Analytical method for hydramethylnon (BAS 315 I) and its metabolites M12, M11, M6, M1a, and M1b in soil

Reports: ECM: EPA MRID No.: 50862503. DeVellis, S.R. 2019. Final Report. Validation of the Analytical Method for the Determination of BAS 315 I and Metabolites in Soil. BASF Document Registration No.: 2018/7005522. BASF Study No.: 828400. Smithers Viscient Study No.: 986.6267. Report prepared by Smithers Viscient, Wareham, Massachusetts, sponsored by BASF Corporation, Research Triangle Park, North Carolina, and submitted by BASF c/o Study Monitor at Landis International, Valdosta, Georgia; 199 pages. Final report issued May 30, 2019.

ILV: EPA MRID No. 50862504. Sharp, S. 2019. Final Report. Independent Laboratory Validation of the Analytical Method for the Determination of BAS 315 I and Metabolites in Soil. BASF Registration Document No.: 2018/7005701. ADPEN Study No.: 18G0604. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, and sponsored and submitted by BASF Crop Protection, Research Triangle Park, North Carolina; 453 pages. Final report issued June 10, 2019.

Document No.: MRIDs 50862503 & 50862504

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with EPA FIFRA (40 CFR Part 160) and OECD Good Laboratory Practice (GLP) standards (p. 3 of MRID 50862503). Signed and dated No Data Confidentiality, GLP, Quality Assurance, Authenticity statements were provided (pp. 2-4). An Authenticity statement was included with the Quality Assurance statement.
ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 50862504). Signed and dated No Data Confidentiality, GLP, Quality Assurance, Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as **Supplemental**. ILV linearity was unsatisfactory for all ions of all analytes, except for M12. ECM linearity was unsatisfactory in the Original ECM for M12, M11, and M1a and in the Updated ECM for M6. For M11, the specificity of the method was not supported by ILV or ECM representative chromatograms. The specificity of the method was not supported by ILV representative chromatograms of M1b. The LOQ is greater than the lowest toxicological level of concern in soil for hydramethylnon. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. ILV sample fortification and sample processing were summarized without many details.

PC Code: 118401

EFED Final Reviewer:	A'ja Duncan, Ph.D. Chemist	Signature: Duncan, Aja Digitally signed by Duncan, Aja Date: 2019.10.28 12:16:50 -04'00' Date: 10/28/2019
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Lisa Muto Date: 08/30/2019
	Mary Samuel, M.S., Environmental Scientist	Signature: Marysamuel Date: 08/30/2019

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, BASF Registration Document No. 2018/7005522, is designed for the quantitative determination of hydramethylnon (BAS 315 I) and its metabolites M12, M11, M6, M1a, and M1b in soil at the LOQ of 50.0 µg/kg using LC/MS/MS. The LOQ is greater than the lowest toxicological level of concern in soil for hydramethylnon (>30 µg/kg; USEPA 2018). In the ECM, the method was performed using characterized loamy sand soil for all analytes (Original ECM), then BAS 315 I and metabolite M6 were re-validated in characterized sandy loam soil using the more abundant carbon isotope (Updated ECM). This updated methodology was more robust and considered the official methodology. The ILV only validated the ECM with the Updated ECM for BAS 315 I and M6, not the Original ECM for BAS 315 I and M6. The ILV was performed using the same characterized sandy loam soil which was used in the ECM. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The ILV successfully validated the ECM in the first trial. In the ILV, the sample fortification and sample processing were summarized without many details. The ECM was reportedly performed as written, except for the use of a mechanical shaker instead of a shaker table, the addition of a calibration standard fortified at 30% of the LOQ, and minor LC/MS instrument and parameter modifications; however, it was not specifically stated that the extractions were performed twice in the sample processing procedures. The ILV recommendations for the ECM included 1) stability evaluation of extracts and standards; 2) the inclusion of a method LOD; 3) discussion of LC/MS/MS optimization for the analysis of compounds containing multiple isomers (M1b, M12, M6, and M1a); 4) the adjustment of fortification volumes of M1a to be ≤10% of the sample weight; and 5) the expansion of the calibration range to include a standard fortified at 30% of the LOQ as the lowest calibration standard, instead of 50% of the LOQ. The ECM included the ILV recommendations which were not necessary for the successful validation but will enhance the reproducibility of the ECM. All submitted ECM and ILV data pertaining to precision, repeatability, and reproducibility was acceptable. ILV linearity was unsatisfactory for all ions of all analytes, except for M12. ECM

linearity was unsatisfactory in the Original ECM for M12, M11, and M1a and in the Updated ECM for M6. For M11, the specificity of the method was not supported by ILV representative chromatograms since the analyte peak was very small compared to baseline noise and only distinguishable from baseline noise by retention time or by ECM representative chromatograms due to significant baseline noise of varied elevation interfered with analyte peak integration and attenuation. ECM and ILV data pertaining to specificity was acceptable for all other analytes, except for ILV representative chromatograms of M1b since enhanced baseline noise at analyte retention time appeared to make quantitation of the analyte peak arbitrary.

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						
Hydramethylnon (BAS 315 I)	50862503 (Original ECM[1])	None submitted		Soil	30/05/2019	BASF Corporation	LC/MS/MS	50.0 µg/kg
M12		50862504[3]						
M11								
M6		None submitted						
M1a		50862504[3]						
M1b								
Hydramethylnon (BAS 315 I)	50862503 (Updated ECM[2])							
M6								

1 In the ECM, all six analytes were fortified in loamy sand soil (SMV Lot No. 012616A; 78% sand, 18% silt, 4% clay; pH 6.8 in 1:1 soil:water ratio; 4.9% organic matter – Walkley Black) from Rochester, Massachusetts (USDA soil texture classification; p. 19; Appendix 3, p. 129 of MRID 50862503). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 In the ECM, BAS 315 I and metabolite M6 were re-validated using the more abundant carbon isotope (^{12}C; p. 26; Appendix 4, pp. 132-136 of MRID 50862503). This updated methodology was more robust and considered the official methodology. For the updated methodology for BAS 315 I and M6, sandy loam soil (SMV Lot No. RMN-SL-PF 0-6" 5-29-18; 75% sand, 18% silt, 7% clay; pH 6.6 in 1:1 soil:water ratio; 3.4% organic matter – Walkley Black) from Rochester, Massachusetts, was used (USDA soil texture classification; p. 19; Appendix 3, p. 130; Appendix 4, p. 132). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

3 The ILV performed the ECM with the Updated ECM for BAS 315 I and M6. The sandy loam soil (Control Matrix No. RMN-SL-PF; SMV Lot No. RMN-SL-PF 0-6" 5-29-18; 75% sand, 18% silt, 7% clay; pH 6.6 in 1:1 soil:water ratio; 3.4% organic matter – Walkley Black) from Rochester, Massachusetts, was used in the study (USDA soil texture classification; p. 17; Appendix A, p. 215 of MRID 50862504; p. 19; Appendix 3, p. 130 of MRID 50862503). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil matrix was provided by and characterized in the ECM. The ILV soil matrix was the same as that used in the ECM trial of the updated methodology for BAS 315 I and M6.

I. Principle of the Method

Original ECM - BAS 315 I, M12, M11, M6, and M1b - Sample Fortification and Processing

Soil (5.00 g dry wt.) was weighed into a 50-mL Nalgene centrifuge tube and fortified with 250 µL of standard solution of BAS 315 I, M12, M11, M6, and M1b to yield final concentrations of 50.0 and 500 µg/kg (pp. 24-27; Figure 1, p. 61 of MRID 50862503). The samples were extracted twice with 20 mL of acetonitrile:purified reagent water (95:5, v:v) via shaking on a shaker table for 20 minutes at 250 rpm. After centrifugation (3000 rpm for 10 minutes), the supernatant was decanted into a 50-mL volumetric flask. The volume of the combined extract was adjusted to 50 mL using acetonitrile:purified reagent water (95:5, v:v). The sample extracts were further diluted into the calibration range using acetonitrile:purified reagent water (50:50, v:v) prior to HPLC/MS/MS analysis**.**

Original ECM - M1a - Sample Fortification and Processing

Soil (5.00 g dry wt.) was weighed into a 50-mL Nalgene centrifuge tube and fortified with 250 µL or 2.50 mL of standard solution of M1a to yield final concentrations of 50.0 and 500 µg/kg (pp. 24-27; Figure 1, p. 61 of MRID 50862503). The samples were extracted twice with 20 mL of acetonitrile via shaking on a shaker table for 20 minutes at 250 rpm. After centrifugation (3000 rpm for 10 minutes), the supernatant was decanted into a 50-mL volumetric flask. The volume of the combined extract was adjusted to 50 mL using acetonitrile. The sample extracts were further diluted into the calibration range using acetonitrile:purified reagent water (50:50, v:v) prior to HPLC/MS/MS analysis**.**

Original ECM - BAS 315 I, M12, M6, and M1b – HPLC/MS/MS

BAS 315 I, M12, M6, and M1b were identified and quantified by LC/MS/MS using a Shimadzu LC-20AD HPLC coupled with an MDS Sciex API 5000 MS (MDS Sciex ESI Turbo V Source) or an AB MDS Sciex 4000 MS (AB MDS Sciex ESI Turbo V Source; pp. 18, 27-29 of MRID 50862503). The following conditions were employed: Agilent Poroshell EC-C8 column (50 mm x 3.0 mm, 2.7 µm particle size; column temperature 40°C) eluted with a gradient mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [time, percent A:B; time 0.50 min. 95.0:5.00, 0.60 min. 50.0:50.0, 3.00-4.00 min. 0.00:100, 4.10-5.00 min. 95.0:5.00] using an injection volume of 50.0 µL and positive (+) ESI ionization MRM scan mode (source temperature 600°C). Analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 496.20→324.10 and *m/z* 496.20→369.10 for BAS 315 I, *m/z* 371.21→159.09 and *m/z* 371.32→199.04 for M12, *m/z* 384.20→364.20 and *m/z* 384.20→151.10 for M6, and *m/z* 511.10→491.10 and *m/z* 511.10→364.10 for M1b (note: *m/z* 496.20→369.10 for BAS 315 I was incorrectly reported as *m/z* 496.20→396.10 for BAS 315 I in the study report - see Product ion spectrum in Figure 2, p. 63, for correct confirmatory ion transition). Expected retention times were 2.03, 2.70, 2.55, and 1.87-1.88 minutes for BAS 315 I, M12, M6, and M1b, respectively.

Updated ECM - BAS 315 I and M6 – Method Re-Evaluation – HPLC/MS/MS

BAS 315 I and metabolite M6 were re-validated using the more abundant carbon isotope (^{12}C; p. 26; Appendix 4, pp. 132-136 of MRID 50862503). This was done in order to provide a more straightforward and efficient method. This updated methodology will be the official methodology in order to provide the most robust methodology for the analysis of samples. Sample fortification and processing was the same as above.

BAS 315 I and M6 were identified and quantified by LC/MS/MS using a Shimadzu LC-20AD HPLC coupled with an AB MDS Sciex 4000 MS (AB MDS Sciex ESI Turbo V Source; Appendix 4, pp. 132, 137-138 of MRID 50862503). The following conditions were employed: Agilent Poroshell EC-C8 column (50 mm x 3.0 mm, 2.7 µm particle size; column temperature 40°C) eluted with a gradient mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [time, percent A:B; time 0.50 min. 95.0:5.00, 0.60 min. 50.0:50.0, 3.00-4.00 min. 0.00:100, 4.10-5.00 min. 95.0:5.00] using an injection volume of 100 µL and positive (+) ESI ionization MRM scan mode (source temperature 650°C). Analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 495.44→323.21 and *m/z* 495.44→368.12 for BAS 315 I and *m/z* 383.19→363.16 and *m/z* 383.19→151.08 for M6 (note: *m/z* 495.44→368.12 for BAS 315 I was incorrectly reported as *m/z* 495.44→386.12 for BAS 315 I in the study report – see Product ion spectrum in Figure 2, p. 63 and Appendix 4, Table 4E, p. 143, for correct confirmatory ion transition). Expected retention times were 1.78 and 2.34 minutes for BAS 315 I and M6, respectively.

Original ECM - M11 – HPLC/MS/MS

M11 was identified and quantified by LC/MS/MS using a Shimadzu LC-20AD HPLC coupled with an MDS Sciex API 5000 MS (MDS Sciex ESI Turbo V Source) or an AB MDS Sciex 4000 MS (AB MDS Sciex ESI Turbo V Source; pp. 18, 29-30 of MRID 50862503). The following conditions were employed: Waters T3 column (100 mm x 4.6 mm, 3.0 µm particle size; column temperature 40°C) eluted with a gradient mobile phase of (A) 0.1% formic acid in water and (B) acetonitrile [time, percent A:B; time 1.00 min. 100:0.00, 1.10 min. 60.0:40.0, 4.00-4.50 min. 0.00:100, 4.60-6.00 min. 100:0.00] using an injection volume of 25.0 µL and positive (+) ESI ionization MRM scan mode (source temperature 600°C). M11 was identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 129.17→69.08 and *m/z* 129.17→70.03. Expected retention time was 2.67 minutes.

Original ECM - M1a – HPLC/MS/MS

M1a was identified and quantified by LC/MS/MS using a Shimadzu LC-20AD HPLC coupled with an MDS Sciex API 5000 MS (MDS Sciex ESI Turbo V Source) or an AB MDS Sciex 4000 MS (AB MDS Sciex ESI Turbo V Source; pp. 18, 31 of MRID 50862503). The following conditions were employed: Agilent Poroshell EC-C8 column (50 mm x 3.0 mm, 2.7 µm particle size; column temperature 40°C) eluted with a gradient mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [time, percent A:B; time 0.50 min. 95.0:5.00, 3.00-4.00 min. 0.00:100, 4.10-5.00 min. 95.0:5.00] using an injection volume of 25.0 µL and positive (+) ESI ionization MRM scan mode (source temperature 600°C). M1a was identified

using two ion transitions (quantitation and confirmation, respectively): *m/z* 511.10→369.10 and *m/z* 511.10→142.20. Expected retention time was 2.59-2.60 minutes.

ILV

In the ILV, the sample fortification and sample processing were summarized without many details (pp. 17, 21, 26-27; Tables 19-22, pp. 48-52 of MRID 50862504). The ECM seemed to be performed as written, except for the use of a mechanical shaker instead of a shaker table, the addition of a calibration standard fortified at 30% of the LOQ, and minor LC/MS instrument and parameter modifications; however, it was not specifically stated that the extractions were performed twice in the sample processing procedures. Analytes were identified and quantified by LC/MS/MS using an Agilent 1290 HPLC (Instrument #25) coupled with an ABSciex 5500 Triple Quad MS. The following conditions were employed for BAS 315 I, M12, M6, and M1b: Agilent Poroshell EC-C8 column (50 mm x 3.0 mm, 2.7 µm particle size; column temperature 40°C) eluted with a gradient mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [time, percent A:B; time 0.00-0.50 min. 95:5, 0.60 min. 50:50, 3.00-4.00 min. 0:100, 4.10-5.00 min. 95:5] using an injection volume of 50 µL and positive (+) ESI ionization MRM scan mode (source temperature 600°C). Analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 495→323 and *m/z* 495→368 for BAS 315 I, *m/z* 371→159 and *m/z* 371→199 for M12, *m/z* 383→363 and *m/z* 383→151 for M6, and *m/z* 511→491 and *m/z* 511→364 for M1b. Expected retention times were *ca*. 1.97, 2.63, 2.53, and 1.81 minutes for BAS 315 I, M12, M6, and M1b, respectively.The following conditions were employed for M11: Waters T3 column (100 mm x 4.6 mm, 3.0 µm particle size; column temperature 40°C) eluted with a gradient mobile phase of (A) 0.1% formic acid in water and (B) acetonitrile [time, percent A:B; time 0.00-1.00 min. 100:0, 1.10 min. 60:40, 4.00-4.50 min. 0:100, 4.60-6.00 min. 100:0] using an injection volume of 25 µL and positive (+) ESI ionization MRM scan mode (source temperature 600°C). M11 was identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 129→69 and *m/z* 129→70. Expected retention time was *ca*. 2.62 minutes. The following conditions were employed for M1a: Agilent Poroshell EC-C8 column (50 mm x 3.0 mm, 2.7 µm particle size; column temperature 40°C) eluted with a gradient mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [time, percent A:B; time 0.50 min. 95:5, 3.00-4.00 min. 0:100, 4.10-5.00 min. 95:5] using an injection volume of 50 µL and positive (+) ESI ionization MRM scan mode (source temperature 600°C). M1a was identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 511→369 and *m/z* 511→142. Expected retention time was *ca*. 2.73 minutes. The LC/MS conditions were generally the same as the ECM with some minor differences in injection volume or MS source temperature. The ECM-modified HPLC/MS/MS ion transitions for BAS 315 I and M6 were used in the ILV. The ILV recommendations for the ECM included 1) stability evaluation for the standards and extracts; 2) the inclusion of a method LOD in addition to the instrument-specific LODs calculated in the ECM; 3) discussion of LC/MS/MS optimization for chromatographic discrepancies which may arise from the analysis of compounds containing multiple isomers (M1b, M12, M6, and M1a – the ILV increased MS dwell time to 200 or 300 milliseconds); 4) the fortification volumes of M1a range 5-50% of sample weight and should be ≤10% of the sample weight; and 5) the calibration range should be expanded to include a standard fortified at 30% of the LOQ as the lowest calibration standard, instead of 50% of the LOQ (pp. 26-27).

LOQ/LOD

The Limit of Quantification (LOQ) for hydramethylnon (BAS 315 I) and its metabolites M12, M11, M6, M1a, and M1b in soil was 50.0 µg/kg in the ECM and ILV (pp. 33, 35, 40; Appendix 4, p. 132 of MRID 50862503; pp. 26-27 of MRID 50862504). In the ECM, the Limit of Determination (LOD) in soil was calculated as 6.34 µg/kg (Q) and 6.58 µg/kg (C) for BAS 315 I, 6.86 µg/kg (Q) and 1.23 µg/kg (C) for M12, 8.50 µg/kg (Q) and 1.96 µg/kg (C) for M11, 5.78 µg/kg (Q) and 9.97 µg/kg (C) for M6, 6.23 µg/kg (Q) and 8.12 µg/kg (C) for M1a, and 1.89 µg/kg (Q) and 2.65 µg/kg (C) for M1b. The LODs were calculated for the Updated ECM for BAS 315 I and M6 as 4.92 µg/kg (Q) and 4.00 µg/kg (C) for BAS 315 I and 6.83 µg/kg (Q) and 5.82 µg/kg (C) for M6. The method LOD for all analytes in soil was reported as 15 µg/kg (30% of the LOQ) in the ILV. In the ECM, the Method Detection Limit (MDL) was calculated as 25.0 µg/kg for all analytes in soil (including the Updated ECM for BAS 315 I and M6).

II. Recovery Findings

ECM (MRID 50862503): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements [means between 70% and 120% and relative standard deviations (RSD) ≤20%] for analysis of hydramethylnon (BAS 315 I), M12, M11, M6, M1a, and M1b at fortification levels of 50.0 µg/kg (LOQ) and 500 µg/kg (10×LOQ) in one loamy sand soil matrix (Tables 1-12, pp. 43-54). BAS 315 I and metabolite M6 were re-validated using the more abundant carbon isotope (^{12}C; p. 26; Appendix 4, pp. 132-136). This updated methodology was more robust and considered the official methodology. Mean recoveries and RSDs were within guideline requirements for analysis of hydramethylnon (BAS 315 I) and M6 at fortification levels of 50.0 µg/kg (LOQ) and 500 µg/kg (10×LOQ) in one sandy loam soil matrix (Appendix 4, Tables 4A-4D, pp. 139-142). All analytes were identified using two ion transitions; performance data (recovery results) for the quantitation and confirmation ion analyses were comparable. The updated methodology for BAS 315 I and M6 produced slightly better performance data based on percent recovery. The loamy sand soil (SMV Lot No. 012616A; 78% sand, 18% silt, 4% clay; pH 6.8 in 1:1 soil:water ratio; 4.9% organic matter – Walkley Black) from Rochester, Massachusetts, was used (USDA soil texture classification; p. 19; Appendix 3, p. 129). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. For the updated methodology for BAS 315 I and M6, sandy loam soil (SMV Lot No. RMN-SL-PF 0-6" 5-29-18; 75% sand, 18% silt, 7% clay; pH 6.6 in 1:1 soil:water ratio; 3.4% organic matter – Walkley Black) from Rochester, Massachusetts, was used (USDA soil texture classification; p. 19; Appendix 3, p. 130; Appendix 4, p. 132). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 50862504): Mean recoveries and RSDs were within guideline requirements for analysis of hydramethylnon (BAS 315 I), M12, M11, M6, M1a, and M1b at fortification levels of 50.0 µg/kg (LOQ) and 500 µg/kg (10×LOQ) in one sandy loam soil matrix (Tables 1-12, pp. 30-41). The ILV performed the ECM with the Updated ECM for BAS 315 I and M6. Analytes were identified using two ion transitions; performance data (recovery results) for the quantitation and confirmation ion analyses were comparable. The sandy loam soil (Control Matrix No. RMN-

SL-PF; SMV Lot No. RMN-SL-PF 0-6" 5-29-18; 75% sand, 18% silt, 7% clay; pH 6.6 in 1:1 soil:water ratio; 3.4% organic matter – Walkley Black) from Rochester, Massachusetts, was used in the study (USDA soil texture classification; p. 17; Appendix A, p. 215 of MRID 50862504; p. 19; Appendix 3, p. 130 of MRID 50862503). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil matrix was provided by and characterized in the ECM. The ILV soil matrix was the same as that used in the ECM trial of the updated methodology for BAS 315 I and M6. The ILV successfully validated the ECM in the first trial (p. 26). In the ILV, the sample fortification and sample processing were summarized without many details (pp. 17, 21, 26-27; Tables 20-22, pp. 50-52). The ECM seemed to be performed as written, except for the use of a mechanical shaker instead of a shaker table, the addition of a calibration standard fortified at 30% of the LOQ, and minor LC/MS instrument and parameter modifications; however, it was not specifically stated that the extractions were performed twice in the sample processing procedures. The ILV recommendations for the ECM included 1) stability evaluation for the standards and extracts; 2) the inclusion of a method LOD in addition to the instrument-specific LODs calculated in the ECM; 3) discussion of LC/MS/MS optimization for chromatographic discrepancies which may arise from the analysis of compounds containing multiple isomers (M1b, M12, M6, and M1a – the ILV increased MS dwell time to 200 or 300 milliseconds); 4) the fortification volumes of M1a range 5-50% of sample weight and should be ≤10% of the sample weight; and 5) the calibration range should be expanded to include a standard fortified at 30% of the LOQ as the lowest calibration standard, instead of 50% of the LOQ. The ECM included the ILV recommendations which were not necessary for the successful validation but will enhance the reproducibility of the ECM (pp. 38-39 of MRID 50862503).

Table 2. Initial Validation Method Recoveries for Hydramethylnon (BAS 315 I) and its Metabolites M12, M11, M6, M1a, and M1b in Soil

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Loamy Sand Soil – Original ECM[1,2]					
	Quantitation Ion Transition					
Hydramethylnon (BAS 315 I)	50.0 (LOQ)	7	82.4-93.1	88.5	4.04	4.56
	500	5	75.4-90.9	81.0	6.03	7.44
M12	50.0 (LOQ)	7	82.0-94.8	89.1	4.37	4.90
	500	5	80.1-86.8	83.5	2.58	3.09
M11	50.0 (LOQ)	7	84.4-101	92.8	5.41	5.83
	500	5	72.0-92.4	80.9	7.81	9.65
M6	50.0 (LOQ)	7	87.1-97.4	92.1	3.68	3.99
	500	5	78.9-88.2	83.7	4.06	4.85
M1a	50.0 (LOQ)	7	89.8-102	96.7	3.97	4.10
	500	5	94.4-98.7	96.2	2.00	2.08
M1b	50.0 (LOQ)	7	81.0-84.6	83.2	1.21	1.45
	500	5	76.0-85.3	80.4	3.49	4.35
	Confirmation Ion Transition					
Hydramethylnon (BAS 315 I)	50.0 (LOQ)	7	87.9-96.5	89.9	4.19	4.66
	500	5	77.2-87.2	82.3	4.01	4.87
M12	50.0 (LOQ)	7	78.1-101	90.0	7.82	8.69
	500	5	81.6-94.1	87.0	4.86	5.58
M11	50.0 (LOQ)	7	79.9-113	99.0	12.5	12.6
	500	5	70.1-85.2	78.7	6.29	7.99
M6	50.0 (LOQ)	7	87.2-105	96.5	6.34	6.57
	500	5	80.2-88.4	84.9	4.00	4.72
M1a	50.0 (LOQ)	7	89.9-104	97.9	5.17	5.28
	500	5	92.6-97.0	95.0	1.67	1.76
M1b	50.0 (LOQ)	7	78.0-83.1	80.9	1.69	2.09
	500	5	75.1-82.9	78.8	2.94	3.73
	Sandy Loam Soil – BAS 315 I and M6 - Updated ECM[3,4]					
	Quantitation Ion Transition					
Hydramethylnon (BAS 315 I)	50.0 (LOQ)	7	95.4-105	99.8	3.13	3.14
	500	5	94.0-101	97.2	2.94	3.03
M6	50.0 (LOQ)	7	93.0-104	98.2	4.35	4.43
	500	5	93.1-97.1	94.5	1.61	1.70
	Confirmation Ion Transition					
Hydramethylnon (BAS 315 I)	50.0 (LOQ)	7	93.4-102	98.6	2.55	2.58
	500	5	92.1-101	96.8	3.19	3.28
M6	50.0 (LOQ)	7	92.7-102	98.1	3.70	3.78
	500	5	91.6-96.4	94.6	2.09	2.21

Data (uncorrected recovery results, pp. 33-35) were obtained from Tables 1-12, pp. 43-54 and Appendix 4, Tables 4A-4D, pp. 139-142 of MRID 50862503.

1 The loamy sand soil (SMV Lot No. 012616A; 78% sand, 18% silt, 4% clay; pH 6.8 in 1:1 soil:water ratio; 4.9% organic matter – Walkley Black) from Rochester, Massachusetts, was used (USDA soil texture classification; p. 19; Appendix 3, p. 129). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 Analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 496.20→324.10 and *m/z* 496.20→369.10 for BAS 315 I, *m/z* 371.21→159.09 and *m/z* 371.32→199.04 for M12, *m/z* 384.20→364.20 and *m/z* 384.20→151.10 for M6, *m/z* 511.10→491.10 and *m/z* 511.10→364.10 for M1b, *m/z* 129.17→69.08 and *m/z* 129.17→70.03 for M11, and *m/z* 511.10→369.10 and *m/z* 511.10→142.20 for M1a.

3 BAS 315 I and metabolite M6 were re-validated using the more abundant carbon isotope (^{12}C; p. 26; Appendix 4, pp. 132-136 of MRID 50862503). This updated methodology was more robust and considered the official methodology. Analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 495.44→323.21 and *m/z* 495.44→368.12 for BAS 315 I and *m/z* 383.19→363.16 and *m/z* 383.19→151.08 for M6.

4 The sandy loam soil (SMV Lot No. RMN-SL-PF 0-6" 5-29-18; 75% sand, 18% silt, 7% clay; pH 6.6 in 1:1 soil:water ratio; 3.4% organic matter – Walkley Black) from Rochester, Massachusetts, was used (USDA soil texture classification; p. 19; Appendix 3, p. 130; Appendix 4, p. 132). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

Table 3. Independent Validation Method Recoveries for Hydramethylnon (BAS 315 I) and its Metabolites M12, M11, M6, M1a, and M1b in Soil[1,2,3]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sandy Loam Soil					
	Quantitation Ion Transition					
Hydramethylnon (BAS 315 I)	50.0 (LOQ)	5	85-93	90	3	4
	500	5	90-96	93	2	2
M12	50.0 (LOQ)	5	75-89	83	6	7
	500	5	81-89	84	3	4
M11	50.0 (LOQ)	5	88-108	98	9	9
	500	5	89-105	96	6	7
M6	50.0 (LOQ)	5	89-98	95	4	4
	500	5	101-107	104	2	2
M1a	50.0 (LOQ)	5	86-90	88	2	2
	500	5	95-106	99	5	5
M1b	50.0 (LOQ)	5	105-115	111	4	4
	500	5	111-121	116	4	4
	Confirmation Ion Transition					
Hydramethylnon (BAS 315 I)	50.0 (LOQ)	5	86-95	90	3	4
	500	5	89-97	92	3	3
M12	50.0 (LOQ)	5	80-88	86	3	4
	500	5	78-88	84	4	4
M11	50.0 (LOQ)	5	65-88	74	9	12
	500	5	95-100	97	2	2
M6	50.0 (LOQ)	5	86-93	90	3	3
	500	5	96-101	98	2	2
M1a	50.0 (LOQ)	5	78-87	83	3	4
	500	5	88-98	93	4	5
M1b	50.0 (LOQ)	5	104-119	112	6	5
	500	5	110-122	116	5	4

Data (uncorrected recovery results, Table 23, pp. 53-54) were obtained from Tables 1-12, pp. 30-41 of MRID 50862504.

1 The sandy loam soil (Control Matrix No. RMN-SL-PF; SMV Lot No. RMN-SL-PF 0-6" 5-29-18; 75% sand, 18% silt, 7% clay; pH 6.6 in 1:1 soil:water ratio; 3.4% organic matter – Walkley Black) from Rochester, Massachusetts, was used in the study (USDA soil texture classification; p. 17; Appendix A, p. 215 of MRID

50862504; p. 19; Appendix 3, p. 130 of MRID 50862503). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil matrix was provided by and characterized in the ECM. The ILV soil matrix was the same as that used in the ECM trial of the updated methodology for BAS 315 I and M6.

2 Analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 495→323 and *m/z* 495→368 for BAS 315 I, *m/z* 371→159 and *m/z* 371→199 for M12, *m/z* 383→363 and *m/z* 383→151 for M6, *m/z* 511→491 and *m/z* 511→364 for M1b, *m/z* 129→69 and *m/z* 129→70 for M11, and *m/z* 511→369 and *m/z* 511→142 for M1a.

3 The ECM was performed by the ILV with the Updated ECM for BAS 315 I and M6.

III. Method Characteristics

The LOQ for BAS 315 I and its metabolites M12, M11, M6, M1a, and M1b in soil was 50.0 µg/kg in the ECM and ILV (pp. 33, 35, 40 of MRID 50862503; pp. 26-27 of MRID 50862504). In the ECM, the LOQ was defined as the lowest fortification level and where blank values (reagent blanks and untreated control samples) did not exceed 30% of the LOQ. In the ILV, the LOQ was defined as the lowest fortification level tested. In the ECM, the LOD was calculated for each analyte and ion transition using the following equation:

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

Where, $t_{0.99}$ is the one-tailed t statistic for n-1 replicates at the 99% confidence level (3.143 for n =7) and SD is the standard deviation of the analyte recovery measurements for n samples at the target LOQ. The LOD in soil was calculated as 6.34 µg/kg (Q) and 6.58 µg/kg (C) for BAS 315 I, 6.86 µg/kg (Q) and 1.23 µg/kg (C) for M12, 8.50 µg/kg (Q) and 1.96 µg/kg (C) for M11, 5.78 µg/kg (Q) and 9.97 µg/kg (C) for M6, 6.23 µg/kg (Q) and 8.12 µg/kg (C) for M1a, and 1.89 µg/kg (Q) and 2.65 µg/kg (C) for M1b. The LODs were calculated for the Updated ECM for BAS 315 I and M6 as 4.92 µg/kg (Q) and 4.00 µg/kg (C) for BAS 315 I and 6.83 µg/kg (Q) and 5.82 µg/kg (C) for M6. The method LOD for all analytes in soil was reported as 15 µg/kg (30% of the LOQ) in the ILV. In the ILV, the LOD was defined as the absolute amount of analyte injected (0.003 ng for BAS 315 I and Metabolites M12, M6, M1b and M1a; 0.0015 ng for Metabolite M11) into the LC-MS/MS when the lowest calibration standard was analyzed (0.060 µg/L) for all analytes with acceptable signal to noise ratio (S/N > 3:1). No method LOD was reported in the ECM. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV; no calculations or comparisons to background levels were reported to justify the LOD for the method in the ILV. In the ECM, the MDL was calculated using the following equation:

$$MDL = MDL_{LCAL} \times DF_{CTRL}$$

Where, MDL_{LCAL} is the lowest concentration calibration standard (i.e., 0.100 µg/L and DF_{CTRL} is the dilution factor of the control samples. The MDL was calculated as 25.0 µg/kg for all analytes in soil (including the Updated ECM for BAS 315 I and M6).

Table 4. Method Characteristics

Analyte			Hydramethylnon (BAS 315 I)	M12	M11	M6	M1a	M1b
Limit of Quantitation (LOQ)			50.0 µg/kg					
Limit of Detection (LOD)	ECM	Method	**Not reported**					
		Calculated - Original	6.34 µg/kg (Q) 6.58 µg/kg (C)	6.86 µg/kg (Q) 1.23 µg/kg (C)	8.50 µg/kg (Q) 1.96 µg/kg (C)	5.78 µg/kg (Q) 9.97 µg/kg (C)	6.23 µg/kg (Q) 8.12 µg/kg (C)	1.89 µg/kg (Q) 2.65 µg/kg (C)
		Calculated - Updated	4.92 µg/kg (Q) 4.00 µg/kg (C)	Not updated		6.83 µg/kg (Q) 5.82 µg/kg (C)	Not updated	
	ILV	Method	15 µg/kg (30% of the LOQ)					
		Calculated	**Not calculated**					
Linearity (calibration curve r^2 and concentration range)[1]	ECM	Original	r^2 = 0.997 (Q) r^2 = 0.995 (C)	r^2 = **0.992** (Q) r^2 = 0.999 (C)	r^2 = **0.993** (Q) r^2 = **0.991** (C)	r^2 = 0.998 (Q & C)	r^2 = **0.994** (Q & C)	r^2 = 0.999 (Q) r^2 = 0.998 (C)
		Updated	r^2 = 0.998 (Q) r^2 = 0.997 (C)	Not updated		r^2 = **0.992** (Q) r^2 = **0.994** (C)	Not updated	
		Range	0.100-1.00 µg/L					
	ILV		r^2 = **0.9934** (Q) r^2 = **0.9900** (C)	r^2 = 0.9980 (Q) r^2 = 0.9976 (C)	r^2 = **0.9860** (Q) r^2 = **0.9853** (C)	r^2 = **0.9936** (Q) r^2 = **0.9928** (C)	r^2 = **0.9823** (Q) r^2 = **0.9839** (C)	r^2 = **0.9926** (Q) r^2 = **0.9928** (C)
		Range	0.060-1.00 µg/L					
Repeatable	ECM	Original[2]	Yes at LOQ and 10×LOQ (one characterized soil)					
		Updated[3]	Yes at LOQ and 10×LOQ (one characterized soil)	Not updated		Yes at LOQ and 10×LOQ (one characterized soil)	Not updated	
	ILV[4,5]		Yes at LOQ and 10×LOQ, using updated ECM for BAS 315 I and M6 (one characterized soil)					
Reproducible			Yes at LOQ and 10×LOQ, using updated ECM for BAS 315 I and M6.					
Specificity	ECM	Original	Yes, matrix interferences were <1% of the LOQ (based on peak area). Peak tailing was observed.	Yes, matrix interferences were <2% (Q) and *ca.* 27% (C)[6] of the LOQ (based on peak area).	**No**, matrix interferences were reported as <LOD and <10% of the LOQ; however, significant baseline noise of varied elevation interfered with analyte peak	Yes, matrix interferences were <2% of the LOQ (based on peak area).	Yes, no matrix interferences were observed.	Yes, matrix interferences were <2% of the LOQ (based on peak area). Minor peak tailing was observed.

Analyte			Hydramethylnon (BAS 315 I)	M12	M11	M6	M1a	M1b
					integration and attenuation.[7]			
		Updated	Yes, no matrix interferences were observed.	Not updated		Yes, no matrix interferences were observed.	Not updated	
	ILV		Yes, no matrix interferences were observed. A nearby minor contaminant was noted near analyte peak RT.	Yes, no matrix interferences were observed.	**No**, matrix interferences were <8% of the LOQ (based on quantified concentration); however, analyte peak was very small compared to baseline noise.[8]	Yes, no matrix interferences were observed.		**No**, no matrix interferences were observed; however, significant baseline noise interfered with analyte peak integration and attenuation.[9]

Data were obtained from pp. 33, 35, 40 (LOQ/LOD); Tables 1-12, pp. 43-54 and Appendix 4, Tables 4A-4D, pp. 139-142 (recovery data); pp. 22, 40 (correlation coefficients); Figures 38-49, pp. 99-110; Appendix 4, Figures 4M-4P, pp. 157-160 (calibration curves); Figures 8-37, pp. 69-98; Appendix 4, Figures 4C-4L, pp. 147-156 (chromatograms) of MRID 50862503; pp. 26-27 (LOQ/LOD); Tables 1-12, pp. 30-41 (recovery data); p. 25; Figure A.1, p. 58; Figure B.1, p. 71; Figure C.1, p. 84; Figure D.1, p. 97; Figure E.1, p. 110; Figure F.1, p. 123; Figure G.1, p. 136; Figure H.1, p. 149; Figure I.1, p. 162; Figure J.1, p. 175; Figure K.1, p. 188; Figure L.1, p. 201 (calibration curves); Figures A.2-L.12, pp. 59-212 (chromatograms) of MRID 50862504; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition.

1 Reported ILV correlation coefficients were reviewer-calculated from r values reported in the study report (Figure A.1, p. 58; Figure B.1, p. 71; Figure C.1, p. 84; Figure D.1, p. 97; Figure E.1, p. 110; Figure F.1, p. 123; Figure G.1, p. 136; Figure H.1, p. 149; Figure I.1, p. 162; Figure J.1, p. 175; Figure K.1, p. 188; Figure L.1, p. 201 of MRID 50862504; DER Attachment 2). In the ECM, solvent-based calibration standards were used for analysis for all analytes in either soil, except M1a for which matrix-matched calibration standards were used (p. 40 of MRID 50862503). In the ILV, solvent-based calibration standards were used for analysis for all analytes (p. 22 of MRID 50862504).

2 In the ECM, all six analytes were fortified in loamy sand soil (SMV Lot No. 012616A; 78% sand, 18% silt, 4% clay; pH 6.8 in 1:1 soil:water ratio; 4.9% organic matter – Walkley Black) from Rochester, Massachusetts (USDA soil texture classification; p. 19; Appendix 3, p. 129 of MRID 50862503). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

3 In the ECM, BAS 315 I and metabolite M6 were re-validated using the more abundant carbon isotope (^{12}C; p. 26; Appendix 4, pp. 132-136 of MRID 50862503). This updated methodology was more robust and considered the official methodology. For the updated methodology for BAS 315 I and M6, sandy loam soil (SMV Lot No. RMN-SL-PF 0-6" 5-29-18; 75% sand, 18% silt, 7% clay; pH 6.6 in 1:1 soil:water ratio; 3.4% organic matter – Walkley Black) from Rochester, Massachusetts, was used (USDA soil texture classification; p. 19; Appendix 3, p. 130; Appendix 4, p. 132). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

4 The ILV performed the ECM with the Updated ECM for BAS 315 I and M6. The sandy loam soil (Control Matrix No. RMN-SL-PF; SMV Lot No. RMN-SL-PF 0-6" 5-29-18; 75% sand, 18% silt, 7% clay; pH 6.6 in 1:1 soil:water ratio; 3.4% organic matter – Walkley Black) from Rochester, Massachusetts, was used in the study (USDA soil texture classification; p. 17; Appendix A, p. 215 of MRID 50862504; p. 19; Appendix 3, p. 130 of MRID 50862503). Soil

characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil matrix was provided by and characterized in the ECM. The ILV soil matrix was the same as that used in the ECM trial of the updated methodology for BAS 315 I and M6.

5 The ILV successfully validated the ECM in the first trial (p. 26). The ILV reportedly performed the ECM as written, except for the use of a mechanical shaker instead of a shaker table, the addition of a calibration standard fortified at 30% of the LOQ, and minor LC/MS instrument and parameter modifications; however, it was not specifically stated that the extractions were performed twice in the sample processing procedures (pp. 17, 21, 26-27; Tables 20-22, pp. 50-52). delete extra period The ILV recommendations for the ECM included 1) stability evaluation for the standards and extracts; 2) the inclusion of a method LOD in addition to the instrument-specific LODs calculated in the ECM; 3) discussion of LC/MS/MS optimization for chromatographic discrepancies which may arise from the analysis of compounds containing multiple isomers (M1b, M12, M6, and M1a – the ILV increased MS dwell time to 200 or 300 milliseconds); 4) the fortification volumes of M1a range 5-50% of sample weight and should be ≤10% of the sample weight; and 5 the calibration range should be expanded to include a standard fortified at 30% of the LOQ as the lowest calibration standard, instead of 50% of the LOQ. The ECM included the ILV recommendations which were not necessary for the successful validation but will enhance the reproducibility of the ECM (pp. 38-39 of MRID 50862503).

6 A confirmatory method is not always necessary when LC/MS or GC/MS is used as the primary method to generate study data.

7 Based on Figures 28-32, pp. 89-93 of MRID 50862503. Values reported in study report based on raw data in Appendix 5, pp. 166-167 of MRID 50862503. Reviewer-calculated matrix interferences based on reported peak areas were *ca*. 30% (Q) of the LOQ (based on peak area).

8 Based on Figures E.9-E.12, pp. 118-121 and Figures F.9-F.12, pp. 131-134 of MRID 50862504. Quantified concentration values based on raw data in Appendix C, pp. 230-231 of MRID 50862504. Reviewer-calculated matrix interferences based on reported peak areas were 30-40% (Q & C) of the LOQ (based on peak area).

9 Based on Figures I.9-I.12, pp. 170-173 and Figures J.9-J.12, pp. 183-186 of MRID 50862504.

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

IV. Method Deficiencies and Reviewer's Comments

1. The ILV performed the ECM with the Updated ECM for BAS 315 I and M6. The Updated ECM for BAS 315 I and metabolite M6 contained the same sample fortification and processing with the adjustment of the LC/MS/MS parameters and monitored ions to quantify the more abundant carbon isotope (^{12}C; p. 26; Appendix 4, pp. 132-136 of MRID 50862503). The ILV did not validate the Original ECM for BAS 315 I and M6; only the ECM report with the Updated ECM for BAS 315 I and M6 was validated in this method validation ECM/ILV set.

2. No method LOD was reported in the ECM. In the ILV, the LOD was defined as the absolute amount of analyte injected (0.003 ng for BAS 315 I and Metabolites M12, M6, M1b and M1a; 0.0015 ng for Metabolite M11) into the LC-MS/MS when the lowest calibration standard was analyzed (0.060 µg/L for all analytes with acceptable signal to noise ratio (S/N > 3:1).

3. The ILV recommendations for the ECM included 1) stability evaluation for the standards and extracts; 2) the inclusion of a method LOD in addition to the instrument-specific LODs calculated in the ECM; 3) discussion of LC/MS/MS optimization for chromatographic discrepancies which may arise from the analysis of compounds containing multiple isomers (M1b, M12, M6, and M1a – the ILV increased MS dwell time to 200 or 300 milliseconds); 4) the fortification volumes of M1a range 5-50% of sample weight and should be ≤10% of the sample weight; and 5) the calibration range should be expanded to include a standard fortified at 30% of the LOQ as the lowest calibration standard, instead of 50% of the LOQ (pp. 26-27 of MRID 50862504). The reviewer noted that the calibration range of the ECM only included one calibration standard below the predicted response of the LOQ sample; the calibration range should include two calibration standards bracketing below the LOQ sample response and above the 10×LOQ sample response for accurate quantitation. The ECM included the ILV recommendations which were not necessary for the successful validation but will enhance the reproducibility of the ECM (pp. 38-39 of MRID 50862503). The ECM reported that the sample extracts were "proven stable for 12 hours,…confirmed by their recoveries" (p. 33).

4. The specificity of the method was not supported by ILV representative chromatograms of M11 and M1b. In representative chromatograms of M11, matrix interferences were <8% of the LOQ (based on quantified concentration); however, analyte peak was very small compared to baseline noise (Figures E.9-E.12, pp. 118-121 and Figures F.9-F.12, pp. 131-134 of MRID 50862504). Analyte peak was only distinguishable from baseline noise by retention time. Quantified matrix interference concentration values were based on raw data in Appendix C, pp. 230-231 of MRID 50862504. Reviewer-calculated matrix interferences based on reported peak areas were 30-40% (Q & C) of the LOQ (based on peak area). In representative chromatograms of M1b, no matrix interferences were observed; however, significant baseline noise interfered with analyte peak integration and attenuation (Figures I.9-I.12, pp. 170-173 and Figures J.9-J.12, pp. 183-186). Enhanced baseline noise at analyte retention time appeared to make quantitation of the analyte peak

arbitrary. Additional sample processing may be required to enhance the specificity of the method for these analytes.

The specificity of the method was not supported by ECM representative chromatograms of M11. In representative chromatograms of M11, matrix interferences were reported as <LOD and <10% of the LOQ; however, significant baseline noise of varied elevation interfered with analyte peak integration and attenuation (Figures 28-32, pp. 89-93 of MRID 50862503). Matrix interference concentration values reported in the study report were based on raw data in Appendix 5, pp. 166-167 of MRID 50862503. Reviewer-calculated matrix interferences based on reported peak areas were *ca*. 30% (Q) of the LOQ (based on peak area). Additional sample processing may be required to enhance the specificity of the method for this analyte.

5. The ILV linearity was unsatisfactory for all ions of all analytes, except for M12: hydramethylnon, r^2 = 0.9934 (Q) and 0.9900 (C); M11, r^2 = 0.9860 (Q) and 0.9853 (C); M6, r^2 = 0.9936 (Q) and 0.9928 (C); M1a, r^2 = 0.9823 (Q) and 0.9839 (C); and M1b, r^2 = 0.9926 (Q) and 0.9928 (C; Figure A.1, p. 58; Figure B.1, p. 71; Figure C.1, p. 84; Figure D.1, p. 97; Figure E.1, p. 110; Figure F.1, p. 123; Figure G.1, p. 136; Figure H.1, p. 149; Figure I.1, p. 162; Figure J.1, p. 175; Figure K.1, p. 188; Figure L.1, p. 201 of MRID 50862504). Linearity is satisfactory when $r^2 \geq 0.995$.

   The ECM linearity was unsatisfactory in the Original ECM for M12 [r^2 = 0.992 (Q)], M11 [r^2 = 0.993 (Q) and 0.991 (C)], and M1a [r^2 = 0.994 (Q & C; Figures 38-49, pp. 99-110; Appendix 4, Figures 4M-4P, pp. 157-160 of MRID 50862503). The ECM linearity was unsatisfactory in the Updated ECM for M6 [r^2 = 0.992 (Q) and 0.994 (C)]. Linearity is satisfactory when $r^2 \geq 0.995$.

6. The LOQ (50.0 µg/kg) is greater than the lowest toxicological level of concern in soil for hydramethylnon (>30 µg/kg; USEPA 2018).

7. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method since only one characterized soil matrix was tested. 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. Even though a certain number of soil matrices is not specified in the OCSPP guidelines, more than one soil matrix would need to be included in an ILV in order to cover the range of soils used in the terrestrial field dissipation studies.

8. In the ILV, the sample fortification and sample processing were summarized without many details (pp. 17, 21, 26-27; Tables 19-22, pp. 48-52 of MRID 50862504). The ECM seemed to be performed as written, except for the use of a mechanical shaker instead of a shaker table, the addition of a calibration standard fortified at 30% of the LOQ, and minor LC/MS instrument and parameter modifications; however, it was not specifically stated that the extractions were performed twice in the sample processing procedures. Although the ILV included the full ECM in its Appendix D and referenced it as the

analytical method which was followed, it is preferred that the ILV report the step-by-step sample processing procedure and full LC/MS/MS equipment and parameters used by the independent laboratory so that it can be accurately compared to the ECM.

9. In the ECM, the LOQ was defined as the lowest fortification level and where blank values (reagent blanks and untreated control samples) did not exceed 30% of the LOQ. In the ILV, the LOQ was defined as the lowest fortification level tested. In the ECM, the LOD was calculated for each analyte and ion transition using the following equation: LOD = ($t_{0.99}$ x SD), where, $t_{0.99}$ is the one-tailed t statistic for n-1 replicates at the 99% confidence level (3.143 for n =7) and SD is the standard deviation of the analyte recovery measurements for n samples at the target LOQ. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV; no calculations or comparisons to background levels were reported to justify the LOD for the method in the ILV. In the ECM, the MDL was calculated using the following equation: MDL = MDL_{LCAL} x DF_{CTRL}, where, MDL_{LCAL} is the lowest concentration calibration standard i.e., 0.100 µg/L and DF_{CTRL} is the dilution factor of the control samples. The LODs and MDL were calculated for the Updated ECM for BAS 315 I and M6, as well. No calculations for the LOQ were provided in the ECM and ILV; no calculations for the LOD were provided in the ILV. Detection limits should not be based on arbitrary values.

10. Matrix effects were studied in the ECM and determined to be insignificant (≤ ±20%) for all analytes in loamy sand soil and BAS 315 I and M6 in sandy loam soil, except M1a in loamy sand soil (p. 40; Tables 13-18, pp. 55-60; Appendix 4, Tables 4E-4F, pp. 143-144 of MRID 50862503). Solvent-based calibration standards were used for analysis for all analytes, except M1a for which matrix-matched calibration standards were used.

    Matrix effects were studied in the ILV and determined to be insignificant (≤ ±20%) for all analytes in sandy loam soil (p. 22; Tables 13-18, pp. 42-47 of MRID 50862504). Solvent-based calibration standards were used for analysis for all analytes.

11. The ILV reported that communication between the ILV Study Director and Study Monitor consisted of the soil test system replacement and the Study Monitor being notified of the successful completion of the ILV trial (pp. 27-28 of MRID 50862504). At no time during the course of the study did anyone from BASF or Landis International visit the testing facility. The raw communications were not included in the study report.

12. The ILV noted that the first control soil sample provided by Smithers Viscient (Control No. 062618B) was used to initiate the independent validation, but then designated as incorrect by the Study Monitor (pp. 26, 28 of MRID 50862504). The independent validation was re-started with the new, correct soil test system. The first control soil sample was reported as a sandy loam soil (p. 17). No additional information about the suitability or lack thereof was provided in the ILV study report.

13. In the ILV, the time required to complete the extraction of one set of 13 samples required ca. 4-6 hours of work, excluding calculation of results and LC/MS/MS analysis (p. 25 of MRID 50862504).

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.

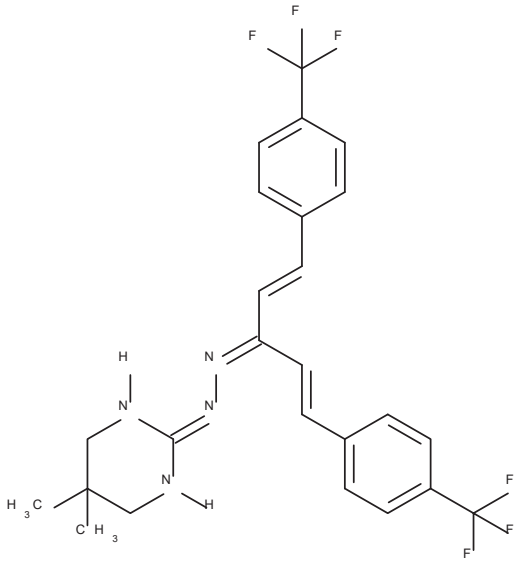
U.S. Environmental Protection Agency. 2018. Hydramethylnon: Preliminary Ecological Risk Assessment for Registration Review. DP barcode 439599. Office of Chemical Safety and Pollution Prevention, Environmental Fate and Effects Division. Memorandum to the Pesticide Re-evaluation Division. Jun. 7, 2018.

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

Attachment 1: Chemical Names and Structures

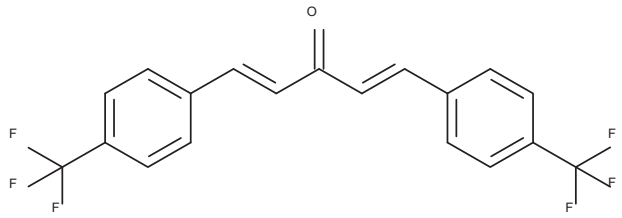
Hydramethylnon (BAS 315 I)

IUPAC Name:	5,5-Dimethylperhydropyrimidin-2-one 4-trifluoromethyl-α-(4-trifluoromethylstyryl)cinnamylidenehydrazone
CAS Name:	Tetrahydro-5,5-dimethyl-2(1H)-pyrimidinone [3-[4-(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl]ethenyl]-2-propen-1-ylidene]hydrazone
CAS Number:	67485-29-4
SMILES String:	N1CC(C)(C)CNC1=NN=C(C=Cc2ccc(C(F)(F)F)cc2)C=Cc3ccc(C(F)(F)F)cc3



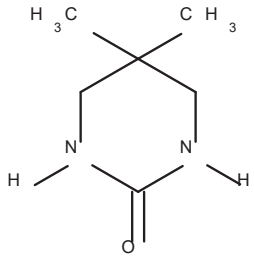
M12 (BAS 255418; Reg. No. 255418)

IUPAC Name: 1,5-Bis[4-(trifluoromethyl)phenyl]penta-1,4-dien-3-one
CAS Name: Not reported
CAS Number: 42160-07-6
SMILES String: FC(F)(F)c1ccc(\C=C\C(=O)\C=C\c2ccc(cc2)C(F)(F)F)cc1



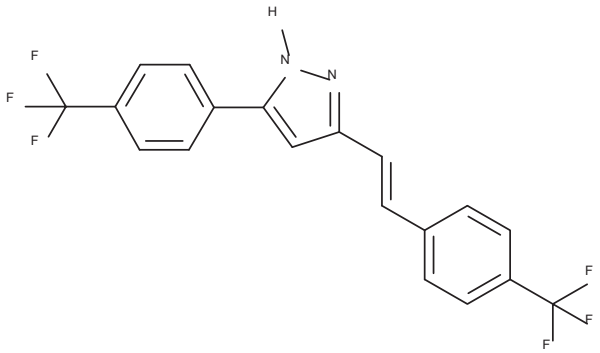
M11 (Hydramethylnon Metabolite P)

IUPAC Name: 5,5-Dimethylhexahydropyrimidin-2-one
CAS Name: Not reported
CAS Number: Not reported
SMILES String: CC1(C)CNC(=O)NC1



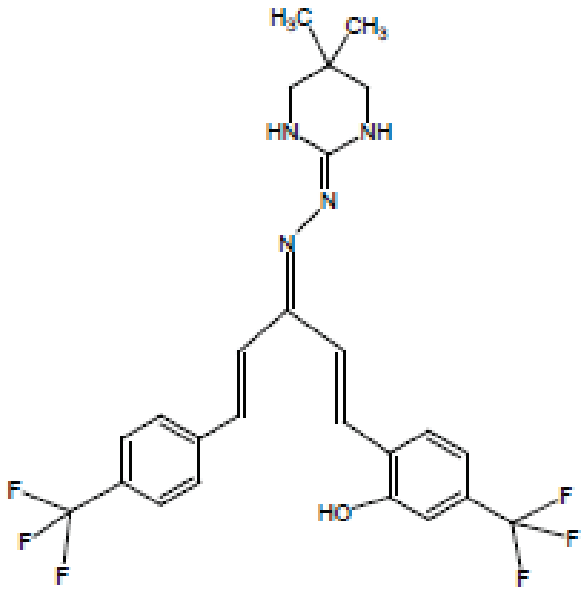
M6 (Hydramethylnon Metabolite M6)

IUPAC Name:	5-[4-(Trifluoromethyl)phenyl]-3-[(E)-2-[4-(trifluoromethyl)phenyl]vinyl]-1H-pyrazole
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	FC(F)(F)c1ccc(\C=C\c2cc([nH]n2)c3ccc(cc3)C(F)(F)F)cc1



M1a (Hydramethylnon Metabolite M1a)

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



M1b (Hydramethylnon Metabolite M1b)

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