

Analytical method for novaluron in water

Reports: ECM: EPA MRID No.: 51561702. Cashmore, A., and O. Idialu. 2020. Validation of the Analytical Method for the Determination of Novaluron in Aqueous matrices by LC-MS/MS. Report prepared by Smithers ERS Limited, North Yorkshire, United Kingdom, sponsored by ADAMA Makhteshim Ltd., Beer Sheva, Israel, and submitted by Agan Chemical Manufacturers, Ltd., c/o Makhteshim-Agan of North America, Inc. (d/b/a ADAMA), Raleigh, North Carolina; 66 pages. Study No.: 3202771. Sponsor Reference No.: 000106386. Final report issued December 1, 2020

ILV: EPA MRID No.: 51561601. Dwamena, A.K. 2021. Independent Laboratory Validation of the Analytical Method For Determination of Novaluron in Aqueous Matrices by LC-MS/MS. Report prepared by Smithers, Wareham, Massachusetts, sponsored by ADAMA Makhteshim Ltd., Beer-Sheva, Israel, and submitted by Agan Chemical Manufacturers, Ltd., c/o Makhteshim-Agan of North America, Inc. (d/b/a ADAMA), Raleigh, North Carolina; 65 pages. Smithers Study No.: 14125.6133. Final report issued April 29, 2021.

Document No.: MRIDs 51561702 & 51561601

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with UK and OECD GLP standards (p. 3; Appendix 4, p. 66 of MRID 51561702). The study was suitable for submission to US FDA, US EPA, and Japanese regulatory authorities. Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5; Appendix 4, p. 66).
ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160; 1989), as accepted by the OECD GLP (1998; p. 3 of MRID 51561601). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). The statement of authenticity was included with the Quality Assurance statement.

Classification: This analytical method is classified as **Acceptable**.


PC Code: 124002

EFED Final He Zhong, Ph.D.

Reviewer: Biologist


Signature: HE ZHONG
Date: 9/22/2021

Lisa Muto, M.S.,
Environmental Scientist

Signature: 
Date: 08/11/2021

**CDM/CSS-
Dynamac JV**

Reviewers: Mary Samuel, M.S.,
Environmental Scientist

Signature: 
Date: 08/12/2021

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

Executive Summary

The analytical method, Smithers ERS Limited Study No. 3202771, based on Smithers Viscient Analytical Method 14125.6100, is designed for the quantitative determination of novaluron in water at the stated LOQ of 0.01 µg/L. The LOQ is less than 0.026 µg/L the lowest toxicological level of concern in water for novaluron. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted in the ILV report and ECM report, the LLMV was equivalent to the ECM reported method LOQ for novaluron in the tested water matrices (0.01 µg/L).

The ECM and ILV each used one characterized ground water and one characterized surface water. The ILV validated Smithers ERS Limited Study No. 3202771 in the first trial for novaluron in both water matrices with insignificant modifications to the analytical equipment and parameters. Based on the communication summary, the reviewer noted that the ground water chromatograms of the confirmation ion were re-integrated. The ILV modifications did not warrant an updated ECM report.

All submitted ILV and ECM data pertaining to repeatability, accuracy, precision, linearity, and specificity were acceptable at the LOQ and 10×LOQ for novaluron in both waters. The LOD was not reported in the ILV.

The ECM reported several method issues for validating novaluron in ground water. The first validation was unsuccessful due to the use of a commercially-prepared LC mobile phase versus a freshly-prepared LC mobile phase. The second validation was unsuccessful due to poor precision at the LOQ, which was improved by increasing the scan rate and analyzing the quantification and confirmation ion transitions in separate instrument runs. The ECM method was re-issued following validation as SMV 3202771-01V, which was the method provided to the ILV for validation.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation					
Novaluron	51561702 ¹	51561601 ²	Water	01/12/2020	Agan Chemical Manufacturers, Ltd. ³	LC/MS/MS	0.01 µg/L

1 In the ECM, the samples of water were sourced by Smithers Viscient (ESG; p. 12; Appendix 2, pp. 53-54 of MRID 51561702). The waters used were CS38/20 Borehole ground water (pH 8.4, hardness 312 mg/L CaCO₃, dissolved organic carbon 3.68 mg/L) and CS01/20 Fountains Abbey surface water (pH 7.51, hardness 132 mg/L CaCO₃, dissolved organic carbon 8.53 mg/L).

2 In the ILV, the ground water (Smithers Batch No.: GROUND WATER 2019; pH 7.6, hardness 92 mg equivalent to CaCO₃/L, conductivity 0.70 mmhos/cm, total dissolved solids 228 ppm) collected as unadulterated water from a 100-meter bedrock well) and surface water (Smithers Batch No. 28Dec20WAT-A WEWEANTIC WATER; pH 6.6, hardness 14 mg equivalent to CaCO₃/L, conductivity 0.10 mmhos/cm, total dissolved solids 52 ppm) collected from the Weweantic River, West Wareham, Massachusetts, were used in the study (pp. 13-14 of MRID 51561601). The test waters were characterized by Agvise Laboratories, Northwood, North Dakota.

3 c/o Makhteshim-Agan of North America, Inc. (d/b/a ADAMA).

I. Principle of the Method

The analytical method was based on Smithers Viscient Analytical Method 14125.6100, which was modified to cover a lower LOQ (p. 13 of MRID 51561702). Water (7 mL) was fortified with fortification solutions (0.07 mL of 1 µg/L or 10 µg/L solution) then diluted with 3 mL of acetonitrile (dilution factor 1.43; pp. 14-17; Appendix 3, pp. 55-65). The sample was stored refrigerated until analyzed by LC/MS/MS.

Samples were analyzed for novaluron using a Shimadzu Nexera series HPLC system coupled with an AB Sciex API 5000 Triple Quadrupole Mass Spectrometer equipped with electrospray ionization (ESI) interface in the positive ion, multiple reaction monitoring (MRM) mode (500°C; pp. 13, 17 of MRID 51561702). The following LC conditions were used: Waters XBridge BEH C18 column (2.1 mm x 50 mm, 2.5 µm; column temperature 40°C), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.0-0.5 min. 70:30, 1.5 min. 40:60, 4.0-5.0 min. 0.0:100, 5.1-6.1 min. 70:30] and injection volume of 50 µL. Two ion pair transitions were monitored for novaluron (quantitation and confirmation, respectively): m/z 493.0→158.1 and m/z 493.0→141.0. Expected retention time was *ca.* 3.4 minutes for novaluron. The ECM noted that LC column and mobile phases, as well as MS/MS polarity and scan type could not be modified.

The ILV performed Smithers ERS Limited Study No. 3202771 as written, with insignificant modifications to the analytical equipment and parameters (pp. 13, 15, 17-20; Appendix 1, pp. 50-58 of MRID 51561601). Samples were analyzed for novaluron using a Shimadzu LC-20ADXR HPLC system coupled with an AB Sciex API 5000 Mass Spectrometer equipped with an ESI Turbo V ion source. The LC/MS/MS parameters were the same as those of the ECM, except for the increase of the injection volume to 75 µL and modifications of MS resolution and autosampler temperature and wash. Two ion pair transitions were monitored for novaluron (quantitation and confirmation, respectively): m/z 493.0→158.1 and m/z 493.0→141.0. These ion transitions were the same as those used in the ECM. Expected retention time was *ca.* 4.1 minutes for novaluron. The ILV modifications did not warrant an updated ECM.

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.01 µg/L for novaluron in water (pp. 10, 20-25 of MRID 51561702; pp. 10, 22-24 of MRID 51561601). The Limit of Detection (LOD) was calculated to be 0.00108-0.00131 µg/L (Q) and 0.00245-0.00431 µg/L (C) for novaluron in the ECM. The LOD was not reported for novaluron in the ILV. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM (MRID 51561702): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD ≤20%) for analysis of novaluron at fortification levels of 0.01 µg/L (LOQ) and 0.10 µg/L (10×LOQ) in two water matrices (Tables 1-4, pp. 28-31). Two ion pair transitions were monitored for novaluron using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable for both matrices. The samples of water were sourced by Smithers Viscient (ESG; p. 12; Appendix 2, pp. 53-54). The waters used were CS38/20 Borehole ground water (pH 8.4, hardness 312 mg/L CaCO₃, dissolved organic carbon 3.68 mg/L) and CS01/20 Fountains Abbey surface water (pH 7.51, hardness 132 mg/L CaCO₃, dissolved organic carbon 8.53 mg/L). Method

issues for validating novaluron in ground water were reported in ECM (pp. 21-22). The first validation was unsuccessful due to the use of a commercially-prepared LC mobile phase versus a freshly-prepared LC mobile phase. The second validation was unsuccessful due to poor precision at the LOQ, which was improved by increasing the scan rate and analyzing the quantification and confirmation ion transitions in separate instrument runs. The reviewer noted that the MS dwell time was 200 ms in the ECM study report and the following statement was included in the Instrument Conditions section of the ECM study report: “the primary and confirmatory transitions can be monitored separately to improve sensitivity” (p. 17). The reviewer did not see a statement in the ECM regarding the importance of freshly-prepared LC mobile phase solvents; however, the Analytical Method did state that “mobile phases used may be prepared in house” and reagents should be given a nominal expiry of 1 month (Appendix 3, p. 57). The ECM method was re-issued following validation as SMV 3202771-01V (Appendix 3, p. 56).

ILV (MRID 51561601): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD $\leq 20\%$) for analysis of novaluron at fortification levels of 0.01 $\mu\text{g/L}$ (LOQ) and 0.100 $\mu\text{g/L}$ (10 \times LOQ) in two water matrices (p. 24; Tables 1-4, pp. 28-31). Two ion pair transitions were monitored for novaluron using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable for both matrices. The ground water (Smithers Batch No.: GROUND WATER 2019; pH 7.6, hardness 92 mg equivalent to CaCO_3/L , conductivity 0.70 mmhos/cm, total dissolved solids 228 ppm) collected as unadulterated water from a 100-meter bedrock well) and surface water (Smithers Batch No. 28Dec20WAT-A WEWEANTIC WATER; pH 6.6, hardness 14 mg equivalent to CaCO_3/L , conductivity 0.10 mmhos/cm, total dissolved solids 52 ppm) collected from the Weweantic River, West Wareham, Massachusetts, were used in the study (pp. 13-14 of MRID 51561601). The test waters were characterized by Agvise Laboratories, Northwood, North Dakota.

The ILV validated Smithers ERS Limited Study No. 3202771 in the first trial for novaluron in both water matrices with insignificant modifications to the analytical equipment and parameters (pp. 10, 13, 15, 17-20, 24; Appendix 1, pp. 50-58; Appendix 3, pp. 62-65 of MRID 51561601). Based on the communication summary, the reviewer noted that the ground water chromatograms of the confirmation ion were re-integrated. The reviewer noted that Smithers ERS Limited Study No. 3202771 was equivalent to the re-issued ECM Method SMV 3202771-01V.

Table 2. Initial Validation Method Recoveries for Novaluron in Water^{1,2}

Analyte	Fortification Level ($\mu\text{g/L}$)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Fountains Abbey Surface Water						
Quantitation ion transition						
Novaluron	0.01 (LOQ)	5	93.8-120	102	11.1	10.9
	0.10	5	93.0-104	100	4.32	4.32
Confirmation ion transition						
Novaluron	0.01 (LOQ)	5	81.0-109	96.9	12.5	12.9
	0.10	5	95.5-108	103	6.27	6.10

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Borehole Ground Water						
Quantitation ion transition						
Novaluron	0.01 (LOQ)	5	84.0-106	95.2	7.90	8.31
	0.10	5	90.2-108	96.3	6.94	7.20
Confirmation ion transition						
Novaluron	0.01 (LOQ)	5	82.5-104	92.8	8.22	8.86
	0.10	5	92.8-105	98.9	5.00	5.06

Data (uncorrected recovery results; p. 18) were obtained from Tables 1-4, pp. 28-31 of MRID 51561702.

1 The samples of water were sourced by Smithers Viscient (ESG; p. 12; Appendix 2, pp. 53-54). The waters used were CS38/20 Borehole ground water (pH 8.4, hardness 312 mg/L CaCO₃, dissolved organic carbon 3.68 mg/L) and CS01/20 Fountains Abbey surface water (pH 7.51, hardness 132 mg/L CaCO₃, dissolved organic carbon 8.53 mg/L).

2 Two ion pair transitions were monitored for novaluron (quantitation and confirmation, respectively): *m/z* 493.0→158.1 and *m/z* 493.0→141.0.

Table 3. Independent Validation Method Recoveries for Novaluron in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water						
Quantitation ion transition						
Novaluron	0.01 (LOQ)	5	72.6-80.7	76.2	3.25	4.27
	0.10	5	71.4-77.2	74.9	2.36	3.16
Confirmation ion transition						
Novaluron	0.01 (LOQ)	5	72.7-75.8	73.9	1.40	1.90
	0.10	5	75.9-81.4	78.9	2.66	3.37
Surface Water						
Quantitation ion transition						
Novaluron	0.01 (LOQ)	5	85.8-98.1	92.8	5.20	5.61
	0.10	5	87.0-94.9	92.0	3.33	3.62
Confirmation ion transition						
Novaluron	0.01 (LOQ)	5	83.3-104	96.5	8.01	8.30
	0.10	5	94.0-97.2	96.0	1.31	1.36

Data (uncorrected recovery results; pp. 22-23) were obtained from p. 24; Tables 1-4, pp. 28-31 of MRID 51561601.

1 The ground water (Smithers Batch No.: GROUND WATER 2019; pH 7.6, hardness 92 mg equivalent to CaCO₃/L, conductivity 0.70 mmhos/cm, total dissolved solids 228 ppm) collected as unadulterated water from a 100-meter bedrock well) and surface water (Smithers Batch No. 28Dec20WAT-A WEWEANTIC WATER; pH 6.6, hardness 14 mg equivalent to CaCO₃/L, conductivity 0.10 mmhos/cm, total dissolved solids 52 ppm) collected from the Weweantic River, West Wareham, Massachusetts, were used in the study (pp. 13-14). The test waters were characterized by Agvise Laboratories, Northwood, North Dakota.

2 Two ion pair transitions were monitored for novaluron (quantitation and confirmation, respectively): *m/z* 493.0→158.1 and *m/z* 493.0→141.0; these were the same as those of the ECM.

III. Method Characteristics

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.01 µg/L for novaluron in water (pp. 10, 18, 20-25 of MRID 51561702; pp. 10, 21-24 of MRID 51561601). In the ECM, the LOQ was defined as the lowest fortification level validated. In the ILV, the LOQ was defined as the lowest fortification level, with mean recoveries 70-110%, and RSD <20%. The LOQ was further defined as having interferences in the reagent blanks and untreated control samples of ≤20% of the LOQ and ≤50% of the MDL peak height response at the retention time of the test substance. No calculations were provided for the LOQ in the ECM or ILV reports. In the ECM report, the LOD was based upon the sample concentration equivalent to three times the baseline noise of a control sample was calculated as follows:

$$\text{LOD} = 3 \times \text{height of control baseline noise} \times \text{control dilution factor} \times \text{calibration standard concentration } (\mu\text{g/mL}) / \text{height of calibration standard peak.}$$

In the ECM report, the LOD was calculated to be 0.00108-0.00131 µg/L (Q) and 0.00245-0.00431 µg/L (C) for novaluron.

In the ILV report, the LOD was reportedly calculated for novaluron using the following equation:

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

Where, LOD is the limit of detection of the analysis, N_{ctl} is the mean signal to noise in height of the control samples (or Blanks), Resp_{LS} is the mean response in height of the two low calibration standards, Conc_{LS} is the concentration of the low calibration standard, and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 1.43).

In the ILV report, the LOD was not reported or calculated.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

Table 4. Method Characteristics in Water

		Novaluron
Limit of Quantitation (LOQ)*	ECM	0.01 µg/L
	ILV	
Limit of Detection (LOD)	ECM (calc)	0.00108-0.00131 µg/L (Q) 0.00245- 0.00431 µg/L (C) ^{1,2}
	ILV (calc)	Not reported
Linearity (calibration curve r and concentration range)	ECM	r = 0.9992 (Q, SW) r = 0.9976 (C, SW) r = 0.9980 (Q, GW) r = 0.9991 (C, GW)
	ILV ³	r = 0.999 (Q & C)
	Range	0.002-0.100 µg/L
Repeatable	ECM ^{4,5}	Yes at LOQ and 10×LOQ. (characterized ground and surface water matrices used)
	ILV ^{6,7}	
Reproducible ⁹		Yes for 0.01 µg/L (LLMV)* and 0.100 µg/L in tested water matrices
Specific	ECM	Yes for surface water and Q in ground water, no matrix interferences were observed, but some minor baseline noise was observed at LOQ which interfered with peak integration and attenuation. No for C in ground water, LOQ peak was very small compared to baseline noise. ^{2,8}
	ILV	Yes for Q, matrix interferences were <10% of the LOQ, but some minor baseline noise was observed. No for C, LOQ peak (RT <i>ca.</i> 4.1 min.) was very small compared to baseline noise and appeared to co-elute with multiple contaminants at RT <i>ca.</i> 3.9-4.2 min. ^{2,9}

Data were obtained from pp. 10, 18, 20-25 (LOQ/LOD); Tables 1-4, pp. 28-31 (recovery results); p. 20 (calibration data); Figures 1-28, pp. 35-50 (calibration curves & chromatograms) of MRID 51561702; pp. 10, 21-24 (LOQ/LOD); p. 24; Tables 1-4, pp. 28-31 (recovery results); p. 24; Figures 15-16, pp. 48-49 (calibration data & curves); Figures 1-14, pp. 34-47 (chromatograms) of MRID 51561601; DER Excel Attachment. Q = quantitation ion; C = confirmation ion. All results reported for Q and C ions unless specified otherwise. GW = Groundwater; SW = Surface water.

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

1 Value is >30% of the LOQ.

2 Does not affect the validity of the linearity/specificity of the method since a confirmation method is not usually required when LC/MS is the primary method used to generate study data.

3 Reported r values were reviewer-calculated from reported r² values (p. 24 of MRID 51561601; DER Excel Attachment). Rules of significant figures were followed.

4 In the ECM, the samples of water were sourced by Smithers Visient (ESG; p. 12; Appendix 2, pp. 53-54 of MRID 51561702). The waters used were CS38/20 Borehole ground water (pH 8.4, hardness 312 mg/L CaCO₃, dissolved organic carbon 3.68 mg/L) and CS01/20 Fountains Abbey surface water (pH 7.51, hardness 132 mg/L CaCO₃, dissolved organic carbon 8.53 mg/L).

5 Method issues for validating novaluron in ground water were reported in ECM (pp. 21-22 of MRID 51561702). The first validation was unsuccessful due to the use of a commercially-prepared LC mobile phase versus a freshly-prepared LC mobile phase. The second validation was unsuccessful due to poor precision at the LOQ, which was improved by increasing the scan rate and analyzing the quantification and confirmation ion transitions in separate instrument runs. The reviewer noted that the MS dwell time was 200 ms in the ECM study report and the following statement was included in the Instrument Conditions section of the ECM study report: “the primary and confirmatory transitions can be monitored separately to improve sensitivity” (p. 17). The reviewer did not see a statement in the ECM regarding the importance of freshly-prepared LC mobile phase solvents; however, the Analytical Method did state that “mobile phases used may be prepared in house” and reagents should be given a nominal expiry of 1 month

- (Appendix 3, p. 57). The ECM method was re-issued following validation as SMV 3202771-01V (Appendix 3, p. 56).
- 6 In the ILV, the ground water (Smithers Batch No.: GROUND WATER 2019; pH 7.6, hardness 92 mg equivalent to CaCO₃/L, conductivity 0.70 mmhos/cm, total dissolved solids 228 ppm) collected as unadulterated water from a 100-meter bedrock well) and surface water (Smithers Batch No. 28Dec20WAT-A WEWEANTIC WATER; pH 6.6, hardness 14 mg equivalent to CaCO₃/L, conductivity 0.10 mmhos/cm, total dissolved solids 52 ppm) collected from the Weweantic River, West Wareham, Massachusetts, were used in the study (pp. 13-14 of MRID 51561601). The test waters were characterized by Agvise Laboratories, Northwood, North Dakota.
- 7 The ILV validated Smithers ERS Limited Study No. 3202771 in the first trial for novaluron in both water matrices with insignificant modifications to the analytical equipment and parameters (pp. 10, 13, 15, 17-20, 24; Appendix 1, pp. 50-58; Appendix 3, pp. 62-65 of MRID 51561601). Based on the communication summary, the reviewer noted that the ground water chromatograms of the confirmation ion were re-integrated. The ILV modifications did not warrant an updated ECM.
- 8 Based on Figure 26, p. 49 of MRID 51561702. Peak response of the confirmation ion transition was significantly (6xs) less than that of the quantitation ion transition.
- 9 Based on Figure 6, p. 39 and Figure 13, p. 46 of MRID 51561601.

IV. Method Deficiencies and Reviewer's Comments

1. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ (pp. 10, 18, 20-25 of MRID 51561702; pp. 10, 21-24 of MRID 51561601). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM authors, the LLMV was equivalent to the ECM-reported method LOQ for novaluron in the tested water matrices (0.01 µg/L).
2. The LOD was not reported in the ILV.

The ECM author-calculated LOD for the ground water confirmation ion transition was >30% of the LOQ; however, this does not affect the validity of the linearity/specificity of the method since a confirmation method is not usually required when LC/MS is the primary method used to generate study data (p. 20 of MRID 51561702).
3. The communications between the ILV Study Director (Amos K. Dwamena of by Smithers, Wareham, Massachusetts) and Study Sponsor [Miriam Frugis of ADAMA Makhteshim Ltd., c/o Makhteshim-Agan of North America, Inc. (d/b/a ADAMA)] were summarized and included the exchange of ILV laboratory study progress and trial results, the discussion of issues and errors in the ILV study report which the Study Sponsor observed, and the discussion of the decision to perform a second trial for ground water (pp. 1-3; Appendix 3, pp. 62-65 of MRID 51561601). The full ECM final report was transferred to the ILV laboratory during this communication. As a result of the communication between the ILV Study Director and ILV Study Sponsor, the ground water chromatograms of the confirmation ion were re-integrated for the final report and the matrix effect calculation was corrected. Detailed communication records were not provided. No technical communication occurred.
4. The reviewer noted that the ECM and ILV laboratories were part of the same company. ECM was performed by Smithers ERS Limited, North Yorkshire, United Kingdom, and the ILV was performed by Smithers, Wareham, Massachusetts (pp. 1, 6, 13 of MRID 51561702; pp. 1, 6, 13 of MRID 51561601). The laboratory location, personnel and equipment differed

between the two laboratories. The only exchange of information was the full ECM study report provided to the ILV via the Study Sponsor Representative (Appendix 3, p. 63 of MRID 51561601).

5. The specificity of the method in one or both water matrices was not well-supported by the ILV and ECM representative chromatograms for the confirmation ion of novaluron since the LOQ peak was very small compared to baseline noise (Figure 26, p. 49 of MRID 51561702; Figure 6, p. 39 and Figure 13, p. 46 of MRID 51561601). Additionally, in the ILV representative chromatograms, the confirmation ion LOQ peak (RT *ca.* 4.1 min.) appeared to co-elute with multiple contaminants (RT *ca.* 3.9-4.2 min.). However, the reviewer noted that deviations in confirmation ion specificity did not affect the validity of the specificity of the method since a confirmation method is not usually required when LC/MS is the primary method used to generate study data.
6. The same batch of novaluron test material appeared to be used by the ECM and ILV laboratories (p. 12 of MRID 51561702; p. 12 of MRID 51561601).
7. Matrix effects were assessed in the ECM and ILV and determined to be insignificant (<20%) in all test water matrices (p. 21; Tables 5-6, pp. 32-33 of MRID 51561702; p. 24; Tables 5-6, pp. 32-33 of MRID 51561601).
8. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 10, 18, 20-25 of MRID 51561702; pp. 10, 22-24 of MRID 51561601). In the ECM, the LOQ was defined as the lowest fortification level validated. In the ILV, the LOQ was defined as the lowest fortification level, with mean recoveries 70-110%, and RSD <20%. The LOQ was further defined as have interferences in the reagent blanks and untreated control samples of $\leq 20\%$ of the LOQ and $\leq 50\%$ of the MDL peak height response at the retention time of the test substance. No calculations were provided for the LOQ in the ECM or ILV reports. In the ECM report, the LOD was based upon the sample concentration equivalent to three times the baseline noise of a control sample was calculated as follows: $LOD = 3 \times \text{height of control baseline noise} \times \text{control dilution factor} \times \text{calibration standard concentration } (\mu\text{g/mL}) / \text{height of calibration standard peak}$. In the ILV report, the LOD was calculated using the following equation: $LOD = (3 \times (SN_{ctl}) / (Res_{PLS}) \times Con_{CLS} \times DF_{CTRL})$, where, LOD is the limit of detection of the analysis, SN_{ctl} is the mean signal to noise in height of the control samples (or Blanks), Res_{PLS} is the mean response in height of the two low calibration standards, Con_{CLS} is the concentration of the low calibration standard, and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 1.43). Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

The method detection limit (MDL) was calculated in the ECM and ILV reports as dependent upon the lowest concentration calibration standard and the dilution factor of the controls (p. 21 of MRID 51561702; pp. 23-24 of MRID 51561601). In the ECM report, the MDL was equivalent to $0.002 \mu\text{g/L} \times 1.43 = 0.00286 \mu\text{g/L}$ for novaluron; in the ILV report, the MDL was also equivalent to $0.00286 \mu\text{g/L}$ for novaluron ($0.002 \mu\text{g/L} \times 1.43$). This MDL

calculation was not in accordance with the EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (2016).

9. The reviewer noted that the cited linearity requirement in ECM was as follows: “correlation coefficient (r) was acceptable if it was ≥ 0.9975 (which is equivalent to a coefficient of determination (r^2) of ≥ 0.9975 ” (p. 19 of MRID 51561702). The reviewer noted that previous linearity requirements were $r^2 \geq 0.995$ based on the EFED template for 850.6100 review; however, DER acceptance criteria for linearity was updated as of 11/2019 to be satisfactory when $r \geq 0.995$ [Linearity criterion is consistent with Superfund analytical methods for inorganic analytes (National Functional Guidelines for Inorganic Superfund Methods Data Review, EPA-540-R-2017-001, January 2017)].
10. The total time required to perform the method was reported in the ILV as one working day (8 hours) for a typical batch of 42 samples with LC-MS/MS analysis performed overnight (p. 22 of MRID 51561601). The total time required to perform the method was reported in the ECM as one working day (8 hours) for a typical batch of thirteen samples (p. 13 of MRID 51561702).

V. References

- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides>.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347, and Revision 2; 2015 and 2016.

Attachment 1: Chemical Names and Structures**Novaluron**

IUPAC Name: N-[(3-Chloro-4-[1,1,2-trifluoro-2-(trifluoromethoxy)ethoxy]phenyl) amino]carbonyl]-2,6-difluorobenzamide.
 (RS)-1-[3-Chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea.

CAS Name: Benzamide, N-[[[3-chloro-4-[1,1,2-trifluoro-2-(trifluoromethoxy)ethoxy]phenyl]amino]carbonyl]-2,6-difluoro-.

CAS Number: 116714-46-6.

SMILES String: C1(C(=O)NC(=O)NC2=CC=C(OC(F)(F)C(F)OC(F)(F)F)C(Cl)=C2)=C(F)C=C
 C=C1F (Epi Suite 4.0).

