Two analytical methods for either spiromesifen (BSN2060) and its metbaolite BSN2060-enol in water or BSN2060 and its four metabolites BSN2060-enol, BSN2060-enol acid, BSN2060-cyclobutyl photoisomer, and BSN2060-enol photoisomer in water

Reports: ECM 1: EPA MRID No.: 45819432. Leimkuehler, W.M., and D.L. Woodard. 2002. Analytical Method for the Determination of BSN2060 and BSN2060-Enol in Water. Bayer Report No.: 200173. Report prepared by Bayer Corporation, Agriculture Division, Environmental Research Section, Stilwell, Kansas, and sponsored and submitted by Bayer Corporation, Agriculture Division, Research and Development Department, Kansas City, Missouri; 23 pages. Final report issued September 26, 2002.

ECM 2: EPA MRID No. 50261205 (Appendix 4, pp. 147-180). Blanchfield, S. 2017. An Analytical Method for the Determination of Residues of Spiromesifen (BSN 2060) and its Degradates: Spiromesifen-Enol, Spiromesifen-Enol Acid, Spiromesifen-Cyclobutyl Photoisomer, and Spiromesifen-Enol Photoisomer in Water Using LC/MS/MS. Analytical Method. Bayer Study ID: ESBSN016. Bayer Method No.: BS-003-W17-01. Report prepared, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 34 pages. Final report issued February 7, 2017.

ILV: EPA MRID No. 50261205. Rutt, D., and F. Li. 2017. Independent Laboratory Validation of Bayer Method BS-003-W17-01: An Analytical Method for the Determination of Residues of Spiromesifen (BSN 2060) and its Degradates: Spiromesifen-Enol, Spiromesifen-Enol Acid, Spiromesifen-Cyclobutyl Photoisomer, and Spiromesifen-Enol Photoisomer in Water Using LC/MS/MS. Final Report. CPS Laboratory Project ID: 106795, Revision 1. Bayer Study ID: ESBSN016. Report prepared by Critical Path Services, LLC (CPS), Garnet Valley, Pennsylvania, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 188 pages. Final report issued March 16, 2017; Revision issued May 1, 2017.

Document No.: MRIDs 45819432 & 50261205

Guideline: 850.6100

Statements: ECM 1 (EPA MRID No.: 45819432): The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, 40 CFR, Part 160 (p. 3 of MRID 45819432). Signed and dated No Data Confidentiality, GLP, and Certification of Authenticity statements were provided (pp. 2-3, 5). The Quality Assurance Statement was not required for analytical method (p. 4). A signed and dated Certification of Availability of Raw Data was included (p. 3).
ECM 2 (Appendix 4 in EPA MRID No. 50261205): The study was not conducted in accordance with GLP standards, since it was not a study (Appendix 4, p. 149 of MRID 50261205). Signed and dated No Data Confidentiality and GLP statements were provided (Appendix 4, pp. 148-149). Authenticity and Quality Assurance statements were not included.

	ILV (EPA MRID No. 50261205): The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (p. 3 of MRID 50261205). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Certification of Authenticity statements were provided (pp. 2-5). The reason for the revision was reported (p. 6).
Classification:	This analytical method, Bayer Report No. 200173, is classified as unacceptable. This analytical method, Bayer Method BS-003-W17-01, is classified as unacceptable. No complete ECM/ILV set was provided for either of the two different Bayer/Bayer CropScience methods reviewed in this DER: Bayer Report No. 200173 and Bayer Method BS-003-W17-01. For both reports, the reported method limit of quantitation (LOQ) was not based on procedures defined in 40 CFR Part 136, and so the reported LOQ is the lowest level of method validation (LLMV) rather than a true LOQ. The following deficiencies were noted for Bayer Report No. 200173: 1) no ILV was submitted; 2) no ECM performance data was provided at 10×LLMV; 3) number of samples was inadequate at all fortification levels; 4) the specificity of the method was not supported by ECM representative chromatograms; 5) the ECM water matrix was not characterized; and 6) the LOD was not specified and justified in the ECM. The following deficiencies were noted for Bayer Method BS-003-W17-01: 1) ECM was a method only with incomplete performance data at the LLMV, with no data at 10xLLMV; 2) ILV linearity was not satisfactory for BSN2060-cyclobutyl photoisomer; 3) ECM linearity was not satisfactory for BSN2060-enol photoisomer and BSN2060-cyclobutyl photoisomer; 4) ILV water matrix was not characterized; 5) the LOD was not reported in the ECM, 6) the LLMV is not adequate to address risk concern; and 7) the ECM was not conducted under USEPA FIFRA GLP standards.
PC Code:	024875

CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Date:	 11/15/2018
	Mary Samuel, M.S., Environmental Scientist	Signature: Date:	 11/16/2018
EPA Reviewer:	Sarah Brazeau, Ph.D. Physical Scientist	Signature: Date:	Sarah Brazeau Digitally signed by Sarah Brazeau Date: 2021.07.06 16:04:45 -04'00' 07/06/2021
EPA Secondary Reviewer:	Ideliz Negrón-Encarnación, Ph.D. Risk Assessment Process Leader	Signature: Date:	Ideliz Negrón-Encarnación Digitally signed by Ideliz Negrón-Encarnación Date: 2021.07.07 18:39:52 -04'00' 07/07/2021

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

Executive Summary

For both analytical methods, the reported method limit of quantitation (LOQ) was not based on procedures defined in 40 CFR Part 136, and so the reported LOQ is the lowest level of method validation (LLMV) rather than a true LOQ. The analytical method, **Bayer Report No. 200173**, is designed for the quantitative determination of spiromesifen (BSN2060) and its metabolite BSN2060-enol in water at the LLMV of 0.025 µg/L using LC/MS. The LLMV of Bayer Report No. 200173 is lower than the lowest toxicological level of concern in water for BSN2060 and BSN2060-enol. The analytical method, **Bayer Method BS-003-W17-01**, is designed for the quantitative determination of spiromesifen (BSN2060) and its four metabolites BSN2060-enol, BSN2060- enol acid, BSN2060-enol acid, and BSN2060-enol photoisomer in water at the LLMV of 0.5 µg/L using LC/MS. The LLMV of Bayer Method BS-003-W17-01is greater than the lowest toxicological level of concern in water for BSN2060 and its four metabolites BSN2060-enol, BSN2060- enol acid, BSN2060-enol acid, and BSN2060-enol photoisomer. The lowest toxicological level of concern in water is for chronic daphnia life cycle, at 0.25 µg/L (USEPA 2020). As a result, Bayer Method BS-003-W17-01 is not adequate to address risk concern. Bayer Report No. 200173 and Bayer Method BS-003-W17-01 could not be considered modifications of each other since they differed significantly in the sample processing, and LLMVs. Also, Bayer Method BS-003-W17-01 included three more spiromesifen metabolites as analytes. **No complete ECM/ILV set was provided for either of the two different Bayer/Bayer CropScience methods.**

For Bayer Report No. 200173 (ECM 1), no ILV was submitted. ECM performance data for Bayer Report No. 200173 was not satisfactory since there were insufficient samples performed at the LLMV, and no 10xLLMV data. The specificity of the method was not supported by ECM representative chromatograms, and the LOD was not specified and justified, described as < 0.05 µg/L without further evidence.

For Bayer Method BS-003-W17-01 (ECM 2), the submitted ECM was a method only with incomplete performance data. For Bayer Method BS-003-W17-01, the ILV validated the ECM method in the first trial with insignificant modifications to the analytical instruments and parameters. All ILV data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory, except for ILV linearity for BSN2060-cyclobutyl photoisomer. ECM 2 provided linearity data, but linearity was not satisfactory for BSN2060-enol photoisomer and BSN2060-cyclobutyl photoisomer. Most significantly, the lowest toxicological level of concern is identical to the LOD. The LOD was also not reported in the ECM. Additionally, Interferences were reported to account for up to 23% of the peak area at the LLMV level (46% of the LOD; 2 times the LOD of 0.25 µg/L). There could be a possible water contamination issue that accounts for this, and so reevaluation is recommended.

No water matrix used in the study reports for Bayer Report No. 200173 and Bayer Method BS-003-W17-01 were characterized.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Method Validation (LLMV)
	Environmental Chemistry Method	Independent Laboratory Validation						
Spiromesifen (BSN2060)	45819432[2]	None Submitted	Sarah Brazeau	Water	26/09/2002 (ECM 1)	Bayer Corporation	LC/MS/MS	0.025 µg/L
BSN2060-enol								
Spiromesifen (BSN2060)	50261205 (Appendix 4)[3]	50261205[4]			07/02/2017[3] (ECM 2) 16/03/2017 (ILV)			0.5 µg/L
BSN2060-enol								
BSN2060-enol acid[1]								
BSN2060-enol photoisomer								
BSN2060-cyclobutyl photoisomer								

1 Also referred to as BSN2060-4-carboxy by Sponsor in accompanying studies.

2 In the ECM 1, water collected from a Bayer Ecotoxicological Study (Bayer Report No. 200062) used in the study (pp. 14, 16 of MRID 45819432). The water characterization data was not provided in this study report, and the water source was not reported.

3 Bayer Method BS-003-W17-01 was provided in Appendix 4 of ILV MRID 50261205 (Appendix 4, pp. 147-180 of MRID 50261205). However, the ECM was a method only; insufficient performance data was provided.

4 In the ILV, water (CPS ID GS-17-27-1) was provided by the Sponsor (Bayer CropScience) and used in the study (p. 20 of MRID 50261205). The water was not characterized, and the water source was not reported.

I. Principle of the Method

ECM 1: Bayer Report No. 200173

Water samples (25 mL) were fortified and mixed with 250 µL of the 0.5 µg/mL internal standard (IS) solution in a test tube (pp. 10-12 of MRID 45819432). The solution was applied to a Mega Bond Elut C18 solid phase extraction (SPE) cartridge [preconditioned with one column volume of acetonitrile containing 0.1% formic acid (*ca*. 20 mL) and two column volumes of water (*ca*. 40 mL) at 5 mL/min.]. The sample was first passed through the cartridge at 2 mL/min. The sample was then washed with one column volume of water (*ca*. 20 mL) at 5 mL/min. After allowing the cartridge to dry for *ca*. 15 seconds, the analytes were eluted with acetonitrile containing 0.1% formic acid (*ca*. 40 mL). The volume of the eluate was reduced to *ca*. 0.5 mL using a Turbovap® set at 40°C. The volume of the residue was adjusted to 1.0 mL with acetonitrile containing 0.1% formic acid. An aliquot was transferred to an autosampler vial for analysis.

Samples were analyzed for analytes using a ThermoFinnigan P-4000 HPLC system coupled to a ThermoFinnigan TSQ 7000 triple quadrupole mass spectrometer (p. 13 of MRID 45819432). The LC/MS conditions consisted of an Eclipse (Zorbax) XDB-C8 column (150 x 4.6 mm, 3.5 µ particle size; column temperature not reported) with a mobile phase gradient of A) 0.1% formic acid in water and B) methanol [percent A:B (v:v) at 0.00 min. 80:20, 6.00 min. 5:95] and Atmospheric Pressure (API) MS detection in electrospray (ESI) mode (ionization temperature not reported). Both analytes were detected in positive ESI mode. Injection volume was 50 µL. One ion transition was monitored for each analyte as follows: *m/z* 370.8→272.8 for spiromesifen (BSN2060) and *m/z* 272.8→186.8 for BSN2060-enol. Expected retention times were 9.5 and 8.4 minutes for spiromesifen, and BSN2060-enol, respectively. BSN2060-enol acid, BSN2060-enol photoisomer, and BSN2060-cyclobutyl photoisomer were not included in the ECM MRID 45819432, Bayer Report No. 200173.

No ILV was submitted for Bayer Report No. 200173.

The LLMV for spiromesifen and BSN2060-enol in water using Bayer Report No. 200173 was 0.025 µg/L in ECM 1 (p. 14 of MRID 45819432). The Limit of Detection (LOD) was listed as < 0.05 µg/L, but more specific information and calculations were not provided.

ECM 2: Bayer Method BS-003-W17-01

ECM 2 was provided in Appendix 4 of ILV MRID 50261205 (Appendix 4, pp. 147-180 of MRID 50261205). However, it was a method only without performance data.

Water samples (50 ± 0.05 mL) were fortified and mixed with 250 µL of the 0.1 µg/mL internal standard (IS) solution in a centrifuge tube (Appendix 4, p. 157 of MRID 50261205). An aliquot was transferred to an autosampler vial, was mixed, and was then ready for analysis.

Samples were analyzed for analytes using a Shimadzu HPLC system coupled to an ABSciex 6500 mass spectrometer (Appendix 4, pp. 152, 157-160 of MRID 50261205). The LC/MS conditions consisted of a Phenomenex® Kinetex® C18 column (50 x 2.1 mm, 1.7 µm particle size; column temperature 40°C) with a mobile phase gradient of A) 0.1% formic acid in water and B) acetonitrile [percent A:B (v:v) at 0.10 min. 90:10, 1.25-2.50 min. 1:99, 2.51 min. 90:10] and MS detection in electrospray (ESI) mode (ionization temperature 300°C). All analytes were detected in positive ESI mode, except for BSN2060-enol acid. Injection volume was 20 µL (adjusted as needed). Two ion transitions were monitored for each analyte as follows (primary and confirmatory, respectively): *m/z* 371.3→273.0 and *m/z* 371.3→255.0 for spiromesifen, *m/z* 273.2→254.9 and *m/z* 273.2→227.0 for BSN2060-enol, *m/z* 301.0→257.0 and *m/z* 301.0→239.0 for BSN2060-enol acid, *m/z* 255.1→209.0 and *m/z* 255.1→179.0 for BSN2060-enol photoisomer, and *m/z* 371.2→209.0 and *m/z* 371.2→169.0 for BSN2060-cyclobutyl photoisomer. Expected retention times were 2.0, 1.5, 1.3, 1.5, and 1.9 minutes for spiromesifen, BSN2060-enol, BSN2060-enol acid, BSN2060-enol photoisomer, and BSN2060-cyclobutyl photoisomer, respectively.

The ILV performed ECM 2 as written with insignificant modifications of the analytical instruments and parameters (pp. 21-22, 24; Tables 3-4, pp. 30-32 of MRID 50261205). Analytes were analyzed for analytes using a Shimadzu Nexera X2 Modular UHPLC system, with a PAL® autosampler, coupled to a Sciex® Triple Quad™ 6500 mass spectrometer with an ESI interface. LC/MS/MS conditions were the same as those of the ECM, except that injection volume was 25 µL.

The LLMV for spiromesifen and its four metabolites in water using Bayer Method BS-003-W17-01 was 0.5 µg/L in the ECM and ILV (p. 26; Tables 1-2, pp. 28-29; Appendix 4, p. 152 of MRID 50261205). The LOD was reported as 0.25 µg/L for all five analytes in the ILV; the LOD was not reported in the ECM.

II. Recovery Findings

ECM 1 (MRID 45819432): Using **Bayer Report No. 200173 (0.025 µg/L LLMV)**, individual recoveries were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of spiromesifen and its metabolite BSN2060-enol in one water matrix at fortification levels of 0.025 µg/L (LLMV), 0.05 µg/L (2×LLMV), 0.1 µg/L (4×LLMV), 1.0 µg/L (40×LLMV), 10 µg/L (400×LLMV), and 25 µg/L (1000×LLMV; Table 1, p. 17). No samples were prepared at 10×LLMV. Means, standard deviations, and RSDs were not be calculated since n = 1 or 2 for all analyses. OCSPP 850.6100 guidelines recommend at least 5 replicates at both the LLMV and 10xLLMV. Analytes were identified using one ion transition; a confirmatory method is not usually required when LC/MS or GC/MS is the primary method used to generate study data. Water collected from a Bayer Ecotoxicological Study (Bayer Report No. 200062) used in the study (pp. 14, 16). The water characterization data was not provided in this study report, and the water source was not reported.

ECM 2 (Appendix 4 of MRID 50261205): Insufficient performance data was provided for **Bayer Method BS-003-W17-01 (LLMV 0.5 µg/L)**. OCSPP 850.6100 guidelines recommend at least 5 replicates at both the LLMV and 10xLLMV, but only two replicates were performed at the LLMV. As a result of this data deficiency, mean recovery and standard deviation were not calculated.

ILV (MRID 50261205): Using **Bayer Method BS-003-W17-01 (LLMV 0.5 µg/L)**, mean recoveries and RSDs were within guideline requirements for analysis of spiromesifen and its metabolites BSN2060-enol, BSN2060-enol acid, BSN2060-enol photoisomer, and BSN2060-cyclobutyl photoisomer in one water matrix at fortification levels of 0.5 µg/L (LLMV) and 5.0 µg/L (10×LLMV; Tables 1-2, pp. 28-29). Analytes were identified using two ion transitions; performance results from primary and confirmatory analyses were comparable. The water (CPS ID GS-17-27-1) was provided by the Sponsor (Bayer CropScience) and used in the study (p. 20). The water was not characterized, and the water source was not reported. The ILV validated ECM 2 method in the first trial with insignificant modifications to the analytical instruments and parameters (pp. 17, 21-22, 24-26).

Table 2a. Initial Validation Method Recoveries for Spiromesifen (BSN2060) and BSN2060-Enol in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Method - Bayer Report No. 200173					
	Water					
Spiromesifen (BSN2060)	0.025 (LLMV)	**2**[4]	111,120	--[3]	--	--
	0.05	**2**	90, 104	--	--	--
	0.1	**2**	110, 116	--	--	--
	1.0	**2**	96, 97	--	--	--
	10	**1**	92	--	--	--
	25	**1**	94	--	--	--
Spiromesifen-enol (BSN2060-enol)	0.025 (LLMV)	**2**	100, 104	--	--	--
	0.05	**2**	106, 108	--	--	--
	0.1	**2**	87, 90	--	--	--
	1.0	**2**	96.4, 97.2	--	--	--
	10	**1**	91.2	--	--	--
	25	**1**	94.3	--	--	--

Data (uncorrected recovery results, pp. 13-14) were obtained from Table 1, p. 17 of MRID 45819432.

1 The water collected from a Bayer Ecotoxicological Study (Bayer Report No. 200062) used in the study (pp. 14, 16). The water characterization data was not provided in this study report, and the water source was not reported.

2 One ion transition was monitored for each analyte as follows: *m/z* 370.8→272.8 for spiromesifen and *m/z* 272.8→186.8 for spiromesifen-enol.

3 Means, standard deviations, and RSDs were not be calculated since n = 1 or 2. OCSPP 850.6100 guidelines recommend at least 5 replicates at both the LLMV and 10xLLMV.

4 Bolded values denote performance data deficiencies.

Table 2b. Initial Validation Method Recoveries for Spiromesifen (BSN2060) and Its Four Metabolites in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Method - Bayer Method BS-003-W17-01					
	Water					
Spiromesifen (BSN2060)	0.5 (LLMV)	**Insufficient performance data submitted.**[1]				
	5.0					
BSN2060-enol	0.5 (LLMV)					
	5.0					
BSN2060-enol acid	0.5 (LLMV)					
	5.0					
BSN2060-enol photoisomer	0.5 (LLMV)					
	5.0					
BSN2060-cyclobutyl photoisomer	0.5 (LLMV)					
	5.0					

Data (uncorrected recovery results, Appendix 4, pp. 160-161) were obtained from ECM 2, Appendix 4 of MRID 50261205. OCSPP 850.6100 guidelines recommend at least 5 replicates at both the LLMV and 10xLLMV

1 Bolded values denote performance data deficiencies.

Table 3. Independent Validation Method Recoveries for Spiromesifen (BSN2060) and Its Four Metabolites in Water[1,2]

Analyte	Fortification Level (µg/L)[3]	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Method - Bayer Method BS-003-W17-01					
	Water					
	Primary Ion Transition					
Spiromesifen (BSN2060)	0.5 (LLMV)	5	93.5-111	101	6.6	6.5
	5.0	5	101-139	116	16	14
BSN2060-enol	0.5 (LLMV)	5	101-105	103	1.8	1.7
	5.0	5	105-108	106	1.2	1.1
BSN2060-enol acid	0.5 (LLMV)	5	90.1-101	94.0	4.4	4.7
	5.0	5	98.8-102	101	1.5	1.5
BSN2060-enol photoisomer	0.5 (LLMV)	5	85.9-91.1	89.3	2.0	2.2
	5.0	5	86.5-90.9	88.6	2.0	2.3
BSN2060-cyclobutyl photoisomer	0.5 (LLMV)	5	73.1-100	83.8	11	13
	5.0	5	108-127	118	7.2	6.1
	Confirmatory Ion Transition					
Spiromesifen (BSN2060)	0.5 (LLMV)	5	101-116	107	5.4	5.0
	5.0	5	94.1-125	104	13	13
BSN2060-enol	0.5 (LLMV)	5	101-106	104	2.4	2.3
	5.0	5	105-109	107	1.5	1.4
BSN2060-enol acid	0.5 (LLMV)	5	80.4-102	92.4	8.0	8.7
	5.0	5	98.4-103	101	2.3	2.3
BSN2060-enol photoisomer	0.5 (LLMV)	5	84.2-89.3	86.5	2.2	2.5
	5.0	5	84.2-87.9	85.6	1.4	1.6
BSN2060-cyclobutyl photoisomer	0.5 (LLMV)	5	70.7-90.5	79.0	7.9	10
	5.0	5	111-125	118	5.7	4.8

Data (uncorrected recovery results) were obtained from Tables 1-2, pp. 28-29 of MRID 50261205.

1 The water (CPS ID GS-17-27-1) was provided by the Sponsor (Bayer CropScience) and used in the study (p. 20). The water was not characterized, and the water source was not reported.

2 Two ion transitions were monitored for each analyte as follows (primary and confirmatory, respectively): *m/z* 371.3→273.0 and *m/z* 371.3→255.0 for spiromesifen, *m/z* 273.2→254.9 and *m/z* 273.2→227.0 for BSN2060-enol, *m/z* 301.0→257.0 and *m/z* 301.0→239.0 for BSN2060-enol acid, *m/z* 255.1→209.0 and *m/z* 255.1→179.0 for BSN2060-enol photoisomer, and *m/z* 371.2→209.0 and *m/z* 371.2→169.0 for BSN2060-cyclobutyl photoisomer.

3 The fortification rates 0.5 and 5.0 µg/L were nominal fortifications; actual fortifications were 0.495 and 4.95 µg/L.

Note that bolded values denote performance data deficiencies (no performance data deficiencies in ILV).

III. Method Characteristics

ECM 1: Bayer Report No. 200173

The LLMV for spiromesifen and BSN2060-enol in water using Bayer Report No. 200173 was 0.025 µg/L in the ECM (p. 14 of MRID 45819432). Because the LOQ was defined as the level at which the method was successfully tested, this value is an LLMV and not a true LOQ. No calculations or comparisons to background levels were reported to justify the LLMV for the method. The LOD was described as < 0.05 µg/L, but no evidence or calculations were provided.

Linearity was satisfactory for spiromesifen and BSN2060-enol in this method (r^2= 0.9975, 0.9998, respectively). Repeatability and specificity were also satisfactory. Reproducibility was not satisfactory, as no ILV was submitted for Bayer Report No. 200173. Even though there was a deficiency in performance data, when n=2, accuracy and precision are satisfactory (cannot be determined when n=1).

ECM 2: Bayer Method BS-003-W17-01

The LLMV for spiromesifen and its four metabolites in water using Bayer Method BS-003-W17-01 was 0.5 µg/L in the ECM and ILV (p. 26; Tables 1-2, pp. 28-29; Appendix 4, p. 152 of MRID 50261205). As with ECM 1, because the evaluated LOQ was defined as the level at which the method was successfully tested, this value is an LLMV and not a true LOQ. The ECM noted that the LLMV could be adjusted as required. The LOD was reported as 0.25 µg/L for all five analytes in the ILV; the LOD was not reported in the ECM. No calculations or comparisons to background levels were reported to justify the LLMV or LOD for the method in the ECM or ILV.

Repeatability was satisfactory. Reproducibility was satisfactory, as the ILV was able to confirm the LLMV despite insufficient sample numbers in the ECM. However, reproducibility could not be confirmed at 10xLLMV. Linearity was satisfactory, except for BSN2060-cyclobutyl photoisomer in the ECM, and BSN2060-enol photoisomer and BSN2060-cyclobutyl photoisomer in the ILV, in which $r^2 < 0.995$.

Table 4. Method Characteristics

Method		**Bayer Report No. 200173**		**Bayer Method BS-003-W17-01**				
Analyte		**Spiromesifen (BSN2060)**	**BSN2060-enol**	**Spiromesifen (BSN2060)**	**BSN2060-enol**	**BSN2060-enol acid**	**BSN2060-enol photoisomer**	**BSN2060-cyclobutyl photoisomer**
Limit of Method Validation (LLMV)	ECM 1	0.025 µg/L		**Not applicable**[9]				
	ECM 2	**Not applicable**		0.5 µg/L				
	ILV							
Limit of Detection (LOD)	ECM 1	< 0.05 µg/L		**Not applicable**				
	ECM 2	**Not applicable**		**Not reported**				
	ILV			0.25 µg/L				
Linearity (calibration curve r^2 and concentration range)	ECM 1	r^2 = 0.9975	r^2 = 0.9998	**Not applicable**				
	ECM 2[1]	**Not applicable**		r^2 = 0.9990	r^2 = 0.9980	r^2 = 0.9950	r^2 = **0.9928**	r^2 = **0.9825**
	ILV			r^2 = 0.9954 (Q) r^2 = **0.9940** (C)	r^2 = 0.9996 (Q) r^2 = 0.9992 (C)	r^2 = 0.9998 (Q) r^2 = 0.9996 (C)	r^2 = 0.9986 (Q) r^2 = 0.9996 (C)	r^2 = **0.9932** (Q) r^2 = **0.9900** (C)
	Concentration Range	0.025-25 µg/mL		0.250-20 µg/mL				
Repeatable	ECM 1[2]	Yes at LLMV, 2×LLMV, 4×LLMV, 40×LLMV, 400×LLMV, and 1000×LLMV, but **n = 1 or 2**. (one uncharacterized water) **No samples prepared at 10×LLMV**		**Not applicable**				
	ECM 2[3]	**Not applicable**		**Insufficient performance data submitted.**				
	ILV[4,5]			Yes at LLMV and 10×LLMV (one uncharacterized water)				
Reproducible		**Could not be determined;** only one set of performance data submitted at LLMV and no performance data submitted at 10×LLMV, No accompanying ILV.		**Could not be determined;** Insufficient performance data submitted at LLMV and 10×LLMV				
Specific	ECM 1	**Could not be determined** at LOD (not reported); matrix interferences were *ca*. 20-21% of		**Not applicable**				

		the LLMV.[6] **No LLMV chromatograms were provided**.					
	ECM 2	**Not applicable**	**Insufficient performance data submitted.**				
	ILV		Yes, matrix interferences were *ca*. 2.3% of the LLMV (based on peak area; 4.6% of LOD). Peak fronting which caused by a contaminant was observed.[7]	Yes, matrix interferences were *ca*. 4.3% of the LLMV (based on peak area; 8.6% of LOD).	Yes, matrix interferences were *ca*. 18-23% of the LLMV (based on peak area; 36-46% of LOD).[8]	Yes, matrix interferences were *ca*. 11% of the LLMV (based on peak area; 22% of LOD). Baseline noise around the LLMV was observed.	Yes, no matrix interferences were observed.

Data were obtained from p. 14 (ECM 1 LLMV/LOD); Table 1, p. 17 (ECM 1 recovery data); p.14 (ECM 1 calibration coefficients); Figures 3-6, pp. 20-23 (ECM 1 chromatograms and calibration curves) of MRID 45819432; p. 26; Appendix 4, p. 152 (ECM 2 & ILV LLMV/LOD); Tables 1-2, pp. 28-29 (ILV recovery data); Figures 1-90, pp. 34-123 (ILV chromatograms and calibration curves); Appendix 1, pp. 125-134 (ILV calibration coefficients); Appendix 4, Appendix 2, pp. 166-170 (ECM 2 correlation coefficients) of MRID 50261205; DER Attachment 2. ECM 1 = MRID 45819432; ECM 2 = Appendix 4 of MRID 50261205; ILV = MRID 50261205. Q = Primary ion transition; C = Confirmatory ion transition.

1 ECM 2 correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Appendix 4, Appendix 2, pp. 166-170 of MRID 50261205; DER Attachment 2). Only one set of linear regressions were provided; the reviewer assumed that the data referred to the primary ion transition.

2 In the ECM 1, water collected from a Bayer Ecotoxicological Study (Bayer Report No. 200062) used in the study (pp. 14, 16 of MRID 45819432). The water characterization data was not provided in this study report, and the water source was not reported.

3 ECM 2 was a method only; no performance data was submitted.

4 In the ILV, water (CPS ID GS-17-27-1) was provided by the Sponsor (Bayer CropScience) and used in the study (p. 20 of MRID 50261205). The water was not characterized, and the water source was not reported.

5 The ILV validated the ECM method in the first trial with insignificant modifications to the analytical instruments and parameters (pp. 17, 21-22, 24-26 of MRID 50261205).

6 Based on data reported on p. 14 of MRID 45819432.

7 Based on Figures 8-9, pp. 41-42 (Q) and Figures 17-18, pp. 50-51 (C) of MRID 50261205.

8 Matrix interferences were <50% of the LOD since the LOD = 0.25 µg/L or 50% of the LLMV.

9 Bolded words/values in table denote data deficiencies or experiments not performed/applicable.

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

IV. Method Deficiencies and Reviewer's Comments

1. This DER summarized two Bayer/Bayer CropScience methods for BSN2060 and its metabolites. Each of the two MRIDs which were submitted contained a distinct method. Typically, a DER should only contain one method which is supported by an ECM and its accompanying ILV, as either one or two MRIDs. The spiromesifen water ECM MRID and ILV MRID were not a match and that the ILV cited a different ECM (contained in the appendix of the ILV), but the reviewer was instructed to review the provided ECM MRID and ILV MRID as one method validation set.

2. For both Bayer Report No. 200173 and Bayer Method BS-003-W17-01, the reported method limit of quantitation (LOQ) was not based on procedures defined in 40 CFR Part 136, and so the reported LOQ is the lowest level of method validation (LLMV) rather than a true LOQ.

3. The two Bayer/Bayer CropScience methods for determination of spiromesifen residues in water, Bayer Report No. 200173 and Bayer Method BS-003-W17-01, differed significantly and could not be considered modifications of each other. Bayer Method BS-003-W17-01 was a direct injection method while Bayer Report No. 200173 involved SPE clean-up prior to analysis. The LLMVs for each method were very different: 0.025 µg/L versus 0.5 µg/L. Finally, Bayer Report No. 200173 was only internally validated for spiromesifen and one of its metabolites (BSN2060-enol) while Bayer Method BS-003-W17-01 was developed for and independently validated for spiromesifen and four of its metabolites (BSN2060-enol, BSN2060-enol acid, BSN2060-cyclobutyl photoisomer, and BSN2060-enol photoisomer). Lastly, Bayer Method BS-003-W17-01 references a different ECM as the source of its method, included in the ILV's Appendix 4.

4. No complete ECM/ILV set was provided for either of the two Bayer/Bayer CropScience methods reviewed in this DER.

   Only one set of performance data was submitted for the analytical method Bayer Report No. 200173 which was contained in MRID 45819432. Only an ECM was submitted.

   Insufficient performance data was submitted for the analytical method Bayer Method BS-003-W17-01 which was contained in MRID 50261205. MRID 50261205 contained the ECM and ILV for Bayer Method BS-003-W17-01, but the ECM (Appendix 4) was a method containing insufficient performance data (n=2) and lacked sufficient supporting chromatograms.

5. The following deficiencies were noted for ECM 1 MRID 45819432 for Bayer Report No. 200173:

   > The reproducibility of the method at 10×LLMV could not be determined since no samples were prepared at 0.25 µg/L (Table 1, p. 17). OCSPP Guideline 850.6100 recommends that minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LLMV and 10×LLMV) for each analyte.

The number of samples was inadequate at all fortification levels, n = 1 or 2 (Table 1, p. 17). OCSPP Guideline 850.6100 recommends that minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LLMV and 10×LLMV) for each analyte.

No LLMV chromatograms were provided (Figures 3-6, pp. 20-23)..

The water characterization data was not provided in this study report, and the water source was not reported (pp. 14, 16). The water was collected from a Bayer Ecotoxicological Study (Bayer Report No. 200062).

The LOD was reported as < 0.05 µg/L, but further justification and calculations were not provided.

6. The following deficiencies were noted for ILV MRID 50261205 for Bayer Method BS-003-W17-01:

The linearity was not satisfactory for BSN2060-cyclobutyl photoisomer (r^2 = 0.9932, Q; r^2 = 0.9900, C; Appendix 1, pp. 125-134). The reviewer noted that the linearity of the confirmatory ion transition for BSN2060 was not satisfactory (r^2 = 0.9940); however, this deviation did not affect the validity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is the primary method used to generate study data. Linearity is satisfactory when $r^2 \geq$ 0.995.

The water was not characterized, and the water source was not reported (p. 20). The water (CPS ID GS-17-27-1) was provided by the Sponsor (Bayer CropScience). It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.

7. The following deficiencies were noted for ECM 2 (Appendix 4) MRID 50261205 for Bayer Method BS-003-W17-01:

Insufficient performance data or representative chromatograms were submitted.

The linearity was not satisfactory for BSN2060-enol photoisomer (r^2 = 0.9928) and BSN2060-cyclobutyl photoisomer (r^2 = 0.9825; Appendix 4, Appendix 2, pp. 166-170; DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$. Only one set of linear regressions were provided; the reviewer assumed that the data referred to the primary ion transition.

The LOD was not reported.

The LLMV was below the lowest toxicological endpoint, making the LLMV invalid for risk assessment.

8. For Bayer Method BS-003-W17-01, communications between the ILV Study Director (Fenn Li, ILV study author) and Bayer CropScience Study Monitor (Chung Lam) were partially reported (pp. 3, 6, 25 of MRID 50261205). The communications between the Study Monitor and ILV involved only the clarification of the study protocol. No technical guidance was provided by the Study Monitor to the ILV during the course of the study.

9. The reviewer determined that the specificity of Bayer Method BS-003-W17-01 for all analytes was acceptably supported by ILV chromatograms, even though matrix interferences were *ca*. 18-23% of the LLMV (based on peak area) for BSN2060-enol acid, since the matrix interferences were <50% of the LOD since the LOD = 0.25 µg/L or 50% of the LLMV (Figures 1-90, pp. 34-123 of MRID 50261205). Although OCSPP 850.6100 indicates that interferences with peak areas that are less than 50 % of the LOD are not considered significant, the LOD is equivalent to the lowest level of toxicological concern (0.25 µg/L).

10. The estimations of LLMV in ECM 1, ECM 2, and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 14 of MRID 45819432; p. 26; Tables 1-2, pp. 28-29; Appendix 4, p. 152 of MRID 50261205). The estimation of LOD in ILV was not based on scientifically acceptable procedures as defined in 40 CFR Part 136. No calculations or comparisons to background levels were reported to justify the LLMV and LOD for the method. Detection limits should not be based on arbitrary values.

11. For Bayer Method BS-003-W17-01, it was reported for the ILV that one sample set of 13 samples required 1 day with *ca*. 1 hour for sample preparation and *ca*. 2 hours for LC/MS/MS analysis (p. 25 of MRID 50261205).

    No timeframe was reported for Bayer Report No. 200173 in ECM 1 MRID 45819432.

V. References

USEPA. 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.

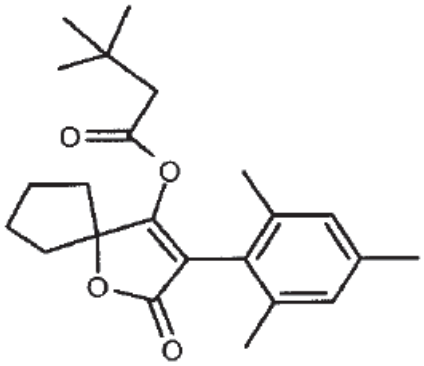
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

USEPA. 2020. Draft Ecological Risk Assessment for Registration Review. DP Barcode 447722. January 31, 2020. Environmental Fate and Effects Division. Office of Pesticide Programs. U.S. Environmental Protection Agency.

Attachment 1: Chemical Names and Structures

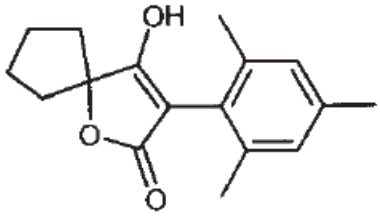
Spiromesifen (BSN2060; K-856; AE 0952850; K-1725)

IUPAC Name: 3-(2,4,6-Trimethylphenyl)-4-(3,3-dimethylbutyl-carbonyloxy)-5(spirocyclopentyl-3-dihydrofuranon-2
CAS Name: 3,3-Dimethyl-2-oxo-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-4-yl ester butanoic acid
CAS Number: 283594-90-1
SMILES String: Not found



BSN2060-enol (K-860; Enol; K-1961; Spiromesifen enol)

IUPAC Name: 4-Hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one
CAS Name: Not reported
CAS Number: 148476-30-6
SMILES String: Not found



BSN2060-4-carboxy (Spiromesifen phenol acid; BSN2060-enol acid; K-912; 4-carboxy)

IUPAC Name:	4-(4-Hydroxy-2-oxo-1-oxaspiro[4.4]non-3-en-3-yl)3-5-spirononyl benzoic acid 4-(4-Hydroxy-2-oxo-1-oxaspiro[4.4]non-3-en-3-yl)-3,5-dimethyl-spirononyl benzoic acid
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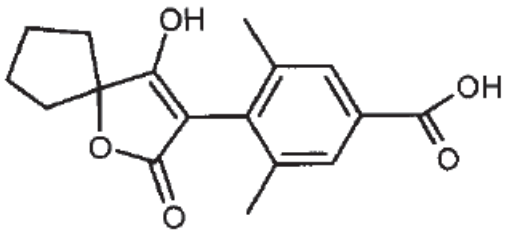

BSN2060-enol photoisomer (K-966; Spiromesifen enol photoisomer)

IUPAC Name:	8',8'a-Dihydro-8'-hydroxy-4',6'-dimethylspiro[cyclopentane-1,1'-[1H]indeno[1,2-c]furan-3'(3'aH)-one
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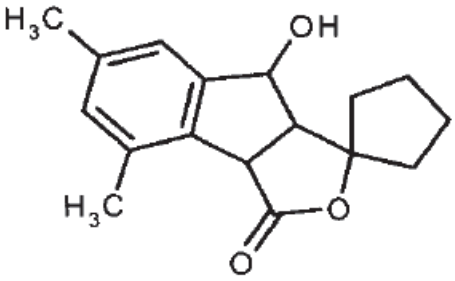
CAS Number:	Not reported
SMILES String:	Not found



BSN2060-cyclobutyl photoisomer (K-957; Spiromesifen cyclobutyl photoisomer)

IUPAC Name:	3,5-Dimethyl-5'-oxospiro[bicyclo[4.2.0]octa-1,3,5-triene-7,4'(5',H)-furan-2'(3',H),1"-cyclopentan]-3'-yl 3,3-dimethylbutanoate
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	Not found