

**Analytical method for pyrasulfotole (AE 0317309) and its degradate AE B197555 in water**

**Reports:** ECM 1: EPA MRID No. 46801820. Netzband, D.J. 2006. AE 0317309: Analytical Method for the Determination of AE 0317309 and its Metabolite AE B197555 in Water by LC/MS/MS. Bayer CropScience Residue Analytical Method No.: AI-003-W05-02. Report prepared by Bayer CropScience, Stillwell, Kansas, and sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 41 pages. Final report (Revision) issued February 21, 2006.

ECM 2: EPA MRID No. 46801821. Netzband, D. 2006. In House Laboratory Validation of an Analytical Method for the Determination of Residues of AE 0317309 and its Metabolite AE B197555 in Water Using LC/MS/MS. Bayer CropScience Study No.: MEAIX018. Report prepared by Bayer CropScience, Stillwell, Kansas, and sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 79 pages. Final report issued February 24, 2006.

ILV: EPA MRID No. 46801822. Krebber, R. 2005. Independent Laboratory Validation of Method AI-003-W05-01 for the Determination of AE 0317309 and its Metabolite AE B197555 in Water. Bayer CropScience AG Report No.: MR-139/05. Laboratory Project ID: P 614 047066. Report prepared by Bayer CropScience AG, Monheim am Rhein, Germany, and sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 29 pages. Final report issued November 25, 2005.

**Document No.:** MRIDs 46801820 & 46801821 & 46801822

**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 46801820). 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 46801821). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Certification of 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 46801822). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5). A statement of the authenticity of the study report was not included. The GLP Certificate of the test facility was provided (Appendix 1, pp. 28-29).

**Classification:** This analytical method is classified as **supplemental**. The submitted final ECM was Method AI-003-W05-02 which had been updated after the ILV validation with ILV results. Only one set of performance data was provided

for the validation of water matrices at 10×LOQ. Only three samples were prepared for each water matrix at the LOQ (0.05 ng/mL) in the ECMs 1 and 2 instead of the recommended five. The characterization of the ECM water matrices was not reported.

<b>PC Code:</b>	000692		
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		Date:	08/24/2020

*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-003-W05-02 (ECM 2), is designed for the quantitative determination of the pyrasulfotole (AE 0317309) and its degradate AE B197555 at 0.05 ng/mL in water using LC/MS/MS. The Limit of Quantitation (LOQ) is less than the the lowest toxicological level of concern in water of 9.57 ng/mL (MRID 46801736). The submitted final ECM was Method AI-003-W05-02 is an update of the original ECM (Method AI-003-W05-01, ECM 1) that included the results of the ILV validation.

ECM 2 successfully validated the original method (Method AI-003-W05-01) using three uncharacterized water matrices: surface, ground and drinking (tap) water. The ILV validated Method AI-003-W05-01 using two characterized water matrices: surface and drinking (tap) water. The method specified the importance of sodium thiosulfate stabilization prior to method performance for finished drinking water matrices (tap water) containing free chlorine. The ILV validated the original ECM method as written except for minor modifications to the analytical parameters and equipment. The ILV findings were communicated to the ECM, and an updated ECM (Method AI-003-W05-02) was prepared. Since the inclusion of the ILV results was the only reported difference between Method AI-003-W05-01 and Method AI-003-W05-02 and this modification would not impact the results of the study, the ECM results from Method AI-003-W05-01 can be use as validation data for Method AI-003-W05-02. The number of ILV trials required to validate the method was not specified, however no ILV modifications or deviations were noted. 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 0.05 ng/mL (LOQ) in water matrices; however, only three samples per each water matrix were prepared in the ECMs 1 and 2. Only ILV performance data was provided for the validation of water matrices 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, precision, and specificity were satisfactory for pyrasulfotole and AE B197555; however, only three replicates were prepared at the 5×LOQ fortification in all matrices. ECM linearity was satisfactory for pyrasulfotole but linearity data was not provided for AE B197555.

**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)	46801820 <sup>1</sup> & 46801821 <sup>2</sup>	46801822 <sup>3</sup>		Water	21/02/2006 (ECM 1) <sup>1</sup>	Bayer CropScience	LC/MS/MS	0.05 ng/mL
AE B197555					24/02/2006 (ECM 2) <sup>2</sup>			

1 MRID 46801820 was designated as ECM 1 which was a summary report using the results of ECM 2 (p. 14 of MRID 46801820).

2 MRID 46801821 was designated as ECM 2 which contained the in-house validation of Method AI-003-W05-01 (the previous version of Method AI-003-W05-02; p. 8 of MRID 46801821). In the ECM 2, three water matrices, surface, ground and treated tap water, were used in the study (pp. 11, 17 of MRID 46801821). The surface water matrix was obtained from the ethoprophos surface water monitoring study Bayer CropScience Study No. 00M19458 (source location: Lodi, California; MRID No. not found). The ground water matrix was obtained from the azinphos-methyl well water survey study Bayer CropScience Study No. MEGUY003 (source location: Biglerville, Pennsylvania; MRID No. not found). The treated tap water matrix was obtained from a treated drinking water tap at Bayer Research Park, Stilwell, Kansas (Bayer CropScience Study No. MEAIX018; MRID No. not found). The matrix characterization was not provided but reportedly archived with the original studies; however, the characterization of the tap water was not included in this report.

3 In the ILV, the two water matrices were surface water (River Rhine Water Hitdorf; pH 7.3, 3 mg/L total organic carbon, 3 mg/L dissolved organic carbon, 535 µS/cm conductivity, 11.2 °dH hardness) sampled in Leverkusen-Hitdorf and drinking (tap) water (Drinking Water Monheim; pH 7.6, <2 mg/L total organic carbon, <2 mg/L dissolved organic carbon, 622 µS/cm conductivity, 14.8 °dH hardness) which were from Germany (p. 12; Tables 7-8, p. 27 of MRID 46801822). The water characterization laboratory was Bayer Industry Services.

## I. Principle of the Method

Prior to method performance, finished drinking water matrices (tap water) containing free chlorine were stabilized by adding 1 mL of a 1000 ppm sodium thiosulfate solution to 100 mL of water sample (Appendix 2, pp. 47, 49 of MRID 46801821).

Samples ( $100 \pm 2$  mL) of water were fortified with the 0.05 or 0.005  $\mu\text{g/mL}$  mixed fortification solutions, as necessary, (pp. 8-12; Appendix 6, p. 40 of MRID 46801820; pp. 7-8; Appendix 2, pp. 45-50, 78 of MRID 46801821). The 0.1  $\mu\text{g/mL}$  mixed deuterated internal standard solution (pyrasulfotole- $\text{d}_3$  and AE B197555- $^{13}\text{C}_6$ ) was added, then the sample was acidified with 6 mL of formic acid. 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 (*ca.* 1 drop/2 second) with *ca.* 1 mL of 0.8% formic acid in water then *ca.* 1 mL of water. The cartridge was not allowed to dry up until the washing but then was dried via vacuum or positive pressure nitrogen for *ca.* 2 minutes (*ca.* 500 Torr pressure can be used). 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  $\mu\text{m}$  syringe filter) prior to LC/MS/MS analysis.

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. 16-17; Appendix 1, pp. 18-21 of MRID 46801820; Appendix 2, Tables 1-2, pp. 54-55; Appendix 2, Appendix 1, pp. 56-59 of MRID 46801821). The following LC conditions were used: Phenomenex Prodigy  $\text{C}_8$  column (2.0 mm x 50 mm, 5  $\mu\text{m}$ ; column temperature ambient), 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 [percent A:B (v:v) at 0.0-1.0 min. 97.0:3.0, 7.0-9.0 min. 3.0:97.0, 9.1-12.0 min. 97.0:3.0], MS temperature 550°C, MS polarity positive (pyrasulfotole) and negative (AE B197555), and injection volume of 30.0  $\mu\text{L}$ . Expected retention times were *ca.* 4.0 and 4.6 minutes for AE 0317309 (pyrasulfotole) and AE B197555, respectively. One ion pair transition was monitored for each analyte:  $m/z$  363 $\rightarrow$ 251 for pyrasulfotole,  $m/z$  366 $\rightarrow$ 254 for pyrasulfotole- $\text{d}_3$ ,  $m/z$  267 $\rightarrow$ 223 for AE B197555, and  $m/z$  273 $\rightarrow$ 229 for AE B197555- $^{13}\text{C}_6$ .

The ILV performed the ECM method as written, except for minor modifications to the analytical parameters and equipment (pp. 13-16 of MRID 46801822). 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-ion-spray interface, MRM mode (performance-enhanced Sciex API-365). The LC/MS/MS parameters were optimized for the system. 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 3-8, pp. 22-23). Observed retention times were *ca.* 5.3 and 5.8 minutes for pyrasulfotole and AE B197555, respectively.

The reported method Limit of Quantification (LOQ) for pyrasulfotole and AE B197555 in water was reported as 0.05 ng/mL in the ECM 1, ECM 2, and ILV (p. 7; Appendix 3, p. 26 of MRID 46801820; pp. 7, 14-16; Table 3, p. 20 of MRID 46801821; pp. 9, 17-18 of MRID 46801822). The method Limit of Detection (LOD) in water was not reported in the ECM 1, ECM 2, or ILV. In the ECM 1 and ECM 2. The LOQ and Method Detection Limit (MDL) were calculated as 10x and 3x the standard deviation of the measured recovered concentrations from the 9 replicate samples. The calculated LOQ and MDL values were 0.018 ng/mL and 0.006 ng/mL for pyrasulfotole, respectively, and 0.035 ng/mL and 0.011 ng/mL for AE B197555.

## II. Recovery Findings

ECMs 1 & 2 (MRIDs 46801820 & 46801821): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of pyrasulfotole and AE B197555 in three water matrices at the LOQ (0.05 ng/mL) and 5 $\times$ LOQ (0.25 ng/mL; Appendix 3, pp. 24-25 of MRID 46801820; Tables 1-2, pp. 18-19 of MRID 46801821; DER Attachment 2). No samples were prepared at 10 $\times$ LOQ (0.5 ng/mL). An insufficient number of samples (n = 3) were prepared at LOQ (0.05 ng/mL) and 5 $\times$ LOQ (0.25 ng/mL). It is recommended that 5 samples are used at each fortification level. Means, standard deviations, and RSDs were reviewer-calculated since means and standard deviations were calculated for combined water 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 at the LOQ were consistently lower than those at 5 $\times$ LOQ. The surface, ground and treated tap water were used in the study (pp. 11, 17 of MRID 46801821). The surface water matrix was obtained from the ethoprophos surface water monitoring study Bayer CropScience Study No. 00M19458 (source location: Lodi, California; MRID No. not found). The ground water matrix was obtained from the azinphos-methyl well water survey study Bayer CropScience Study No. MEGUY003 (source location: Biglerville, Pennsylvania; MRID No. not found). The treated tap water matrix was obtained from a treated drinking water tap at Bayer Research Park, Stilwell, Kansas (Bayer CropScience Study No. MEAIX018; MRID No. not found). The matrix characterization was not provided but reportedly archived with the original studies; however, the characterization of the tap water was not included in this report. ECM 1 data was a repetition of ECM 2 data.

ILV (MRID 46801822): Mean recoveries and RSDs met requirements for analysis of pyrasulfotole and AE B197555 in two water matrices at the LOQ (0.05 ng/mL) and 10 $\times$ LOQ (0.5 ng/mL; Tables 5-6, pp. 17-18 of MRID 46801822). 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 $\times$ LOQ. The two water matrices were surface water (River Rhine Water Hitdorf; pH 7.3, 3 mg/L total organic carbon, 3 mg/L dissolved organic carbon, 535  $\mu$ S/cm conductivity, 11.2  $^{\circ}$ dH hardness) sampled in Leverkusen-Hitdorf and drinking (tap) water (Drinking Water Monheim; pH 7.6, <2 mg/L total organic carbon, <2 mg/L dissolved organic carbon, 622  $\mu$ S/cm conductivity, 14.8  $^{\circ}$ dH hardness) which were from Germany (p. 12; Tables 7-8, p. 27). The water characterization laboratory was Bayer Industry Services. ECM 1 (Method AI-003-W05-01) was validated by the ILV as written except for minor modifications to the analytical parameters and

equipment (pp. 13-16). The ILV findings were communicated to the ECM authors, and an updated ECM (Method AI-003-W05-02) was prepared which included the ILV results (p. 16; Appendix 2, p. 52; Appendix 7, p. 79 of MRID 46801821; pp. 9-10, 19 of MRID 46801822). Since the inclusion of the ILV results was the only reported difference between Method AI-003-W05-01 and Method AI-003-W05-02 and this modification would not impact the results of the study, the ECM results from Method AI-003-W05-01 can be used as validation data for Method AI-003-W05-02. The number of ILV trials required to validate the method was not specified, however no ILV modifications or deviations were noted (p. 16 of MRID 46801822).

**Table 2. Initial Validation Method Recoveries for Pyrasulfotole (AE 0317309) and AE B197555 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>Surface Water</b>						
Pyrasulfotole (AE 0317309)	0.05 (LOQ)	<b>3</b>	71-78	75	4	5
	0.25	<b>3</b>	86-106	98	11	11
AE B197555	0.05 (LOQ)	<b>3</b>	80-93	85	7	9
	0.25	<b>3</b>	89-106	97	9	9
<b>Ground Water</b>						
Pyrasulfotole (AE 0317309)	0.05 (LOQ)	<b>3</b>	72-81	75	5	7
	0.25	<b>3</b>	90-112	103	11	11
AE B197555	0.05 (LOQ)	<b>3</b>	70-75	73	3	4
	0.25	<b>3</b>	78-84	81	3	4
<b>Treated Tap Water</b>						
Pyrasulfotole (AE 0317309)	0.05 (LOQ)	<b>3</b>	78-80	79	1	1
	0.25	<b>3</b>	90-92	91	1	1
AE B197555	0.05 (LOQ)	<b>3</b>	71-75	73	2	3
	0.25	<b>3</b>	87-92	89	3	3

Data (uncorrected results, pp. 12-13 of MRID 46801821) were obtained from Appendix 3, pp. 24-25 of MRID 46801820; Tables 1-2, pp. 18-19 of MRID 46801821; DER Attachment 2.

Values in **Bold** are less than the guideline recommended value.

- Three water matrices, surface, ground and treated tap water, were used in the study (pp. 11, 17 of MRID 46801821). The surface water matrix was obtained from the ethoprophos surface water monitoring study Bayer CropScience Study No. 00M19458 (source location: Lodi, California; MRID No. not found). The ground water matrix was obtained from the azinphos-methyl well water survey study Bayer CropScience Study No. MEGUY003 (source location: Biglerville, Pennsylvania; MRID No. not found). The treated tap water matrix was obtained from a treated drinking water tap at Bayer Research Park, Stilwell, Kansas (Bayer CropScience Study No. MEAIX018; MRID No. not found). The matrix characterization was not provided but reportedly archived with the original studies; however, the characterization of the tap water was not included in this report. ECM 1 data was a repetition of ECM 2 data.
- One ion pair transition was monitored for each analyte:  $m/z$  363→251 for pyrasulfotole and  $m/z$  267→223 for AE B197555.
- 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 water matrices at each fortification.

**Table 3. Independent Validation Method Recoveries for Pyrasulfotole (AE 0317309) and AE B197555 in Water<sup>1,2</sup>**

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>3</sup>	Relative Standard Deviation (%)
<b>River Rhine Water</b>						
Pyrasulfotole (AE 0317309)	0.05 (LOQ)	10	89-115	98	8	8.2
	0.50	10	89-94	93	2	1.7
AE B197555	0.05 (LOQ)	10	82-97	87	5	5.3
	0.50	10	84-95	89	4	4.5
<b>Drinking (Tap) Water</b>						
Pyrasulfotole (AE 0317309)	0.05 (LOQ)	10	88-100	94	4	3.9
	0.50	10	91-96	93	1	1.4
AE B197555	0.05 (LOQ)	10	88-96	92	3	3.4
	0.50	10	86-97	91	4	4.0

Data (uncorrected results, p. 15) were obtained from Tables 5-6, pp. 17-18 of MRID 46801822; DER Attachment 2.

1 The water matrices were surface water (River Rhine Water Hitdorf; pH 7.3, 3 mg/L total organic carbon, 3 mg/L dissolved organic carbon, 535  $\mu$ S/cm conductivity, 11.2  $^{\circ}$ dH hardness) sampled in Leverkusen-Hitdorf and drinking (tap) water (Drinking Water Monheim; pH 7.6, <2 mg/L total organic carbon, <2 mg/L dissolved organic carbon, 622  $\mu$ S/cm conductivity, 14.8  $^{\circ}$ dH hardness) which were from Germany (p. 12; Tables 7-8, p. 27). The water characterization laboratory was Bayer Industry Services.

2 One ion pair transition was monitored for each analyte:  $m/z$  363 $\rightarrow$ 251 for pyrasulfotole and  $m/z$  267 $\rightarrow$ 223 for AE B197555; the monitored ion transitions of the ILV were the same as 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 water was reported as 0.05 ng/mL in the ECM 1, ECM 2, and ILV (p. 7; Appendix 3, p. 26 of MRID 46801820; pp. 7, 14-16; Table 3, p. 20 of MRID 46801821; pp. 9, 17-18 of MRID 46801822). No justification was provided for this LOQ in the ECM 1, ECM 2, or ILV. The method LOD in water was not reported 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 MDL was calculated as the sum of 3 times the standard deviation at the method LOQ. The calculations were based on the overall data (all three waters, n = 9). In the ECM 1 and ECM 2, the calculated LOQ and MDL were 0.018 ng/mL and 0.006 ng/mL for pyrasulfotole, respectively, and 0.035 ng/mL and 0.011 ng/mL for AE B197555, respectively. The calculated LOQs supported the method LOQ for both analytes in water and sediment matrices. The ECM 1/ECM 2 study report noted that the MDL can vary between instruments and conditions.

**Table 4. Method Characteristics**

		Pyrasulfotole (AE 0317309)	AE B197555
Limit of Quantitation (LOQ)*	ECM 1 <sup>1</sup> & ECM 2 <sup>3</sup>	0.05 ng/mL (method reported)	
		0.018 ng/mL (calc)	0.035 ng/mL (calc)
	ILV	0.05 ng/mL	
Limit of Detection (LOD)	ECM 1 <sup>1</sup> & ECM 2 <sup>3</sup>	0.006 ng/mL (calc)	0.011 ng/mL (calc)
	ILV	<b>Not reported</b>	
Linearity (calibration curve r and concentration range)	ECM 1 <sup>1</sup> & ECM 2 <sup>3</sup>	r = 0.99957 <sup>2</sup>	<b>Not reported</b>
		0.0-20.0 µg/L	
	ILV	r = 0.9996 (surface) r = 0.9984 (drinking)	r = 0.9957 (surface) r = 0.9961 (drinking)
		0.2-20.0 µg/L	
Repeatable	ECM 1 <sup>1</sup> & ECM 2 <sup>3</sup>	Yes for LOQ and 5×LOQ in three uncharacterized water matrices, but n = <b>3</b> for each matrix. <b>No</b> samples prepared at 10×LOQ.	
	ILV <sup>4,5</sup>	Yes for LOQ and 10×LOQ in two characterized water matrices.	
Reproducible		Yes for 0.05 ng/mL (LOQ) in water matrices. <b>Could not be determined</b> at 0.50 ng/mL or 0.25 ng/mL in water matrices; only one set of performance data.	
Specific	ECM 1 <sup>1</sup> & ECM 2 <sup>3</sup>	Yes, no matrix interferences were quantified, and only minor matrix interferences were observed at the analyte retention time. Some contamination/baseline noise and peak tailing was observed in water.	Yes; matrix interferences were not observed in the surface water and were <25% of the LOQ in the ground and tap water matrices (based on quantified residues). A large nearby contaminant peak was observed. Analyte peak was broad and small in ground water.
	ILV	Yes, matrix interferences were <4% of the LOQ (based on peak area).	Yes, no matrix interferences were observed.

Data were obtained from p. 7; Appendix 3, p. 26 (ECM 1 LOQ/LOD); Appendix 3, pp. 24-25 (ECM 1 recovery data); Appendix 4, p. 30 (calibration curves); Appendix 5, pp. 34-39 (chromatograms) of MRID 46801820; pp. 7, 14-16; Table 3, p. 20 (ECM 2 LOQ/LOD); Tables 1-2, pp. 18-19 (ECM 2 recovery data); p. 15; Figure 1, p. 21 (calibration data); Appendix 1, pp. 25-37 (chromatograms) of MRID 46801821; pp. 9, 17-18 (LOQ/LOD); p. 16 (linearity coefficients); Tables 5-6, pp. 17-18 (recovery data); Figures 1-2, p. 21; Figures 9-10, p. 24 (calibration curves); Figures 3-16, pp. 22-26 (chromatograms) of MRID 46801822; DER Attachment 2.

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

1 MRID 46801820 was designated as ECM 1 which was a summary report using the results of ECM 2 (p. 14 of MRID 46801820).

2 Only the calibration data for pyrasulfotole in tap water matrix was provided. Solvent-based calibration standards were prepared (p. 15; Figure 1, p. 21; Appendix 2, p. 49 of MRID 46801821).

3 MRID 46801821 was designated as ECM 2 which contained the in-house validation of Method AI-003-W05-01 (the previous version of Method AI-003-W05-02; p. 8 of MRID 46801821). In the ECM 2, three water matrices, surface, ground and treated tap water, were used in the study (pp. 11, 17 of MRID 46801821). The surface water matrix was obtained from the ethoprophos surface water monitoring study Bayer CropScience Study No. 00M19458 (source location: Lodi, California; MRID No. not found). The ground water matrix was obtained from the azinphos-methyl well water survey study Bayer CropScience Study No. MEGUY003 (source location: Biglerville, Pennsylvania; MRID No. not found). The treated tap water matrix was obtained from a treated drinking water tap at Bayer Research Park, Stilwell, Kansas (Bayer CropScience Study No. MEAIX018; MRID No. not found). The matrix characterization was not provided but reportedly archived with the original studies; however, the characterization of the tap water was not included in this report.



- 4 In the ILV, the two water matrices were surface water (River Rhine Water Hitdorf; pH 7.3, 3 mg/L total organic carbon, 3 mg/L dissolved organic carbon, 535  $\mu$ S/cm conductivity, 11.2  $^{\circ}$ dH hardness) sampled in Leverkusen-Hitdorf and drinking (tap) water (Drinking Water Monheim; pH 7.6, <2 mg/L total organic carbon, <2 mg/L dissolved organic carbon, 622  $\mu$ S/cm conductivity, 14.8  $^{\circ}$ dH hardness) which were from Germany (p. 12; Tables 7-8, p. 27 of MRID 46801822). The water characterization laboratory was Bayer Industry Services.
- 5 The ILV validated the original ECM method (Method AI-003-W05-01) as written except for insignificant modifications to the analytical parameters and equipment (pp. 13-16 of MRID 46801822). The ILV findings were communicated to the ECM, and an updated ECM (Method AI-003-W05-02) was prepared which included the ILV results (p. 16; Appendix 2, p. 52; Appendix 7, p. 79 of MRID 46801821; pp. 9-10, 19 of MRID 46801822). Since the inclusion of the ILV results was the only reported difference between Method AI-003-W05-01 and Method AI-003-W05-02 and this modification would not impact the results of the study, the ECM results from Method AI-003-W05-01 can be use as validation data for Method AI-003-W05-02. 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 trial since no ILV modifications or deviations were noted (p. 16 of MRID 46801822).

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

1. The calculated method LOQ was based on scientifically acceptable procedures defined in 40 CFR Part 136 in the ECM (p. 7; Appendix 3, p. 26 of MRID 46801820; pp. 7, 14-16; Table 3, p. 20 of MRID 46801821; pp. 9, 17-18 of MRID 46801822).
2. The submitted final ECM was Method AI-003-W05-02 (pp. 1, 14; Appendix 7, p. 41 of MRID 46801820). The ILV was performed based on the original ECM Method AI-003-W05-01 (pp. 9, 13 of MRID 46801822). After the ILV validation, the ILV findings were communicated to the ECM, and an updated ECM (Method AI-003-W05-02) was prepared which included the ILV results (p. 16; Appendix 2, p. 52; Appendix 7, p. 79 of MRID 46801821; pp. 9-10, 19 of MRID 46801822). Since the inclusion of the ILV results was the only reported difference between Method AI-003-W05-01 and Method AI-003-W05-02 and this modification would not impact the results of the study, the ECM results from Method AI-003-W05-01 can be use as validation data for Method AI-003-W05-02.
3. The reproducibility of the method could not be determined for analyses at 0.50 ng/mL in water matrices since no water samples were prepared at 0.50 ng/mL (10 $\times$ LOQ) in the ECMs 1/2. Water samples were prepared at 0.250 ng/mL (5 $\times$ 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 $\times$  LOQ) for each analyte.
4. An insufficient number of samples (n = 3) were prepared for each of the three water matrices at the LOQ (0.05 ng/mL) in the ECMs 1/2. OCSPP guidelines state that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10 $\times$  LOQ) for each analyte. In the study report, means and standard deviations were calculated for combined water matrices (n = 9) at each fortification.

5. The characterization of the ECMs 1/2 water matrices was not reported. The matrix characterization was not provided but reportedly archived with the original studies; however, the characterization of the tap water was not included in this report (which was its original study; pp. 11, 17 of MRID 46801821).
6. In the ECMs 1/2, calibration curve and correlation coefficient were not provided for AE B197555; therefore, the acceptability of the linearity of the method could not be determined for AE B197555 for the ECMs 1/2. The ECMs 1/2 calibration curve and correlation coefficient for pyrasulfotole was associated with the tap water analysis; however, since solvent-based calibrants were used, the ECMs 1/2 calibration curve and correlation coefficient for pyrasulfotole in tap water was considered to apply to all tested water matrices.
7. 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 trial since no ILV modifications or deviations were noted (p. 16 of MRID 46801822).
8. The LC/MS/MS parameters were not reported in detail in the ILV.
9. In Method AI-003-W05-01 and Method AI-003-W05-02, prior to method performance, finished drinking water matrices (tap water) containing free chlorine were stabilized by adding sodium thiosulfate (Appendix 2, pp. 47, 49 of MRID 46801821). The effects of chlorine on analyte recoveries was studied to show the importance of the sodium thiosulfate step (Appendix 3, p. 27 of MRID 46801820; Appendix 2, p. 65 of MRID 46801821).

One of the ILV matrices was drinking (tap) water; however, the water characterization did not include chlorine content (p. 12; Tables 7-8, p. 27 of MRID 46801922). It was not reported if the ILV performed the sodium thiosulfate stabilization prior to method performance (pp. 13-14).

10. The reviewer determined that the determinations of the calculated MDL 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 46801820; pp. 7, 14-16; Table 3, p. 20 of MRID 46801821; pp. 9, 17-18 of MRID 46801822). No justification was provided for reported method LOQ 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 MDL was calculated as the sum of 3 times the standard deviation at the method LOQ. The calculations were based on the overall data (all three waters, n = 9). The calculated LOD was termed MDL in the study report. These LOQ and LOD calculations appeared to follow the method of Keith *et al.* 1983 (pp. 14-15; Table 3, p. 20 of MRID 46801821). The calculated LOQs supported the method LOQ for both analytes in water matrices.

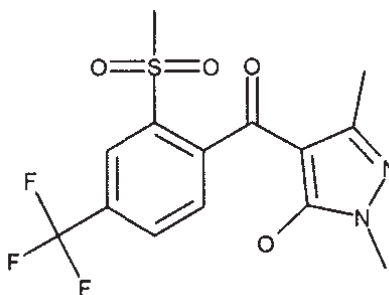
11. Solvent-based calibration standards were prepared in Method AI-003-W05-01 and Method AI-003-W05-02 (p. 15; Appendix 2, p. 49 of MRID 46801821). Matrix effects were not studied in the ECMs 1/2 or ILV.
12. The time requirement for the method was reported in the ILV as *ca.* one calendar day for each method trial of 12 samples with *ca.* 3 hours for preparation and *ca.* 20 hours for LC/MS/MS analysis (p. 18 of MRID 46801822). This time requirement was similar to that reported in the ECM 2 (p. 15 of MRID 46801821).

## V. References

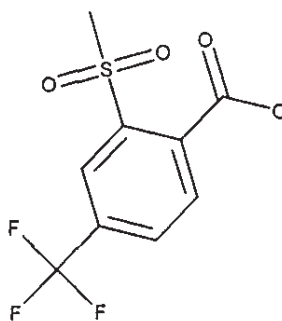
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition 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\_46801820+\_8  
50.6100\_Calculations.x