

**Analytical method for benzobicyclon and its metabolites 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076 in soil**

**Reports:** ECM: EPA MRID No.: 51086513 (Appendix 3, pp. 326-1189). Mitchell, J., and M.T. Boatwright. 2017. GWN-10235: Aquatic Field Dissipation of the Herbicide Benzobicyclon at Two Rice Production Locations (Louisiana and California). WEI Study No.: 469.13. GPL Study No.: 140544. Report prepared by Waterborne Environmental, Inc. (WEI), Leesburg, Virginia, and Golden Pacific Laboratories, LLC (GPL), Fresno, California (analytical phase), and sponsored and submitted by Gowan Company, Yuma, Arizona; 864 pages (Analytical Method study). Final report issued January 12, 2017.

ILV: EPA MRID No.: 51086515. Bentley, K. 2018. Independent Laboratory Validation (ILV) of the Analytical Method for the Determination of Benzobicyclon and its Metabolites 1315P-070, 1315P-076, 1315P-570, 1315P-683, and 1315P-960 in Soil by LC-MS/MS. Smithers Viscient Study No.: 12791.6295. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored and submitted by Gowan Company, Yuma, Arizona; 205 pages. Final report issued February 7, 2018.

**Document No.:** MRIDs 51086513 & 51086515



**Guideline:** 850.6100

**Statements:** ECM: The AFD and Analytical Method study were conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3; Appendix 3, p. 327 of MRID 51086513). The following GLP exception was noted for the analytical phase: the metabolites 1315P-076, 1315P-570, 1315P-683, 1315P-960, 1315P-962, and 1315P-966 were certified by SDS Biotech K.K. and certificates of analysis were received with the standards, however, the analysis was not conducted according to GLPs. For the AFD, signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-5); an authenticity statement was included with the QA statement. For the Analytical Method, signed and dated GLP and Quality Assurance statements were provided (Appendix 3, pp. 327-328); a Data Confidentiality statement was not provided. An authenticity statement was included with the QA statement.

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 51086515). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An authenticity statement was included with the QA statement.

**Classification:** This analytical method is classified as **supplemental**. 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 LOQ. **The LLMV is greater than the most sensitive toxicological endpoint.** No ECM representative chromatograms were submitted for the method validation samples.

**PC Code:** 215101

<b>EFED Final Reviewer:</b>	Joshua Antoline, Ph. D., Chemist	Signature:	
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		Date:	10/15/2020

*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 entitled “Analytical Method for the Determination of Benzobicyclon and its Metabolites 1315P-070, 1315P-076, 1315P-570, 1315P-683, and 1315P-960 in Soil by LC-MS/MS” (GPL-MTH-088, Revision 1), is designed for the quantitative determination of benzobicyclon and its five metabolites 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076 in soil at the stated LOQ of 0.005 mg/kg using LC/MS/MS. The reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the Lowest Level of Method Validation (LLMV) rather than an LOQ. The LLMV is greater than the lowest toxicological level of concern in soil for benzobicyclon and its five metabolites 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076, based on a 6-inch soil depth, a soil density of 1.5 g/cm<sup>3</sup>, and a No Observable Adverse Effects Concentration for lettuce seedling emergence of 0.0017 mg/kg (MRID 49541205).<sup>1</sup> Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the method LOQ for benzobicyclon and its five metabolites in the tested soil matrices (0.005 mg/kg). The ECM and ILV were based on the analytical method reported in Golden Pacific Laboratories Method GPL-MTH-088 Revision 1; however, Golden Pacific Laboratories Method GPL-MTH-088 Revision 1 was not included in the submitted ECM or ILV.

The ECM validated the method using characterized clay loam and loam soil matrices which were sourced from the two sites in the benzobicyclon aquatic field dissipation studies which was also the submitted ECM. The ILV validated the method using characterized, locally-sourced clay loam and loam soil matrices. The soils used in the ILV covered the major USDA textural classes found in soil samples from the aquatic field dissipation test sites. It could not be determined if the ILV was

<sup>1</sup> USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides> (Accessed January 29, 2019).

provided with the most difficult matrices with which to validate the method. Communications between authors of the ECM and ILV were documented in the ILV study report.

The ILV validated the method for benzobicyclon and its five metabolites 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076 in the first trial; however, the communication log included discussions of the validation being re-performed with matrix-matched calibrants and some samples further re-analyzed. The ILV performed the ECM method as written, except for the use of a different shaker type, the use of interspersed matrix-matched (*i.e.*, same solvent as test samples) calibration standard to generate the calibration curve data, and insignificant modifications to the analytical parameters. The validation of benzobicyclon in loam soil was sensitive to the storage conditions/times of the sample extracts and control extracts used for preparing the calibration standards. Following ILV recommendations/issues, the ECM should be updated to include a matrix effect assessment and interspersed calibration standards, as well as precautions about the sample and control extract storage conditions/times.

All ILV data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for benzobicyclon and its five metabolites in tested soil matrices. ECM data from the method validation samples and aquatic field dissipation laboratory fortification samples were assessed. All ECM data regarding repeatability, accuracy, precision, and linearity were satisfactory for benzobicyclon and its five metabolites in tested soil matrices. The specificity of the method was not well-supported by submitted ECM representative chromatograms. No ECM representative chromatograms were submitted for the method validation samples. Insufficient ECM representative chromatograms were submitted for the aquatic field dissipation laboratory fortification samples.

**Table 1. Analytical Method Summary**

Analytes	MRID		EPA Review	Matrix	Method Date	Registrant	Analytical Technique	Lowest Level of Method Validation (LLMV)
	Environmental Chemistry Method	Independent Laboratory Validation						
Benzobicyclon	51086513 <sup>1,2</sup>	51086515 <sup>3</sup>	Supplemental	Soil	09/06/2017 (AFD)	Gowan Company	LC/MS/MS	0.005 mg/kg
1315P-070					12/01/2017 (Analytical Report of AFD)			
1315P-570								
1315P-683								
1315P-960								
1315P-076								

AFD = aquatic field dissipation

- In the ECM, clay loam soil (Sample ID: PA. CA2. K. 0-6. A; 40% sand, 22% silt, 38% clay; pH 7.7; 1.70% organic matter; cation exchange capacity 30.8 meq/100 g) collected from California and loam soil (Sample ID: PA. LA. K. 0-6. A; 38% sand, 37% silt, 25% clay; pH 6.9; 1.50% organic matter; cation exchange capacity 15.8 meq/100 g) collected from Louisiana were used in the method validation study (USDA soil texture classification; pp. 18-19; Tables 3-4, pp. 44-45; Appendix B, pp. 542-553 of MRID 51086513). The soils were obtained from this aquatic field dissipation study with sites in California and Louisiana and characterized by Agvise Laboratories, Northwood, North Dakota. Soil characterization was not reported in the method validation report, but reviewer-determined based on the Sample ID of the method validation samples and the 0-6 in. soil classification. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.
- The DER included ECM data from the method validation samples and aquatic field dissipation laboratory fortification samples (p. 34; Appendix 3, Tables 16-27, pp. 383-394; Tables 45-56, pp. 416-427; Appendix 3, Appendix B, pp. 542-553 of MRID 51086513). Soil samples used for the aquatic field dissipation laboratory fortification samples were mostly the same as those of the method validation and were taken from the 0-6 in. soil core, although several were taken from the 6-12 in. soil core (Appendix 3, Appendix B, pp. 798-859).
- In the ILV, clay loam soil (Soil ID: SMV 14Dec16 Soil-B; 40% sand, 28% silt, 32% clay; pH 5.4 in 0.01M CaCl<sub>2</sub>; 5.6% organic matter Walkley-Black; cation exchange capacity 19.2 meq/100 g) and loam soil (Soil ID: SMV 29Mar17 Soil-B; 44% sand, 36% silt, 20% clay; pH 6.6 in 0.01M CaCl<sub>2</sub>; 12.0% organic matter Walkley-Black; cation exchange capacity 23.0 meq/100 g) were used in the study (p. 24 of MRID 51086515). The soil characterization laboratory was not reported. According to ILV communications, the ILV soil matrices were not those used in the aquatic field dissipation, but locally sourced soils which had the same texture as those used in the aquatic field dissipation study (Appendix 3, pp. 178, 194). The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

## I. Principle of the Method

Soil samples were analyzed using Golden Pacific Laboratories Method GPL-MTH-088 Revision 1 entitled “*Analytical Method for the Determination of Benzobicyclon and its Metabolites 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076 in Soil by LC-MS/MS*” (M. Boatwright, January 2015; Appendix 3, pp. 349-350 of MRID 51086513; Appendix 1, p. 163 of MRID 51086515).

Soil samples (*ca.* 10 g) in 250-mL Nalgene® bottles were fortified at 0.005 mg/kg or 0.05 mg/kg and extracted by adding 90 mL of acetonitrile:0.55M citric acid (80:20, v:v) and shaking on a platform shaker for 30 minutes (Appendix 3, pp. 349-350, 356; Appendix 3, Appendix A, p. 525 of MRID 51086513). To separate the extract, the sample was filtered by vacuum using a 70-mm Whatman #5 filter in a Buchner funnel with *ca.* 1 cm thick layer of celite on the filter. The extract was collected in a 100-mL graduated cylinder fitted with a vacuum side arm. The filter cake was rinsed with 10 mL of acetonitrile: 0.55M Citric Acid (80:20, v:v). The rinsate and extract were combined and brought to a final volume of 100 mL using acetonitrile: 0.55M Citric Acid (80:20, v:v). An aliquot of sample extract was filtered through a 0.45- $\mu$ m PTFE filter attached to a plastic syringe and diluted 1:1 with water for analysis by LC-MS/MS. Samples requiring further dilutions were diluted with acetonitrile:water (40:60, v:v).

Samples were analyzed for benzobicyclon and its metabolites using a Shimadzu LC-20AD XR HPLC coupled with a Sciex API5000 MS with TurboIonSpray (ESI) ion source operated in the positive ion mode with multiple reaction monitoring (MRM; Appendix 3, pp. 350-351 of MRID 51086513). The following LC conditions were used: Phenomenex Luna 3 $\mu$  C18 100 $\text{\AA}$  column, 150 mm x 2.0 mm, 3  $\mu$  particle size; column temperature not reported), mobile phase of (A) 0.2% formic acid in acetonitrile and (B) 0.2% formic acid in water [mobile gradient phase of percent A:B (v:v) at 0.0 min. 10:90, 7.00-8.00 min. 95:5, 8.10-9.50 min. 10:90] and injection volume of 10.0  $\mu$ L. MS source temperature was not reported. One ion pair transition was monitored for each analyte:  $m/z$  447.1 $\rightarrow$ 257.1 for benzobicyclon,  $m/z$  355.0 $\rightarrow$ 165.0 for 1315P-070,  $m/z$  354.0 $\rightarrow$ 164.0 for 1315P-570,  $m/z$  319.1 $\rightarrow$ 240.1 for 1315P-683,  $m/z$  412.1 $\rightarrow$ 176.0 for 1315P-960, and  $m/z$  398.1 $\rightarrow$ 208.1 for 1315P-076. Reported retention times were *ca.* 4.78, 3.75, 2.56, 2.38, 2.61, and 2.40 minutes for benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076, respectively.

The ILV was conducted to independently validate “*Analytical Method for the Determination of Benzobicyclon and its Metabolites 1315P-070, 1315P-076, 1315P-570, 1315P-683, and 1315P-960 in Soil by LC-MS/MS*” (Boatwright, 2015; p. 15 of MRID 51086515). The ILV performed the ECM method as written, except for the use of an orbital shaker (250 rpm) in the place of a platform shaker, matrix-matched calibration standards which were interspersed with the test samples, and insignificant modifications to the analytical parameters (pp. 23, 27-33). The sample weight was reported based on wet weight (12.20 g for clay loam soil and 12.99 g for loam soil). According to the communication log, the ECM sample weight was not corrected for moisture content (Appendix 3, p. 196). A confirmatory ion transition was added to the LC/MS/MS method (pp. 23, 31-32). Samples were analyzed for benzobicyclon using Shimadzu LC-20AD HPLC coupled with an AB Sciex API 5000 MS with ESI Turbo V ion source operated in the positive ion mode with multiple reaction monitoring (MRM). The LC/MS/MS parameters were the same as those of the ECM; however, the column temperature was reported as 40°C and the MS source temperature was reported as 550°C. Two ion pair transitions were monitored for each analyte (quantitation and

confirmation, respectively):  $m/z$  447.44→257.07 and  $m/z$  447.44→229.10 for benzobicyclon,  $m/z$  355.35→165.09 and  $m/z$  355.35→183.09 for 1315P-070,  $m/z$  354.34→164.10 and  $m/z$  354.34→318.10 for 1315P-570,  $m/z$  319.33→240.12 and  $m/z$  319.33→212.10 for 1315P-683,  $m/z$  412.33→176.15 and  $m/z$  412.33→222.12 for 1315P-960, and  $m/z$  398.30→208.14 and  $m/z$  398.3→319.10 for 1315P-076. The monitored quantitation ion transitions were similar to those of the ECM. Reported retention times were *ca.* 4.54, 3.46, 2.25, 2.08, 2.24, and 2.16 minutes for benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076, respectively. The ILV identified critical steps and had recommendations for the ECM based on ILV modifications (p. 35).

The reported Limit of Quantification (LOQ) for benzobicyclon and its five metabolites 1315P-070, 1315P-076, 1315P-570, 1315P-683, and 1315P-960 in soil was 0.005 mg/kg in the ECM and ILV (pp. 27, 34; Appendix 3, pp. 347-348; Appendix 3, Appendix B, pp. 542-553 of MRID 51086513; pp. 15-17, 36-39 of MRID 51086515). In the ECM, the Limits of Detection (LODs) were reported as 0.002 mg/kg for all analytes. In the ILV, the LODs were calculated as 0.3-0.9 µg/L for benzobicyclon, 0.2-1.2 µg/L for 1315P-070, 0.1-0.4 µg/L for 1315P-570, 0.1 µg/L for 1315P-683, 0.3-0.5 µg/L for 1315P-960, and 0.05-0.3 µg/L for 1315P-076. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

## II. Recovery Findings

ECM (MRID 51086513): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of benzobicyclon and its five metabolites 1315P-070, 1315P-076, 1315P-570, 1315P-683, and 1315P-960 at fortification levels of 0.005 mg/kg (LOQ) and 0.05 mg/kg (10×LOQ) in two soil matrices (p. 34; Appendix 3, Tables 16-27, pp. 383-394; Tables 45-56, pp. 416-427; Appendix 3, Appendix B, pp. 542-553). Data from the method validation samples and aquatic field dissipation laboratory fortification samples were evaluated. Actual fortification levels ranged 0.00498-0.00505 mg/kg and 0.0498-0.0505 mg/kg. One ion pair transition was monitored and quantified. Results from the method validation samples were comparable to results from the aquatic field dissipation laboratory fortification samples, except for analysis of benzobicyclon in loam soil at the LLMV. Clay loam soil (Sample ID: PA. CA.2. K. 0-6. A; 40% sand, 22% silt, 38% clay; pH 7.7; 1.70% organic matter; cation exchange capacity 30.8 meq/100 g) collected from California and loam soil (Sample ID: PA. LA. K. 0-6. A; 38% sand, 37% silt, 25% clay; pH 6.9; 1.50% organic matter; cation exchange capacity 15.8 meq/100 g) collected from Louisiana were used in the method validation study (USDA soil texture classification; pp. 18-19; Tables 3-4, pp. 44-45; Appendix B, pp. 542-553). The soils were obtained from this aquatic field dissipation study with sites in California and Louisiana and characterized by Agvise Laboratories, Northwood, North Dakota. Soil characterization was not reported in the method validation report, but reviewer-determined based on the Sample ID of the method validation samples and the 0-6 in. soil classification. Soil samples used for the aquatic field dissipation laboratory fortification samples were mostly the same as those of the method validation and were taken from the 0-6 in. soil core, although several were taken from the 6-12 in. soil core (Appendix 3, Appendix B, pp. 798-859).

ILV (MRID 51086515): Mean recoveries and RSDs were within guidelines for analysis of benzobicyclon and its five metabolites 1315P-070, 1315P-076, 1315P-570, 1315P-683, and 1315P-960 at fortification levels of 0.005 mg/kg (LOQ) and 0.05 mg/kg (10×LOQ) in two soil matrices

(Tables 1-24, pp. 46-69). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. Clay loam soil (Soil ID: SMV 14Dec16 Soil-B; 40% sand, 28% silt, 32% clay; pH 5.4 in 0.01M CaCl<sub>2</sub>; 5.6% organic matter Walkley-Black; cation exchange capacity 19.2 meq/100 g) and loam soil (Soil ID: SMV 29Mar17 Soil-B; 44% sand, 36% silt, 20% clay; pH 6.6 in 0.01M CaCl<sub>2</sub>; 12.0% organic matter Walkley-Black; cation exchange capacity 23.0 meq/100 g) were used in the study (USDA soil texture classification not reported; p. 24). The soil characterization laboratory was not reported and no certificates of analysis were provided for the soil samples. According to ILV communications, the ILV soil matrices were not those used in the aquatic field dissipation, but locally sourced soils which had the same texture as those used in the aquatic field dissipation study (Appendix 3, pp. 178, 194). The method for benzobicyclon and its five metabolites 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076 in soil was reportedly validated in the first trial; however, according to the communication log, the validation was “re-performed and analyzed against matrix-matched standards” and the “first attempt...[was]...repeated” (p. 15; Appendix 3, pp. 188, 198). The ILV performed the ECM method as written, except for the use of an orbital shaker (250 rpm) in the place of a platform shaker, matrix-matched calibration standards which were interspersed with the test samples, and insignificant modifications to the analytical parameters (pp. 23, 27-33). The validation of benzobicyclon in loam soil appeared to be sensitive to the storage conditions/times of the sample extracts and control extracts used for preparing the calibration standards (Appendix 3, p. 198). Following ILV recommendations/issues, the ECM should be updated to include a matrix effect assessment and interspersed calibration standards, as well as precautions about the storage conditions/times of the sample extracts and control extracts used for preparing the calibration standards (pp. 33, 35; Appendix 3, p. 198).

**Table 2. Initial Validation Method Recoveries for Benzobicyclon and its five metabolites 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076 in Soil<sup>1</sup>**

Analyte	Fortification Level (mg/kg) <sup>2</sup>	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Method Validation Data<sup>3</sup></b>						
<b>California Clay Loam Soil</b>						
Quantitation ion transition						
Benzobicyclon	0.005 (LOQ)	5	81.3-95.8	89.1	6.88	7.72
	0.05	5	96.6-103	99.7	2.47	2.48
1315P-070	0.005 (LOQ)	5	89.6-102	96.6	6.00	6.21
	0.05	5	85.6-96.4	91.4	3.83	4.19
1315P-570	0.005 (LOQ)	5	94.0-99.0	96.1	2.09	2.17
	0.05	5	85.5-98.6	92.8	4.70	5.06
1315P-683	0.005 (LOQ)	5	82.7-96.6	88.4	5.49	6.21
	0.05	5	82.3-90.7	87.3	3.41	3.91
1315P-960	0.005 (LOQ)	5	89.7-105	98.8	5.62	5.69
	0.05	5	86.7-100	93.7	5.56	5.93
1315P-076	0.005 (LOQ)	5	88.4-108	93.8	8.13	8.67
	0.05	5	88.8-99.6	84.8	4.76	5.02
<b>Louisiana Loam Soil</b>						
Quantitation ion transition						
Benzobicyclon	0.005 (LOQ)	5	91.2-97.6	93.6	2.45	2.62
	0.05	5	96.4-101	98.6	2.11	2.14
1315P-070	0.005 (LOQ)	5	96.4-105	100	3.98	3.98
	0.05	5	93.6-97.2	95.0	1.34	1.41
1315P-570	0.005 (LOQ)	5	89.6-97.2	93.8	2.75	2.93
	0.05	5	88.4-91.6	90.2	1.46	1.62
1315P-683	0.005 (LOQ)	5	80.8-86.0	84.1	2.20	2.62
	0.05	5	79.6-85.2	82.2	2.15	2.62
1315P-960	0.005 (LOQ)	5	99.4-116	107	6.40	5.98
	0.05	5	92.7-98.2	95.0	2.15	2.26
1315P-076	0.005 (LOQ)	5	90.9-99.0	95.7	3.68	3.85
	0.05	5	87.9-95.4	90.7	3.52	3.88
<b>AFD Laboratory Fortification Data<sup>4</sup></b>						
<b>California Clay Loam Soil</b>						
Quantitation ion transition						
Benzobicyclon	0.005 (LOQ)	5	74.9-103	86.3	11.4	13.2
	0.05	5	100-105	102	2.12	2.08
1315P-070	0.005 (LOQ)	5	92.8-98.4	94.9	2.30	2.42
	0.05	5	85.5-92.0	89.6	2.68	2.99
1315P-570	0.005 (LOQ)	5	93.2-100	96.7	2.83	2.93
	0.05	5	89.6-101	94.7	4.44	4.69
1315P-683	0.005 (LOQ)	5	82.8-92.7	86.8	3.83	4.41
	0.05	5	81.1-88.7	84.6	3.36	3.97
1315P-960	0.005 (LOQ)	5	91.5-103	96.5	5.17	5.36
	0.05	5	89.1-105	97.6	5.99	6.14
1315P-076	0.005 (LOQ)	5	97.0-106	101	3.28	3.25
	0.05	5	87.9-102	96.5	5.44	5.64



Analyte	Fortification Level (mg/kg) <sup>2</sup>	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Louisiana Loam Soil</b>						
Quantitation ion transition						
Benzobicyclon	0.005 (LOQ)	5	66.9-87.1	79.5	7.51	9.45
	0.05	5	94.2-110	101	6.03	5.97
1315P-070	0.005 (LOQ)	5	86.0-100	92.5	5.91	6.39
	0.05	5 <sup>5</sup>	92.4-96.0	94.5	1.51	1.60
1315P-570	0.005 (LOQ)	5	82.4-102	95.5	7.96	8.34
	0.05	5	94.2-105	99.8	3.89	3.90
1315P-683	0.005 (LOQ)	5	77.2-95.0	88.7	7.22	8.14
	0.05	5	80.0-98.6	91.7	7.03	7.67
1315P-960	0.005 (LOQ)	5	97.0-104	101	3.63	3.59
	0.05	5	92.3-105	100	5.03	5.03
1315P-076	0.005 (LOQ)	5	88.4-110	97.2	8.04	8.27
	0.05	5	96.4-105	99.2	3.44	3.47

Data (uncorrected recovery results; Appendix 3, Appendix B, pp. 542-553) were obtained from p. 34; Appendix 3, Tables 16-27, pp. 383-394; Tables 45-56, pp. 416-427; Appendix 3, Appendix B, pp. 542-553 of MRID 51086513. AFD = Aquatic Field Dissipation.

- 1 One ion pair transition was monitored for each analyte:  $m/z$  447.1→257.1 for benzobicyclon,  $m/z$  355.0→165.0 for 1315P-070,  $m/z$  354.0→164.0 for 1315P-570,  $m/z$  319.1→240.1 for 1315P-683,  $m/z$  412.1→176.0 for 1315P-960, and  $m/z$  398.1→208.1 for 1315P-076. A confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.
- 2 Nominal fortification level reported. Actual fortification levels ranged 0.00498-0.00505 mg/kg and 0.0498-0.0505 mg/kg (Tables 16-27, pp. 383-394; Tables 45-56, pp. 416-427).
- 3 The clay loam soil (Sample ID: PA. CA2. K. 0-6. A; 40% sand, 22% silt, 38% clay; pH 7.7; 1.70% organic matter; cation exchange capacity 30.8 meq/100 g) collected from California and loam soil (Sample ID: PA. LA. K. 0-6. A; 38% sand, 37% silt, 25% clay; pH 6.9; 1.50% organic matter; cation exchange capacity 15.8 meq/100 g) collected from Louisiana were used in the method validation study (USDA soil texture classification; pp. 18-19; Tables 3-4, pp. 44-45; Appendix B, pp. 542-553). The soils were obtained from this aquatic field dissipation study with sites in California and Louisiana and characterized by Agvise Laboratories, Northwood, North Dakota. Soil characterization was not reported in the method validation report, but reviewer-determined based on the Sample ID of the method validation samples and the 0-6 in. soil classification. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.
- 4 Soil samples used for the aquatic field dissipation laboratory fortification samples were mostly the same as those of the method validation and were taken from the 0-6 in. soil core, although several were taken from the 6-12 in. soil core (Appendix 3, Appendix B, pp. 798-859).
- 5 One sample was re-analyzed to demonstrate extract stability, but the result of the re-analysis was not included in the statistical calculations (Table 52, p. 423).

**Table 3. Independent Validation Method Recoveries for Benzobicyclon and its five metabolites 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076 in Soil<sup>1,2</sup>**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Clay Loam Soil</b>						
Quantitation ion transition						
Benzobicyclon	0.005 (LOQ)	5	85.6-102	93.7	6.62	7.07
	0.05	5	95.5-107	99.2	4.84	4.88
1315P-070	0.005 (LOQ)	5	76.7-108	88.1	13.1	14.9
	0.05	5	88.3-101	95.1	5.07	5.34
1315P-570	0.005 (LOQ)	5	93.5-101	97.6	3.16	3.24
	0.05	5	87.6-99.9	94.1	5.02	5.33
1315P-683	0.005 (LOQ)	5	80.5-101	90.2	9.10	10.1
	0.05	5	72.1-87.8	81.6	6.09	7.47
1315P-960	0.005 (LOQ)	5	92.2-98.0	96.0	2.37	2.47
	0.05	5	95.8-107	102	4.20	4.12
1315P-076	0.005 (LOQ)	5	92.9-110	100	6.57	6.54
	0.05	5	78.7-98.0	90.4	7.33	8.11
Confirmation ion transition						
Benzobicyclon	0.005 (LOQ)	5	79.9-113	99.2	12.6	12.7
	0.05	5	90.3-110	98.8	7.62	7.72
1315P-070	0.005 (LOQ)	5	86.4-98.5	93.1	5.13	5.51
	0.05	5	83.3-106	92.1	8.34	9.05
1315P-570	0.005 (LOQ)	5	84.2-105	95.3	8.80	9.23
	0.05	5	86.1-99.1	91.8	4.84	5.27
1315P-683	0.005 (LOQ)	5	88.8-97.8	94.0	4.56	4.85
	0.05	5	73.1-90.2	82.7	6.16	7.46
1315P-960	0.005 (LOQ)	5	96.6-110	102	5.18	5.05
	0.05	5	98.0-115	104	6.57	6.32
1315P-076	0.005 (LOQ)	5	85.6-111	100	11.7	11.7
	0.05	5	78.8-97.2	90.1	6.81	7.55
<b>Loam Soil</b>						
Quantitation ion transition						
Benzobicyclon	0.005 (LOQ)	5	77.8-92.9	84.6	6.12	7.24
	0.05	5	85.8-101	93.3	5.64	6.05
1315P-070	0.005 (LOQ)	5	89.5-110	103	7.90	7.69
	0.05	5	86.9-95.2	90.9	4.88	5.37
1315P-570	0.005 (LOQ)	5	91.7-101	96.2	3.94	4.09
	0.05	5	93.5-103	96.7	4.43	4.58
1315P-683	0.005 (LOQ)	5	71.3-94.3	84.8	8.80	10.4
	0.05	5	85.2-102	91.9	7.77	8.46
1315P-960	0.005 (LOQ)	5	75.4-104	91.8	12.4	13.6
	0.05	5	100-117	111	6.30	5.69
1315P-076	0.005 (LOQ)	5	72.6-102	90.8	12.0	13.2
	0.05	5	87.7-105	94.9	7.90	8.32
Confirmation ion transition						
Benzobicyclon	0.005 (LOQ)	5	89.2-104	94.7	5.72	6.04
	0.05	5	93.9-103	99.2	4.20	4.24

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
1315P-070	0.005 (LOQ)	5	85.8-102	90.9	6.51	7.17
	0.05	5	94.1-105	101	5.06	5.02
1315P-570	0.005 (LOQ)	5	78.9-104	92.3	8.98	9.73
	0.05	5	94.3-106	102	4.78	4.70
1315P-683	0.005 (LOQ)	5	74.5-89.5	82.7	6.47	7.82
	0.05	5	85.4-107	93.9	10.2	10.9
1315P-960	0.005 (LOQ)	5	93.8-105	99.1	4.43	4.47
	0.05	5	110-117	115	2.72	2.38
1315P-076	0.005 (LOQ)	5	70.7-99.4	88.6	11.5	13.0
	0.05	5	85.7-105	94.6	8.75	9.25

Data (uncorrected recovery results; pp. 35-36) were obtained from Tables 1-24, pp. 46-69 of MRID 51086515.

1 The clay loam soil (Soil ID: SMV 14Dec16 Soil-B; 40% sand, 28% silt, 32% clay; pH 5.4 in 0.01M CaCl<sub>2</sub>; 5.6% organic matter Walkley-Black; cation exchange capacity 19.2 meq/100 g) and loam soil (Soil ID: SMV 29Mar17 Soil-B; 44% sand, 36% silt, 20% clay; pH 6.6 in 0.01M CaCl<sub>2</sub>; 12.0% organic matter Walkley-Black; cation exchange capacity 23.0 meq/100 g) were used in the study (USDA soil texture classification not reported; p. 24). The soil characterization laboratory was not reported. According to ILV communications, the ILV soil matrices were not those used in the aquatic field dissipation, but locally sourced soils which had the same texture as those used in the aquatic field dissipation study (Appendix 3, pp. 178, 194). The soil textures were verified by the reviewer using USDA-NRCS technical support tools.

2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively):  $m/z$  447.44→257.07 and  $m/z$  447.44→229.10 for benzobicyclon,  $m/z$  355.35→165.09 and  $m/z$  355.35→183.09 for 1315P-070,  $m/z$  354.34→164.10 and  $m/z$  354.34→318.10 for 1315P-570,  $m/z$  319.33→240.12 and  $m/z$  319.33→212.10 for 1315P-683,  $m/z$  412.33→176.15 and  $m/z$  412.33→222.12 for 1315P-960, and  $m/z$  398.30→208.14 and  $m/z$  398.3→319.10 for 1315P-076. The monitored quantitation ion transitions were similar to those of the ECM.

### III. Method Characteristics

The reported LOQ for benzobicyclon and its five metabolites 1315P-070, 1315P-076, 1315P-570, 1315P-683, and 1315P-960 in soil was 0.005 mg/kg in the ECM and ILV (pp. 27, 34; Appendix 3, pp. 347-348; Appendix 3, Appendix B, pp. 542-553 of MRID 51086513; pp. 15-17, 36-39 of MRID 51086515). Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the LLMV rather than a LOQ.

In the ECM, the LODs were reported as 0.002 mg/kg for all analytes, also without justification.

In the ILV, the LODs were calculated from the signal-to-noise response of each analyte in matrix at the LOQ level using the following equation:

$$\text{LOD} = (3 \times (\text{SN}_{\text{ctl}}) / (\text{Resp}_{\text{LS}})) \times \text{Conc}_{\text{LS}} \times \text{DF}_{\text{Ctl}}$$

Where, LOD is the method limit of detection ( $\mu\text{g/L}$ ),  $\text{SN}_{\text{ctl}}$  is the mean signal to noise in height of the control samples (or blanks),  $\text{Resp}_{\text{LS}}$  is the mean response in height of the two low calibration standards (0.100  $\mu\text{g/L}$ ),  $\text{Conc}_{\text{LS}}$  is the concentration of the low calibration standard ( $\mu\text{g/L}$ ), and  $\text{DF}_{\text{Ctl}}$  is the dilution factor of the control samples.

The ILV LODs were calculated as 0.3-0.9  $\mu\text{g/L}$  for benzobicyclon, 0.2-1.2  $\mu\text{g/L}$  for 1315P-070, 0.1-0.4  $\mu\text{g/L}$  for 1315P-570, 0.1  $\mu\text{g/L}$  for 1315P-683, 0.3-0.5  $\mu\text{g/L}$  for 1315P-960, and 0.05-0.3  $\mu\text{g/L}$  for 1315P-076.

**Table 4. Method Characteristics in Soil**

		Benzobicyclon	1315P-070	1315P-570	1315P-683	1315P-960	1315P-076
Limit of Quantitation (LOQ)*	ECM	0.005 mg/kg					
	ILV						
Limit of Detection (LOD)	ECM	0.002 mg/kg					
	ILV (calc)	0.3 µg/L (Q & C, CL) 0.5 µg/L (Q, L) 0.9 µg/L (C, L)	0.6 µg/L (Q, CL) 0.2 µg/L (C, CL) 1.2 µg/L (Q, L) 0.2 µg/L (C, L)	0.1 µg/L (Q, CL) 0.4 µg/L (C, CL) 0.1 µg/L (Q, L) 0.2 µg/L (C, L)	0.1 µg/L (Q & C, CL) 0.1 µg/L (Q & C, L)	0.4 µg/L (Q, CL) 0.3 µg/L (C, CL) 0.5 µg/L (Q, L) 0.3 µg/L (C, L)	0.05 µg/L (Q, CL) 0.2 µg/L (C, CL) 0.06 µg/L (Q, L) 0.3 µg/L (C, L)
Linearity (calibration curve r and concentration range)	ECM <sup>2</sup>	r = 0.9994 (Q, CL) r = 1.0000 (Q, L) r = 0.9995 (AFD)	r = 0.9998 (Q, CL) r = 0.9999 (Q, L) r = 0.9998 (AFD)	r = 0.9997 (Q, CL) r = 1.0000 (Q, L) r = 0.9999 (AFD)	r = 0.9995 (Q, CL) r = 0.9997 (Q, L) r = 0.9998 (AFD)	r = 0.9997 (Q, CL) r = 0.9998 (Q, L) r = 0.9994 (AFD)	r = 0.9993 (Q, CL) r = 0.9998 (Q, L) r = 0.9995 (AFD)
	ILV	r = 0.9973 (Q, CL) r = 0.9951 (C, CL) r = 0.9998 (Q, L) r = 0.9995 (C, L)	r = 0.9959 (Q, CL) r = 0.9988 (C, CL) r = 0.9978 (Q, L) <sup>3</sup> r = 0.9995 (C, L)	r = 0.9993 (Q, CL) r = 0.9987 (C, CL) r = 0.9972 (Q, L) r = 0.9986 (C, L)	r = 0.9982 (Q & C, CL) r = 0.9966 (Q, L) r = 0.9957 (C, L)	r = 0.9991 (Q, CL) r = 0.9996 (C, CL) r = 0.9952 (Q, L) r = 0.9978 (C, L)	r = 0.9983 (Q, CL) r = 0.9974 (C, CL) r = 0.9958 (Q, L) r = 0.9970 (C, L)
	Range	0.101-5.05 ng/mL or 0.100-5.00 ng/mL					
Repeatable	ECM <sup>4,5</sup>	Yes at LOQ (0.005 mg/kg) and 10×LOQ (0.050 mg/kg) (two characterized soil matrices: clay loam and loam soils)					
	ILV <sup>6,7</sup>						
Reproducible <sup>6</sup>	Yes for 0.005 mg/kg (LLMV)* and 0.050 mg/kg in tested soil matrices						
Specific	ECM	No representative chromatograms were submitted for the method validation samples. Representative chromatograms were submitted for the laboratory fortification samples of the AFD, but only one matrix per analyte was submitted.					
		Yes, based on chromatograms submitted for the laboratory fortification samples of the AFD. No matrix interferences were observed.					
	ILV	Yes, matrix interferences were <5% of the LOQ (based on peak area).  Analyte peak small compared to baseline noise in loam soil.	Yes, matrix interferences were <5% of the LOQ (based on peak area).  LOQ analyte peak small compared to baseline noise.  Minor contaminant was observed (RT ca. 2.25 min.). <sup>8</sup>	Yes, matrix interferences were <5% of the LOQ (based on peak area).	Yes, matrix interferences were <5% of the LOQ (based on peak area).  Insignificant contaminant or baseline noise was observed near analyte peak (RT ca. 2.25 min.). <sup>8</sup>	Yes, matrix interferences were <5% of the LOQ (based on peak area).  LOQ peak small compared to baseline noise.	Yes, no matrix interferences were observed.

Data were obtained from pp. 27, 34; Appendix 3, pp. 347-348; Appendix 3, Appendix B, pp. 542-553 (LOQ/LOD); p. 34; Appendix 3, Tables 16-27, pp. 383-394; Tables 45-56, pp. 416-427; Appendix 3, Appendix B, pp. 542-553 (recovery results & calibration coefficients); Appendix 3, Appendix C, Figures 161-170, pp. 1070-1079; Figures 181-190, pp. 1090-1099; Figures 201-210, pp. 1110-1119; Figures 221-230, pp. 1130-1139; Figures 241-250, pp. 1150-1159; Figures 261-270, pp. 1170-1179 (chromatograms and calibration curves) of MRID 51086513; pp. 15-17, 36-39 (LOQ/LOD); Tables 1-24, pp. 46-69 (recovery results); p. 40; Figures 1-84, pp. 70-153 (calibration coefficients); Figures 1-84, pp. 70-153 (chromatograms and calibration curves) of MRID 51086515. Q = quantitation ion transition; C = confirmation ion transition; CL = Clay Loam Soil; L = Loam Soil; AFD = Aquatic Field Dissipation.

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

- 1 In the ECM, one ion pair transition was monitored for each analyte (Appendix 3, pp. 350-351 of MRID 51086513). A confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.
- 2 Data from the method validation samples and aquatic field dissipation laboratory fortification samples were included. Aquatic field dissipation laboratory fortification sample data denoted with AFD, if necessary. It could not be determined if calibration standards were matrix-matched or solvent-based (see Reviewer's Comment #2). The ILV reported that solvent-based calibration standards were used in the ECM (p. 33 of MRID 51086515).
- 3 The correlation coefficient was reported from Figure 55, pp. 124 of MRID 51086515. The value reported in the table on p. 40 of MRID 51086515 was rounded incorrectly to 0.997.
- 4 In the ECM, clay loam soil (Sample ID: PA. CA2. K. 0-6. A; 40% sand, 22% silt, 38% clay; pH 7.7; 1.70% organic matter; cation exchange capacity 30.8 meq/100 g) collected from California and loam soil (Sample ID: PA. LA. K. 0-6. A; 38% sand, 37% silt, 25% clay; pH 6.9; 1.50% organic matter; cation exchange capacity 15.8 meq/100 g) collected from Louisiana were used in the method validation study (USDA soil texture classification; pp. 18-19; Tables 3-4, pp. 44-45; Appendix B, pp. 542-553 of MRID 51086513). The soils were obtained from this aquatic field dissipation study with sites in California and Louisiana and characterized by Agvise Laboratories, Northwood, North Dakota. Soil characterization was not reported in the method validation report, but reviewer-determined based on the Sample ID of the method validation samples and the 0-6 in. soil classification. The soil textures were verified by the reviewer using USDA-NRCS technical support tools.
- 5 The DER included ECM data from the method validation samples and aquatic field dissipation laboratory fortification samples (p. 34; Appendix 3, Tables 16-27, pp. 383-394; Tables 45-56, pp. 416-427; Appendix 3, Appendix B, pp. 542-553 of MRID 51086513). Soil samples used for the aquatic field dissipation laboratory fortification samples were mostly the same as those of the method validation and were taken from the 0-6 in. soil core, although several were taken from the 6-12 in. soil core (Appendix 3, Appendix B, pp. 798-859).
- 6 In the ILV, clay loam soil (Soil ID: SMV 14Dec16 Soil-B; 40% sand, 28% silt, 32% clay; pH 5.4 in 0.01M CaCl<sub>2</sub>; 5.6% organic matter Walkley-Black; cation exchange capacity 19.2 meq/100 g) and loam soil (Soil ID: SMV 29Mar17 Soil-B; 44% sand, 36% silt, 20% clay; pH 6.6 in 0.01M CaCl<sub>2</sub>; 12.0% organic matter Walkley-Black; cation exchange capacity 23.0 meq/100 g) were used in the study (USDA soil texture classification not reported; p. 24 of MRID 51086515). The soil characterization laboratory was not reported. According to ILV communications, the ILV soil matrices were not those used in the aquatic field dissipation, but locally sourced soils which had the same texture as those used in the aquatic field dissipation study (Appendix 3, pp. 178, 194). The soil textures could not be verified by the reviewer using USDA-NRCS technical support tools.
- 7 The ILV reportedly validated the method for benzobicyclon and its five metabolites 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076 in the first trial; however, the communication log included discussions of the validation being re-performed with matrix-matched calibrants and some samples further re-analyzed (p. 15; Appendix 3, pp. 188, 198 of MRID 51086515). The ILV performed the ECM method as written, except for the use of an orbital shaker (250 rpm) in the place of a platform shaker, matrix-matched calibration standards which were interspersed with the test samples, and insignificant modifications to the analytical parameters (pp. 23, 27-33). The validation of benzobicyclon in loam soil appeared to be sensitive to the storage conditions/times of the sample extracts and control extracts used for preparing the calibration standards (Appendix 3, p. 198). Following ILV recommendations/issues, the ECM should be updated to include a matrix effect assessment and interspersed calibration standards, as well as precautions about the storage conditions/times of the sample extracts and control extracts used for preparing the calibration standards.
- 8 Based on Figures 10-12, pp. 79-81; Figure 32, p. 101; Figures 52-54, pp. 121-123; Figure 74, p. 143 of MRID 51086515. 1315P-570 occurred at RT *ca.* 2.25 min.

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

1. Since the reported method 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 an LOQ (pp. 27, 34; Appendix 3, pp. 347-348; Appendix 3, Appendix B, pp. 542-553 of MRID 51086513; pp. 15-17, 36-39 of MRID 51086515). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the method LOQ for benzobicyclon and its five metabolites 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076 in the tested soil matrices (0.005 mg/kg).
2. The submitted ECM was a benzobicyclon aquatic field dissipation study which contained method validation data of the analytical method used to analyze the field samples. The original method was Golden Pacific Laboratories Method GPL-MTH-088 Revision 1 entitled "*Analytical Method for the Determination of Benzobicyclon and its Metabolites 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076 in Soil by LC-MS/MS*" (M. Boatwright, January 2015; Appendix 3, pp. 349-350 of MRID 51086513). The submitted ILV was performed using the protocol which was based on that same method, Golden Pacific Laboratories Method GPL-MTH-088 Revision 1 (M. Boatwright, 2015; Appendix 1, p. 163 of MRID 51086515). However, the original method Golden Pacific Laboratories Method GPL-MTH-088 Revision 1 was not included in the submitted ECM or ILV. It was also noted that the author of Golden Pacific Laboratories Method GPL-MTH-088 Revision 1 was also the author of the analytical phase of the submitted ECM (benzobicyclon aquatic field dissipation study).

The analytical method for Golden Pacific Laboratories Method GPL-MTH-088 Revision 1 was only provided in summary and diagram form in the ECM (Appendix 3, pp. 349-350, 356; Appendix 3, Appendix A, p. 525 of MRID 51086513). Details of the fortification and calibration solution preparation was not included. Some sample processing details and LC/MS/MS parameters were not included. The ILV noted the lack of detail in the ECM method (p. 33 of MRID 51086515).

3. The ILV study author (Kristen Bentley, Smithers Viscient) communicated directly via phone conference with the ECM study author (Megan Boatwright, Golden Pacific Laboratories, LLC) regarding ILV technical issues (p. 1; Appendix 3, p. 326 of MRID 51086513; p. 1; Appendix 3, pp. 191-192 of MRID 51086515). The communications between the ILV study author (Kristen Bentley, Smithers Viscient), ILV Study Monitors (Premjit Halarnkar and Chris Waid, Gowan Company), ECM study author (Megan Boatwright, Golden Pacific Laboratories, LLC), and Robert Testman (President, Golden Pacific Laboratories, LLC) were summarized in the ILV study report and communication details were provided in Appendix 3 of the ILV (pp. 1, 5, 34; Appendix 1, p. 164; Appendix 3, pp. 177-205 of MRID 51086515). Communications were in the form of email and phone conferences. Phone conference agendas and minutes were generally provided. Reported communications summary included: protocol and method issue, matrix differences, ILV issues with diverging calibration curves, and exchange of the results of the first attempt of the ILV. The correspondence details also involved ILV soil matrix choice, ILV request for supporting chromatograms from the ECM, deadline changes, repetition and re-analysis of samples from the first trial, and questions about moisture content correction. The ILV question about soil moisture content from Kristen Bentley was answered by Megan Boatwright via email

forwarding by Premjit Halarnkar (Appendix 3, p. 196 of MRID 51086515).

4. Matrix-matched calibrants was necessary for the ILV successful validation of the ECM (pp. 33, 35; Appendix 3, p. 198 of MRID 51086515). Additionally, the validation of benzobicyclon in loam soil appeared to be sensitive to the storage conditions/times of the sample extracts and control extracts used for preparing the calibration standards. So, the ECM could be updated with cautions about the storage conditions/times of the sample extracts and control extracts used for preparing the calibration standards. The ILV also “recommended that the analysis be run with interspersed calibration standards” (p. 35).
5. The ILV soil textures were reported as clay loam and loam, but the original Certificates of Analysis for each ILV soil were not provided in the study report, and the soil characterization laboratory was not reported. A clay loam soil and loam soil were used in the ECM (USDA soil texture classification; pp. 18-19; Tables 3-4, pp. 44-45; Appendix B, pp. 542-553 of MRID 51086513). According to ILV communications, the ILV soil matrices were not those used in the aquatic field dissipation, but locally sourced soils which had the same texture as those used in the aquatic field dissipation study (Appendix 3, pp. 178, 194 of MRID 51086515). The ILV soil matrices had lower percent clay but higher percent organic matter than the ECM soil matrices. OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs.

The reviewer noted that ECM soil characterization data was not reported in the method validation report. Soil characterization for the soils used in the ECM method validation study were reviewer-determined based on the Sample ID of the method validation samples and the 0-6 in. soil classification.

6. No ECM representative chromatograms were submitted for the method validation samples. Representative chromatograms were submitted for the laboratory fortification samples of the AFD, but only one matrix per analyte was submitted (Appendix 3, Appendix C, Figures 161-170, pp. 1070-1079; Figures 181-190, pp. 1090-1099; Figures 201-210, pp. 1110-1119; Figures 221-230, pp. 1130-1139; Figures 241-250, pp. 1150-1159; Figures 261-270, pp. 1170-1179 of MRID 51086513). The reviewer’s evaluation of the specificity of the method for the ECM was based on chromatograms submitted for the laboratory fortification samples of the AFD.
7. The DER included ECM data from the method validation samples and aquatic field dissipation laboratory fortification samples (p. 34; Appendix 3, Tables 16-27, pp. 383-394; Tables 45-56, pp. 416-427; Appendix 3, Appendix B, pp. 542-553 of MRID 51086513). Soil samples used for the aquatic field dissipation laboratory fortification samples were mostly the same as those of the method validation and were taken from the 0-6 in. soil core, although several were taken from the 6-12 in. soil core (Appendix 3, Appendix B, pp. 798-859). Data from the aquatic field dissipation laboratory fortification samples was included in the DER since the only submitted representative chromatograms were from the aquatic field dissipation laboratory fortification samples.
8. No reagent blank was included in the ECM.



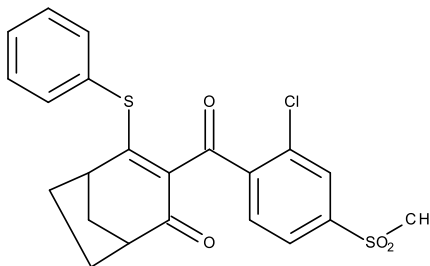
9. The ILV reported that the ECM was validated for all analytes in the first trial; however, according to the communication log, the validation was “re-performed and analyzed against matrix-matched standards” and the “first attempt...[was]...repeated” (p. 15; Appendix 3, pp. 188, 198). The number of ILV trials required to validate the ECM should be clear. The reviewer noted that, even with a re-performance, the number of trials would have still been acceptable ( $\leq 3$ ).
10. In the ILV, the sample weight was reported based on wet weight (12.20 g for clay loam soil and 12.99 g for loam soil; pp. 28-29 of MRID 51086515). According to the communication log, the ECM sample weight was not corrected for moisture content (Appendix 3, p. 196 of MRID 51086513). This deviation did not appear to affect the validity of the results.
11. The reviewer noted that a minor contaminant at RT *ca.* 2.25 min. was observed in ILV representative chromatograms of 1315P-070 and 1315P-683 (Figures 10-12, pp. 79-81; Figure 32, p. 101; Figures 52-54, pp. 121-123; Figure 74, p. 143 of MRID 51086515). The reviewer proposed that this contaminant could have been the metabolite 1315P-570 since it occurred at RT 2.25 min.
12. The determinations of the LOD and LOQ in the ECM and ILV were not based on procedures as defined in 40 CFR Part 136 (pp. 27, 34; Appendix 3, pp. 347-348; Appendix 3, Appendix B, pp. 542-553 of MRID 51086513; pp. 15-17, 36-39 of MRID 51086515). No justification or calculations were provided to support the LOQ in the ECM or ILV. In the ECM, the LOD was reported without justification. In the ILV, the LOD was calculated using the following equation:  $LOD = (3 \times (SN_{ctl}) / (Res_{PLS}) \times Conc_{LS} \times DF_{ctl})$ , where, LOD is the limit of detection of the analysis,  $SN_{ctl}$  is the mean noise in height of the control samples (or blanks),  $Res_{PLS}$  is the mean response in height of the two low calibration standards (0.100  $\mu\text{g/L}$ ),  $Conc_{LS}$  is the concentration of the low calibration standard, and  $DF_{ctl}$  is the dilution factor of the control samples. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.  
  
Since the reported LOQ of 0.005 mg/kg was not based on procedures defined in 40 CFR Part 136, the reported LOQ is the Lowest Level of Method Validation (LLMV) rather than an LOQ.
13. The storage stability was assessed in the ECM as part of the aquatic field dissipation study.
14. The matrix interferences were observed, and matrix-matched calibration standards were used in the ILV (p. 33 of MRID 51086515). No data was provided. Matrix effect assessment was not included in the ECM.
15. The total time required to complete one set (one matrix) of 25 samples was reported as two calendar days total, with one working day (8 hours) for sample processing and LC/MS/MS analysis performed overnight (4 hours) in the ILV (p. 34 of MRID 51086515).

## V. References

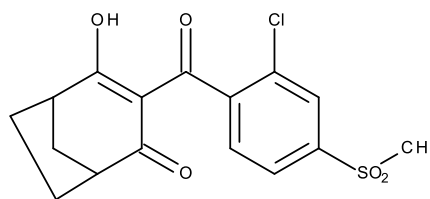
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides>.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319, and Revision 2; 1994 and 2016.

**Attachment 1: Chemical Names and Structures****Benzobicyclon (GWN-10037, SAN1315H, SB-500)**

**IUPAC Name:** 3-(2-Chloro-4-mesylbenzoyl)-2-phenylthiobicyclo[3.2.1]oct-2-en-4-one  
**CAS Name:** 3-[2-Chloro-4-(methylsulfonyl)benzoyl]-4-(phenylthio)bicyclo[3.2.1]oct-3-en-2-one  
**CAS Number:** 156963-66-5  
**SMILES String:** CS(=O)(=O)c1ccc(c(c1)Cl)C(=O)C2=C(C3CCC(C3)C2=O)Sc4ccccc4

**1315P-070 (Metabolite B)**

**IUPAC Name:** 3-(2-Chloro-4-methylsulfonyl-benzoyl)-2-hydroxy-bicyclo[3.2.1]oct-2-en-4-one  
**CAS Name:** Not reported  
**CAS Number:** 126656-88-0  
**SMILES String:** CS(=O)(=O)c1ccc(c(c1)Cl)C(=O)C2=C(C3CCC(C3)C2=O)O



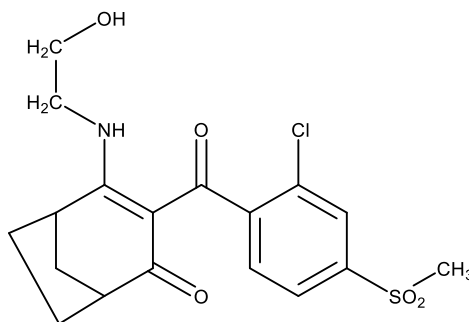
**1315P-076**

**IUPAC Name:** 3-(2-Chloro-4-(methylsulfonyl)benzoyl)-4-((2-hydroxyethyl)amino)bicyclo[3.2.1]oct-3-en-2-one

**CAS Name:** Not reported

**CAS Number:** Not reported

**SMILES String:** CS(C1=CC=C(C(C2=C(NCCO)C(C3)CCC3C2=O)=O)C(Cl)=C1)(=O)=O

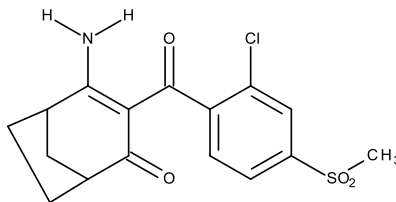
**1315P-570**

**IUPAC Name:** 2-Amino-3-(2-chloro-4-methylsulfonyl-benzoyl)bicyclo[3.2.1]oct-2-en-4-one

**CAS Name:** Not reported

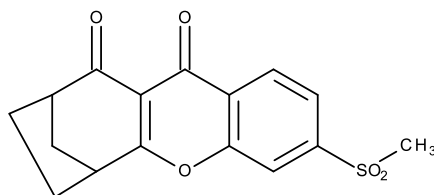
**CAS Number:** Not reported

**SMILES String:** [H]N([H])C1=C(C(=O)C2CCC1C2)C(=O)c3ccc(cc3Cl)S(=O)(=O)C



**1315P-683**

**IUPAC Name:** 3,4-Dihydro-2,4-ethylene-6-methylsulfonyl-1H-xanthane-1,9(2H)-dione  
**CAS Name:** Not reported  
**CAS Number:** Not reported  
**SMILES String:** CS(=O)(=O)c1ccc2c(c1)oc3c(c2=O)C(=O)C4CCC3C4

**1315P-960**

**IUPAC Name:** 2-[[3-(2-Chloro-4-methylsulfonyl-benzoyl)-4-oxo-2-bicyclo[3.2.1]oct-2-enyl]amino]acetic acid  
**CAS Name:** Not reported  
**CAS Number:** Not reported  
**SMILES String:** CS(=O)(=O)c1ccc(c(c1)Cl)C(=O)C2=C(C3CCC(C3)C2=O)NCC(=O)O

