Analytical method for pyrasulfotole (AE 0317309) and its degradate AE B197555 in soil and sediment

Reports: ECM 1: EPA MRID No. 46801814. Netzband, D.J., and D.M. Smith. 2006. AE 0317309: Analytical Method for the Determination of AE 0317309 and its Metabolite AE B197555 in Soil and Sediment by LC/MS/MS. Bayer CropScience Residue Analytical Method No.: AI-002-S05-02. Report prepared by Bayer CropScience, Stillwell, Kansas, and sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 52 pages. Final report (Revision) issued February 21, 2006.

ECM 2: EPA MRID No. 46801815. Netzband, D.J. 2006. In House Laboratory Validation of an Analytical Method for the Determination of Residues of AE 0317309 and its Metabolite AE B197555 in Soil And Sediment Using LC/MS/MS. Bayer CropScience Study No.: 04MEAIX017. Report prepared by Bayer CropScience, Stillwell, Kansas, and sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 94 pages. Final report issued February 24, 2006.

ILV: EPA MRID No. 46801816. Brumhard, B. 2005. Independent Laboratory Validation of Method AI002-S05-01 for the Determination of AE 0317309 and its Metabolite AE B197555 in Soil and Sediment by LC/MS/MS. Bayer CropScience AG Report No.: MR-112/05. Laboratory Project ID: P611050012. Report prepared by Bayer CropScience AG, Monheim am Rhein, Germany, and sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 30 pages. Final report issued November 16, 2005.

Document No.: MRIDs 46801814 & 46801815 & 46801816

Guideline: 850.6100

Statements: ECM 1: The study was not conducted in accordance with USEPA FIFRA (40 CFR Part 160) Good Laboratory Practices (GLP) since it was not a study (p. 3 of MRID 46801814). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Authenticity and Quality Assurance statements were not provided.
ECM 2: The study was conducted in accordance with USEPA FIFRA GLP (p. 3 of MRID 46801815). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).
ILV: The study was conducted in accordance with OECD GLP standards which also meet requirements of German, USEPA FIFRA, and Japanese (JMAFF) GLP (p. 3 of MRID 46801816). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4, 6). A statement of the authenticity of the study report was not included. The GLP Certificate of the test facility was provided (Appendix 1, pp. 29-30).

Classification: This analytical method is classified as **Supplemental**. The submitted final ECM was Method AI-002-S05-02 which had been updated after the ILV

validation with a minor modification to Accelerated Solvent Extraction (ASE) flush volume. Sediment matrices were not included in the ILV. It could not be determined if the ILV was conducted independently from the ECMs 1 and 2. The classification and characterization of ECM 2 soil and sediment matrices were not reported. The reproducibility of the method was only validated for 0.5 µg/kg (LOQ) in soil matrices, and insufficient performance data was provided for the validation of soil matrices at 10×LOQ.

PC Code:	000692		
Reviewer:	Joshua Antoline, Ph.D., Chemist	Signature:	JOSHUA ANTOLINE Digitally signed by JOSHUA ANTOLINE Date: 2020.10.08 12:33:22 -04'00'
	Karen Milians, Ph.D. Chemist	Signature:	Digitally signed by KAREN MILIANS Date: 2020.10.08 13:11:39 -04'00'
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature:	
		Date:	08/10/2020
	Mary Samuel, M.S., Environmental Scientist	Signature:	
		Date:	08/10/2020

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

Executive Summary

This analytical method, Bayer CropScience Residue Analytical **Method AI-002-S05-02** (ECM 2), is designed for the quantitative determination of the pyrasulfotole (AE 0317309) and its degradate AE B197555 at 0.5 µg/kg in soil and sediment using LC/MS/MS. The LOQ is greater the most sensitive toxicological endpoint of 0.0078 µg/kg in soil based on a 6-inch soil depth and a soil density of 1.5 g/cm^3 (MRID 46801937).

The submitted final ECM, Method AI-002-S05-02, was an update of the original ECM (Method AI-002-S05-01, ECM 1) with a modification to ASE flush volume to prevent collection cell overflow based on the findings of the ILV validation. This method validated the original method (Method AI-002-S05-01) using two uncharacterized soil and two uncharacterized sediment matrices. The ILV validated Method AI-002-S05-01 using two characterized soil matrices; sediment matrices were not included in the ILV. The ILV validated the original ECM method as written except for the reduction of ASE flush volume due to collection cell overflow and for minor modifications to the analytical parameters and equipment. The ILV findings were communicated to the ECM study authors, and an updated ECM (Method AI-002-S05-02) was prepared. The ASE flush volume modification was the only reported difference between Method AI-002-S05-01 and Method AI-002-S05-02. This modification is not expected to impact the

results of the study. The number of ILV trials required to validate the method was not specified, but the reviewer assumed that the ILV validated the method in the first or second trial based on the ILV modification of ASE flush volume. It could not be determined if the ILV was conducted independently from the ECMs 1 and 2 since there was direct communication between the ILV and ECM study authors, but communications were only summarized.

The reproducibility of the method was only validated for 0.5 µg/kg (LOQ) in soil matrices. Only ILV performance data was provided for the validation of soil matrices at 10×LOQ. Only ECM performance data was provided for the validation of sediment matrices at the LOQ. No sediment samples were fortified at 10×LOQ. All submitted ILV data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for pyrasulfotole and AE B197555. The LOD was not reported in the ILV. All submitted ECM data regarding repeatability, accuracy, and precision were satisfactory for pyrasulfotole and AE B197555; however, only three replicates were prepared at the 5×LOQ fortification in all matrices. All submitted ECM data regarding linearity and specificity were satisfactory for pyrasulfotole and AE B197555; however, data was not provided for all test matrices.

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						
Pyrasulfotole (AE 0317309)	46801814[1] & 46801815[2]	46801816[3]		Soil	21/02/2006 (ECM 1)[1]	Bayer CropScience	LC/MS/MS	0.5 µg/kg
AE B197555		None submitted		Sediment	24/02/2006 (ECM 2)[2]			

1 MRID 46801814 was designated as ECM 1 which was a summary report using the results of ECM 2 (p. 8 of MRID 46801814).

2 MRID 46801815 was designated as ECM 2 which contained the in-house validation of Method AI-002-S05-01 (the previous version of Method AI-002-S05-02; p. 10 of MRID 46801815). In the ECM 2, two soil matrices, Brunisolic Gray Brown Luvisol (Sample ID: 03BCS01-B) from Ontario, Canada, and Black Chernozem. (Sample ID: 03BCS01-C) from Manitoba, Canada, and two sediment matrices, Alabama (Sample ID: LT) from Lake Tuscaloosa, Alabama, and South Carolina (Sample ID: SR-L) from Sandhill Research Station Lake, Columbia, South Carolina, were used in the study (p. 13 of MRID 46801815). The soil matrices were obtained from the terrestrial field dissipation study Bayer CropScience Study No. 03BCS01 (MRID 46801719). The sediment matrices were obtained from Bayer CropScience Study No. EBFIY003 which studied the toxicity of fipronil to sediment dwelling organisms in the field (MRID 47152301). USDA soil texture classification was not provided.

2 In the ILV, the two soil matrices were silt loam (Höfchen; pH 7.4 (in water) and 6.7 (in $CaCl_2$); 4.3% sand, 76.3% silt, 19.4% clay, 0.92% organic carbon, 12.4 meq/100 g cation exchange capacity) and sandy loam (Laacher Hof; pH 7.4 (in water) and 6.8 (in $CaCl_2$); 69.7% sand, 18.3% silt, 12.0% clay, 1.20% organic carbon, 9.8 meq/100 g cation exchange capacity) which were from Germany (USDA soil texture classification; p. 11; Tables 7-8, pp. 27-28 of MRID 46801816). The soil characterization laboratory was not reported. Sediment matrices were not included.

I. Principle of the Method

Samples (25 ± 0.1 g) of soil or sediment were mixed with 3.0 ± 0.1 g of hydromatrix (Varian Part No. 0019-8003) via manual hand-shaking (pp. 9-14; Appendix 6, p. 51 of MRID 46801814; pp. 10, 16-17; Appendix 2, pp. 49-55 of MRID 46801815). The step was repeated if significant quantities of water were present in the sample. After the sample was transferred to a 33-mL Dionex extraction cell, the sample was fortified with the 0.05 or 0.005 µg/mL mixed fortification solutions, as necessary, at the top of the soil column contained in the extraction cell. The extraction cell was topped-off completely with sand then immediately loaded onto the Accelerated Solvent Extractor (ASE) 200 system. The ASE settings were as follows: preheat 0 min., heat 5 min., static time 15 min., flush volume 80%, purge time 150 seconds, 2 cycles, 1500 psi, 100°C, and extraction solvents (A) 65% acetonitrile and (B) 35% deionized water. The 0.1 µg/mL mixed deuterated internal standard solution (pyrasulfotole-d_3 and AE B197555-$^{13}C_6$) was added to collection vials. An aliquot (*ca*. 20 mL) of the ASE collection flask was reduced to *ca*. 5 mL using a Turbovap® II evaporator at 50°C. The residue was mixed with 60 µL of formic acid via sonication for 2-3 minutes. An aliquot (*ca*. 1.0 mL) of the sample was applied to the Applied Separations 200 mg/3 mL RP-102 Resin Spe-ed solid phase extraction (SPE) Cartridge which was pre-conditioned with one column volume each of acetonitrile:water (50:50, v:v) and HPLC water. The cartridge was washed with *ca*. 1 mL of water (*ca*. 1 drop/2 second). The cartridge was not allowed to dry up until the washing but then was dried via vacuum for *ca*. 2 minutes under pressure (*ca*. 20 inches in Hg), if necessary. The analytes were eluted using *ca*. 1.0 mL of acetonitrile:methanol (50:50, v:v), after allowing cartridge to soak in elution solvent for 1-2 minutes. Eluate was mixed with *ca*. 4 mL of 0.1% acetic acid in deionized water. Sample was filtered (Acrodisc® 0.45 µm syringe filter) prior to LC/MS/MS analysis. The Original ECM Method AI-002-S05-01 used an ASE flush volume of 100%; however, the ILV reported that the ASE collection cells overflowed so the ASE flush volume reduced to 80% in the Revised ECM Method AI-002-S05-02 (p. 16 of MRID 46801814; pp. 16-17 of MRID 46801815).

Samples were analyzed for pyrasulfotole and AE B197555 using two Shimadzu LC-10ADVP HPLC coupled to a Perkin Elmer Sciex API 3000 mass spectrometer equipped with a PE Sciex Turbo Ion Spray electrospray interface with multiple reaction monitoring (MRM; Tables 1-2, pp. 18-19; Appendix 1, pp. 20-23 of MRID 46801814; Tables 1-2, pp. 59-60; Appendix 2, Appendix 1, pp. 61-64 of MRID 46801815). The following LC conditions were used: Phenomenex Prodigy C_8 column (2.0 mm x 50 mm, 5 µm; column temperature not reported), Javelin-Direct Connect Column Filter (2.1 mm i.d.) guard column, mobile phase of (A) 0.1% acetic acid in water and (B) acetonitrile:water (85:15, v:v) + 0.03% formic acid in [mobile gradient phase of percent A:B (v:v) at 0.0-1.0 min. 97.0:3.0, 4.0-13.0 min. 7.0:93.0, 13.0-15.0 min. 97.0:3.0], MS temperature 550°C, MS polarity positive (pyrasulfotole) and negative (AE B197555), and injection volume of 30.0 µL. Expected retention times were *ca*. 3.4 and 3.8 minutes for AE 0317309 (pyrasulfotole) and AE B197555, respectively. One ion pair transition was monitored for each analyte: *m/z* 363→251 for pyrasulfotole, *m/z* 366→254 for pyrasulfotole-d_3, *m/z* 267→223 for AE B197555, and *m/z* 273→229 for AE B197555-$^{13}C_6$. Alternative LC/MS/MS conditions were reported in the ECM 2 as follows: Waters SymmetryShield RP8 column (3.0 mm x 150 mm, 5 µm; column temperature not reported), Javelin-Direct Connect Column Filter (2.1 mm i.d.) guard column, and mobile phase of (A) acetonitrile and (B) 0.1% acetic acid in water [mobile gradient phase of percent A:B (v:v) at 0.00-1.00 min. 20.0:80.0, 8.00-12.50 min. 90.0:10.0,

12.60-15.00 min. 20.0:80.0] with expected retention times of *ca*. 8.2 and 10.6 minutes for AE 0317309 (pyrasulfotole) and AE B197555, respectively (Appendix 2, Appendix 7, p. 94 of MRID 46801815).

The ILV performed the ECM method as written, except for the modification that the ASE flush volume was reduced from 100% to 80% due to collection cell overflow and for the minor modifications to the analytical parameters and equipment (pp. 12-14, 16 of MRID 46801816). The same SPE column was used as in the ECM. Samples were analyzed for pyrasulfotole and AE B197555 using Agilent HP 1100 HPLC system coupled with an IONICS EP 10+ mass spectrometer equipped with turbo-ionspray interface, MRM mode (performance-enhanced Sciex API-365). The LC/MS/MS parameters were optimized for the system and reportedly not identical to those in the ECM. The LC/MS/MS parameters were not reported in detail, but the LC column was the same as that of the ECM. One ion pair transition was monitored for each analyte; the monitored ion transitions of the ILV were the same as those of the ECM (Figures 3-7, pp. 22-26). Observed retention times were *ca*. 4.2 and 4.7 minutes for pyrasulfotole and AE B197555, respectively.

The method Limit of Quantification (LOQ) for pyrasulfotole and AE B197555 in soil was reported as 0.5 µg/kg in the ECM 1, ECM 2, and ILV (p. 7; Appendix 3, p. 26 of MRID 46801814; pp. 8-9, 15-16; Tables 5-6, pp. 23-24 of MRID 46801815; pp. 8, 17 of MRID 46801816). In the ECM 1 and ECM 2, the LOQ and Method Detection Limit (MDL) were calculated as 0.22-0.34 µg/kg and 0.07-0.11 µg/kg for pyrasulfotole, respectively, and 0.30-0.31 µg/kg and 0.09-0.10 µg/kg for AE B197555, respectively.

The time requirement for the method was reported in the ILV as *ca*. two calendar days for each method trial of 12 samples with *ca*. 2 hours for preparation, ca. 4 hours for subsample preparation, and *ca*. 9 hours for LC/MS/MS analysis (p. 18 of MRID 46801816). This time requirement was similar to that reported in the ECM 2 (p. 16 of MRID 46801815).

II. Recovery Findings

ECMs 1 & 2 (MRIDs 46801814 & 46801815): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of pyrasulfotole and AE B197555 in two soil and two sediment matrices at the LOQ (0.5 µg/kg) and 5×LOQ (2.5 µg/kg; Appendix 3, p. 26 of MRID 46801814; Tables 1-4, pp. 19-22 of MRID 46801815; DER Attachment 2). No samples were prepared at 10×LOQ (5.0 µg/kg). Only three replicates were prepared at 5×LOQ (2.5 µg/kg). Means, standard deviations, and RSDs were reviewer-calculated since means and standard deviations were calculated for combined soil and sediment matrices at each fortification in the study report. Only one ion transition was monitored for each analyte; a confirmatory method is not usually required when LC/S or GC/MS is used as the primary method to generate study data. Recoveries in the sediment were lower than those of the soil for both analytes. Two soil matrices, Brunisolic Gray Brown Luvisol (Sample ID: 03BCS01-B) from Ontario, Canada, and Black Chernozem. (Sample ID: 03BCS01-C) from Manitoba, Canada, and two sediment matrices, Alabama (Sample ID: LT) from Lake Tuscaloosa, Alabama, and South Carolina (Sample ID: SR-L) from Sandhill Research Station Lake, Columbia, South Carolina, were used in the study (p. 13 of MRID 46801815). The soil matrices were obtained from the

terrestrial field dissipation study Bayer CropScience Study No. 03BCS01 (MRID 46801719). The sediment matrices were obtained from Bayer CropScience Study No. EBFIY003 which studied the toxicity of fipronil to sediment dwelling organisms in the field (MRID 47152301). USDA soil texture classification was not provided; the soil texture could not be reported or verified by the reviewer using USDA-NRCS technical support tools. ECM 1 data was a repetition of ECM 2 data.

ILV (MRID 46801816): Mean recoveries and RSDs met requirements for analysis of pyrasulfotole and AE B197555 in two soil matrices at the LOQ (0.5 µg/kg) and 10×LOQ (5.0 µg/kg; Tables 5-6, pp. 17-18 of MRID 46801816). Only one ion transition was monitored for each analyte; a confirmatory method is not usually required when LC/S or GC/MS is used as the primary method to generate study data. Recoveries at the LOQ were slightly lower than those at 10×LOQ. The two soil matrices were silt loam (Höfchen; pH 7.4 (in water) and 6.7 (in $CaCl_2$); 4.3% sand, 76.3% silt, 19.4% clay, 0.92% organic carbon, 12.4 meq/100 g cation exchange capacity) and sandy loam (Laacher Hof; pH 7.4 (in water) and 6.8 (in $CaCl_2$); 69.7% sand, 18.3% silt, 12.0% clay, 1.20% organic carbon, 9.8 meq/100 g cation exchange capacity) which were from Germany (USDA soil texture classification; p. 11; Tables 7-8, pp. 27-28). The soil characterization laboratory was not reported. Sediment matrices were not included. The original ECM method (Method AI-002-S05-01) was validated by the ILV as written except for the minor modification that the ASE flush volume was reduced from 100% to 80% due to collection cell overflow and for the insignificant modifications to the analytical parameters and equipment (pp. 12-14, 16). The ILV findings were communicated to the ECM, and an updated ECM (Method AI-002-S05-02) was prepared in which the ASE flush volume was reduced from 100% to 80% (pp. 16-17; Appendix 7, p. 52 of MRID 46801815; pp. 8-9, 19 of MRID 46801816). Since the ASE flush volume modification was the only reported difference between Method AI-002-S05-01 and Method AI-002-S05-02 and this modification would not impact the results of the study, the ECM results from Method AI-002-S05-01 can be use as validation data for Method AI-002-S05-02. The number of ILV trials required to validate the method was not specified, but the reviewer assumed that the ILV validated the method in the first or second trial based on the ILV modification of ASE flush volume (p. 16 of MRID 46801816).

Table 2. Initial Validation Method Recoveries for Pyrasulfotole (AE 0317309) and AE B197555 in Soil[1,2,3]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ontario Soil						
Pyrasulfotole (AE 0317309)	0.5 (LOQ)	6	79-98	89	8	9
	2.5	**3**	96-100	98	2	2
AE B197555	0.5 (LOQ)	7	85-107	94	8	8
	2.5	**3**	97-100	98	2	2
Manitoba Soil						
Pyrasulfotole (AE 0317309)	0.5 (LOQ)	7	80-96	86	6	7
	2.5	**3**	93-104	99	6	6
AE B197555	0.5 (LOQ)	7	88-100	92	4	5
	2.5	**3**	90-96	93	3	3
Alabama Sediment						
Pyrasulfotole (AE 0317309)	0.5 (LOQ)	7	68-77	70	3	4
	2.5	**3**	73-85	80	6	8
AE B197555	0.5 (LOQ)	7	70-86	80	7	8
	2.5	**3**	79-90	85	6	7
South Carolina Sediment						
Pyrasulfotole (AE 0317309)	0.5 (LOQ)	7	64-80	72	6	8
	2.5	**3**	80-86	84	3	4
AE B197555	0.5 (LOQ)	7	72-89	79	6	8
	2.5	**3**	71-82	77	6	7

Values in **bold** indicate that number of test is below the guideline recommended number

Data (uncorrected results, p. 14 of MRID 46801815) were obtained from Appendix 3, p. 26 of MRID 46801814; Tables 1-4, pp. 19-22 of MRID 46801815; DER Attachment 2.

1 Two soil matrices, Brunisolic Gray Brown Luvisol (Sample ID: 03BCS01-B) from Ontario, Canada, and Black Chernozem. (Sample ID: 03BCS01-C) from Manitoba, Canada, and two sediment matrices, Alabama (Sample ID: LT) from Lake Tuscaloosa, Alabama, and South Carolina (Sample ID: SR-L) from Sandhill Research Station Lake, Columbia, South Carolina, were used in the study (p. 13 of MRID 46801815). The soil matrices were obtained from the terrestrial field dissipation study Bayer CropScience Study No. 03BCS01 (MRID 46801719). The sediment matrices were obtained from Bayer CropScience Study No. EBFIY003 which studied the toxicity of fipronil to sediment dwelling organisms in the field (MRID 47152301). USDA soil texture classification was not provided; the soil texture could not be reported or verified by the reviewer using USDA-NRCS technical support tools. ECM 1 data was a repetition of ECM 2 data.

2 One ion pair transition was monitored for each analyte: *m/z* 363→251 for pyrasulfotole and *m/z* 267→223 for AE B197555.

3 Means, standard deviations, and RSDs were reviewer-calculated since these values were not reported in the study report (see DER Attachment 2). Rules of significant figures were followed. In the study report, means and standard deviations were calculated for combined soil and sediment matrices at each fortification.

Table 3. Independent Validation Method Recoveries for Pyrasulfotole (AE 0317309) and AE B197555 in Soil[1,2]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
Höfchen Soil						
Pyrasulfotole (AE 0317309)	0.5 (LOQ)	5	89-95	92	2	2.7
	5.00	5	94-99	97	2	2.4
AE B197555	0.5 (LOQ)	5	85-94	89	4	4.6
	5.00	5	100-112	107	5	4.4
Laacher Hof Soil						
Pyrasulfotole (AE 0317309)	0.5 (LOQ)	5	91-96	93	2	1.9
	5.00	5	96-98	97	1	0.9
AE B197555	0.5 (LOQ)	5	81-99	88	7	8.7
	5.00	5	100-108	105	3	3.1

Data (uncorrected results, p. 21) were obtained from Tables 5-6, pp. 17-18 of MRID 46801816; DER Attachment 2.

1 The soil matrices were silt loam (Höfchen; pH 7.4 (in water) and 6.7 (in $CaCl_2$); 4.3% sand, 76.3% silt, 19.4% clay, 0.92% organic carbon, 12.4 meq/100 g cation exchange capacity) and sandy loam (Laacher Hof; pH 7.4 (in water) and 6.8 (in $CaCl_2$); 69.7% sand, 18.3% silt, 12.0% clay, 1.20% organic carbon, 9.8 meq/100 g cation exchange capacity) which were from Germany (USDA soil texture classification; p. 11; Tables 7-8, pp. 27-28). The soil characterization laboratory was not reported. The soil texture was verified by the reviewer using USDA-NRCS technical support tools. Sediment matrices were not included.

2 Two ion pair transitions were monitored (primary and confirmatory, respectively): *m/z* 218.9→174.4 and *m/z* 220.9→176.7 for pyrasulfotole, and *m/z* 205.0→161.0 and *m/z* 205.0→125.0 for AE B197555; the monitored ion transitions of the ILV were similar to those of the ECM.

3 Standard deviations were reviewer-calculated from the reported data since these values were not reported in the study report. Rules of significant figures were followed.

III. Method Characteristics

The LOQ for pyrasulfotole and AE B197555 in soil was reported as 0.5 µg/kg in the ECM 1, ECM 2, and ILV (p. 7; Appendix 3, p. 26 of MRID 46801814; pp. 8-9, 15-16; Tables 5-6, pp. 23-24 of MRID 46801815; pp. 8, 17 of MRID 46801816). No justification was provided for the LOQ of 0.5 µg/kg in the ECM 1, ECM 2, or ILV. In the ECM 1 and ECM 2, the LOQ was also calculated as the sum of 10 times the standard deviation at the method LOQ and the average apparent residue in the untreated control, and the LOD/MDL was calculated as the sum of 3 times the standard deviation at the method LOQ and the average apparent residue in the untreated control. The calculations were based on the overall data (both soils, n = 14). In the ECM 1 and ECM 2, the calculated LOQ and MDL were 0.22-0.34 µg/kg and 0.07-0.11 µg/kg for pyrasulfotole, respectively, and 0.30-0.31 µg/kg and 0.09-0.10 µg/kg for AE B197555, respectively. The calculated LOQs supported the method LOQ for both analytes in soil and sediment matrices. The ECM 1/ECM 2 study report noted that the LOD/MDL can vary between instruments and conditions.

Table 4. Method Characteristics

		Pyrasulfotole (AE 0317309)	AE B197555
Limit of Quantitation (LOQ)*	ECM 1[1] & ECM 2[3]	0.5 µg/kg (method)	
		0.34 µg/kg (calc soil) 0.22 µg/kg (calc sediment)	0.31 µg/kg (calc soil) 0.30 µg/kg (calc sediment)
	ILV	0.5 µg/kg	
Limit of Detection (LOD)	ECM 1[1] & ECM 2[3]	0.11 µg/kg (calc soil) 0.07 µg/kg (calc sediment)	0.10 µg/kg (calc soil) 0.09 µg/kg (calc sediment)
	ILV	**Not reported**	
Linearity (calibration curve r and concentration range)	ECM 1[1] & ECM 2[3]	r = 0.99988 (sediment)[2]	r = 0.99849 (sediment)[2]
		0.0-20.0 µg/L	
	ILV	r = 0.9993016	r = 0.9996467
		0.2-20.0 µg/L	
Repeatable	ECM 1[1] & ECM 2[3]	Yes for LOQ and 5×LOQ in two uncharacterized soil and two uncharacterized sediment matrices, but n = **3** for 5×LOQ. **No** samples prepared at 10×LOQ.	
	ILV[4,5]	Yes for LOQ and 10×LOQ in two characterized soil matrices. **No** samples prepared with sediment matrices.	
Reproducible		Yes for 0.5 µg/kg (LOQ) in soil matrices. **Could not be determined** at 5.00 µg/kg in soil matrices; only one set of performance data. **Could not be determined** at 0.5 µg/kg (LOQ) in sediment matrices; only one set of performance data. **No** at 5.00 µg/kg in sediment matrices.	
Specific	ECM 1[1] & ECM 2[3]	Yes, matrix interferences were <6% of the LOQ in soil and <10% of the LOQ in sediment (based on quantified residues). Some contamination/baseline noise and peak tailing was observed in soil.	Yes, matrix interferences were <6% of the LOQ in soil and not observed in sediment (based on quantified residues).
		Representative chromatograms were only provided for one soil and one sediment matrix.	
	ILV	Yes, matrix interferences were <4% of the LOQ (based on peak area).	Yes, no matrix interferences were observed. Minor baseline noise was observed near the analyte peak.

Data were obtained from p. 7; Appendix 3, p. 26 (ECM 1 LOQ/LOD); Appendix 3, p. 26 (ECM 1 recovery data); Appendix 4, pp. 28-29 (calibration curves); Appendix 5, pp. 33-49 (chromatograms) of MRID 46801814; pp. 8-9, 15-16; Tables 5-6, pp. 23-24 (ECM 2 LOQ/LOD); Tables 1-4, pp. 19-22 (ECM 2 recovery data); p. 16; Appendix 2, Appendix 4, pp. 69-72 (calibration data); Appendix 1, pp. 29-40 (chromatograms) of MRID 46801815; pp. 8, 17 (LOQ/LOD); p. 16 (linearity coefficients); Tables 5-6, pp. 17-18 (recovery data); Figures 1-2, pp. 21 (calibration curves); Figures 3-7, pp. 22-26 (chromatograms) of MRID 46801816; DER Attachment 2.

* The LOQ was based on scientifically acceptable procedures defined in 40 CFR Part 136.

1 MRID 46801814 was designated as ECM 1 which was a summary report using the results of ECM 2 (p. 8 of MRID 46801814).

2 Only the calibration data for one of the sediment matrices (Sample ID:LT) was provided. Solvent-based calibration standards were prepared (Appendix 2, p. 52 of MRID 46801815).

3 MRID 46801815 was designated as ECM 2 which contained the in-house validation of Method AI-002-S05-01 (the previous version of Method AI-002-S05-02; p. 10 of MRID 46801815). In the ECM 2, two soil matrices, Brunisolic Gray Brown Luvisol (Sample ID: 03BCS01-B) from Ontario, Canada, and Black Chernozem. (Sample

ID: 03BCS01-C) from Manitoba, Canada, and two sediment matrices, Alabama (Sample ID: LT) from Lake Tuscaloosa, Alabama, and South Carolina (Sample ID: SR-L) from Sandhill Research Station Lake, Columbia, South Carolina, were used in the study (p. 13 of MRID 46801815). The soil matrices were obtained from the terrestrial field dissipation study Bayer CropScience Study No. 03BCS01 (MRID 46801719). The sediment matrices were obtained from Bayer CropScience Study No. EBFIY003 which studied the toxicity of fipronil to sediment dwelling organisms in the field (MRID 47152301). USDA soil texture classification was not provided.

4 In the ILV, the two soil matrices were silt loam (Höfchen; pH 7.4 (in water) and 6.7 (in $CaCl_2$); 4.3% sand, 76.3% silt, 19.4% clay, 0.92% organic carbon, 12.4 meq/100 g cation exchange capacity) and sandy loam (Laacher Hof; pH 7.4 (in water) and 6.8 (in $CaCl_2$); 69.7% sand, 18.3% silt, 12.0% clay, 1.20% organic carbon, 9.8 meq/100 g cation exchange capacity) which were from Germany (USDA soil texture classification; p. 11; Tables 7-8, pp. 27-28 of MRID 46801816). The soil characterization laboratory was not reported. Sediment matrices were not included.

5 The ILV validated the original ECM method (Method AI-002-S05-01) as written except for the minor modification that the ASE flush volume was reduced from 100% to 80% due to collection cell overflow and for the modifications to the analytical parameters and equipment (pp. 12-14, 16 of MRID 46801816). The ILV findings were communicated to the ECM, and an updated ECM (Method AI-002-S05-02) was prepared in which the ASE flush volume was reduced from 100% to 80% (pp. 16-17; Appendix 7, p. 52 of MRID 46801815; pp. 8-9, 19 of MRID 46801816). Since the ASE flush volume modification was the only reported difference between Method AI-002-S05-01 and Method AI-002-S05-02 and this modification would not impact the results of the study, the ECM results from Method AI-002-S05-01 can be use as validation data for Method AI-002-S05-02. The number of ILV trials required to validate the method was not specified, but the reviewer assumed that the ILV validated the method in the first or second trial based on the ILV modification of ASE flush volume (p. 16 of MRID 46801816).

IV. Method Deficiencies and Reviewer's Comments

1. The reported method LOQ of 0.5 µg/kg was the target LOQ, not the limit based on a scientifically acceptable procedure. The calculated method LOQs were based on scientifically acceptable procedures defined in 40 CFR Part 136 in the ECM (p. 7; Appendix 3, p. 26 of MRID 46801814; pp. 8-9, 15-16; Tables 5-6, pp. 23-24 of MRID 46801815).

2. The submitted final ECM was Method AI-002-S05-02 (pp. 1, 18; Appendix 7, p. 52 of MRID 46801814). The ILV was performed based on the original ECM Method AI-002-S05-01 (pp. 8, 12 of MRID 46801816). After the ILV validation, the ILV findings/modifications were communicated to the ECM, and an updated ECM (Method AI-002-S05-02) was prepared in which the ASE flush volume was reduced from 100% to 80% (pp. 16-17; Appendix 7, p. 52 of MRID 46801815; pp. 8-9, 19 of MRID 46801816). Since the ASE flush volume modification was the only reported difference between Method AI-002-S05-01 and Method AI-002-S05-02 and this modification would not impact the results of the study, the ECM results from Method AI-002-S05-01 (in ECMs 1/2) can be use as validation data for Method AI-002-S05-02.

3. It could not be determined if the ILV was conducted independently from the ECMs 1/2 since the ILV study author (Björn Brumhard) communicated directly with Derek Netzband, who was the study author of ECM 2 and one of the study authors of ECM 1 (p. 1 of MRID 46801814; pp. 1, 16-17; Appendix 7, p. 52 of MRID 46801815; pp. 8-9, 19 of MRID 46801816). The communications were only summarized but included transfer of the ECM method and ILV protocol prior to the ILV validation, and ILV results and

modification after to ILV validation (p. 19 of MRID 46801816). The summary of the communications did not appear to involve the transfer of technical guidance during the ILV validation, but Derek Netzband reportedly provided "some minor comments to the protocol" (p. 19).

4. The reproducibility of the method could not be determined for analyses at 5.00 µg/kg in soil matrices since no soil samples were prepared at 5.00 µg/kg (10×LOQ) in the ECMs 1/2. Soil samples were prepared at 2.50 µg/kg (5×LOQ) in the ECMs 1/2, but only three replicates were prepared for each matrix. OCSPP guidelines state that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

5. The reviewer determined that the determinations of the LOD and LOQ in the ECM were based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 7; Appendix 3, p. 26 of MRID 46801814; pp. 8-9, 15-16 of MRID 46801815; pp. 8, 17 of MRID 46801816). No justification was provided for the LOQ of 0.5 µg/kg in the ECM 1, ECM 2, or ILV. In the ECM 1 and ECM 2, the LOQ was calculated as the sum of 10 times the standard deviation at the method LOQ and the average apparent residue in the untreated control, and the LOD/MDL was calculated as the sum of 3 times the standard deviation at the method LOQ and the average apparent residue in the untreated control. The calculations were based on the overall data (both soils, n = 13-14). These LOQ and MDL calculations appeared to follow the method of Keith *et al*. 1983, except for the inclusion of the average apparent residue in the untreated control (pp. 15-16; Tables 5-6, pp. 23-24 of MRID 46801815). No average apparent residue in the untreated control was included in the LOD/LOQ calculations for AE B197555 since average recovery was 0.0000 µg/kg in both soil and sediment. The calculated LOQs supported the method LOQ for both analytes in soil and sediment matrices.

6. No LOD was reported in the ILV.

   The MDL is calculated as S x $t_{(N-1,1-\infty=.99)}$, where S is the Standard deviation of the matrix-spiked sample set concentrations (n must be ≥ 7) and $t_{(N-1,1-\infty=.99)}$ = Critical t value from a student t-test table at 99% confidence.

7. The reproducibility of the method could not be determined for analyses at 0.5 µg/kg (LOQ) in sediment matrices since no sediment matrices were included in the ILV. The applicability of the validation in soil matrices to sediment matrices could not be determined, and it was noted the ECM 1/2 recoveries in the sediment were lower than those of the soil for both analytes (Appendix 3, p. 26 of MRID 46801814; Tables 1-4, pp. 19-22 of MRID 46801815; DER Attachment 2).

8. The method was not validated at 5.00 µg/kg (10×LOQ) in sediment since no samples were prepared in the ECMs 1/2 or ILV.

9. OCSPP 850.6100 guidance states that, if the laboratory that conducted the validation belonged to the same organization as the originating laboratory, 1) the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion, and 2) the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies.

10. The classification and characterization of the ECM soil and sediment matrices were not reported in the study, but soil types were reported for the soil matrices (pp. 13, 18 of MRID 46801815). The soil matrices were obtained from the terrestrial field dissipation study Bayer CropScience Study No. 03BCS01 (MRID 46801719). The sediment matrices were obtained from Bayer CropScience Study No. EBFIY003 which studied the toxicity of fipronil to sediment dwelling organisms in the field (MRID 47152301).

11. The number of ILV trials required to validate the method was not specified, but the reviewer assumed that the ILV validated the method in the first or second trial based on the ILV modification of ASE flush volume (p. 16 of MRID 46801816).

12. In the ECMs 1/2, representative chromatograms were only provided for one soil and one sediment matrix. Representative chromatograms for all analytes/matrices should be submitted to access the specificity of the method.

13. The LC/MS/MS parameters were not reported in detail in the ILV.

14. Solvent-based calibration standards were prepared in Method AI-002-S05-01 and Method AI-002-S05-02 (p. 14; Appendix 2, p. 52 of MRID 46801815). Matrix effects were not studied in the ECMs 1/2 or ILV.

15. In the ECM 2, the stability of the calibration solutions was reportedly assessed during another study and found to be stable for up to 6 months when stored in the dark at <5°C (pp. 17-18 of MRID 46801815).

V. References

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319, and Revision 2; 1994 and 2016.

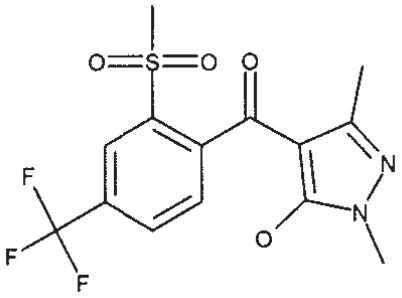
Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.*

1983, *55*, 2210-2218.

Attachment 1: Chemical Names and Structures

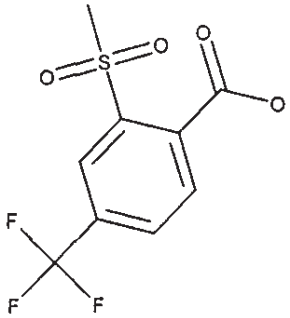
Pyrasulfotole (AE 0317309)

IUPAC Name: Not reported
CAS Name: (5-Hydroxy-1,3-dimethyl-1H-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone
CAS Number: 365400-11-9
SMILES String: Not found



AE B197555

IUPAC Name: Not reported
CAS Name: 2-(Methylsulfonyl)-4-(trifluoromethyl)benzoic acid
CAS Number: 142994-06-7
SMILES String: Not found



Attachment 2: Calculations Spreadsheet

000692_46801814+_8
50.6100_Calculations.x

Attachment 2: Calculations Spreadsheet

000692_46801814+_8
50.6100_Calculations.x