

Analytical method for novaluron and its degradates CPU and CLA in water

Reports: ECM: EPA MRID No.: 50610215. Reibach, P. 2018. Validation of the Analytical Method for the Determination of Novaluron and its Degradates in Aqueous Matrices by LC-MS/MS. Report prepared by Smithers Viscient, Wareham, Massachusetts, sponsored by ADAMA Agricultural Solutions, Ltd., Israel, and submitted by Agan Chemical Manufacturers, Ltd., c/o Makhteshim-Agan of North America, Inc. (d/b/a ADAMA), Raleigh, North Carolina; 121 pages. Smithers Viscient Study No.: 14125.6100. Sponsor Protocol/Project No.: R-38356. Final report issued May 22, 2018.

ILV: EPA MRID No.: 51561701. Cashmore, A., and O. Idialu. 2020. Independent Laboratory Validation of Analytical Method 14125.6100 for the Determination of Novaluron Degradates CPU and CLA in Water. 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; 95 pages. Study No.: 3202770. Final report issued November 10, 2020.

Document No.: MRIDs 50610215 & 51561701

Guideline: 850.6100

Statements: ECM: 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 50610215). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The statement of authenticity was included with the Quality Assurance statement.

ILV: The study was conducted in compliance with UK and OECD GLP standards (p. 3; Appendix 6, p. 95 of MRID 51561701). 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 6, p. 95).

Classification: This analytical method is classified as **Acceptable** for novaluron degradates CPU and CLA. Novaluron was not included as an analyte in the ILV MRID 51561701, even though it was included as an analyte in ECM MRID 50610215.


PC Code: 124002

**EFED Final
Reviewer:** He Zhong, Ph.D.
Biologist


Signature:
Date: 9/22/2021

**CDM/CSS-
Dynamac JV**

Reviewers: Lisa Muto, M.S.,
Environmental Scientist

Signature: 
Date: 08/10/2021

Mary Samuel, M.S.,
Environmental Scientist

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Date: 08/10/2021

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 Viscient Analytical Method 14125.6100, is designed for the quantitative determination of novaluron and its metabolites CPU and CLA in water at the stated LOQ of 0.100 µg/L. The LOQs for CPU and CLA are less than the human health benchmark of 65 µg/L¹ and irrelevant for ecological risk assessment since the degradates are not residues of concern. The LOQ for novaluron is greater than 0.026 µg/L, the lowest toxicological level of concern in water for estuarine/marine invertebrates (MRID 45638212). Novaluron was not included as an analyte in ILV report MRID 51561701. It was included in ILV report MRID 50691104, which was reviewed separately. Therefore, this DER focuses on the degradate analytes included in ILV report MRID 51561701.

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 by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for CPU and CLA in the tested water matrices (0.100 µg/L).

The ECM validation used one uncharacterized ground water and one characterized surface water; the ILV used characterized ground and surface waters. The ILV validated Smithers Viscient Analytical Method 14125.6100 in the first trial for CPU and CLA in both water matrices with insignificant modifications to the analytical equipment and parameters. The original samples of ground water fortified with CPU were re-injected with freshly-prepared calibration standards after the response of the lowest calibrant was not acceptable; no modification was noted for ground water re-injection. The ILV modifications did not warrant an updated ECM.

All submitted ILV data pertaining to repeatability, accuracy, precision, linearity, and specificity were acceptable at the LOQ and 10×LOQ for CPU and CLA in both waters. Although ILV linearity and specificity were acceptable for CPU in both waters, the reviewer noted that one calibration result was excluded to meet linearity standards and a contamination (RT *ca.* 2.67 min.; peak height <10% of LOQ) in both test water matrices was very close to the analyte peak (RT 2.55 min).

All submitted ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for novaluron and its degradates CPU and CLA.

¹ Human health benchmarks are at: <https://ordspub.epa.gov/ords/pesticides/f?p=HHBP:home:6256672777531:>

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						
Novaluron	50610215 ¹	Not included ²		Water	22/05/2018	Agan Chemical Manufacturers, Ltd. ⁴	LC/MS/MS	0.100 µg/L (ECM only)
CPU		51561701 ³						0.100 µg/L (ECM/ILV)
CLA								

1 In the ECM, the ground water was filtered Town of Wareham, Massachusetts, well water; the surface water (pH 7.03, dissolved oxygen 5.22 mg/L, total organic carbon 6.49 mg/L) was collected from the Taunton River in Taunton, Massachusetts (p. 16 of MRID 50610215). The ground water characterization data was not provided.

2 The analytical method for novaluron in water was supported by the concurrent submission of ECM MRID 51561702 and ILV MRID 51561601.

3 In the ILV, the samples of water were sourced by Smithers Viscient (ESG; p. 15; Appendix 2, pp. 80-81 of MRID 51561701). 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).

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

I. Principle of the Method

Water (5 mL) was fortified with mixed fortification solutions (0.05 or 0.5 mL of 0.1 mg/L solution) then diluted with 5 mL of acetonitrile (pp. 17-21 of MRID 50610215). The sample was further diluted 10x (LOQ) or 25x (10×LOQ) with acetonitrile:purified reagent water (50:50, v:v) before aliquots were analyzed by LC/MS/MS.

Samples were analyzed for novaluron, CPU, and CLA using a Shimadzu LC-20ADXR coupled to a Sciex 6500+ QTRAP mass spectrometer equipped with electrospray ionization (ESI) interface in the positive ion, multiple reaction monitoring (MRM) mode (500°C; pp. 15, 21-22 of MRID 50610215). 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 reagent grade water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.01-0.50 min. 70.0:30.0, 1.50 min. 40.0:60.0, 4.00-5.00 min. 0.00:100.00, 5.10-6.10 min. 70.0:30.0] and injection volume of 20 µL. Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 493.1→158.0 and m/z 493.1→140.9 for novaluron, m/z 353.0→275.2 and m/z 353.0→108.1 for CPU, and m/z 310.1→108.0 and m/z 310.1→127.2 for CLA. Expected retention times were *ca.* 3.8, 2.9, and 3.2 minutes for novaluron, CPU, and CLA, respectively.

The ILV performed Smithers Viscient Analytical Method 14125.6100 as written, with the significant modification that novaluron was not included as an analyte and the insignificant modifications to the analytical equipment and parameters (pp. 12, 15-20; Appendix 3, pp. 82-92 of MRID 51561701). Samples were analyzed for CPU and CLA using a Shimadzu Nexera series HPLC system coupled with an AB Sciex API 5000 Triple Quadrupole Mass Spectrometer. The LC/MS/MS parameters were the same as those of the ECM. Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 353.0→275.1 and m/z 353.0→108.0 for CPU, and m/z 310.0→108.0 and m/z 310.0→127.0 for CLA. These ion transitions were similar to those used in the ECM. Expected retention times were *ca.* 2.5 and 2.9 minutes for CPU and CLA,

respectively. The ILV noted that LC column and mobile phases, as well as MS/MS polarity and scan type could not be modified. The ILV modifications did not warrant an updated ECM.

In the ECM, the Limit of Quantification (LOQ) was 0.100 µg/L for novaluron, CPU and CLA in water (pp. 12, 23-26 of MRID 50610215). The Limit of Detection (LOD) was calculated to be 0.004-0.010 µg/L for novaluron, 0.016-0.020 µg/L (Q) and 0.055-0.072 µg/L (C) for CPU, and 0.009-0.011 µg/L for CLA. In the ILV, novaluron was not included as an analyte. The LOQ was 0.100 µg/L for CPU and CLA in water (pp. 22-25 of MRID 51561701). The LOD was calculated to be 0.01001-0.02317 µg/L for CPU and 0.00302-0.00870 µg/L for CLA. 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 50610215): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD ≤20%) for analysis of novaluron, CPU, and CLA at fortification levels of 0.100 µg/L (LOQ) and 1.00 µg/L (10×LOQ) in two water matrices (Tables 1-12, pp. 37-48). Two ion pair transitions were monitored for novaluron, CPU and CLA using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable for all analytes/matrices, except CPU at the LOQ in both water matrices. The ground water was filtered Town of Wareham, Massachusetts, well water; the surface water (pH 7.03, dissolved oxygen 5.22 mg/L, total organic carbon 6.49 mg/L) was collected from the Taunton River in Taunton, Massachusetts (p. 16). The ground water characterization data was not provided.

ILV (MRID 51561701): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD ≤20%) for analysis of CPU and CLA at fortification levels of 0.100 µg/L (LOQ) and 1.00 µg/L (10×LOQ) in two water matrices (p. 22; Tables 1-8, pp. 32-39). Novaluron was not included as an analyte. Two ion pair transitions were monitored for CPU and CLA using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable for all analytes/matrices, except CPU at the LOQ in surface water. The samples of water were sourced by Smithers Viscient (ESG; p. 15; Appendix 2, pp. 80-81 of MRID 51561701). 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). The ILV validated Smithers Viscient Analytical Method 14125.6100 in the first trial for CPU and CLA in both water matrices with insignificant modifications to the analytical equipment and parameters (pp. 12, 15-20, 24; Appendix 4, p. 93). The original samples of ground water fortified with CPU were re-injected with freshly-prepared calibration standards after the response of the lowest calibrant was not acceptable; no modification was noted for ground water re-injection. Smithers Viscient Analytical Method 14125.6100 for novaluron was not validated by the ILV.

Table 2. Initial Validation Method Recoveries for Novaluron, CPU, and CLA 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	0.100 (LOQ)	5	104-110	108	2.73	2.54
	1.00	5	105-114	109	3.76	3.44
CPU	0.100 (LOQ)	5	98.0-111	103	5.63	5.44
	1.00	5	105-113	109	3.22	2.96
CLA	0.100 (LOQ)	5	97.5-110	103	4.60	4.45
	1.00	5	107-115	110	2.75	2.49
Confirmation ion transition						
Novaluron	0.100 (LOQ)	5	97.1-123	109	10.6	9.75
	1.00	5	107-114	110	2.51	2.58
CPU	0.100 (LOQ)	5	95.6-137	113	17.2	15.2
	1.00	5	110-128	116	7.77	6.71
CLA	0.100 (LOQ)	5	99.7-119	107	7.01	6.55
	1.00	5	104-111	109	2.90	2.66
Surface Water						
Quantitation ion transition						
Novaluron	0.100 (LOQ)	5	98.1-104	99.7	2.53	2.54
	1.00	5	95.3-103	101	3.23	3.22
CPU	0.100 (LOQ)	5	86.7-97.7	92.6	4.10	4.43
	1.00	5	89.6-101	97.2	4.45	4.58
CLA	0.100 (LOQ)	5	89.9-97.8	94.1	3.28	3.49
	1.00	5	91.7-101	97.9	3.65	3.73
Confirmation ion transition						
Novaluron	0.100 (LOQ)	5	96.4-110	99.9	6.19	6.19
	1.00	5	93.9-102	99.3	3.15	3.18
CPU	0.100 (LOQ)	5	100-126	110	10.4	9.47
	1.00	5	97.0-108	103	3.93	3.81
CLA	0.100 (LOQ)	5	96.4-103	99.4	2.92	2.94
	1.00	5	91.5-102	97.8	4.39	4.49

Data (uncorrected recovery results; pp. 24-25) were obtained from Tables 1-12, pp. 37-48 of MRID 50610215.

1 The ground water was filtered Town of Wareham, Massachusetts, well water; the surface water (pH 7.03, dissolved oxygen 5.22 mg/L, total organic carbon 6.49 mg/L) was collected from the Taunton River in Taunton, Massachusetts (p. 16). The ground water characterization data was not provided.

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 493.1→158.0 and m/z 493.1→140.9 for novaluron, m/z 353.0→275.2 and m/z 353.0→108.1 for CPU, and m/z 310.1→108.0 and m/z 310.1→127.2 for CLA.

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

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Borehole Ground Water						
Quantitation ion transition						
CPU	0.100 (LOQ)	5	98.7-113	105	5.14	4.90
	1.00	5	93.2-105	99.6	4.26	4.28
CLA	0.100 (LOQ)	5	101-112	107	4.22	3.96
	1.00	5	95.8-107	99.6	4.34	4.36
Confirmation ion transition						
CPU	0.100 (LOQ)	5	101-113	109	4.60	4.23
	1.00	5	97.3-112	101	6.40	6.31
CLA	0.100 (LOQ)	5	95.8-104	101	3.17	3.15
	1.00	5	92.3-99.2	94.8	2.61	2.75
Fountains Abbey Surface Water						
Quantitation ion transition						
CPU	0.100 (LOQ)	5	85.7-106	95.5	7.19	7.53
	1.00	5	100-106	102	2.30	2.25
CLA	0.100 (LOQ)	5	93.6-112	105	7.36	7.02
	1.00	5	90.8-101	96.9	3.80	3.92
Confirmation ion transition						
CPU	0.100 (LOQ)	5	92.6-116	107	10.1	9.50
	1.00	5	89.7-99.1	95.1	3.83	4.03
CLA	0.100 (LOQ)	5	92.3-108	97.4	6.67	6.85
	1.00	5	89.8-95.6	93.3	2.52	2.70

Data (uncorrected recovery results; p. 20) were obtained from p. 22; Tables 1-8, pp. 32-39 of MRID 51561701.

1 Novaluron was not included as an analyte.

2 The samples of water were sourced by Smithers Viscient (ESG; p. 15; Appendix 2, pp. 80-81 of MRID 51561701).

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).

3 Two ion pair transitions were monitored (quantitation and confirmation, respectively): *m/z* 353.0→275.1 and *m/z* 353.0→108.0 for CPU, and *m/z* 310.0→108.0 and *m/z* 310.0→127.0 for CLA; these were similar to those of the ECM.

III. Method Characteristics

In the ECM, the LOQ was 0.100 µg/L for novaluron, CPU, and CLA in water (pp. 12, 23-26 of MRID 50610215). In the ILV, novaluron was not included as an analyte. The LOQ was 0.100 µg/L for CPU and CLA in water (pp. 20, 22-25 of MRID 51561701). In the ECM, the LOQ was defined as the lowest fortification level. In the ILV, the LOQ was reported as the lowest level validated. No calculations were provided for the LOQ in the ECM or ILV. In the ECM, the LOD was calculated using three times the signal-to-noise value of the control samples. The LOD was calculated for each analyte 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., 10).

In the ECM, the LOD was calculated to be 0.004-0.010 µg/L for novaluron, 0.016-0.020 µg/L (Q) and 0.055-0.072 µg/L (C) for CPU, and 0.009-0.011 µg/L for CLA.

In the ILV, the LOD 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 ILV, the LOD was calculated to be 0.01001-0.02317 µg/L for CPU and 0.00302-0.00870 µg/L for CLA.

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	CPU	CLA
Limit of Quantitation (LOQ)*	ECM	0.100 µg/L		
	ILV	Not included ¹	0.100 µg/L	
Limit of Detection (LOD)	ECM (calc)	0.004-0.010 µg/L (Q & C)	0.016-0.020 µg/L (Q) 0.055-0.072 µg/L (C) ^{2,3}	0.009-0.011 µg/L (Q & C)
	ILV (calc)	Not included	0.01001-0.02317 µg/L (Q & C)	0.00302-0.00339 (Q) 0.00751-0.00870 µg/L (C)
Linearity (calibration curve r and concentration range)	ECM ⁴	r = 0.998 (Q & C)	r = 0.997 (Q & C)	r = 0.997 (Q) r = 0.996 (C)
		0.005-0.050 µg/L		
	ILV	Not included	r = 0.9984 (Q, GW) r = 0.9979 (C, GW) ^{3,5} r = 0.9981 (Q, SW) ⁶ r = 0.9979 (C, SW)	r = 0.9996 (Q, GW) r = 0.9991 (C, GW) r = 0.9985 (Q, SW) r = 0.9978 (C, SW) ^{3,7}
		0.003-0.05 µg/L		
Repeatable	ECM ⁸	Yes at LOQ and 10×LOQ. (characterized surface and uncharacterized ground water matrices used)		
	ILV ^{9,10}	Not included	Yes at LOQ and 10×LOQ. (characterized surface and ground water matrices used)	
Reproducible ⁹		No , only one set of performance data was submitted.	Yes for 0.100 µg/L (LLMV)* and 1.00 µg/L in tested water matrices	
Specific	ECM	Yes, no matrix interferences were observed, but some minor baseline noise was observed at LOQ.	Yes for Q, no matrix interferences were observed, but some minor baseline noise was observed at LOQ. No for C, LOQ peak was very small and surrounded by significant matrix interferences (3xLOQ peak height). ^{3,11}	Yes, no matrix interferences were observed, but some minor baseline noise was observed at LOQ.
	ILV	Not included	Yes, no matrix interferences were observed, but some minor baseline noise was observed at LOQ which interfered with peak integration and attenuation [this interference (RT ca. 2.67 min.; peak height <10% of LOQ) was integrated with the analyte (RT 2.55 min.) at 10×LOQ]. ¹²	Yes, no matrix interferences were observed.

Data were obtained from pp. 12, 23-26, 29-34 (LOQ/LOD); Tables 1-12, pp. 37-48 (recovery results); Figures 1-30, pp. 61-90 (chromatograms); Figures 31-36, pp. 91-96 (calibration curve) of MRID 50610215; pp. 22-25 (LOQ/LOD); p. 22; Tables 1-8, pp. 32-39 (recovery results); p. 22 (calibration data); Figures 1-56, pp. 45-76 (calibration curves & chromatograms) of MRID 51561701; 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 Ranges include Q and C LODs unless separated.
 - 2 Value is >30% of the LOQ.
 - 3 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.
 - 4 Reported r values were reviewer-calculated from reported r² values (p. 27 of MRID 50610215; DER Excel Attachment). Rules of significant figures were followed.
 - 5 The study authors excluded two of the sixteen calibration points “to meet linearity criteria $r \geq 0.9975$ ” (p. 22; Figure 2, p. 46 of MRID 51561701).
 - 6 The study authors excluded one of the sixteen calibration points “to meet linearity criteria $r \geq 0.9975$ ” (p. 22; Figure 29, p. 61 of MRID 51561701). One of two 0.003 µg/L analyte peak values was excluded.
 - 7 The study authors excluded one of the sixteen calibration points “to meet linearity criteria $r \geq 0.9975$ ” (p. 22; Figure 44, p. 70 of MRID 51561701).
 - 8 In the ECM, the ground water was filtered Town of Wareham, Massachusetts, well water; the surface water (pH 7.03, dissolved oxygen 5.22 mg/L, total organic carbon 6.49 mg/L) was collected from the Taunton River in Taunton, Massachusetts (p. 16 of MRID 50610215). The ground water characterization data was not provided.
 - 9 In the ILV, the samples of water were sourced by Smithers Viscient (ESG; p. 15; Appendix 2, pp. 80-81 of MRID 51561701). 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).
 - 10 The ILV validated Smithers Viscient Analytical Method 14125.6100 in the first trial for CPU and CLA in both water matrices with insignificant modifications to the analytical equipment and parameters (pp. 12, 15-20, 24; Appendix 4, p. 93 of MRID 51561701). The original samples of ground water fortified with CPU were re-injected with freshly-prepared calibration standards after the response of the lowest calibrant was not acceptable; no modification was noted for ground water re-injection. Smithers Viscient Analytical Method 14125.6100 for novaluron was not validated by the ILV. The ILV modifications did not warrant an updated ECM.
 - 11 Based on Figure 16, p. 76 of MRID 50610215.
 - 12 Based on Figure 13, p. 52 and Figure 41, p. 68 of MRID 51561701.

IV. Method Deficiencies and Reviewer’s Comments

1. Smithers Viscient Analytical Method 14125.6100 (MRID 50610215) was previously submitted and reviewed with MRID 50691103 as the supporting independent validation study. The CDM/CSS-Dynamac review cited the following issues: 1) ILV linearity was not satisfactory for CPU; 2) the specificity of the method for CPU in both matrices and CLA in ground water was not supported by ILV representative chromatograms; and 3) the ECM ground water matrix was not characterized.

The reviewer noted that linearity requirements at the time of review 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)].

2. Smithers Viscient Analytical Method 14125.6100 for novaluron was validated by the ILV report MRID 50691103 but not by the ILV report MRID 51561701. Novaluron was not included as an analyte in ILV report MRID 51561701, even though it was included as an analyte in ECM report MRID 50610215.
3. 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. 12, 23-26 of MRID 50610215; pp. 20, 22-25 of MRID 51561701). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for novaluron degradates CPU and CLA in the tested water matrices (0.100 µg/L). Novaluron was not included in the ILV report as an analyte.

4. The ECM ground water matrix was not characterized (p. 16 of MRID 50610215). The water characterization was reportedly retained in raw data.
5. The reviewer noted that the ILV representative chromatograms for CPU contained a contamination (RT *ca.* 2.67 min.; peak height <10% of LOQ) in both test water matrices which was very close to the analyte peak (RT 2.55 min.; Figure 13, p. 52 and Figure 41, p. 68 of MRID 51561701). This contamination interfered with peak integration and attenuation and was integrated with the analyte at 10×LOQ.

The specificity of the method in both water matrices was not well-supported by the ECM representative chromatograms for the confirmation ion of CPU since the LOQ peak was very small and surrounded by significant matrix interferences (3×LOQ peak height); however, the reviewer noted that this deviation 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 (Figure 16, p. 76 of MRID 50610215).

6. The linearity was acceptable for all ECM and ILV analyses; however, the reviewer noted that the ILV study authors excluded one or two of the sixteen calibration points “to meet linearity criteria $r \geq 0.9975$ ” for three CPU calibration curves (p. 22; Figure 2, p. 46; Figure 29, p. 61; Figure 44, p. 70 of MRID 51561701). Only one of these three calibration curves was for the quantitation ion analysis: CPU in surface water. Data should not be excluded, in general; however, the reviewer noted that the only excluded data point for the quantitation ion analysis of CPU in surface water was one of two 0.003 µg/L analyte peak values.

The ECM calculated LODs for the CPU confirmation ion transition of both water matrices were >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. 26 of MRID 50610215).

7. ECM and ILV performance data were comparable between the quantitation and confirmation analyses, except for the LOQ analysis of CPU in either surface water or both matrices (Tables 1-12, pp. 37-48 of MRID 51561701; p. 22; Tables 1-8, pp. 32-39 of MRID 51561701).
8. In the ECM, the CPU test material chemical purity was 86.9% (p. 14 of MRID 50610215).
9. The communications between the ILV and Study Sponsor (ADAMA Makhteshim Ltd., Beer Sheva, Israel) were reportedly limited to study progress and trial results, including the exchange of the ILV chromatograms of both water matrices “to confirm the absence of significant interference at the retention time of CPU and CLA” (p. 1; Appendix 5, p. 94 of MRID 51561701). Detailed communication records were not provided. The ILV study was monitored by Chaitail Roy of ADAMA. No technical communication occurred.

10. The reviewer noted that the ECM and ILV laboratories were part of the same company. The ECM validation was performed by Smithers Viscient, Wareham, Massachusetts, and the ILV was performed by Smithers Viscient (ESG) Ltd., North Yorkshire, United Kingdom (pp. 1, 5, 15 of MRID 50610215; pp. 1, 6, 15 of MRID 51561701). The laboratory location, personnel and equipment differed between the two laboratories. The only exchange of information was the ECM Method/Protocol provided to the ILV via the Sponsor Representative (p. 15; Appendix 3, pp. 82-92 of MRID 51561701).
11. Matrix effects were assessed in the ECM and ILV and determined to be insignificant (<20%) in all test water matrices (p. 27; Tables 13-24, pp. 49-60 of MRID 50610215; p. 23; Tables 9-12, pp. 40-43 of MRID 51561701). The ILV study report noted that the initial matrix assessment for the CPU quantitative ion transition was inconclusive due to unacceptable precision in both test water matrices, and the matrix assessment was repeated after re-tuning of the instrument to get acceptable results.
12. 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. 12, 23-26, 29-34 of MRID 50610215; pp. 20, 22-25 of MRID 51561701). In the ECM, the LOQ was defined as the lowest fortification level. In the ILV, the LOQ was reported as the lowest level validated. No calculations were provided for the LOQ in the ECM or ILV. In the ECM, the LOD was calculated using three times the signal-to-noise value of the control samples. The LOD was calculated for each analyte using the following equation: $LOD = (3 \times (SN_{ctl}) / (Res_{PLS}) \times Conc_{CLS} \times DF_{CTRL})$, 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, $Conc_{CLS}$ 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., 10). In the ILV, the LOD based upon the sample concentration equivalent to three times the baseline noise of a control sample was calculated as follows: $LOD = 3 \times \text{height of control baseline noise} \times \text{control dilution factor} \times \text{calibration standard concentration } (\mu\text{g/mL}) / \text{height of calibration standard peak}$. 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 as dependent upon the lowest concentration calibration standard and the dilution factor of the controls (p. 25 of MRID 50610215; pp. 20-21, 23 of MRID 51561701). In the ECM, the MDL was equivalent to $0.005 \mu\text{g/L} \times 10.0 = 0.0500 \mu\text{g/L}$ for novaluron and its degradates CPU and CLA; in the ILV, the MDL was equivalent to $0.03 \mu\text{g/L}$ for novaluron degradates CPU and CLA ($0.003 \mu\text{g/L} \times 10$). This MDL calculation was not in accordance with the EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (2016).

13. The ECM noted that an extension for the calibration curve to include a $0.0025 \mu\text{g/L}$ standard was conducted, but no peak for the $0.00250 \mu\text{g/L}$ CPU confirmatory standard was seen. Thus this transition does not meet the SANCO/8256/00 rev. 8.1 guideline that the lowest standard be 30% of the LOQ (pp. 26-27 of MRID 50610215). This does not invalidate the method as this result is for the CPU confirmatory ion only.

14. The total time required to perform the method was reported in the ILV as one working day (8 hours) for a typical batch of thirteen samples (p. 15 of MRID 51561701).

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.

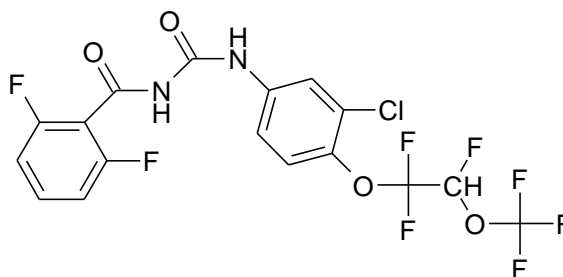
Attachment 1: Chemical Names and Structures**Novaluron**

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).

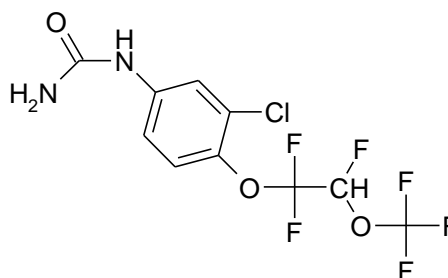
**CPU**

IUPAC Name: N-{3-chloro-4-[1,1,2-trifluoro-2-(trifluoromethoxy)ethoxy]phenyl}urea.

CAS Name: 1-[3-Chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)phenyl]urea.

CAS Number: 554-187-04

SMILES String: Not found



CLA

IUPAC Name: 3-Chloro-4-[1,1,2-trifluoro-2- (trifluoromethoxy)ethoxy]aniline.
CAS Name: 3-Chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)aniline.
CAS Number: 554-136-01
SMILES String: Not found

