Analytical method for spiromesifen (BSN2060) and its four metabolites BSN2060-enol, BSN2060-4-carboxy, BSN2060-enol photoisomer, and BSN2060-cyclobutyl photoisomer in soil

Reports: ECM: EPA MRID No.: 45819415. Leimkuehler, W.M., and R. J. Ripperger. 2002. Analytical Method for the Determination of BSN2060 and Metabolite Residues in Soil. Bayer Report No.: 110478. 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; 37 pages. Final report issued September 26, 2002.

ILV: EPA MRID No. 45819430. Bauer, M.R. 2002. Independent Laboratory Validation of "Determination of BSN2060 and Four Metabolites in Soil by LC-MS/MS". Battelle Study No.: AG010018. Bayer Study No.: BS112101 and Report No.: 200168. Report prepared by Battelle, Columbus, Ohio, sponsored and submitted by Bayer Corporation, Agriculture Division, Stilwell, Kansas; 97 pages. Final report issued July 8, 2002.

Document No.: MRIDs 45819415 & 45819430

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, 40 CFR, Part 160 (p. 3 of MRID 45819415). 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).
ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (p. 3 of MRID 45819430). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Certification of Authenticity statements were provided (pp. 2-5). A signed and dated Certification of Availability of Raw Data was included (p. 3).

Classification: This analytical method is classified as supplemental. Communications between the Sponsor and the ILV lab need to be clarified, specifically including what data and information was shared. Because the reported method limit of quantitation (LOQ) was not based on procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than a true LOQ. The LLMV was higher than the lowest toxicological level of concern. 10xLLMV data was not reported in the ECM. The specificity of the method could not be determined by the ECM representative chromatograms, and it is unclear whether these chromatograms represent the LOQ. ILV linearity was not satisfactory for BSN2060-cyclobutyl photoisomer. The ILV soil matrix was not characterized, and more clarity is needed on whether the soil is similar to that described in the corresponding Terrestrial Field Dissipation (TFD) studies. The LOD was not reported in the ILV.

PC Code: 024875

CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature:	Lisa Muto
		Date:	11/09/2018
	Mary Samuel, M.S., Environmental Scientist	Signature:	Marysamuel
		Date:	11/16/2018
EPA Reviewer:	Sarah Brazeau, Ph.D. Physical Scientist	Signature:	Sarah Brazeau Digitally signed by Sarah Brazeau Date: 2021.07.14 16:39:14 -04'00'
		Date:	07/14/2021
EPA Secondary Reviewer:	Ideliz Negrón-Encarnación, Ph.D. Risk Assessment Process Leader	Signature:	Ideliz Negrón-Encarnación Digitally signed by Ideliz Negrón-Encarnación Date: 2021.07.15 11:41:41 -04'00'
		Date:	07/14/2021

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

The analytical method, Bayer Report No.: 110478, is designed for the quantitative determination of spiromesifen (BSN2060) and its four metabolites BSN2060-enol, BSN2060-4-carboxy, BSN2060-enol photoisomer, and BSN2060-cyclobutyl photoisomer in soil at the limit of quantitation (LOQ) of 10 µg/kg-soil using LC/MS. As the LOQ was not based on procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than a true LOQ. The experimentally evaluated LLMV is higher than the lowest toxicological level of concern for plants and terrestrial invertebrates. In this case, the lowest toxicological level of concern is the No Observed Adverse Effect Concentration (NOAEC) at 0.012 lb a.i./A (5.9 µg a.i./kg-soil) for the monocot plant ryegrass (USEPA 2020, USEPA 2012b).
Two LLMVs are listed in the ECM, the level at which the method is evaluated, and the statistically determined LOQ of 3.3 times the LOD (not evaluated experimentally). The statistically determined LOQ (1.5-2 µg a.i./kg-soil) is less than the lowest toxicological level of concern suggesting that the method could be upgraded if validation is conducted at that level.

The ECM and ILV were performed using one soil each which were both not characterized or described in texture. Soil descriptions are included in Spiromesifen Terrestrial Field Dissipation (TFD), but clarification is needed on whether the soils are identical to those evaluated in the TFDs. In the ILV, the soil chosen, a California soil, was selected because it was described as the most difficult. After reviewing the TFDs, it is not clear why the California soil was the most

difficult matrix with which to validate the method, and so additional information on the California soil would reduce uncertainty for the method.

The ILV validated the ECM method in the first trial with insignificant modifications to the analytical instruments and parameters. Reported communications demonstrated that technical guidance was provided to the ILV by the Bayer Study Director who was also one of the ECM study authors. Additionally, communications note that the Sponsor sent data, and approved data included in the ILV. More specific information on communications and what data was shared would increase confidence in the reproducibility of the method.

Only one ion transition was monitored; a confirmatory method is not usually required when LC/MS or GC/MS is the primary method used to generate study data. Only one set of performance data was provided at 10×LLMV in the ILV; no samples were prepared at 100 µg/kg in the ECM. All ECM and ILV data regarding repeatability, accuracy, and precision were satisfactory for spiromesifen and its four metabolites. All ECM and ILV data regarding linearity were satisfactory for spiromesifen and its four metabolites, except for ILV linearity for BSN2060-cyclobutyl photoisomer. ILV representative chromatograms supported the method; however, the specificity of the method could not be determined by the ECM representative chromatograms. The LOD was not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Lowest Level of Method Validation (LLMV)
	Environmental Chemistry Method	Independent Laboratory Validation						
Spiromesifen (BSN2060)	45819415	45819430		Soil[2,3]	26/09/2002 (ECM); 08/07/2002 (ILV)	Bayer Corporation	LC/MS/MS	10 µg/kg
BSN2060-enol								
BSN2060-4-carboxy[1]								
BSN2060-enol photoisomer								
BSN2060-cyclobutyl photoisomer								

1 Also referred to as Spiromesifen phenol acid or BSN2060-enol acid by Sponsor in accompanying studies.
2 In the ECM, Florida soil collected from a Bayer Terrestrial Field Dissipation (TFD; Bayer Report No. 110978) used in the study (pp. 9, 20 of MRID 45819415). The soil characterization data was not provided in this study report, and the soil texture was not reported.
3 In the ILV, California soil was provided by the Sponsor (Bayer) and used in the study (p. 20 of MRID 45819430). The soil was not characterized, and the soil texture was not reported. The study report indicated that the soil was selected because it represented a difficult matrix; however, the properties which made this soil difficult were not reported.

I. Principle of the Method

Soil samples (20 g) were fortified and mixed with 4 g of Hydromatrix™ in a 100-mL beaker (pp. 13-14 of MRID 45819415). The mixture was transferred to a 33-mL stainless steel Dionex™ extraction tube. The Dionex™ tube was placed on a Dionex™ Accelerated Solvent Extraction (ASE) apparatus and processed with the following conditions: preheat 0 min., flush volume 60%, pressure 1500 psi, heat 5 min., purge 5 min., temperature 80°C, static 5 min., cycles 1 min., and solvent system acetonitrile:water (7:3, v:v). After processing, 1 mL of the internal standard (IS) solution was added to the eluate in the glass collection vessels. The volume of the eluate was then adjusted to 50 mL with acetonitrile:water (8:2, v:v) containing 0.05% formic acid. After mixing by inverting twice, an aliquot (*ca*. 1.5 mL) was transferred to an autosampler vial for analysis.

Samples were analyzed for analytes using a ThermoFinnigan P-4000 HPLC system, with a ThermoFinnigan degasser and a 3000 autosampler attached, coupled to a ThermoFinnigan TSQ triple quadrupole mass spectrometer (pp. 14-16 of MRID 45819415). The LC/MS conditions consisted of an Eclipse (Zorbax) column (150 x 4.6 mm, 3.5 µ particle size; column temperature 30°C) with a mobile phase gradient of A) 0.1% formic acid in Millipore water and B) methanol [percent A:B (v:v) at 0.00-1.00 min. 40:60, 6.00-11.00 min. 20:80, 15.00-20.00 min. 5:95, 21.00-

25.00 min. 40:60; all with a flow rate of 0.8 mL/min] and Atmospheric Pressure (API II) MS detection in electrospray (ESI) mode (ionization temperature 325°C). All analytes were detected in positive ESI mode, except for BSN2060-4-carboxy and its IS. Injection volume was 50 µL. One ion transition was monitored for each analyte as follows: *m/z* 371→273 for spiromesifen, *m/z* 273→255 for BSN2060-enol, *m/z* 301→195 for BSN2060-4-carboxy, *m/z* 255→209 for BSN2060-enol photoisomer, and *m/z* 371→209 for BSN2060-cyclobutyl photoisomer. Expected retention times were 15.2, 8.3, 6.9, 8.1, and 14.6 minutes for spiromesifen, BSN2060-enol, BSN2060-4-carboxy, BSN2060-enol photoisomer, and BSN2060-cyclobutyl photoisomer, respectively.

In the ILV, the ECM was performed as written, except for the use of a different LC/MS system (pp. 20-23; Figure 1, p. 30 of MRID 45819430). A Hewlett Packard 1090 HPLC system coupled to an Micromass Quarttro LC mass spectrometer with Z-Spray® interface was used. All LC/MS conditions were the same, except that the mobile phase gradient was slightly modified [percent A:B (v:v) at 0-1 min. 40:60, 6-11 min. 20:80, 14-15 min. 5:95, 16-20 min. 40:60] and injection volume was 15 µL (since significant fronting was seen in the BSN2060-4-carboxy chromatogram with 50 µL). Ion transitions were the same as those in the ECM. Approximate expected retention times were 13.3, 7.1, 4.7, 6.8 and 12.5 minutes for spiromesifen, BSN2060-enol, BSN2060-4-carboxy, BSN2060-enol photoisomer, and BSN2060-cyclobutyl photoisomer, respectively. For the ECM, no safety information was provided, nor description of time for analysis.

The LLMV for spiromesifen and its four metabolites in soil was 10 µg/kg in the ECM and ILV (pp. 18-19 of MRID 45819415; p. 13 of MRID 45819430). Determination of the LLMV was neither demonstrated nor explained. The Limit of Detection (LOD) for spiromesifen and its four metabolites in soil was calculated as 0.38-0.79 µg/kg in the ECM; the LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 45819415): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of spiromesifen and its metabolites BSN2060-enol, BSN2060-4-carboxy, BSN2060-enol photoisomer, and BSN2060-cyclobutyl photoisomer in one soil matrix at fortification levels of 10 µg/kg (LLMV) and 50 µg/kg (5×LLMV; Table 1, pp. 21-22; DER Attachment 2). No samples were prepared at 10×LLMV. 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. Individual recoveries as % of applied and standard deviations were reviewer-calculated since these values were not provided in the study report. The lone soil used in this study was a Florida soil collected from a Bayer Terrestrial Field Dissipation (TFD; Bayer Report No. 110978) (pp. 9, 20). The soil characterization data was not provided in this study report, and the soil texture was not reported. The ECM reports that this method was also validated in soils from dissipation sites in California, Texas, and Washington, but clarification on soil properties is requested. Including data for other soil samples in the ECM would also reduce uncertainty in the method.

ILV (MRID 45819430): Mean recoveries and RSDs were within guideline requirements for analysis of spiromesifen and metabolites BSN2060-enol, BSN2060-4-carboxy, BSN2060-enol photoisomer, and BSN2060-cyclobutyl photoisomer in one soil matrix at fortification levels of 10 µg/kg (LLMV) and 100 µg/kg (10×LLMV; Table 1, p. 21). Analytes were identified using the same single ion transition followed in the ECM. The soil matrix used was called a Fresno, California soil in the ILV and was provided by the Sponsor (Bayer) and used in the study (p. 20). The soil was not characterized, and the soil texture was not reported. Details on soil properties can be found in the TFD, but confirmation is needed on whether the soil samples were identical. The study report indicated that the soil was selected because it represented a difficult matrix; however, the properties which made this soil difficult were not reported. This information was also not found in the TFD. The ILV validated the ECM method in the first trial with insignificant modifications to the analytical instrumentation and parameters (pp. 13, 20-23; Figure 1, p. 30).

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

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)[3]	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Florida Soil					
Spiromesifen (BSN2060)	10 (LLMV)	7	88.0-92.0	91	1.5	1.5
	50	5	86.4-88.8	87.2	1.0	1.1
BSN2060-enol	10 (LLMV)	7	88.0-92.0	90	1.4	1.4
	50	5	88.0-90.4	89.2	1.0	1.1
BSN2060-4-carboxy	10 (LLMV)	7	96.0-100.0	97	1.7	1.6
	50	5	94.0-96.0	94.6	0.8	0.8
BSN2060-enol photoisomer	10 (LLMV)	7	84.0-90.0	87	2.4	2.5
	50	5	84.8-86.8	86	0.8	0.9
BSN2060-cyclobutyl photoisomer	10 (LLMV)	7	86.0-94.0	89	2.6	2.8
	50	5	77.8-83.2	80.6	2.0	2.5

Data (uncorrected recovery results, pp. 16-17) were obtained from Table 1, pp. 21-22 of MRID 45819415 and DER Attachment 2.

1 The Florida soil collected from a Bayer Terrestrial Field Dissipation (TFD; Bayer Report No. 110978) used in the study (pp. 9, 20). The soil characterization data was not provided in this study report, and the soil texture was not reported.

2 One ion transition was monitored for each analyte as follows: *m/z* 371→273 for spiromesifen, *m/z* 273→255 for BSN2060-enol, *m/z* 301→195 for BSN2060-4-carboxy, *m/z* 255→209 for BSN2060-enol photoisomer, and *m/z* 371→209 for BSN2060-cyclobutyl photoisomer.

3 Individual recoveries as % of applied and standard deviations were reviewer-calculated since these values were not provided in the study report. Rules of significant figures was followed for standard deviations. For recoveries, values were reported to the tenth decimal place in accordance with the recovered residues, even though the fortification level was a whole number.

Bolded font in table denotes data deficiencies (no performance data deficiencies in this ECM).

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

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	California Soil					
Spiromesifen (BSN2060)	10 (LLMV)	5	86-91	89	2	3
	100	5	103-110	107	3	3
BSN2060-enol	10 (LLMV)	5	84-95	93	5	5
	100	5	106-113	109	3	2
BSN2060-4-carboxy	10 (LLMV)	5	103-110	106	3	3
	100	5	96-101	99	2	2
BSN2060-enol photoisomer	10 (LLMV)	5	93-110	101	6	6
	100	5	104-114	109	4	4
BSN2060-cyclobutyl photoisomer	10 (LLMV)	5	98-116	109	7	6
	100	5	98-106	103	3	3

Data (uncorrected recovery results, pp. 23-24) were obtained from Table 3, p. 29 of MRID 45819430.

1 The California soil was provided by the Sponsor (Bayer) and used in the study (p. 20). The soil was not characterized, and the soil texture was not reported. The study report indicated that the soil was selected because it represented a difficult matrix; however, the properties which made this soil difficult were not reported.

2 One ion transition was monitored for each analyte as follows: *m/z* 371→273 for spiromesifen, *m/z* 273→255 for BSN2060-enol, *m/z* 301→195 for BSN2060-4-carboxy, *m/z* 255→209 for BSN2060-enol photoisomer, and *m/z* 371→209 for BSN2060-cyclobutyl photoisomer.

Bolded font in table denotes data deficiencies (no performance data deficiencies in this ILV).

III. Method Characteristics

The LLMV for spiromesifen and its four metabolites in soil was 10 µg/kg in the ECM and ILV (pp. 18-19; Appendix 1, pp. 36-37 of MRID 45819415; p. 13 of MRID 45819430). No justification for this LLMV was provided besides the statement in the ECM that this was chosen because this is the level at which the method was validated. As previously discussed, this LLMV is higher than the lowest toxicological level of concern. In the ECM, the LLMV was defined as the level at which the method was successfully tested; however, the study report noted that the LOQ is also statistically *ca*. 3.3 times the LOD. The LOD for spiromesifen and its four metabolites in soil was calculated in the ECM using the following equation:

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

Where, $t_{0.99}$ is the one-tailed t statistic for n = 7 (3.3) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. The calculated LODs were *ca*. 0.42, 0.38, 0.49, 0.79, and 0.68 µg/kg for spiromesifen, BSN2060-enol, BSN2060-4-carboxy, BSN2060-enol photoisomer, and BSN2060-cyclobutyl photoisomer, respectively. The study authors also noted that the statistical LOQs based on the calculated LODs were 1.5-2 µg/kg. This was not further explored or discussed. However, as this statistical LOQ is lower than the lowest toxicological endpoint, this would be a valid LOQ for further analysis and risk characterization. No calculations or comparisons to background levels were reported to justify the LLMV for the method in the ILV. The ILV LLMV was reported from the ECM; the LOD was not reported in the ILV.

Satisfactory repeatability, accuracy, and precision were achieved for parent Spiromesifen and degradates BSN2060-enol, BSN2060-4-carboxy, BSN2060-enol photoisomer, and BSN2060-cyclobutyl photoisomer. Reproducibility was not satisfactory because the ILV did not provide determination of the LLMV, as described above, and data on 10xLLMV not provided in the ECM. Linearity was nearly satisfactory ($r^2 \geq 0.995$), with the exception of BSN2060-cyclobutyl photoisomer. This r^2 value was reviewer-calculated as 0.9945, despite being reported in the ILV as 0.997258. Also, while there is no interference in ECM chromatograms, no matrix and solvent controls were given, and so specificity could not be determined for the ECM. Lastly, it is unclear whether the ECM chromatograms represent the LLMV or 5xLLMV.

Table 4. Method Characteristics

Analyte		Spiromesifen (BSN2060)	BSN2060-enol	BSN2060-4-carboxy	BSN2060-enol photoisomer	BSN2060-cyclobutyl photoisomer
Lowest Level of Method Validation (LLMV)	ECM	10 µg/kg 1.5-2 µg/kg (calculated)				
	ILV	10 µg/kg				
Limit of Detection (LOD)	ECM (calculated)	*ca*. 0.42 µg/kg	*ca*. 0.38 µg/kg	*ca*. 0.49 µg/kg	*ca*. 0.79 µg/kg	*ca*. 0.68 µg/kg
	ILV	Not reported				
Linearity (calibration curve r^2 and concentration range)[1]	ECM	$r^2 = 1.0000$	$r^2 = 0.9999$	$r^2 = 0.9999$	$r^2 = 0.9993$	$r^2 = 0.9998$
	ILV	$r^2 = 0.9960$	$r^2 = 0.9961$	$r^2 = 0.9997$	$r^2 = 0.9975$	$r^2 =$ **0.9945**[6]
	Concentration Range	0.0-0.4 µg/mL (0-1000 µg/kg)				
Repeatable	ECM[2]	Yes at LLMV and 5×LLMV (one soil uncharacterized in ECM) **No samples prepared at 10×LLMV**				
	ILV[3,4]	Yes at LLMV and 10×LLMV (one uncharacterized soil)[5]				
Reproducible		Yes at LLMV **Could not be determined at 10×LLMV**				
Specific	ECM	**Could not be determined** Representative chromatograms were **not** labeled as fortified soil sample chromatograms. Analyte peaks were well-defined, and baseline noise was minimal; however, **no** control chromatograms were included to access matrix interferences at the analyte retention times.				
	ILV	Yes, no matrix interferences were observed.				

Data were obtained from pp. 18-19; Appendix 1, pp. 36-37 (LLMV/LOD); Table 1, pp. 21-22 (recovery data); Figures 8-10, pp. 30-32 (calibration curves); Figures 11-13, pp. 33-35 (chromatograms) of MRID 45819415; p. 13 (LLMV/LOD); Table 1, p. 27 (linearity data); Table 3, p. 29 (recovery data); Appendix II, pp. 75-83 (chromatograms); Appendix III, pp. 85-89 (calibration curves) of MRID 45819430; DER Attachment 2.

1 ILV correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Appendix III, pp. 85-89 of MRID 45819430; DER Attachment 2). Although r values were reported to six significant figures, the reviewer only reported correlation coefficients to four significant figures.

2 In the ECM, Florida soil collected from a Bayer Terrestrial Field Dissipation (TFD; Bayer Report No. 110978) used in the study (pp. 9, 20 of MRID 45819415). The soil characterization data was not provided in this study report, and the soil texture was not reported.

3 In the ILV, California soil was provided by the Sponsor (Bayer) and used in the study (p. 20 of MRID 45819430). The soil was not characterized, and the soil texture was not reported. The study report indicated that the soil was selected because it represented a difficult matrix; however, the properties which made this soil difficult were not reported.

4 The ILV validated the ECM method in the first trial with insignificant modifications to the analytical instrumentation and parameters (pp. 13, 20-23; Figure 1, p. 30 of MRID 45819430).

5 While no soil characterization details are provided in the ECM and ILV, there is soil characterization in TFDs (Bayer Report No. 110978 and 110348). Confirmation is needed from the Sponsor that the soil properties match those listed in the TFD.

6 Bolded font in table denotes performance data deficiency.

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

IV. Method Deficiencies and Reviewer's Comments

1. It is not clear whether ILV was conducted independently of the ECM. Communications between the ILV Study Director (ILV study author) and Bayer Study Monitor (Bill Leimkuehler) were partially reported and demonstrated that technical guidance was provided by the Bayer Study Monitor to the ILV study author (Mark R. Bauer; p. 1 of MRID 45819415; pp. 1, 3, 26; Appendix V, p. 97 of MRID 45819430). Communications suggest that the Study Monitor was involved in clarification of the study protocol, exchange of first trial study results, advice to repeat the injection of the first trial samples with a modified calibration range, and final approval of ILV results. More details on specifically what information and data was exchanged is required to improve confidence in the reproducibility of the method.

2. The reproducibility of the method at 10×LLMV could not be determined since only one set of performance data was provided at the fortification level of 100 µg/kg; no samples were prepared at 100 µg/kg in the ECM. 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. Instead of providing 10xLLMV, the ECM included 5xLLMV.

3. The specificity of the method could not be determined by the ECM representative chromatograms since chromatograms were not identified as matrix-fortified samples or control samples (Figures 11-13, pp. 33-35 of MRID 45819415).

4. ILV linearity was not satisfactory for BSN2060-cyclobutyl photoisomer ($r^2 = 0.9945$; Appendix III, pp. 85-89 of MRID 45819340; DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$.

5. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method since the ILV soils were not characterized and the soil texture was not reported. California soil was provided by the Sponsor (Bayer) and used in the study (p. 20 of MRID 45819430). The soil was not characterized, and the soil texture was not reported. Clarification is needed on whether the properties of the soil match that of the corresponding TFD study. The study report indicated that the soil was selected for the ILV because it represented a difficult matrix; however, the properties which made this soil difficult were not reported. While the TFD gives more insight onto California soil properties, the TFD does not clarify why the California soil is a difficult matrix (TFD; Bayer Report No. 110348). Additionally, it could not be determined if the ILV matrices covered the range of soils used in the TFD studies since no TFD studies accompanied the Method Validation. The reviewer noted that the ECM soil matrix was Florida soil collected from a Bayer Terrestrial Field Dissipation (TFD; Bayer Report No. 110978; pp. 9, 20 of MRID 45819415). The soil characterization data was not provided in this study report, and the soil texture was not reported.

6. The estimations of LLMV and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 18-19; Appendix 1, pp. 36-37

of MRID 45819415; p. 13 of MRID 45819430). In the ECM, the LOQ was defined as the level at which the method was successfully tested, and as a result is the lowest level of method validation (LLMV) as opposed to a true LOQ. However, the study report noted that the LOQ is also statistically *ca*. 3.3 times the LOD. In the ECM, the LOD was calculated for each matrix using the following equation: LOD = ($t_{0.99}$ x SD), where, $t_{0.99}$ is the one-tailed t statistic for n = 7 (3.3) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. The study authors also noted that the statistical LOQs based on the calculated LODs were 1.5-2 µg/kg. This value supported the method LOQ, and also is less than the lowest toxicological level of concern, which suggests that the method could be upgraded if validation is conducted at that level. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ILV. The ILV LOQ was reported from the ECM; the LOD was not reported in the ILV. Detection limits should not be based on arbitrary values.

7. The matrix effects were found to be insignificant (< ±20%) for spiromesifen in the ILV by comparison of the linear regression response for solvent calibration standards and matrix-based calibration standards (pp. 24-25 of MRID 45819430).

8. It was reported for the ILV that one sample set of 13 samples required *ca*. 8 hours of work over the course of three work days, with ASE run overnight, LC/MS/MS analysis occurring overnight, and the results calculated the next morning (p. 25 of MRID 45819430). No time of analysis was reported in the ECM.

9. The ECM is dated after the ILV. Per OCSPP 850.6100 guidelines, the ECM should be sent to an independent laboratory for ILV to verify the method. The methods in the ECM, ILV, and TFDs all are consistent. To decrease method uncertainty, further information is needed on the development of the method, including raw data and methods sent out by the Sponsor for completion of the ILV and TFDs.

V. References

USEPA. 2012a. 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. 2012b. Environmental Chemistry Methods Guidance. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. U.S. Environmental Protection Agency.

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.

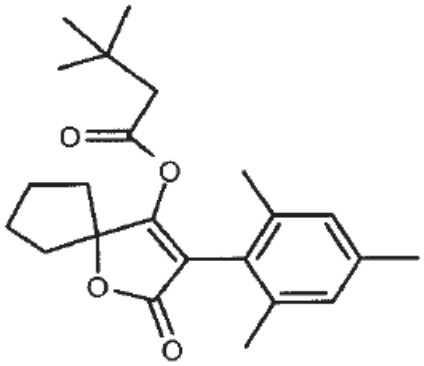
Dyer, D.G., S.E. Wood, W.M. Leimkuehler and R.J. Ripperger. 2002. Terrestrial Field Dissipation of BSN2060 on Florida soil, 1999. Unpublished study performed by Bayer Corporation, Stilwell, KS (soil processing and analysis). A&L Great Lakes Laboratories, Inc., Fort Wayne, IN (soil characterization) and Weed Systems, Inc., Hawthorne, FL (field portion), and submitted by Bayer Corporation, Kansas City, MO. Bayer Study No. BS022103. Bayer Report No. 110978. MRID 45819812.

Dyer, D.G., S.E. Wood, W.M. Leimkuehler and R.J. Ripperger. 2002. Terrestrial Field Dissipation of BSN2060 on California soil, 1999. Unpublished study performed by Bayer Corporation, Stilwell, KS (soil processing and analysis). A&L Great Lakes Laboratories, Inc., Fort Wayne, IN (soil characterization) and Bayer Research Farm, Fresno, CA (field portion), and submitted by Bayer Corporation, Kansas City, MO. Bayer Study No. BS022102. Bayer Report No. 110348. MRID 45819807.

Attachment 1: Chemical Names and Structures

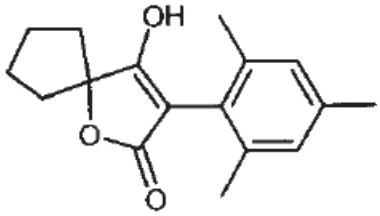
Spiromesifen (BSN2060; K-856; AE 0952850; K-1725)

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



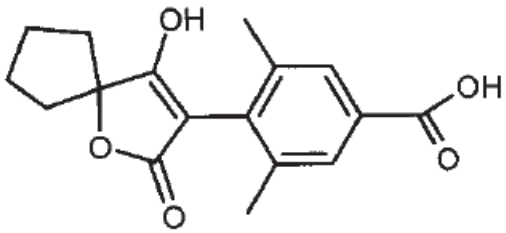
BSN2060-enol (K-860; Enol; K-1961; Spiromesifen enol)

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



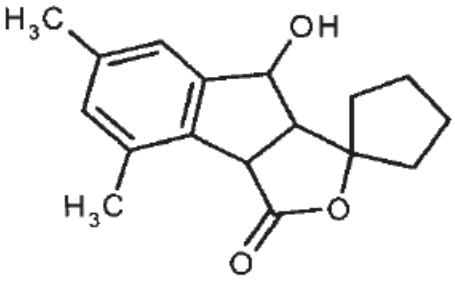
BSN2060-4-carboxy (Spiromesifen phenol acid; BSN2060-enol acid; K-912; 4-Carboxy)

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
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	Not found



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
CAS Name:	Not reported
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