ethanone<br><br><b>CAS No.:</b> 1360819-11-9<br><br><b>Formula:</b> C <sub>25</sub> H <sub>24</sub> ClF <sub>4</sub> N <sub>5</sub> O <sub>5</sub> S <sub>2</sub><br><b>MW:</b> 650.1g/mol<br><b>SMILES:</b><br>FC(F)C1=NN(CC(N2CCCC(C3=NC(C4=NOC(C5=C(Cl)C=C(C=C5OS(C)(=O)=O)C4)=CS3)CC2)=O)C(C(F)F)=C1 |  | 850.6100<br>ECM Soil  | 51820515<br>51820516 | PRT                     | PRT                               |
|                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                     | 850.6100<br>ILV Soil  | 51820519             |                         |                                   |
|                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                     | 850.6100<br>ECM Water | 51820517<br>52029302 |                         |                                   |
|                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                     | 850.6100<br>ILV Water | 51820518             |                         |                                   |

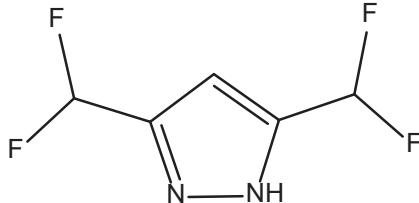
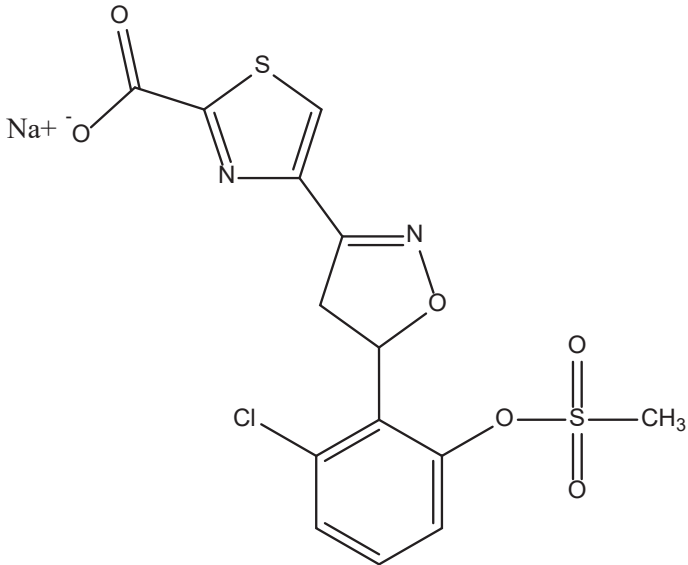


| Code Name/<br>Synonym | Chemical Name                                                                                                                                                                                                                                                                                                                                                                                                                                             | Chemical Structure                                                                  | Study Type               | MRID                 | Maximum<br>%AR<br>(day) | Final<br>%AR<br>(study<br>length) |
|-----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------|----------------------|-------------------------|-----------------------------------|
| <b>BCS-CY96288</b>    | <b>IUPAC:</b> 2-(3-(2-(1-(2-(3,5-bis(difluoromethyl)-1H-pyrazol-1-yl)acetyl)-4-hydroxypiperidin-4-yl)thiazol-4-yl)-4,5-dihydroisoxazol-5-yl)-3-chlorophenyl methanesulfonate<br><br><b>Formula:</b> C <sub>25</sub> H <sub>24</sub> ClF <sub>4</sub> N <sub>5</sub> O <sub>6</sub> S <sub>2</sub><br><b>MW:</b> 666.1 g/mol<br><b>SMILES:</b><br><chem>FC(F)C1=NN(CC(N2CCCC(C3=NC(C4=NOC(C5=C(Cl)C=C(C=C5OS(C)(=O)=O)C4)=CS3)(O)CC2)=O)C(C(F)F)=C1</chem> |  | 850.6100<br>ECM Soil     | 51820515<br>51820516 | PRT                     | PRT                               |
|                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                     | 850.6100<br>ILV Soil     | 51820519             |                         |                                   |
|                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                     | 850.6100<br>ECM<br>Water | 51820517<br>52029302 |                         |                                   |
|                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                     | 850.6100<br>ILV<br>Water | 51820518             |                         |                                   |

| Code Name/<br>Synonym | Chemical Name                                                                                                                                                                                                                                                                                                                                                          | Chemical Structure                                                                  | Study Type               | MRID                 | Maximum<br>%AR<br>(day) | Final<br>%AR<br>(study<br>length) |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------|----------------------|-------------------------|-----------------------------------|
| <b>BCS-CU97237</b>    | <b>IUPAC:</b> 4-(4-(5-(2-Chloro-6-((methylsulfonyl)oxy)phenyl)-4,5-dihydroisoxazol-3-yl)thiazol-2-yl)piperidin-1-ium chloride<br><br><b>Formula:</b> C <sub>18</sub> H <sub>21</sub> ClN <sub>3</sub> O <sub>4</sub> S <sub>2</sub> Cl<br><b>MW:</b> 478.4 g/mol<br><b>SMILES:</b><br><chem>CS(OC1=CC=CC(Cl)=C1C2C(C(C3=CSC(C4CC[NH2]CC4)=N3)=NO2)(=O)=O.[Cl-])</chem> |  | 850.6100<br>ECM Soil     | 51820515<br>51820516 | PRT                     | PRT                               |
|                       |                                                                                                                                                                                                                                                                                                                                                                        |                                                                                     | 850.6100<br>ILV Soil     | 51820519             |                         |                                   |
|                       |                                                                                                                                                                                                                                                                                                                                                                        |                                                                                     | 850.6100<br>ECM<br>Water | 51820517<br>52029302 |                         |                                   |
|                       |                                                                                                                                                                                                                                                                                                                                                                        |                                                                                     | 850.6100<br>ILV<br>Water | 51820518             |                         |                                   |

| Code Name/<br>Synonym | Chemical Name                                                                                                                                                                                                                                                                                                                                      | Chemical Structure                                                                 | Study Type            | MRID                 | Maximum<br>%AR<br>(day) | Final<br>%AR<br>(study<br>length) |
|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-----------------------|----------------------|-------------------------|-----------------------------------|
| <b>BCS-DA63612</b>    | <b>IUPAC:</b> 3-Chloro-2-(3-(2-(2-oxopiperidin-4-yl)thiazol-4-yl)-4,5-dihydroisoxazol-5-yl)phenyl methanesulfonate<br><br><b>Formula:</b> C <sub>18</sub> H <sub>18</sub> ClN <sub>3</sub> O <sub>5</sub> S <sub>2</sub><br><b>MW:</b> 455.94 g/mol<br><b>SMILES:</b><br><chem>CS(OC1=CC=CC(Cl)=C1C2C(C(C3=CSC(C(C4)CCNC4=O)=N3)=NO2)(=O)=O</chem> |  | 850.6100<br>ECM Soil  | 51820515<br>51820516 | PRT                     | PRT                               |
|                       |                                                                                                                                                                                                                                                                                                                                                    |                                                                                    | 850.6100<br>ILV Soil  | 51820519             |                         |                                   |
|                       |                                                                                                                                                                                                                                                                                                                                                    |                                                                                    | 850.6100<br>ECM Water | 51820517<br>52029302 |                         |                                   |
|                       |                                                                                                                                                                                                                                                                                                                                                    |                                                                                    | 850.6100<br>ILV Water | 51820518             |                         |                                   |
| <b>BCS-CC26101</b>    | <b>IUPAC:</b> 2-(3,5-bis(difluoromethyl)-1H-pyrazol-1-yl)acetic acid<br><br><b>Formula:</b> C <sub>7</sub> H <sub>6</sub> F <sub>4</sub> N <sub>2</sub> O <sub>2</sub><br><b>MW:</b> 226.13 g/mol<br><b>SMILES:</b><br><chem>FC(F)C1=NN(CC(O)=O)C(C(F)F)=C1</chem>                                                                                 |                                                                                    | 850.6100<br>ECM Soil  | 51820515<br>51820516 | PRT                     | PRT                               |
|                       |                                                                                                                                                                                                                                                                                                                                                    |                                                                                    | 850.6100<br>ILV Soil  | 51820519             |                         |                                   |
|                       |                                                                                                                                                                                                                                                                                                                                                    |                                                                                    | 850.6100<br>ECM Water | 51820517<br>52029302 |                         |                                   |

|                    |                                                                                                                                                                                                                                                     |   | 850.6100<br>ILV<br>Water | 51820518             |     |     |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------|----------------------|-----|-----|
| <b>BCS-CZ38260</b> | <b>IUPAC:</b> 5-(Difluoromethyl)-1H-pyrazole-3-carboxylic acid<br><br><b>Formula:</b> C <sub>5</sub> H <sub>4</sub> F <sub>2</sub> N <sub>2</sub> O <sub>2</sub><br><b>MW:</b> 162.1 g/mol<br><b>SMILES:</b><br><chem>O=C(O)C1=NNC(C(F)F)=C1</chem> |  | 850.6100<br>ECM Soil     | 51820515<br>51820516 | PRT | PRT |
|                    |                                                                                                                                                                                                                                                     |                                                                                     | 850.6100<br>ILV Soil     | 51820519             |     |     |
|                    |                                                                                                                                                                                                                                                     |                                                                                     | 850.6100<br>ECM<br>Water | 51820517<br>52029302 |     |     |
|                    |                                                                                                                                                                                                                                                     |                                                                                     | 850.6100<br>ILV<br>Water | 51820518             |     |     |

| Code Name/<br>Synonym                             | Chemical Name                                                                                                                                                                                                                                                                                                                                | Chemical Structure                                                                  | Study Type               | MRID                 | Maximum<br>%AR<br>(day) | Final<br>%AR<br>(study length) |
|---------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------|----------------------|-------------------------|--------------------------------|
| BCS-BP32808                                       | <b>IUPAC:</b> 3,5-bis(difluoromethyl)-1H-pyrazole<br><br><b>Formula:</b> C <sub>5</sub> H <sub>4</sub> F <sub>4</sub> N <sub>2</sub><br><b>MW:</b> 168.1 g/mol<br><b>SMILES:</b><br>FC(F)C1=NNC(C(F)F)=C1                                                                                                                                    |   | 850.6100<br>ECM Soil     | 51820515<br>51820516 | PRT                     | PRT                            |
|                                                   |                                                                                                                                                                                                                                                                                                                                              |                                                                                     | 850.6100<br>ILV Soil     | 51820519             |                         |                                |
|                                                   |                                                                                                                                                                                                                                                                                                                                              |                                                                                     | 850.6100<br>ECM<br>Water | 51820517<br>52029302 |                         |                                |
|                                                   |                                                                                                                                                                                                                                                                                                                                              |                                                                                     | 850.6100<br>ILV<br>Water | 51820518             |                         |                                |
| BCS-DH17585                                       | <b>IUPAC:</b> Sodium 4-(5-(2-chloro-6-((methylsulfonyl)oxy)phenyl)-4,5-dihydroisoxazol-3-yl)thiazole-2-carboxylate<br><br><b>Formula:</b> C <sub>14</sub> H <sub>10</sub> ClN <sub>2</sub> O <sub>6</sub> S <sub>2</sub> Na<br><b>MW:</b> 424.8 g/mol<br><b>SMILES:</b> [O-]<br>[C(C1=NC(C2=NOC(C3=C(Cl)C=CC=C3OS(C)(=O)=O)C2)=CS1)=O].[Na+] |  | 850.6100<br>ECM Soil     | 51820515<br>51820516 | PRT                     | PRT                            |
|                                                   |                                                                                                                                                                                                                                                                                                                                              |                                                                                     | 850.6100<br>ILV Soil     | 51820519             |                         |                                |
|                                                   |                                                                                                                                                                                                                                                                                                                                              |                                                                                     | 850.6100<br>ECM<br>Water | 51820517<br>52029302 |                         |                                |
|                                                   |                                                                                                                                                                                                                                                                                                                                              |                                                                                     | 850.6100<br>ILV<br>Water | 51820518             |                         |                                |
| MAJOR (>10%) TRANSFORMATION PRODUCTS              |                                                                                                                                                                                                                                                                                                                                              |                                                                                     |                          |                      |                         |                                |
| No major transformation products were identified. |                                                                                                                                                                                                                                                                                                                                              |                                                                                     |                          |                      |                         |                                |
| MINOR (<10%) TRANSFORMATION PRODUCTS              |                                                                                                                                                                                                                                                                                                                                              |                                                                                     |                          |                      |                         |                                |

| Code Name/<br>Synonym                                      | Chemical Name | Chemical Structure | Study<br>Type | MRID | Maximum<br>%AR<br>(day) | Final<br>%AR<br>(study<br>length) |
|------------------------------------------------------------|---------------|--------------------|---------------|------|-------------------------|-----------------------------------|
| No minor transformation products were identified.          |               |                    |               |      |                         |                                   |
| <b>REFERENCE COMPOUNDS NOT IDENTIFIED</b>                  |               |                    |               |      |                         |                                   |
| All compounds used as reference compounds were identified. |               |                    |               |      |                         |                                   |

<sup>A</sup> AR means "applied radioactivity". MW means "molecular weight". PRT means "parent". ECM means "environmental chemical methods". ILV means "independent laboratory validation".