Analytical method for bromadiolone in soil

Reports:	ECM: EPA MRID No. 51185102. Volker, S.F. 2019. Validation of the Environmental Analytical Method 184A for Bromadiolone Residues in Soil. Laboratory Project ID: QA-3078. Report prepared by National Wildlife Research Center, USDA/APHIS/WS, Fort Collins, Colorado, and sponsored and submitted by Liphatech, Inc., Milwaukee, Wisconsin; 86 pages. Final report issued September 30, 2019 (Submitter/Sponsor signatures dated November 27, 2019). ILV: EPA MRID No. 51185103. Whiting, S. 2019. Independent Laboratory Validation of "Determination of Bromadiolone Residues in Soil". Eurofins Study No.: 88377. Report prepared by Eurofins EAG Agroscience, LLC, Columbia, Missouri, and sponsored and submitted by Liphatech, Inc., Milwaukee, Wisconsin; 87 pages. Final report issued June 11, 2019.
Document No.:	MRIDs 51185102 & 51185103
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR Part 160) Good Laboratory Practices (GLP; p. 3 of MRID 51185102). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the Quality Assurance statement. ILV: The study, excluding characterization of the test substance and internal standard, was conducted in accordance with USEPA FIFRA (40 CFR Part 160) GLP, which are compatible with OECD GLP [ENV/MC/CHEM (98) 17; p. 3 of MRID 51185103]. Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-4, 6). A statement of the authenticity of the study report was also included with the Quality Assurance statement. A Summary of QA Audits for Agvise Laboratories (soil characterization) was provided (p. 5)
Classification:	This analytical method is classified as **supplemental**. The LOQ (10 µg/kg) is greater than the toxicological level of concern in soil (2.8 µg/kg). While the ILV was not conducted independently, the method was shown to be robust under a variety of laboratory conditions. No 10×LOQ representative chromatograms were provided in the ECM. The ECM report should have been updated with the precautions for stock and calibration solution storage and more information regarding the use of the glassware cleaning procedure.
PC Code:	112001
Reviewer:	A'ja Duncan, Ph.D. Chemist Signature: AJA DUNCAN Digitally signed by AJA DUNCAN Date: 2021.04.22 07:58:10 -04'00' Date: 4/22/2020
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist Signature: Lisa Muto Date: 07/30/2020
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Date: 07/30/2020

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

Executive Summary

This analytical method, National Wildlife Research Center (NWRC) Analytical Method 184A, is designed for the quantitative determination of bromadiolone at 10 µg/kg in soil using LC/MS/MS. The LOQ is greater than the toxicological level of concern in soil (*i.e.,* 2.8 µg ai/kg-soil). The ECM and ILV validated the method using slightly different sandy clay loam soil matrices, one per study. Since the soil type used by the ECM and ILV were similar, it could not be determined if the ILV soil matrix was the most difficult soil matrix with which to validate the method.

The ILV validated the method with the first trial as written with minor modifications to the sample processing procedure (shaking/mixing steps excluded or performed longer) and insignificant modifications to the analytical parameters and equipment. No critical steps were noted by the ILV, but two method deviations were documented and reported as having no impact on the integrity of the study. Sponsor-facilitated communications occurred between the ECM and ILV staff, which indicates the ILV was not conducted independently. While these communications did not involve method performance issues, important additional method information was provided to the ILV staff. Since the method was shown to be robust under a variety of laboratory conditions, the lack of ILV independence was considered less of a major deficiency.The ECM report should have been updated with the precautions for stock and calibration solution storage and more information regarding the use of the prescribed intensive glassware cleaning procedure.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for bromadiolone; however, no 10×LOQ representative chromatograms were provided in the ECM.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Bromadiolone	51185102[1]	51185103[2]		Soil	30/09/2019	Liphatech, Inc.	LC/MS/MS	10 µg/kg

1 In the ECM, the sandy loam soil (Sample ID: MSL-PF 0-6"; pH 6.6 (1:1 soil:water ratio); 63% sand, 17% silt, 20% clay; 3.5% organic matter) was characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 8; Appendix IV, p. 68 of MRID 51185102). The soil texture was determined to be sandy clay loam by the reviewer using USDA-NRCS technical support tools.

2 In the ILV, the sandy clay loam soil (Sample ID: MSL-PF; pH 6.6 (1:1 soil:water ratio); 59% sand, 20% silt, 21% clay; 3.8% organic matter) was obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 13; Appendix III, p. 68 of MRID 51185103).

I. Principle of the Method

Samples (10 g, dry weight) of soil were fortified at 0.01 and 0.10 mg bromadiolone/kg soil (fortification solution volume and concentration not specified), as necessary, and with 150 µL of 40X Surrogate Stock (5000 ng/mL d_5-bromadiolone in acetonitrile), then extracted with 12.0 mL of acetonitrile and 2 g of NaCl for 15 minutes on a flatbed shaker (*ca*. 300 rpm) then centrifuged (3500 RCF for 2 minutes; p. 9; Appendix V, pp. 70-71, 74 of MRID 51185102). The extraction solution was transferred into a 15-mL polypropylene tube via a 10-mL syringe fitted with a 25-mm diameter/0.45-µm glass fiber filter. In a separate 15-mL polypropylene tube, a 5-mL aliquot of the filtered extract was mixed with 2.5 mL of 0.1% formic acid. An aliquot (3 mL) of the diluted extract was applied to a Strata-X solid phase extraction (SPE) cartridge (60 mg/ 3 mL) which was pre-conditioned with 1 mL each of acetonitrile then 0.1% aqueous formic acid. The cartridge was washed with 1 mL wash solution (methanol:ultra-pure deionized water in 0.1% formic acid (15:85; v:v), then the analyte was eluted with 3 mL of acetone into a 15-mL polypropylene tube. The eluate was evaporated to dryness under a gentle flow of nitrogen in a 50-60°C nitrogen evaporator. The sample was reconstituted with 0.800 mL of acetonitrile with vortex mixing for 4-5 seconds, then 0.200 mL of 0.1% aqueous formic acid was added via vortex mixing for 4-5 seconds prior to analysis.

Samples were analyzed for bromadiolone using an Agilent 1290 UPLC coupled to an Agilent 6470 Triple Quadrupole MS/MS equipped with electrospray ionization (ESI) in the negative ion, multiple reaction monitoring (MRM) mode (p. 9; Appendix V, pp. 75-76 of MRID 51185102). The following LC conditions were used: Waters Xbridge BEH C18 column (2.1 mm x 50 mm, 2.5 µm; column temperature 45°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.00-0.50 min. 60:40, 2.00-2.50 min. 0:100, 2.51-3.00 min. 60:40], MS temperature 300°C, and injection volume of 10 µL. Two ion pair transitions were monitored (primary and confirmatory, respectively) for bromadiolone: *m/z* 525.1→250.0 and *m/z* 525.1→180.9. One ion transition was monitored for d_5-bromadiolone: *m/z* 529.9→254.9. Observed retention time was *ca*. 1.49-1.5 minutes for bromadiolone and d_5-bromadiolone.

The ILV performed the ECM method as written, except for modifications to the sample processing procedure (shaking/mixing steps excluded or performed longer) and the analytical parameters and equipment (pp. 12, 14-18, 23-24; Appendix I, pp. 45-51 of MRID 51185103). Samples were analyzed for bromadiolone using UPLC tandem mass spectrometric (MS/MS) detection with an Applied Biosystems/Sciex API5500 Q-Trap with Waters Acquity Column Manager. The LC/MS/MS parameters were the same as those of the ECM, except that MS temperature was 650°C and the injection volume was reduced to 1.0 µL. Four ion pair transitions were monitored (primary, confirmatory 1, confirmatory 2, and confirmatory 3, respectively) for bromadiolone: *m/z* 525.2→250.0, *m/z* 525.2→181.0, *m/z* 527.1→250.1, and *m/z* 527.1→181.0. The primary and confirmatory 1 monitored ion transitions of the ILV were similar to those of the ECM. Four ion pair transitions were monitored (primary, confirmatory 1, confirmatory 2, and

confirmatory 3, respectively) for d_5-bromadiolone: *m/z* 530.2→255.0, *m/z* 530.2→181.0, *m/z* 532.2→255.0, and *m/z* 532.2→181.0. Expected retention time was *ca*. 1.7 minutes for bromadiolone and d_5-bromadiolone. Results were calculated based on the ratio of the analyte and internal standard (Appendix V, pp. 77-78).

The method Limit of Quantification (LOQ) for bromadiolone in soil was reported as 0.01 mg/kg in the ECM and ILV (pp. 8, 11-13; Appendix IV, pp. 61-62, 65; Appendix V, p. 81 of MRID 51185102; pp. 11-12, 22-23; Table 3, p. 29 of MRID 51185103). The LOQ was calculated in the ECM as 0.000209 mg/kg for the quantitation ion transition; confirmation ion transition result was not reported. Additionally, LOQs were calculated in the ILV as 0.00460 and 0.00939 mg/kg for the quantitation and confirmation ion transitions, respectively. The Limits of Detection (LODs) in soil were calculated in the ILV as 0.00153 and 0.00313 mg/kg for the quantitation and confirmation ion transitions, respectively. The LOD was calculated in the ECM as 0.000063 mg/kg for the quantitation ion transition; confirmation ion transition result was not reported.

II. Recovery Findings

ECM (MRID 51185102): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of bromadiolone in one soil matrix at the LOQ (0.01 mg/kg) and 10×LOQ (0.1 mg/kg; Table 2, p. 16; Table 5, p. 18; Appendix V, p. 82). Confirmation ion transition recoveries were not reported; confirmation ion transition recoveries were reviewer-calculated using quantitation ion recoveries (Table 2) and confirmation ion transition qualifier match % (Table 5; see DER Attachment 2). Recovery results of the quantitative and confirmatory ion transitions were comparable. The sandy loam soil (Sample ID: MSL-PF 0-6"; pH 6.6 (1:1 soil:water ratio); 63% sand, 17% silt, 20% clay; 3.5% organic matter) was characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 8; Appendix IV, p. 68). The soil texture was determined to be sandy clay loam by the reviewer using USDA-NRCS technical support tools.

ILV (MRID 51185103): Mean recoveries and RSDs met requirements for analysis of bromadiolone in one soil matrix at the LOQ (0.01 mg/kg) and 10×LOQ (0.1 mg/kg; Table 1, p. 27). Recovery results of the quantitative and confirmatory ion transitions were comparable. The sandy clay loam soil (Sample ID: MSL-PF; pH 6.6 (1:1 soil:water ratio); 59% sand, 20% silt, 21% clay; 3.8% organic matter) was obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 13; Appendix III, p. 68). The method was validated by the ILV with the first trial as written with minor modifications to the sample processing procedure (shaking/mixing steps excluded or performed longer) and insignificant modifications to the analytical parameters and equipment (pp. 23-24; Appendix VI, p. 81). No critical steps were noted by the ILV, but two method deviations were documented: 1) the modifications to the sample processing procedure; and 2) not sonicating the bromadiolone reference standard when preparing the stock solution for the calibration standards (Appendix I, pp. 62-63). Both method deviations were reported as having no impact on the integrity of the study.

Table 2. Initial Validation Method Recoveries for Bromadiolone in Soil[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Clay Loam Soil						
Quantitation ion transition						
Bromadiolone	0.01 (LOQ)	7	103-106	103	1.1	1.1
	0.10	7	101-104	102	1.1	1.1
Confirmation ion transition[3]						
Bromadiolone	0.01 (LOQ)	7	95-106	102	3	3
	0.10	7	101-105	103	1	1

Data (uncorrected results, p. 10; Appendix V, pp. 77-79) were obtained from Table 2, p. 16; Table 5, p. 18; Appendix V, p. 82 of MRID 51185102.

1 The sandy loam soil (Sample ID: MSL-PF 0-6"; pH 6.6 (1:1 soil:water ratio); 63% sand, 17% silt, 20% clay; 3.5% organic matter) was characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 8; Appendix IV, p. 68). The soil texture was determined to be sandy clay loam by the reviewer using USDA-NRCS technical support tools.

2 Two ion pair transitions were monitored (primary and confirmatory, respectively) for bromadiolone: *m/z* 525.1→250.0 and *m/z* 525.1→180.9.

3 Confirmation ion transition recoveries were not reported; confirmation ion transition recoveries provided above were reviewer-calculated using quantitation ion recoveries (Table 2, p. 16) and confirmation ion transition qualifier match % (Table 5, p. 18; see DER Attachment 2). Means, standard deviations, and RSDs were reviewer-calculated using that data. Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Bromadiolone in Soil[1]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Clay Loam Soil						
Quantitation ion transition						
Bromadiolone	0.01 (LOQ)	5	95-106	102	4.0	4.0
	0.10	5	98-105	103	2.9	2.8
Confirmation ion transition 1						
DCSA	0.01 (LOQ)	5	84-105	98	8.3	8.5
	0.10	5	100-105	103	2.3	2.2

Data (uncorrected results, p. 20) were obtained from Table 1, p. 27 of MRID 51185103.

1 The sandy clay loam soil (Sample ID: MSL-PF; pH 6.6 (1:1 soil:water ratio); 59% sand, 20% silt, 21% clay; 3.8% organic matter) was obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 13; Appendix III, p. 68). The soil texture was verified by the reviewer using USDA-NRCS technical support tools.

2 Four ion pair transitions were monitored (primary, confirmatory 1, confirmatory 2, and confirmatory 3, respectively) for bromadiolone: *m/z* 525.2→250.0, *m/z* 525.2→181.0, *m/z* 527.1→250.1, and *m/z* 527.1→181.0; the primary and confirmatory 1 monitored ion transitions of the ILV were similar to those of the ECM. Results were only reported form the primary and confirmatory 1 monitored ion transitions.

III. Method Characteristics

The LOQ for bromadiolone in soil was reported as 0.01 mg/kg in the ECM and ILV (pp. 8, 11-13; Appendix IV, pp. 61-62, 65; Appendix V, p. 81 of MRID 51185102; pp. 11-12, 22-23; Table 3, p. 29 of MRID 51185103). The LOQ was justified as the fortification level at which acceptable accuracy and precision (mean 70 to 110% and RSD ≤20%) was obtained. In the ECM, the LOD and LOQ were calculated in the ECM as 3xs and 10xs the average baseline noise (measured peak to peak) observed in the control soil, respectively. The calculated LOQ and LOD were 0.000209 mg/kg and 0.000063 mg/kg, respectively, for the quantitation ion transition; confirmation ion transition results were not reported. In the ILV, the LOD was calculated using the following equation:

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

Where, $t_{0.99}$ is the one-tailed t statistic for n = 5 (3.747) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. The LODs in soil were calculated as 0.00153 and 0.00313 mg/kg for the quantitation and confirmation ion transitions, respectively. Additionally, LOQs were calculated in the ILV as 3 × LOD (calculated) which was equivalent to 0.00460 and 0.00939 mg/kg for the quantitation and confirmation ion transitions, respectively. No comparisons to background levels were reported to justify the LOQ and LOD for the method in the ILV.

Calculated ECM and ILV LOQs supported the stated method LOQ.

Table 4. Method Characteristics

		Bromadiolone
Limit of Quantitation (LOQ)	ECM	0.01 mg/kg (method)
		0.000209 mg/kg (Q, calc)[1]
	ILV	0.01 mg/kg (method)
		0.00460 mg/kg (Q, calc) 0.00939 mg/kg (C, calc)
Limit of Detection (LOD)	ECM	0.000063 mg/kg (Q, calc)[1]
	ILV	0.00153 mg/kg (Q, calc) 0.00313 mg/kg (C, calc)
Linearity (calibration curve r and concentration range)	ECM[1,2]	r = 0.9995 (Q) 2.42-244 ng/mL
	ILV[3]	r = 0.99947968 (Q) r = 0.99942468 (C) 2.50-250 ng/mL
Repeatable	ECM[4,5]	Yes for LOQ and 10×LOQ in one characterized sandy clay loam soil matrices
	ILV[6,7]	
Reproducible		Yes for 0.01 mg/kg (LOQ) and 0.10 mg/kg in sandy clay loam soil
Specific	ECM	Yes, matrix interferences were <1% of the LOQ (based on quantified residues). **No** 10×LOQ representative chromatograms were provided.
	ILV	Yes, no matrix interferences were observed. Minor baseline noise affected peak integration.

Data were obtained from pp. 8, 11-13; Appendix IV, pp. 61-62, 65; Appendix V, p. 81 (LOQ/LOD); pp. 10-11 (linearity data); Table 2, p. 16; Table 5, p. 18; Appendix V, p. 82 (recovery data); Figure 1, p. 19 (calibration curves); Figures 2-5, pp. 20-23 (chromatograms) of MRID 51185102; pp. 11-12, 22-23; Table 3, p. 29 (LOQ/LOD); Table 1, p. 27 (recovery data); Figure 1, p. 31; Figure 7, p. 37 (calibration curves); Figures 2-12, pp. 32-42 (chromatograms); Appendix IV, p. 71 (linearity data) of MRID 51185103; DER Attachment 2. Q = quantitative ion transition; C = confirmatory ion transition.

1 Only quantitation ion transition results reported.

2 Quadratic equation used because correlation at lower concentrations was not good with a linear regression equation (Table 1, p. 15 of MRID 51185102). Reported r value was reviewer-calculated from r^2 value reported in the study reports (Figure 1, p. 19 of MRID 51185102; DER Attachment 2). Values were reported to four significant figures.

3 Linear regression equation used.

4 In the ECM, the sandy loam soil (Sample ID: MSL-PF 0-6"; pH 6.6 (1:1 soil:water ratio); 63% sand, 17% silt, 20% clay; 3.5% organic matter) was characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 8; Appendix IV, p. 68 of MRID 51185102). The soil texture was determined to be sandy clay loam by the reviewer using USDA-NRCS technical support tools.

5 Based on quantitation and confirmation ion transition recoveries. Confirmation ion transition recoveries were not reported in the study report but were reviewer-calculated using quantitation ion recoveries (Table 2, p. 16) and confirmation ion transition qualifier match % (Table 5, p. 18; see DER Attachment 2). Means, standard deviations, and RSDs were reviewer-calculated using that data. Rules of significant figures were followed

6 In the ILV, the sandy clay loam soil (Sample ID: MSL-PF; pH 6.6 (1:1 soil:water ratio); 59% sand, 20% silt, 21% clay; 3.8% organic matter) was obtained from and characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 13; Appendix III, p. 68 of MRID 51185103). The soil texture was verified by the reviewer using USDA-NRCS technical support tools.

7 The ILV validated the method for bromadiolone in the tested soil with the first trial as written with minor modifications to the sample processing procedure (shaking/mixing steps excluded or performed longer) and insignificant modifications to the analytical parameters and equipment (pp. 23-24; Appendix VI, p. 81 of MRID 51185103). No critical steps were noted by the ILV, but two method deviations were documented: 1) the modifications to the sample processing procedure; and 2) not sonicating the bromadiolone reference standard when preparing the stock solution for the calibration standards (Appendix I, pp. 62-63). Both method deviations were reported as having no impact on the integrity of the study.

IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136 (pp. 8, 11-13; Appendix IV, pp. 61-62, 65; Appendix V, p. 81 of MRID 51185102) (pp. 11-12, 22-23; Table 3, p. 29 of MRID 51185103). In the ECM, the LOD and LOQ were calculated as 3x and 10x the average baseline noise (measured peak to peak) observed in the control soil, respectively. The calculated ECM LOQs supported the stated method LOQ. In the ILV, the LOD was calculated using the following equation: LOD = ($t_{0.99}$ × SD), where $t_{0.99}$ is the one-tailed t statistic for n = 5 (3.747) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. Additionally, LOQs were calculated in the ILV as 3 × LOD (calculated). No comparisons to background levels were reported to justify the LOQ and LOD for the method in the ILV; detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. The calculated ILV LOQs supported the stated method LOQ. The reviewer noted that the LOD calculation was not equivalent to the accepted MDL calculation since n < 7.

2. No 10×LOQ representative chromatograms were provided in the ECM.

3. While the ILV was not independent, the method was shown to be robust under a variety of laboratory conditions. The ILV study author (Sara Whitting of Eurofins EAG Agroscience, LLC) communicated with the ECM study author (Steven Volker of National Wildlife Research Center) via the Sponsor Representative (Katherine/Katie Swift of Liphatech, Inc.; p. 1 of MRID 51185102; p. 1; Appendix VI, pp. 79-80 of MRID 51185103). Most of these ECM/ILV Sponsor-facilitated communications occurred prior to the first ILV trial and involved the stability of soil extracts and stock and calibration solutions, laboratory equipment suitability, and necessity of intensive prescribed glassware cleaning procedure. These communications did not involve method performance issues. This communication provided additional guidance to the ILV regarding the importance of the prescribed 4°C storage (see Reviewer's Comment #4) and cautions with the intensive prescribed glassware cleaning procedure (see Reviewer's Comment #5). Due to this communication, the ILV did not implement the glassware cleaning procedure.

   Post-trial, one ECM/ILV Sponsor-facilitated communication occurred regarding the ILV omission of the sonication of the bromadiolone reference standard when preparing the stock solution for the calibration standards (Appendix VI, p. 86 of MRID 51185103). The ECM study staff directed the ILV study author to create a method deviation for this omission since the method stated that the step could be modified but not omitted (see Reviewer's Comment #8). A summary of the email communications between the ILV study author, ECM study staff, and Sponsor was provided (p. 24; Appendix VI, pp. 76-87 of MRID 51185103). Other communication involved method and test material exchanges between the ILV study author and Sponsor, as well as ILV trial outcome and final ILV report exchange. The method deviations from the protocol were initiated by the ILV. The ILV method deviations were successful for pre- and post-trials, which indicate the ECM method is successful under variable laboratory conditions.

4. It could not be determined if the ILV was provided with the most difficult soil matrix with which to validate the method since only one characterized soil matrix was tested. The ILV soil matrix was characterized as sandy clay loam soil (21% clay), but this was the same soil texture matrix as that of the ECM (20% clay; p. 8; Appendix IV, p. 68 of MRID 51185102; p. 13; Appendix III, p. 68 of MRID 51185103). The reviewer also noted that the Sample IDs of the ECM and ILV soil matrices were very similar.

   The reviewer noted that the texture of the ECM soil was reported as sandy loam soil (63% sand, 17% silt, 20% clay) using USDA soil texture classification by Agvise Laboratories (p. 8; Appendix IV, p. 68 of MRID 51185102). However, the reviewer determined that the soil texture was sandy clay loam using USDA-NRCS technical support tools. Throughout the DER, the soil texture was reported as sandy clay loam.

5. No ECM stability data for the stock and calibration solutions were reported; however, these solutions were to be stored at 4°C (Appendix V, pp. 71-73 of MRID 51185102). The ILV staff submitted questions about the stability of the standards and extracts to which the ECM staff responded that 6 months at 4°C was the standard for stock solutions

of anticoagulant rodenticides but no data was necessary for the extract stability since the validation was completed within 3 days (Appendix VI, pp. 79-80 of MRID 51185103). In this correspondence, the ECM staff cautioned against stock solution and standard storage at <4°C due to the possibility of precipitating bromadiolone. The ECM staff also noted in this correspondence that the storage at 4°C was necessary to prevent the evaporation of the solvent and consequential increase in test material concentration. The ECM report should be updated with these precautions for stock and calibration solution storage.

6. The ILV staff submitted questions about the intensive prescribed glassware cleaning procedure in the method to which the ECM staff responded that the procedure was not necessary if the ILV laboratory has not had problems with contamination (Appendix I, p. 46; Appendix VI, pp. 79-80 of MRID 51185103). Furthermore, the ECM staff reported that their initial failed attempt to validate the method was attributed to residual detergents in the glassware. Due to this communication, the ILV staff did not implement the glassware cleaning procedure. The ECM report should have been updated with more information regarding the use of the glassware cleaning procedure.

7. It was unclear if the provided bromadiolone Certificate of Analysis (COA) corresponded to the bromadiolone test material used in the ILV (Appendix II, p. 65 of MRID 51185103). No expiration date was reported in the bromadiolone COA, only an analysis date (March 20, 2013). The ILV reported that the bromadiolone test material should not be used beyond March 13, 2021, but this date is difficult to attribute to the COA analysis date (p. 12). The reviewer noted that the ILV reported that the first "reference material" provided to them by the Sponsor had an expired COA (communication dated March 5, 2019; Appendix VI, p. 77). A new reference/test material with COA was sent (communication dated March 11, 2019).

8. Two ILV method deviations were documented: 1) the modifications to the sample processing procedure (shaking/mixing steps performed longer or not documented); and 2) not sonicating the bromadiolone reference standard when preparing the stock solution for the calibration standards (Appendix I, pp. 62-63 of MRID 51185103). Both method deviations were reported as having no impact on the integrity of the study. Due to the method importance of the sonication of the bromadiolone reference standard when preparing the stock solution for the calibration standards, the ILV demonstrated the fact that the omission had no impact on the solubility of bromadiolone by preparing a second bromadiolone standard solution (with sonication) for fortifications and a QC check.

9. In the ILV, no significant matrix effects were observed (<20%; p. 22; Table 2, p. 28 of MRID 51185103).

10. In the ILV, it was reported that additional criteria from the method were met: 1) the recovery of d_5-bromadiolone surrogate analyte was ≥20% when compared to the average d_5-bromadiolone peak area response observed in the calibration standards; and 2) the peak area response ratio of the confirmation transition divided by the quantitation transition was ±20% of the average ratio determined for the calibration standards (pp. 18-

22 of MRID 51185103). Also, the 125 ng/mL (0.0750 mg/kg equivalent) bromadiolone quality control standard had back-calculated accuracies of 101% (Q) and 103% (C).

11. The time requirement for the method was reported in the ILV as *ca*. 6.25 person-hours for preparation of the standards and solutions, *ca*. 1.5 person-hours for weighing samples and NaCl, *ca*. 5.5 person-hours for sample fortification and extraction, and *ca*. 4 hours for a 28-injection run sequence (p. 24 of MRIRD 51185103). Overall, a total of *ca*. 11 hours or 2 business days was required to complete one set of 13 samples.

12. The reviewer calculated the toxicological level of concern (LOC) of 2.8 µg ai/kg-soil as follows:

    a. Using the mammalian acute oral LD_{50} (0.6 mg ai/kg-bw; MRID 00241703), the LD_{50}-sq ft method in the T-REX model (version 1.5.2; USEPA 2012*a*), and the acute LOC, the application rate at the acute risk LOC (*i.e.*, where the highest acute mammalian risk quotient equals the LOC of 0.5) was calculated at 0.00095 lb ai/A.
    b. This application rate was divided by a soil depth of 1 inch (0.0254 m), which is considered relevant to a rodenticide spill (EFED ECM guidance recommends a default 6-inch depth that is relevant to terrestrial plant toxicity studies (USEPA 2012)) and converted to metric units as follows:

$$\frac{0.00095\ lb\ ai}{acre} \times \frac{4.536 \times 10^{8})\ \mu g}{1\ lb} \times \frac{1\ acre}{4047\ m^2} \times \frac{1}{0.0254\ m} \times \frac{1\ m^3}{1000\ L} = 4.2\ \frac{\mu g\ ai}{L\ soil}$$

    c. Using a default soil bulk density of 1.5 kg-soil/L (based on ECM guidance; USEPA 2012 *a*), the toxicological LOC from the above equation was converted to a value with units of µg ai/kg-soil:

$$\frac{4.2\ \mu g\ ai}{L\ soil} \times \frac{1\ L}{1.5\ kg\ soil} = 2.8\ \frac{\mu g\ ai}{kg\ soil}$$

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.

U.S. Environmental Protection Agency. 2012*a*. T-REX Version 1.5 User's Guide for Calculating Pesticide Residues on Avian and Mammalian Food Items. Office of Pesticide Programs, Environmental Fate and Effects Division. March 22, 2012.

U.S. Environmental Protection Agency. 2016. Bromadiolone Problem Formulation for Environmental Fate, Ecological Risk, Endangered Species, and Drinking Water Exposure Assessments in Support of Registration Review. Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, Environmental Fate and Effects Division. DP 429381.

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

Bromadiolone

IUPAC Name: 3-[3-[4-(4-Bromophenyl)phenyl]-3-hydroxy-1-phenylpropyl]-4-hydroxychromen-2-one
CAS Name: 3-[3-(4'-Bromo-4-biphenylyl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-chromen-2-one
CAS Number: 28772-56-7
SMILES String: Not found