

Analytical method for hydramethylnon (BAS 315 I) and its metabolites Compound E, Compound UK, Compound R, Compound C, and Compound F in groundwater and surface water

Reports: ECM: EPA MRID No.: 50862501. DeVellis, S.R. 2019. Final report. Validation of the Analytical Method for the Determination of BAS 315 I and Metabolites in Groundwater and Surface Water. BASF Document Registration No.: 2018/7005607. BASF Study No.: 828400-1. Smithers Viscient Study No.: 986.6265. 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; 337 pages. Final report issued March 25, 2019.

ILV: EPA MRID No. 50862502. Sharp, S. 2019. – Final Report. Independent Laboratory Validation of the Analytical Method for the Determination of BAS 315 I and Metabolites in Groundwater and Surface Water. BASF Registration Document No.: 2018/7005700. ADPEN Study No.: 18G0704. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, and sponsored and submitted by BASF Crop Protection, Research Triangle Park, North Carolina; 816 pages. Final report issued April 29, 2019.

Document No.: MRIDs 50862501 & 50862502

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, except for the characterization of Compound UK (p. 3 of MRID 50862501). 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, except for the characterization of Compound UK and that the water characterization reports were maintained at Smithers Viscient (p. 3 of MRID 50862502). 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 in both water matrices of hydramethylnon, Compound UK, and Compound C. ECM linearity was unsatisfactory in the Original ECM for Compound C and Compound F in ground water and in the Updated ECM for hydramethylnon in surface water. For Compound R, the specificity of the method was not supported by ILV representative chromatograms. Compound UK was not identified. ILV water matrices were not characterized. ILV sample fortification and sample processing were summarized without many details.

PC Code: 118401

**EFED Final
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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. 2018/7005607, is designed for the quantitative determination of hydramethylnon (BAS 315 I) and its metabolites Compound E, Compound UK, Compound R, Compound C, and Compound F in groundwater and surface water at the LOQ of 0.100 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern for hydramethylnon (0.2 µg/L; USEPA 2018) in groundwater and surface water for all analytes. In the ECM, the method was performed using characterized groundwater and surface water for all analytes (Original ECM), then BAS 315 I and metabolite Compound UK (structure unknown) were re-validated in the same water matrices 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 Compound UK, not the Original ECM for BAS 315 I and Compound UK. The ILV was performed using uncharacterized groundwater and surface water which was provided by the ECM. 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 seemed to be performed as written, except for the use of Waters Oasis MCS SPE columns instead of Waters Oasis MCX SPE columns and minor LC/MS instrument and parameter modifications. The ILV recommendations for the ECM included 1) matrix effect evaluation; 2) the fortification volumes range 1-10% of sample weight and should be ≤1% of the sample weight; 3) the use of consistent volumes for the sample processing of Compound R; 4) discussion of LC/MS/MS optimization for chromatographic discrepancies which may arise from the analysis of compounds containing multiple isomers (BAS 315 I, Compound E, and Compound UK); and 5) extract stability evaluation. The ECM included the ILV recommendations which were not necessary for the successful validation but will enhance the reproducibility of the ECM. Regarding the multiple isomers, it was noted that ILV identified a mixture of isomers for BAS 315 I, Compound E, and Compound UK, but the ECM only identified multiple isomers for Compound E. All submitted ECM and ILV data pertaining to precision, repeatability, and reproducibility was acceptable for

all analytes. ILV linearity was unsatisfactory for all ions in both water matrices of hydramethylnon, Compound UK, and Compound C. ECM linearity was unsatisfactory in the Original ECM for Compound C and Compound F in ground water and in the Updated ECM for hydramethylnon in surface water. All submitted ECM and ILV data pertaining to specificity was acceptable for all analytes, except Compound R in the ILV. For Compound R, the specificity of the method was not supported due to a nearby significant, broad contaminant (RT *ca.* 3.1 min.; peak height *ca.* 40% of the LOQ peak height) noted in ILV quantitation ion transition representative chromatograms in both water matrices (significant contaminant also in confirmation ion transition representative chromatograms).

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)	50862501 (Original ECM ¹)	None submitted		Ground water and Surface Water	25/03/2019	BASF Corporation	LC/MS/MS	0.100 µg/L
Compound E		50862502 ³						
Compound UK								
Compound R		None submitted						
Compound C		50862502 ³						
Compound F								
Hydramethylnon (BAS 315 I)								
Compound R	50862501 (Updated ECM ²)							

1 In the ECM, groundwater (pH 6.31; dissolved oxygen concentration 5.62 mg/L) obtained as unfiltered well water, and surface water (pH 6.2; dissolved oxygen concentration 6.2 mg/L) collected from Taunton River (collected from an area of the river with *ca.* 30 to 60 cm of overlying water) were used (pp. 23-24; Appendix 4, pp. 213-214 of MRID 50862501). The water characterization was performed by Smithers Viscient. For the surface water, SMV Lot No.12 Jul 17 Wat-B, collected on 12 July 2017, was used for the Original ECM, and SMV Lot No.19 Mar 18 Wat-A, collected on 19 March 2018, was used for the Updated ECM.

2 In the ECM, BAS 315 I and metabolite Compound UK were re-validated using the more abundant carbon isotope (¹²C; p. 34; Appendix 5, pp. 216-220 of MRID 50862501). This updated methodology was more robust and considered the official methodology. The re-evaluation was performed with the same water matrices as the Original ECM.

3 The ILV performed the ECM with the Updated ECM for BAS 315 I and Compound UK. The groundwater and surface water were provided by Smithers Viscient, but characterization data was not reported in the study (p. 24 of MRID 50862502). The water characterization was maintained by Smithers Viscient.

I. Principle of the Method

Original ECM - BAS 315 I, Compound E, and Compound UK - Sample Fortification and Processing

Water samples were fortified with 0.0500 or 0.500 mL of 0.01 mg/L standard solution of BAS 315 I, Compound E, and Compound UK to yield final volumes of 5.00 mL and final concentrations of 0.100 and 1.00 µg/L (pp. 30-31, 34-35; Figure 1, p. 92 of MRID 50862501). All samples were diluted to 10.0 mL using methanol. The 10×LOQ samples (2.00 mL aliquots) were further diluted into the calibration range using methanol:test water (50:50, v:v) to a final volume of 10.0 mL. Aliquots of the samples were taken for HPLC/MS/MS analysis.

Original ECM - Compound R - Sample Fortification and Processing

Water samples were fortified with 0.0250 or 0.500 mL of 0.100 mg/L standard solution of Compound R to yield a final volume of 25.0 mL and final concentration of 0.100 µg/L and final volume of 5.00 mL and final concentration of 1.00 µg/L (pp. 31-32, 35-36; Figure 1, p. 92 of MRID 50862501). The samples were mixed with formic acid (0.100 mL for LOQ samples and 0.0200 mL for the 10×LOQ samples). Waters Oasis MCX SPE columns (150 mg, 6 cc) were conditioned by rinsing with two column volumes of methanol followed by two column volumes of purified reagent water. The acidified sample was applied to the column under low vacuum (<1 drop/second). Methanol:ammonium hydroxide (95:5, v:v; 2.50 mL) was added to the column to saturation and allowed to sit for thirty seconds before low vacuum (<1 drop/second) was applied to collect the analyte. The volume of the eluate was reduced to 0.200 mL under a gentle stream of nitrogen at 50.0°C. The sample was reconstituted to 5.00 mL in caustic methanol:purified reagent water (20:80, v:v) with vortexing. Aliquots of the samples were taken for HPLC/MS/MS analysis.

Original ECM - Compound C and Compound F - Sample Fortification and Processing

Water samples were fortified with 0.080 or 0.800 mL of 0.010 mg/L standard solution of Compound C and Compound F to yield a final volume 8.00 mL and final concentrations of 0.100 and 1.00 µg/L (pp. 33, 36-37; Figure 1, p. 92 of MRID 50862501). All samples were diluted to 10.0 mL using methanol. The 10×LOQ samples (4.00 mL aliquots) were further diluted into the calibration range using methanol:test water (50:50, v:v) to a final volume of 10.0 mL. Aliquots of the samples were taken for HPLC/MS/MS analysis.

Original ECM - BAS 315 I, Compound E, and Compound UK – HPLC/MS/MS

BAS 315 I, Compound E, and Compound UK 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. 23, 37-39 of MRID 50862501). The following conditions were employed: Phenomenex Synergi Fusion RP 80Å column (5 mm x 2.0 mm, 4.0 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 0.01-0.50 min. 85.0:15.0, 3.00-4.00 min. 0.00:100, 4.10-

5.00 min. 85.0:15.0] using an injection volume of 100 μ L 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 \rightarrow 324.10 and m/z 496.20 \rightarrow 369.10 for BAS 315 I, m/z 351.44 \rightarrow 331.08 and m/z 351.44 \rightarrow 275.05 for Compound E, and m/z 512.16 \rightarrow 324.21 and m/z 512.16 \rightarrow 315.29 for Compound UK. Expected retention times were *ca.* 2.7, 2.4-2.6, and 2.5 minutes for BAS 315 I, Compound E, and Compound UK, respectively.

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

BAS 315 I and metabolite Compound UK were re-validated using the more abundant carbon isotope (^{12}C ; p. 34; Appendix 5, pp. 216-220 of MRID 50862501). 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 Compound UK 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 5, pp. 217, 220-222 of MRID 50862501). The following conditions were employed: Phenomenex Synergi Fusion RP 80Å column (5 mm x 2.0 mm, 4.0 μ m 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 0.01-0.50 min. 85.0:15.0, 3.00-4.00 min. 0.00:100, 4.10-5.00 min. 85.0:15.0] using an injection volume of 100 μ L 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 \rightarrow 323.21 and m/z 495.44 \rightarrow 368.12 for BAS 315 I and m/z 511.36 \rightarrow 323.10 and m/z 511.36 \rightarrow 314.21 for Compound UK. Expected retention times were *ca.* 2.6 and 2.3 minutes for BAS 315 I and Compound UK, respectively.

Original ECM - Compound R – HPLC/MS/MS

Compound R was identified and quantified by LC/MS/MS using a Shimadzu LC-20AD HPLC coupled with an MDS Sciex API 1.000 MS (MDS Sciex ESI Turbo V Source) or an AB MDS Sciex 4000 MS (AB MDS Sciex ESI Turbo V Source; pp. 23, 39-40 of MRID 50862501). The following conditions were employed: Waters Atlantis T3 column (100 mm x 4.6 mm, 3.0 μ m 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 0.01-0.50 min. 100:0.00, 6.00-6.50 min. 40.0:60.0, 6.60-8.00 min. 100:0.00] using an injection volume of 100 μ L and positive (+) ESI ionization MRM scan mode (source temperature 600°C). Compound R was identified using two ion transitions (quantitation and confirmation, respectively): m/z 142.13 \rightarrow 70.02 and m/z 142.13 \rightarrow 72.17. Expected retention time was *ca.* 3.4 minutes.

Original ECM - Compound C and Compound F – HPLC/MS/MS

Compound C and Compound F were identified and quantified by LC/MS/MS using a Shimadzu LC-20AD HPLC coupled with an MDS Sciex API 1.000 MS (MDS Sciex ESI Turbo V Source) or an AB MDS Sciex 4000 MS (AB MDS Sciex ESI Turbo V Source; pp. 23, 40-41 of MRID

50862501). The following conditions were employed: Waters Xbridge BEH C18 column (50 mm x 2.1 mm, 2.5 μ m 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 0.01-0.50 min. 95.0:5.00, 3.50-4.00 min. 0.00:100, 4.10-5.00 min. 95.0:5.00] using an injection volume of 100 μ L and negative (-) ESI ionization MRM scan mode (source temperature 650°C). Analytes were identified using two ion transitions (quantitation and confirmation, respectively): m/z 189.01 \rightarrow 144.86 and m/z 235.08 \rightarrow 188.82 for Compound C and m/z 215.04 \rightarrow 170.98 and m/z 261.09 \rightarrow 214.88 for Compound F. Expected retention times were *ca.* 3.2 and 3.3 minutes for Compound C and Compound F, respectively.

ILV

In the ILV, the sample fortification and sample processing were summarized without many details (pp. 7, 24, 27, 36-37; Tables 37-40, pp. 73-77 of MRID 50862502). The ECM seemed to be performed as written, except for the use of Waters Oasis MCS SPE columns instead of Waters Oasis MCX SPE columns and minor LC/MS instrument and parameter modifications. 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, Compound E, and Compound UK: Phenomenex Synergi Fusion RP column (5 mm x 2.0 mm, 4.0 μ m 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. 85:15, 3.00-4.00 min. 0:100, 4.10-5.00 min. 85:15] using an injection volume of 100 μ L 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 \rightarrow 323 and m/z 495 \rightarrow 368 for BAS 315 I, m/z 351 \rightarrow 331 and m/z 351 \rightarrow 275 for Compound E, and m/z 511 \rightarrow 323 and m/z 511 \rightarrow 314 for Compound UK (the reviewer noted that the ion transitions for Compound UK were incorrectly reported as m/z 512 \rightarrow 323 and m/z 512 \rightarrow 314 in Table 38, p. 75 - see pp. 10, 27, 34 for correct ion transition). Expected retention times were *ca.* 2.53, 2.34, and 2.27 minutes for BAS 315 I, Compound E, and Compound UK, respectively. The following conditions were employed for Compound R: Waters Atlantis T3 column (100 mm x 4.6 mm, 3.0 μ m 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. 100:0, 6.00-6.50 min. 40:60, 6.60-8.00 min. 100:0] using an injection volume of 100 μ L and positive (+) ESI ionization MRM scan mode (source temperature 600°C). Compound R was identified using two ion transitions (quantitation and confirmation, respectively): m/z 142 \rightarrow 70 and m/z 142 \rightarrow 72. Expected retention time was *ca.* 3.32 minutes. The following conditions were employed for Compound C and Compound F: Waters Xbridge BEH C18 column (50 mm x 2.1 mm, 2.5 μ m 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, 3.50-4.00 min. 0:100, 4.10-5.00 min. 95:5] using an injection volume of 100 μ L and negative (-) ESI ionization MRM scan mode (source temperature 600°C). Analytes were identified using two ion transitions (quantitation and confirmation, respectively): m/z 189 \rightarrow 145 and m/z 235 \rightarrow 189 for Compound C and m/z 215 \rightarrow 171 and m/z 261 \rightarrow 215 for Compound F. Expected retention times were *ca.* 3.30 and 3.40 minutes for Compound C and Compound F, respectively. The LC/MS conditions were generally the same as the ECM with some minor differences in MS source temperature. The ECM-

modified HPLC/MS/MS ion transitions for BAS 315 I and Compound UK were used in the ILV. The ILV recommendations for the ECM included 1) matrix effect evaluation; 2) the fortification volumes range 1-10% of sample weight and should be $\leq 1\%$ of the sample weight; 3) the use of consistent volumes for the sample processing of Compound R; 4) discussion of LC/MS/MS optimization for chromatographic discrepancies which may arise from the analysis of compounds containing multiple isomers (BAS 315 I, Compound E, and Compound UK); and 5) extract stability evaluation (pp. 36-37).

LOQ/LOD

The Limit of Quantification (LOQ) for hydramethylnon (BAS 315 I) and its metabolites Compound E, Compound UK, Compound R, Compound C, and Compound F in groundwater and surface water was 0.100 $\mu\text{g/L}$ in the ECM and ILV (pp. 42-43, 45-47, 52-53; Appendix 5, p. 216 of MRID 50862501; pp. 36-37 of MRID 50862502). In the ECM, the Limit of Determination (LOD) in groundwater and surface water was calculated as 0.002-0.01 $\mu\text{g/L}$ (Q) and 0.01-0.02 $\mu\text{g/L}$ (C) for BAS 315 I, 0.004-0.007 $\mu\text{g/L}$ (Q) and 0.009-0.01 $\mu\text{g/L}$ (C) for Compound E, 0.005-0.01 $\mu\text{g/L}$ (Q) and 0.01-0.02 $\mu\text{g/L}$ (C) for Compound UK, 0.01 $\mu\text{g/L}$ (Q) and 0.01-0.02 $\mu\text{g/L}$ (C) for Compound R, 0.007-0.008 $\mu\text{g/L}$ (Q) and 0.01-0.02 $\mu\text{g/L}$ (C) for Compound C, and 0.02-0.03 $\mu\text{g/L}$ (Q & C) for Compound F. The LODs were calculated in the Updated ECM as 0.01 $\mu\text{g/L}$ (Q & C) for BAS 315 I and 0.009-0.01 $\mu\text{g/L}$ (Q) and 0.01-0.02 $\mu\text{g/L}$ (C) for Compound UK. The method LOD for all analytes in groundwater and surface water was reported as 0.03 $\mu\text{g/L}$ (30% of the LOQ) in the ILV. In the ECM, the Method Detection Limit (MDL) in groundwater and surface water was calculated as 0.0600 $\mu\text{g/L}$ for BAS 315 I, Compound E, and Compound UK, 0.0500 $\mu\text{g/L}$ for Compound R, and 0.0625 $\mu\text{g/L}$ for Compound C and Compound F (including the Updated ECM for BAS 315 I and Compound UK).

II. Recovery Findings

ECM (MRID 50862501): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements [means between 70% and 120% and relative standard deviations (RSD) $\leq 20\%$] for analysis of hydramethylnon (BAS 315 I), Compound E, Compound UK, Compound R, Compound C, and Compound F at fortification levels of 0.100 $\mu\text{g/L}$ (LOQ) and 1.00 $\mu\text{g/L}$ (10 \times LOQ) in groundwater and surface water matrix (Tables 1-24, pp. 56-79). BAS 315 I and metabolite Compound UK were re-validated using the more abundant carbon isotope (^{12}C ; p. 34; Appendix 5, pp. 216-220). 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 Compound UK at fortification levels of 0.100 $\mu\text{g/L}$ (LOQ) and 1.00 $\mu\text{g/L}$ (10 \times LOQ) in groundwater and surface water matrix (Appendix 5, Tables 4A-4H, pp. 223-230). 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 Compound UK produced similar performance data based on percent recovery. The groundwater (pH 6.31; dissolved oxygen concentration 5.62 mg/L) obtained as unfiltered well water, and surface water (pH 6.2; dissolved oxygen concentration 6.2 mg/L) collected from Taunton River (collected from an area of the river with *ca.* 30 to 60 cm of overlying water) were used (pp. 23-24; Appendix 4, pp. 213-214). The water

characterization was performed by Smithers Viscient. For the surface water, SMV Lot No.12 Jul 17 Wat-B, collected on 12 July 2017, was used for the Original ECM, and SMV Lot No.19 Mar 18 Wat-A, collected on 19 March 2018, was used for the Updated ECM.

ILV (MRID 50862502): Mean recoveries and RSDs were within guideline requirements for analysis of hydramethylnon (BAS 315 I), Compound E, Compound UK, Compound R, Compound C, and Compound F at fortification levels of 0.100 µg/L (LOQ) and 1.00 µg/L (10×LOQ) in groundwater and surface water matrix, except for the confirmation ion transition of BAS 315 I in surface water at the LOQ (mean 68%; Tables 1-24, pp. 40-63). The ILV performed the ECM with the Updated ECM for BAS 315 I and Compound UK. Analytes were identified using two ion transitions; performance data (recovery results) for the quantitation and confirmation ion analyses were comparable. The groundwater and surface water were provided by Smithers Viscient, but characterization data was not reported in the study (p. 24). The water characterization was maintained by Smithers Viscient. The ILV successfully validated the ECM in the first trial (p. 36). In the ILV, the sample fortification and sample processing were summarized without many details (pp. 7, 24, 27, 36-37; Tables 37-40, pp. 73-77). The ECM seemed to be performed as written, except for the use of Waters Oasis MCS SPE columns instead of Waters Oasis MCX SPE columns and minor LC/MS instrument and parameter modifications. The ILV recommendations for the ECM included 1) matrix effect evaluation; 2) the fortification volumes range 1-10% of sample weight and should be ≤1% of the sample weight; 3) the use of consistent volumes for the sample processing of Compound R; 4) discussion of LC/MS/MS optimization for chromatographic discrepancies which may arise from the analysis of compounds containing multiple isomers (BAS 315 I, Compound E, and Compound UK); and 5) extract stability evaluation (pp. 36-37). The ECM included the ILV recommendations which were not necessary for the successful validation but will enhance the reproducibility of the ECM (pp. 50-51 of MRID 50862501).

Table 2. Initial Validation Method Recoveries for Hydramethylnon (BAS 315 I) and its Metabolites Compound E, Compound UK, Compound R, Compound C, and Compound F in Groundwater and surface water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Groundwater – Original ECM^{1,2}						
Quantitation Ion Transition						
Hydramethylnon (BAS 315 I)	0.100 (LOQ)	7	103-105	105	0.782	0.749
	1.00	5	96.2-100	98.3	1.43	1.45
Compound E	0.100 (LOQ)	7	102-109	105	2.31	2.21
	1.00	5	101-105	103	1.37	1.33
Compound UK	0.100 (LOQ)	7	103-106	105	1.44	1.38
	1.00	5	96.3-99.9	98.2	1.63	1.66
Compound R	0.100 (LOQ)	7	90.5-101	96.7	3.42	3.53
	1.00	5	77.5-87.5	81.9	5.15	6.29
Compound C	0.100 (LOQ)	7	96.5-103	99.7	2.16	2.16
	1.00	5	104-111	107	2.58	2.40
Compound F	0.100 (LOQ)	7	86.4-106	94.3	7.37	7.81
	1.00	5	99.5-108	104	3.18	3.04
Confirmation Ion Transition						
Hydramethylnon (BAS 315 I)	0.100 (LOQ)	7	96.4-107	102	3.28	3.21
	1.00	5	95.7-101	97.5	1.97	2.02
Compound E	0.100 (LOQ)	7	99.8-108	103	3.02	2.92
	1.00	5	96.9-102	99.7	2.01	2.02
Compound UK	0.100 (LOQ)	7	98.7-113	107	4.62	4.31
	1.00	5	93.8-104	99.0	3.60	3.64
Compound R	0.100 (LOQ)	7	93.1-106	101	4.80	4.76
	1.00	5	77.1-90.6	84.7	6.04	7.13
Compound C	0.100 (LOQ)	7	97.3-106	101	3.86	3.82
	1.00	5	98.3-111	104	4.85	4.67
Compound F	0.100 (LOQ)	7	95.9-109	102	5.30	5.19
	1.00	5	94.4-104	99.8	4.01	4.02
Groundwater – BAS 315 I and Compound UK - Updated ECM^{1,3}						
Quantitation Ion Transition						
Hydramethylnon (BAS 315 I)	0.100 (LOQ)	7	103-114	107	4.05	3.79
	1.00	5	90.9-98.8	94.9	3.15	3.32
Compound UK	0.100 (LOQ)	7	102-113	107	4.42	4.12
	1.00	5	92.5-98.3	96.1	2.27	2.37
Confirmation Ion Transition						
Hydramethylnon (BAS 315 I)	0.100 (LOQ)	7	100-111	106	3.71	3.50
	1.00	5	93.6-100	96.8	2.52	2.60
Compound UK	0.100 (LOQ)	7	91.0-105	101	4.73	4.68
	1.00	5	90.4-104	97.0	5.39	5.55
Surface Water – Original ECM^{1,2}						
Quantitation Ion Transition						
Hydramethylnon (BAS 315 I)	0.100 (LOQ)	7	101-110	105	3.81	3.63
	1.00	5	101-110	104	3.71	3.55

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Compound E	0.100 (LOQ)	7	82.9-86.6	85.1	1.26	1.48
	1.00	5	79.9-84.1	82.1	1.54	1.88
Compound UK	0.100 (LOQ)	7	98.2-108	103	3.71	3.60
	1.00	5	100-104	102	1.50	1.47
Compound R	0.100 (LOQ)	7	89.3-97.8	93.9	3.05	3.25
	1.00	5	87.6-95.1	90.9	3.14	3.45
Compound C	0.100 (LOQ)	7	92.1-99.7	96.7	2.39	2.47
	1.00	5	99.4-107	102	2.97	2.90
Compound F	0.100 (LOQ)	7	100-121	109	8.47	7.77
	1.00	5	105-109	107	1.48	1.39
Confirmation Ion Transition						
Hydramethylnon (BAS 315 I)	0.100 (LOQ)	7	96.5-111	102	4.82	4.75
	1.00	5	103-105	104	0.606	0.583
Compound E	0.100 (LOQ)	7	97.9-109	104	4.39	4.23
	1.00	5	93.7-101	96.5	2.66	2.75
Compound UK	0.100 (LOQ)	7	84.2-105	92.8	7.43	8.00
	1.00	5	93.7-106	99.1	5.19	5.24
Compound R	0.100 (LOQ)	7	92.8-105	97.6	3.95	4.05
	1.00	5	83.5-93.9	88.9	4.32	4.86
Compound C	0.100 (LOQ)	7	97.7-113	105	5.37	5.13
	1.00	5	98.2-110	104	4.57	4.39
Compound F	0.100 (LOQ)	7	78.5-98.4	92.0	8.35	9.07
	1.00	5	90.9-112	100	8.00	8.00
Surface Water – BAS 315 I and Compound UK - Updated ECM ^{1,3}						
Quantitation Ion Transition						
Hydramethylnon (BAS 315 I)	0.100 (LOQ)	7	91.4-105	101	4.72	4.70
	1.00	5	96.9-99.9	98.7	1.1	1.43
Compound UK	0.100 (LOQ)	7	98.2-107	101	2.82	1.78
	1.00	5	93.5-98.7	96.2	2.32	2.42
Confirmation Ion Transition						
Hydramethylnon (BAS 315 I)	0.100 (LOQ)	7	97.3-107	101	3.95	3.92
	1.00	5	95.7-99.6	97.2	1.73	1.78
Compound UK	0.100 (LOQ)	7	84.2-106	97.5	6.78	6.95
	1.00	5	90.7-99.5	93.9	3.56	3.79

Data (uncorrected recovery results, pp. 43-45) were obtained from Tables 1-24, pp. 56-79 and Appendix 5, Tables 4A-4H, pp. 223-230 of MRID 50862501.

1 The groundwater (pH 6.31; dissolved oxygen concentration 5.62 mg/L) obtained as unfiltered well water, and surface water (pH 6.2; dissolved oxygen concentration 6.2 mg/L) collected from Taunton River (collected from an area of the river with *ca.* 30 to 60 cm of overlying water) were used (pp. 23-24; Appendix 4, pp. 213-214). The water characterization was performed by Smithers Viscient. For the surface water, SMV Lot No.12 Jul 17 Wat-B, collected on 12 July 2017, was used for the Original ECM, and SMV Lot No.19 Mar 18 Wat-A, collected on 19 March 2018, was used for the Updated ECM.

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* 351.44→331.08 and *m/z* 351.44→275.05 for Compound E, *m/z* 512.16→324.21 and *m/z* 512.16→315.29 for Compound UK, *m/z* 142.13→70.02 and *m/z* 142.13→72.17 for Compound R, *m/z* 189.01→144.86 and *m/z* 235.08→188.82 for Compound C, and *m/z* 215.04→170.98 and *m/z* 261.09→214.88 for Compound F.

3 BAS 315 I and metabolite Compound UK were re-validated using the more abundant carbon isotope (^{12}C ; p. 34; Appendix 5, pp. 216-220 of MRID 50862501). This updated methodology was more robust and considered the official methodology. The re-evaluation (Updated ECM) was performed with the same water matrices as the Original ECM. Analytes were identified using two ion transitions (quantitation and confirmation, respectively): m/z 495.44 \rightarrow 323.21 and m/z 495.44 \rightarrow 368.12 for BAS 315 I and m/z 511.36 \rightarrow 323.10 and m/z 511.36 \rightarrow 314.21 for Compound UK.

Table 3. Independent Validation Method Recoveries for Hydramethylnon (BAS 315 I) and its Metabolites Compound E, Compound UK, Compound R, Compound C, and Compound F in Groundwater and surface water^{1,2,3}

Analyte	Fortification Level ($\mu\text{g/L}$)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Groundwater						
Quantitation Ion Transition						
Hydramethylnon (BAS 315 I)	0.100 (LOQ)	5	80-101	88	9	10
	1.00	5	92-118	101	10	10
Compound E	0.100 (LOQ)	5	109-120	114	4	4
	1.00	5	104-118	111	6	5
Compound UK	0.100 (LOQ)	5	76-87	83	4	5
	1.00	5	94-105	99	5	5
Compound R	0.100 (LOQ)	5	75-89	79	6	7
	1.00	5	81-90	85	3	4
Compound C	0.100 (LOQ)	5	90-101	94	5	5
	1.00	5	87-112	95	10	10
Compound F	0.100 (LOQ)	5	94-103	98	4	5
	1.00	5	92-116	101	10	10
Confirmation Ion Transition						
Hydramethylnon (BAS 315 I)	0.100 (LOQ)	5	80-103	91	8	9
	1.00	5	96-107	104	5	4
Compound E	0.100 (LOQ)	5	105-109	107	1	1
	1.00	5	98-112	104	5	5
Compound UK	0.100 (LOQ)	5	78-86	84	3	4
	1.00	5	95-110	102	6	6
Compound R	0.100 (LOQ)	5	74-94	85	8	9
	1.00	5	81-94	88	5	6
Compound C	0.100 (LOQ)	5	85-99	92	6	7
	1.00	5	83-108	93	10	10
Compound F	0.100 (LOQ)	5	89-102	96	6	6
	1.00	5	91-116	99	10	10
Surface Water						
Quantitation Ion Transition						
Hydramethylnon (BAS 315 I)	0.100 (LOQ)	5	61-79	71	7	10
	1.00	5	93-112	103	7	7
Compound E	0.100 (LOQ)	5	107-120	114	5	4
	1.00	5	109-123	114	6	5
Compound UK	0.100 (LOQ)	5	85-105	98	8	9

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	1.00	5	104-125	115	8	7
Compound R	0.100 (LOQ)	5	74-82	78	3	4
	1.00	5	77-89	80	5	7
Compound C	0.100 (LOQ)	5	85-124	98	15	15
	1.00	5	99-104	101	2	2
Compound F	0.100 (LOQ)	5	92-131	104	16	15
	1.00	5	102-110	107	3	3
Confirmation Ion Transition						
Hydramethylnon (BAS 315 I)	0.100 (LOQ)	5	61-76	68	7	11
	1.00	5	89-100	96	4	5
Compound E	0.100 (LOQ)	5	104-114	108	4	4
	1.00	5	102-113	106	5	4
Compound UK	0.100 (LOQ)	5	87-107	98	8	8
	1.00	5	109-128	116	8	7
Compound R	0.100 (LOQ)	5	72-78	76	2	3
	1.00	5	74-85	79	4	5
Compound C	0.100 (LOQ)	5	80-120	95	15	16
	1.00	5	95-101	98	2	2
Compound F	0.100 (LOQ)	5	92-132	105	16	15
	1.00	5	105-111	108	3	3

Data (uncorrected recovery results, Table 41, pp. 78-81) were obtained from Tables 1-24, pp. 40-63 of MRID 50862502.

- 1 The groundwater and surface water were provided by Smithers Viscient, but characterization data was not reported in the study (p. 24). The water characterization was maintained by Smithers Viscient.
- 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 351→331 and m/z 351→275 for Compound E, and m/z 511→323 and m/z 511→314 for Compound UK, m/z 142→70 and m/z 142→72 for Compound R, m/z 189→145 and m/z 235→189 for Compound C, and m/z 215→171 and m/z 261→215 for Compound F.
- 3 The ECM was performed by the ILV with the Updated ECM for BAS 315 I and Compound UK.

III. Method Characteristics

The LOQ for BAS 315 I and its metabolites Compound E, Compound UK, Compound R, Compound C, and Compound F in groundwater and surface water was 0.100 µg/L in the ECM and ILV (pp. 42-43, 45-47, 52-53; Appendix 5, p. 216 of MRID 50862501; pp. 36-37 of MRID 50862502). 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:

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

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 groundwater and surface water was calculated as 0.002-0.01 µg/L (Q)

and 0.01-0.02 µg/L (C) for BAS 315 I, 0.004-0.007 µg/L (Q) and 0.009-0.01 µg/L (C) for Compound E, 0.005-0.01 µg/L (Q) and 0.01-0.02 µg/L (C) for Compound UK, 0.01 µg/L (Q) and 0.01-0.02 µg/L (C) for Compound R, 0.007-0.008 µg/L (Q) and 0.01-0.02 µg/L (C) for Compound C, and 0.02-0.03 µg/L (Q & C) for Compound F. The LODs were calculated in the Updated ECM as 0.01 µg/L (Q & C) for BAS 315 I and 0.009-0.01 µg/L (Q) and 0.01-0.02 µg/L (C) for Compound UK. The method LOD for all analytes in groundwater and surface water was reported as 0.03 µg/L (30% of the LOQ) in the ILV. In the ILV, the LOD was defined as the absolute amount of analyte injected (0.0015 ng for BAS 315 I, Compound E and Compound UK; 0.015 ng for Compound R; 0.0024 ng for Compound C and Compound F) into the LCMS/MS when the lowest calibration standard (0.015 µg/L for BAS 315 I, Compound E and Compound UK; 0.15 µg/L for Compound R; 0.024 µg/L for Compound C and Compound F) 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., 0.0300 µg/L) and DF_{CTRL} is the dilution factor of the control samples. The MDL was calculated as 0.0600 µg/L for BAS 315 I, Compound E, and Compound UK, 0.0500 µg/L for Compound R, and 0.0625 µg/L for Compound C and Compound F (including the Updated ECM for BAS 315 I and Compound UK).

Table 4. Method Characteristics

Analyte		Hydramethylnon (BAS 315 I)	Compound E	Compound UK	Compound R	Compound C	Compound F	
Limit of Quantitation (LOQ)		0.100 µg/L						
Limit of Detection (LOD)	ECM	Method	Not reported					
		Calculated - Original	0.002-0.01 µg/L (Q) 0.01-0.02 µg/L (C)	0.004-0.007 µg/L (Q) 0.009-0.01 µg/L (C)	0.005-0.01 µg/L (Q) 0.01-0.02 µg/L (C)	0.01 µg/L (Q) 0.01-0.02 µg/L (C)	0.007-0.008 µg/L (Q) 0.01-0.02 µg/L (C)	0.02-0.03 µg/L (Q & C)
		Calculated - Updated	0.01 µg/L (Q & C)	Not updated	0.009-0.01 µg/L (Q) 0.01-0.02 µg/L (C)	Not updated		
	ILV	Method	0.03 µg/L (30% of the LOQ)					
		Calculated	Not calculated					
Linearity (calibration curve r^2 and concentration range) ¹	ECM	Original	$r^2 = 1.00$ (Q, GW) $r^2 = 0.999$ (C, GW) $r^2 = 1.00$ (Q, SW) $r^2 = 0.998$ (C, SW)	$r^2 = 0.998$ (Q, GW) $r^2 = 0.997$ (C, GW) $r^2 = 1.00$ (Q & C, SW)	$r^2 = 1.00$ (Q, GW) $r^2 = 0.998$ (C, GW) $r^2 = 0.999$ (Q, SW) $r^2 = 0.996$ (C, SW)	$r^2 = 0.996$ (Q & C, GW) $r^2 = 0.997$ (Q, SW) $r^2 = \mathbf{0.993}$ (C, SW)	$r^2 = \mathbf{0.992}$ (Q & C, GW) $r^2 = 0.994$ (Q, SW) $r^2 = 0.998$ (C, SW)	$r^2 = \mathbf{0.993}$ (Q, GW) $r^2 = \mathbf{0.992}$ (C, GW) $r^2 = 0.996$ (Q, SW) $r^2 = \mathbf{0.990}$ (C, SW)
		Updated	$r^2 = 0.999$ (Q, GW) $r^2 = 0.9989$ (C, GW) $r^2 = \mathbf{0.993}$ (Q, SW) $r^2 = 0.996$ (C, SW)	Not updated	$r^2 = 0.999$ (Q, GW) $r^2 = 0.995$ (C, GW) $r^2 = 0.998$ (Q, SW) $r^2 = 0.999$ (C, SW)	Not updated		
		Range	0.0300-0.300 µg/L			0.250-2.00 µg/L	0.0500-0.50 µg/L	
	ILV		$r^2 = \mathbf{0.9874}$ (Q, GW) $r^2 = \mathbf{0.9928}$ (C, GW) $r^2 = \mathbf{0.9894}$ (Q, SW) $r^2 = \mathbf{0.9862}$ (C, SW)	$r^2 = 0.9958$ (Q, GW) $r^2 = 0.9968$ (C, GW) $r^2 = 0.9966$ (Q, SW) $r^2 = 0.9952$ (C, SW)	$r^2 = \mathbf{0.9876}$ (Q, GW) $r^2 = \mathbf{0.9904}$ (C, GW) $r^2 = \mathbf{0.9870}$ (Q, SW) $r^2 = \mathbf{0.9849}$ (C, SW)	$r^2 = 0.9952$ (Q, GW) $r^2 = \mathbf{0.9894}$ (C, GW) $r^2 = 0.9978$ (Q, SW) $r^2 = 0.9976$ (C, SW)	$r^2 = \mathbf{0.9928}$ (Q, GW) $r^2 = \mathbf{0.9920}$ (C, GW) $r^2 = \mathbf{0.9936}$ (Q, SW) $r^2 = \mathbf{0.9926}$ (C, SW)	$r^2 = 0.9972$ (Q, GW) $r^2 = 0.9962$ (C, GW) $r^2 = 0.9976$ (Q, SW) $r^2 = 0.9964$ (C, SW)
		Range	0.015-0.30 µg/L			0.150-2.00 µg/L	0.024-0.50 µg/L	
Repeatable	ECM	Original ²	Yes at LOQ and 10×LOQ (characterized groundwater and surface water matrices)					
		Updated ³	Yes at LOQ and 10×LOQ (characterized groundwater and surface water matrices)	Not updated	Yes at LOQ and 10×LOQ (characterized groundwater and surface water matrices)	Not updated		

Analyte		Hydramethylnon (BAS 315 I)	Compound E	Compound UK	Compound R	Compound C	Compound F	
	ILV ^{4,5}	Using updated ECM for BAS 315 I and Compound UK (uncharacterized groundwater and surface water matrices)						
		Yes at LOQ and 10×LOQ, except for C ion at LOQ in surface water (mean 68%) ⁶	Yes at LOQ and 10×LOQ					
Reproducible		Yes at LOQ and 10×LOQ, using updated ECM for BAS 315 I and Compound UK.						
Specificity	ECM	Original	Yes, matrix interferences were <5% of the LOQ (based on peak area). Peak tailing was observed.	Yes, matrix interferences were <5% of the LOQ (based on peak area). Cis/trans isomer peaks observed.	Yes, matrix interferences were <5% of the LOQ (based on peak area).	Yes, matrix interferences were <2% of the LOQ (based on peak area). Baseline noise and contaminants interfered with C peak. ⁶	Yes, no matrix interferences were observed, but analyte peak was small compared to baseline noise.	Yes, matrix interferences were <7% (Q) and <15% (C) of the LOQ (based on peak area). Nearby minor contaminant (Q) and baseline noise (C) interfered with analyte peak integration and attenuation.
		Updated	Yes, matrix interferences were <1% of the LOQ (based on peak area). Minor baseline noise interference was observed.	Not updated	Yes, matrix interferences were <1% of the LOQ (based on peak area).	Not updated		
	ILV	Yes, matrix interferences were <2% of the LOQ (based on peak area). Peak tailing was observed. Cis/trans isomer peaks observed.	Yes, no matrix interferences were observed. Cis/trans isomer peaks observed.	Yes, matrix interferences were <15% of the LOQ (based on peak area). Isomer peaks observed.	No , no matrix interferences were observed, but nearby significant, broad contaminant (RT <i>ca.</i> 3.1 min.) noted in Q ion. ⁷ Nearby significant contaminant interfered with C peak. ⁶	Yes, matrix interferences were <5% of the LOQ (based on peak area).	Yes, no matrix interferences were observed. Significant baseline noise was observed in the C chromatograms. ⁶	

Data were obtained from pp. 42-43, 45-47, 52-53; Appendix 5, p. 216 (LOQ/LOD); Tables 1-24, pp. 56-79 and Appendix 5, Tables 4A-4H, pp. 223-230 (recovery data); pp. 52-53 (correlation coefficients); pp. 27-28; Figures 68-91, pp. 161-184; Appendix 5, Figures 4W-4AD, pp. 253-260 (calibration curves); Figures 8-67, pp. 101-160; Appendix 5, Figures 4C-4V, pp. 233-252 (chromatograms) of MRID 50862501; pp. 36-37 (LOQ/LOD); Tables 1-24, pp. 40-63 (recovery data); p. Figure A.1, p. 84; Figure B.1, p. 97; Figure C.1, p. 110; Figure D.1, p. 123; Figure E.1, p. 136; Figure F.1, p. 149; Figure G.1, p. 162; Figure H.1, p. 175; Figure I.1, p. 188; Figure J.1, p. 201; Figure K.1, p. 214; Figure L.1, p. 227; Figure M.1, p. 240; Figure N.1, p. 253; Figure O.1, p. 266; Figure P.1, p. 279; Figure Q.1, p. 292; Figure R.1, p. 305; Figure S.1, p. 318; Figure T.1, p. 331; Figure U.1, p. 344; Figure V.1, p. 357; Figure W.1, p. 370; Figure X.1, p. 383 (calibration curves); Figures A.2-X.12, pp. 85-394 (chromatograms) of MRID 50862502; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition; GW = Groundwater; SW = Surface water.

1 Reported ILV correlation coefficients were reviewer-calculated from r values reported in the study report (Figure A.1, p. 84; Figure B.1, p. 97; Figure C.1, p. 110; Figure D.1, p. 123; Figure E.1, p. 136; Figure F.1, p. 149; Figure G.1, p. 162; Figure H.1, p. 175; Figure I.1, p. 188; Figure J.1, p. 201; Figure K.1, p. 214; Figure L.1, p. 227; Figure M.1, p. 240; Figure N.1, p. 253; Figure O.1, p. 266; Figure P.1, p. 279; Figure Q.1, p. 292; Figure R.1, p. 305; Figure S.1, p. 318; Figure T.1, p. 331; Figure U.1, p. 344; Figure V.1, p. 357; Figure W.1, p. 370; Figure X.1, p. 383 of MRID 50862502; DER Attachment 2). In the ECM, solvent-based calibration standards were used for analysis for all analytes in either groundwater and surface water, except Compound E for which matrix-matched calibration standards were used (pp. 52-53 of MRID 50862501). In the ILV, solvent-based calibration standards were used for analysis for all analytes (p. 29 of MRID 50862502).

2 In the ECM, groundwater (pH 6.31; dissolved oxygen concentration 5.62 mg/L) obtained as unfiltered well water, and surface water (pH 6.2; dissolved oxygen concentration 6.2 mg/L) collected from Taunton River (collected from an area of the river with *ca.* 30 to 60 cm of overlying water) were used (pp. 23-24; Appendix 4, pp. 213-214 of MRID 50862501). The water characterization was performed by Smithers Viscient. For the surface water, SMV Lot No.12 Jul 17 Wat-B, collected on 12 July 2017, was used for the Original ECM, and SMV Lot No.19 Mar 18 Wat-A, collected on 19 March 2018, was used for the Updated ECM.

3 In the ECM, BAS 315 I and metabolite Compound UK were re-validated using the more abundant carbon isotope (^{12}C ; p. 34; Appendix 5, pp. 216-220 of MRID 50862501). This updated methodology was more robust and considered the official methodology. The re-evaluation (Updated ECM) was performed with the same water matrices as the Original ECM.

4 The ILV performed the ECM with the Updated ECM for BAS 315 I and Compound UK. The groundwater and surface water were provided by Smithers Viscient, but characterization data was not reported in the study (p. 24 of MRID 50862502). The water characterization was maintained by Smithers Viscient.

5 The ILV successfully validated the ECM in the first trial (p. 36 of MRID 50862502). In the ILV, the sample fortification and sample processing were summarized without many details (pp. 7, 24, 27, 36-37; Tables 37-40, pp. 73-77). The ECM seemed to be performed as written, except for the use of Waters Oasis MCS SPE columns instead of Waters Oasis MCX SPE columns and minor LC/MS instrument and parameter modifications. The ILV recommendations for the ECM included 1) matrix effect evaluation; 2) the fortification volumes range 1-10% of sample weight and should be $\leq 1\%$ of the sample weight; 3) the use of consistent volumes for the sample processing of Compound R; 4) discussion of LC/MS/MS optimization for chromatographic discrepancies which may arise from the analysis of compounds containing multiple isomers (BAS 315 I, Compound E, and Compound UK); and 5) extract stability evaluation (pp. 36-37). The ECM included the ILV recommendations which were not necessary for the successful validation but will enhance the reproducibility of the ECM (pp. 50-51 of MRID 50862501).

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 G.11-G.12, pp. 172-173 and Figures S.11-S.12, pp. 328-329 of MRID 50862502. Contaminants were noted in reagent blanks and control samples, but not in calibration standards.

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

IV. Method Deficiencies and Reviewer's Comments

1. The specificity of the method was not supported by ILV representative chromatograms of Compound R. No matrix interferences were observed, but nearby significant, broad contaminant (RT *ca.* 3.1 min.; peak height *ca.* 40% of the LOQ peak height) noted in quantitation ion transition representative chromatograms (Figures G.11-G.12, pp. 172-173 and Figures S.11-S.12, pp. 328-329 of MRID 50862502. Contaminants were noted in reagent blanks and control samples, but not in calibration standards. In the confirmation ion transition chromatograms, a nearby significant contaminant (RT *ca.* 3.25 min.; peak height *ca.* 50-70% of the LOQ peak height) interfered with C peak. The contaminants were persistent in both water matrices and fortification levels. Additional sample processing may be required to enhance the specificity of the method for this analyte.
2. The ILV recommendations for the ECM included 1) matrix effect evaluation; 2) the fortification volumes range 1-10% of sample weight and should be $\leq 1\%$ of the sample weight; 3) the use of consistent volumes for the sample processing of Compound R; 4) discussion of LC/MS/MS optimization for chromatographic discrepancies which may arise from the analysis of compounds containing multiple isomers (BAS 315 I, Compound E, and Compound UK); and 5) extract stability evaluation (pp. 36-37 of MRID 50862502). Regarding the multiple isomers, it was noted that the ILV identified a mixture of isomers for BAS 315 I, Compound E, and Compound UK, but the ECM only identified multiple isomers for Compound E. The ECM reported that the sample extracts were "proven stable for 12 hours, ... confirmed by their recoveries" (p. 43).
3. The ILV performed the ECM with the Updated ECM for BAS 315 I and Compound UK. The Updated ECM for BAS 315 I and metabolite Compound UK 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. 34; Appendix 5, pp. 216-220 of MRID 50862501). The ILV did not validate the Original ECM for BAS 315 I and Compound UK; only the ECM with the Updated ECM for BAS 315 I and Compound UK was validated in this method validation ECM/ILV set.
4. The ILV linearity was unsatisfactory for all ions in both water matrices of hydramethylnon [$r^2 = 0.9874$ (Q, GW) and 0.9928 (C, GW); $r^2 = 0.9894$ (Q, SW) and 0.9862 (C, SW)], Compound UK [$r^2 = 0.9876$ (Q, GW) and 0.9904 (C, GW); $r^2 = 0.9870$ (Q, SW) and 0.9849 (C, SW)], and Compound C [$r^2 = 0.9928$ (Q, GW) and 0.9920 (C, GW); $r^2 = 0.9936$ (Q, SW) and 0.9926 (C, SW); Figure A.1, p. 84; Figure B.1, p. 97; Figure C.1, p. 110; Figure D.1, p. 123; Figure E.1, p. 136; Figure F.1, p. 149; Figure G.1, p. 162; Figure H.1, p. 175; Figure I.1, p. 188; Figure J.1, p. 201; Figure K.1, p. 214; Figure L.1, p. 227; Figure M.1, p. 240; Figure N.1, p. 253; Figure O.1, p. 266; Figure P.1, p. 279; Figure Q.1, p. 292; Figure R.1, p. 305; Figure S.1, p. 318; Figure T.1, p. 331; Figure U.1, p. 344; Figure V.1, p. 357; Figure W.1, p. 370; Figure X.1, p. 383 of MRID 50862502). ILV linearity was also unsatisfactory for the confirmation ion transition of Compound R in ground water, $r^2 = 0.9894$ (C, GW). The reviewer noted that this deviation does not affect the linearity acceptability of Compound R in ground water since a confirmatory method is not always necessary when LC/MS or GC/MS is used as the

primary method to generate study data. Linearity is satisfactory when $r^2 \geq 0.995$.

The ECM linearity was unsatisfactory in the Original ECM for Compound C in ground water [$r^2 = 0.992$ (Q & C, GW)] and Compound F in ground water [$r^2 = 0.993$ (Q, GW) and 0.992 (C, GW)]; pp. 27-28; Figures 68-91, pp. 161-184; Appendix 5, Figures 4W-4AD, pp. 253-260 of MRID 50862501]. The ECM linearity was unsatisfactory in the Updated ECM for hydramethylnon in surface water [$r^2 = 0.993$ (Q, SW)]. ECM linearity was also unsatisfactory in the Original ECM for the confirmation ion transitions in surface water of Compound R, $r^2 = 0.993$ (C, SW), and Compound F, $r^2 = 0.990$ (C, SW). The reviewer noted that this deviation does not affect the linearity acceptability of Compound R and Compound F in surface water since a confirmatory method is not always necessary when LC/MS or GC/MS is used as the primary method to generate study data. Linearity is satisfactory when $r^2 \geq 0.995$.

5. Performance data was not acceptable for the confirmation ion transition analysis of hydramethylnon in surface water at the LOQ (mean 68%; Tables 1-24, pp. 40-63 of MRID 50862502). OCSPP guidelines state that recovery means should be between 70% and 120%. The reviewer noted that this deviation does not affect the repeatability or reproducibility of the method for hydramethylnon in surface water since a confirmatory method is not always necessary when LC/MS or GC/MS is used as the primary method to generate study data. Additionally, the study author attributed the low recoveries to matrix suppression since solvent-based calibration standards were used for quantitation (matrix effect for BAS 315 I (confirmation transition) was found to be -19%; p. 29).
6. The reviewer noted that Compound UK was a metabolite with an unknown structure (no proposed structure was provided also; p. 21; Appendix 3, pp. 205-210 of MRID 50862501). The chemical purity was determined at Smithers Viscient using HPLC with diode array detection. No structure determination was performed in the ECM or ILV. The reviewer noted that the ILV reportedly observed isomers for Compound UK in the HPLC/MS/MS (Figures E.11-E.12, pp. 146-147, Figures F.11-F.12, pp. 159-160, Figures Q.11-Q.12, pp. 302-303 and Figures R.11-R.12, pp. 315-316 of MRID 50862502). Isomers were not observed in the ECM.
7. The ILV groundwater and surface water matrices were not characterized.
8. In the ILV, the sample fortification and sample processing were summarized without many details (pp. 7, 24, 27, 36-37; Tables 37-40, pp. 73-77 of MRID 50862502). The ECM seemed to be performed as written, except for the use of Waters Oasis MCS SPE columns instead of Waters Oasis MCX SPE columns and minor LC/MS instrument and parameter modifications. 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: $LOD = (t_{0.99} \times SD)$, 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. In the ILV was defined as the absolute amount of analyte injected (0.0015 ng for BAS 315 I, Compound E and Compound UK; 0.015 ng for Compound R; 0.0024 ng for Compound C and Compound F) into the LCMS/MS when the lowest calibration standard (0.015 µg/L for BAS 315 I, Compound E and Compound UK; 0.15 µg/L for Compound R; 0.024 µg/L for Compound C and Compound F) 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: $MDL = MDL_{LICAL} \times DF_{CTRL}$, where, MDL_{LICAL} 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 Compound UK, 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 ($\leq \pm 20\%$) for all analytes in groundwater and surface water, except Compound E for which matrix-matched calibration standards were used (pp. 52-53 of MRID 50862501).

Matrix effects were studied in the ILV and determined to be insignificant ($\leq \pm 20\%$) for all analytes in groundwater and surface water (p. 29; Tables 25-30, pp. 64-69 of MRID 50862502). 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 Study Monitor being notified of the successful completion of the ILV trial (pp. 37-38 of MRID 50862502). 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. Extract stability of the final sample extracts was studied in the ILV (pp. 35-36; Tables 31-36, pp. 70-72 of MRID 50862502). For BAS 315 I, Compound E and Compound UK, extracts in groundwater were not stable (average recovery >120%) after 11 days of refrigerated storage (both transitions). In surface water, the stored extracts were not stable (average recovery >120%) after 11 days of refrigerated storage for BAS 315 I (quantitation transition), Compound E (both transitions) or Compound UK (both transitions). During the ILV, all sample extracts were analyzed for BAS 315 I, Compound E and Compound UK within 1 day of extraction, and acceptable recovery for fortified samples was used to suggest at least 1 day of extract stability. For Compound R,

extracts in groundwater and surface water were stable (average recovery 70-120%) after 8 days of refrigerated storage. For Compound C and Compound F, extracts in groundwater and surface water were stable (average recovery 70-120%) after 8 days of refrigerated storage.

13. In the ILV, the time required to complete the extraction of one set of 13 samples required *ca.* 7-8 hours of work, excluding calculation of results and LC/MS/MS analysis (p. 35 of MRID 50862502).

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.

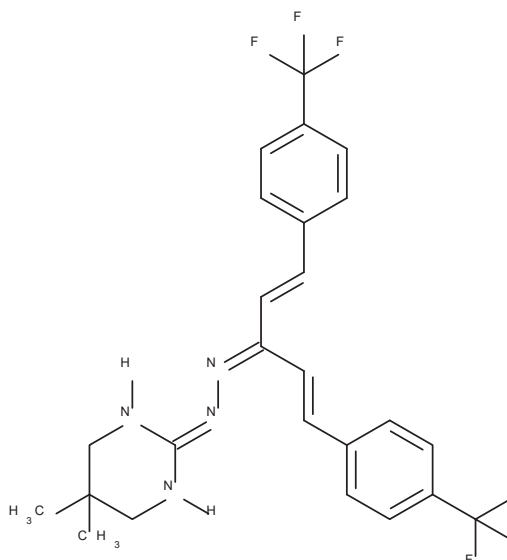
Attachment 1: Chemical Names and Structures**Hydramethylnon (BAS 315 I; Compound A)**

IUPAC Name: 5,5-Dimethylperhydropyrimidin-2-one 4-(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

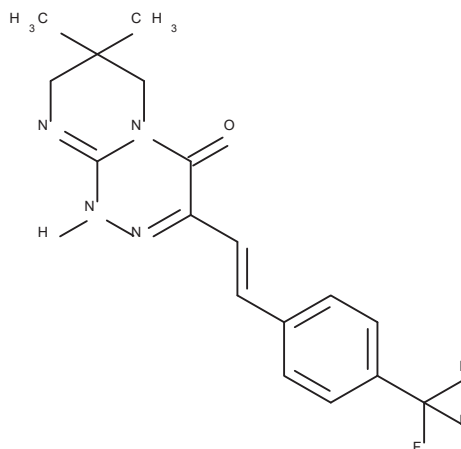
CAS Number: 67485-29-4

SMILES String: N1CC(C)(C)CNC1=NN=C(C=Cc2ccc(C(F)(F)F)cc2)C=Cc3ccc(C(F)(F)F)cc3



COMPOUND E (BAS 4435553; Reg. No. 4435553)

IUPAC Name: 7,7-Dimethyl-3-{(E)-2-[4-(trifluoromethyl)phenyl]ethenyl}-1,6,7,8-tetrahydro-4H-pyrimido[2,1-c][1,2,4]triazin-4-one
CAS Name: Not reported
CAS Number: Not reported
SMILES String: CC1(C)CN=C2NN=C(\C=C\c3ccc(cc3)C(F)(F)F)C(=O)N2C1

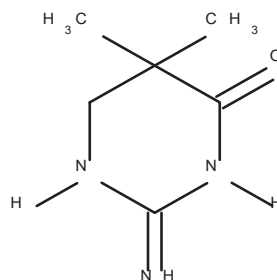
**COMPOUND UK**

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

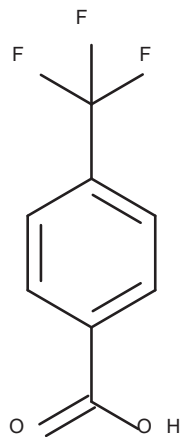
Structure unknown

COMPOUND R

IUPAC Name: 2-Imino-5,5-dimethyltetrahydro-pyrimidin-4-one
CAS Name: Not reported
CAS Number: Not reported
SMILES String: CC1(C)CNC(=N)NC1=O

**Compound C**

IUPAC Name: 4-(Trifluoromethyl)benzoic acid
CAS Name: Not reported
CAS Number: 455-24-3
SMILES String: OC(=O)c1ccc(cc1)C(F)(F)F



Compound F (trans-4-(Trifluoromethyl)cinnamic acid)**IUPAC Name:** Not reported**CAS Name:** Not reported**CAS Number:** 16642-92-5**SMILES String:** Not found