

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

Reports: ECM: EPA MRID No.: MRID 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, and 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.: MRID 50691103. Cashmore, A. 2018. Novaluron – Independent Laboratory Validation of Analytical Method 14125.6100 for the Determination of Novaluron and its Degradates in Water. Report prepared by Smithers Viscient (ESG) Ltd., North Yorkshire, United Kingdom, and 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; 103 pages. Study No.: 3201701. Sponsor Study ID: R-38357. Final report issued July 24, 2018 (p. 3).

Document No.: MRIDs 50610215 & 50691103

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR Part 160; 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 3, p. 90 of MRID 50691103). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5; Appendix 3, p. 90).

Classification: This analytical method is classified as **Supplemental**. The specificity of the method for CPU in both matrices and CLA in ground water was not supported by ILV representative chromatograms. The LOQ is greater than the lowest toxicological level of concern for aquatic organisms.

PC Code: 124002

EFED Final A'ja Duncan, Ph.D.

Signature:

Reviewer: Chemist

Date: 03/25/2019

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**CDM/CSS-
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Date: 02/21/2019

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

Executive Summary

The analytical method, 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 LOQ is greater than the lowest toxicological level of concern (0.03 µg/L; USEPA, 2014) in water for all three analytes. The ECM used one uncharacterized ground water and one characterized surface water; the ILV used characterized ground and surface waters. The ILV validated the ECM in the first trial for all three analytes in both water matrices with the further dilution of a majority of the 10×LOQ samples and insignificant modifications to the analytical equipment and parameters; however, a few samples [novaluron (10×LOQ ground water) and CPU (10×LOQ ground water and LOQ/10×LOQ surface water)] passed in the second injection. No modification was noted for ground water re-injection; for surface water CPU samples, the integration smoothing points were changed from 3 to 1 for the CPU primary transition. All submitted ILV and ECM data pertaining to precision, repeatability, and reproducibility was acceptable at the LOQ and 10×LOQ for all three analytes in both waters. ILV linearity was acceptable for novaluron and CLA, but not CPU. The specificity of the method for CPU in both matrices and CLA in ground water was not supported by ILV representative chromatograms; however, the specificity of novaluron in both matrices was acceptable. ECM linearity and specificity were acceptable for all three analytes in both waters. Based on the ILV chromatograms, the reviewer believed that additional clean-up steps may need to be applied to the method.

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	MRID 50610215 ¹	MRID 50691103 ²		Water	22/05/2018	Agan Chemical Manufacturers, Ltd. ³	LC/MS/MS	0.100 µg/L
CPU								
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 In the ILV, the samples of water were sourced by Smithers Viscient (ESG; pp. 14-15; Appendix 2, pp. 88-89 of MRID 50691103). The waters used were CS 14/18 Fountains Abbey surface water (pH 7.44, hardness 86 mg/L CaCO₃, dissolved organic carbon 11.2 mg/L) and CS 13/18 Borehole ground water (pH 8.0, hardness 349 mg/L CaCO₃, dissolved organic carbon 0.00 mg/L).

3 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 the ECM method as written, except for the further dilution of a majority of the 10×LOQ samples and insignificant modifications to the analytical equipment and parameters (pp. 15, 19-21 of MRID 50691103). Samples were analyzed for novaluron, 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 493.1→158.0 and m/z 493.1→141.1 for novaluron, m/z 353.0→275.4 and m/z 353.0→108.3 for CPU, and m/z 310.1→108.0 and m/z 310.1→127.1 for CLA. Expected retention times were *ca.* 3.0, 2.2, and 2.6 minutes for novaluron, CPU, and CLA, respectively. The ILV noted that novaluron stocks should be prepared directly into disposable glass vials (p. 26).

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.100 µg/L for novaluron, CPU and CLA in water (pp. 12, 23-26 of MRID 50610215; pp. 22-25 of MRID 50691103). In the ECM, 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, the LOD was calculated to be 0.00798-0.0471 µg/L for novaluron, 0.0148-0.0390 µg/L for CPU, and 0.00907-0.0443 µg/L for CLA.

II. Recovery Findings

ECM (MRID 50610215): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of novaluron, CPU, and CLA at fortification levels of 0.100 $\mu\text{g/L}$ (LOQ) and 1.00 $\mu\text{g/L}$ (10 \times 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 surface water. 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 50691103): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of novaluron, CPU, and CLA at fortification levels of 0.100 $\mu\text{g/L}$ (LOQ) and 1.00 $\mu\text{g/L}$ (10 \times LOQ) in two water matrices (Tables 1-12, pp. 30-41). 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. The samples of water were sourced by Smithers Viscient (ESG; pp. 14-15; Appendix 2, pp. 88-89). The waters used were CS 14/18 Fountains Abbey surface water (pH 7.44, hardness 86 mg/L CaCO_3 , dissolved organic carbon 11.2 mg/L) and CS 13/18 Borehole ground water (pH 8.0, hardness 349 mg/L CaCO_3 , dissolved organic carbon 0.00 mg/L). The ILV validated the ECM in the first trial for all three analytes in both water matrices with the further dilution of a majority of the 10 \times LOQ samples and insignificant modifications to the analytical equipment and parameters; however, a few samples [novaluron (10 \times LOQ ground water) and CPU (10 \times LOQ ground water and LOQ/10 \times LOQ surface water)] passed in the second injection (pp. 19-21, 25-26; Appendix 4, p. 91). No modification was noted for ground water re-injection; for surface water CPU samples, the integration smoothing points were changed from 3 to 1 for the CPU primary transition.

Table 2. Initial Validation Method Recoveries for Novaluron, CPU and CLA in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water¹						
Quantitation ion ²						
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 ²						
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 Water¹						
Quantitation ion ²						
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 ²						
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 Novaluron, CPU, and CLA in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Borehole Ground Water¹						
Quantitation ion ²						
Novaluron	0.100 (LOQ)	5	103-111	106	3.8	3.6
	1.00	5	95-118	111	9.1	8.3
CPU	0.100 (LOQ)	5	105-132	117	12.0	10.3
	1.00	5	106-127	114	10.0	8.8
CLA	0.100 (LOQ)	5	86-102	95	6.4	6.7
	1.00	5	102-116	111	5.6	5.1
Confirmation ion ²						
Novaluron	0.100 (LOQ)	5	91-115	105	9.5	9.1
	1.00	5	90-119	108	10.8	10.0
CPU	0.100 (LOQ)	5	94-105	99	3.9	3.9
	1.00	5	102-123	113	9.4	8.3
CLA	0.100 (LOQ)	5	81-109	96	11.7	12.2
	1.00	5	106-123	112	7.6	6.8
Fountain Abbey Surface Water¹						
Quantitation ion ²						
Novaluron	0.100 (LOQ)	5	97-110	105	5.3	5.0
	1.00	5	91-112	103	8.2	8.0
CPU	0.100 (LOQ)	5	84-104	94	9.7	10.3
	1.00	5	76-118	94	17.2	18.2
CLA	0.100 (LOQ)	5	98-103	100	2.3	2.3
	1.00	5	100-108	105	3.2	3.0
Confirmation ion ²						
Novaluron	0.100 (LOQ)	5	89-101	93	5.1	5.5
	1.00	5	95-107	99	7.1	7.2
CPU	0.100 (LOQ)	5	90-124	107	12.2	11.4
	1.00	5	83-111	99	10.1	10.2
CLA	0.100 (LOQ)	5	95-105	100	4.1	4.1
	1.00	5	92-109	103	6.9	6.7

Data (uncorrected recovery results; p.14; Appendix 4, pp. 51-52) were obtained from Tables 1-12, pp. 30-41 of MRID 50691103.

1 The samples of water were sourced by Smithers Viscient (ESG; pp. 14-15; Appendix 2, pp. 88-89). The waters used were CS 14/18 Fountains Abbey surface water (pH 7.44, hardness 86 mg/L CaCO₃, dissolved organic carbon 11.2 mg/L) and CS 13/18 Borehole ground water (pH 8.0, hardness 349 mg/L CaCO₃, dissolved organic carbon 0.00 mg/L).

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 493.1→158.0 and m/z 493.1→141.1 for novaluron, m/z 353.0→275.4 and m/z 353.0→108.3 for CPU, and m/z 310.1→108.0 and m/z 310.1→127.1 for CLA; these were similar to those of the ECM.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.100 µg/L for novaluron, CPU, and CLA in water (pp. 12, 23-26 of MRID 50610215; pp. 22-25 of MRID 50691103). 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:

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

Where, LOD is the limit of detection of the analysis, SN_{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 0.00798-0.0471 µg/L for novaluron, 0.0148-0.0390 µg/L for CPU, and 0.00907-0.0443 µg/L for CLA.

Table 4. Method Characteristics for Novaluron, CPU and CLA in Water

Parameter		Novaluron (HEC-5725)	CPU	CLA	
Limit of Quantitation (LOQ)	ECM	0.100 µg/L			
	ILV				
Limit of Detection (LOD) ¹	ECM	0.004-0.010 µg/L	0.016-0.020 µg/L (Q) 0.055-0.072 µg/L (C)	0.009-0.011 µg/L	
	ILV	Ground	0.0249-0.0471 µg/L	0.0148-0.0390 µg/L	0.0371-0.0443 µg/L
		Surface	0.00798-0.0209 µg/L	0.0166-0.0305 µg/L	0.00907-0.0130 µg/L
Linearity (calibration curve r ² and concentration range)	ECM	r ² = 0.996 (Q & C)			
		0.005-0.050 µg/L			
	ILV ³	Ground	r ² = 0.9968-0.9984 (Q) r ² = 0.9910-0.9912 (C) ²	r ² = 0.9904-0.9926 (Q) r ² = 0.9948-0.9974 (C) ²	r ² = 0.9978 (Q) r ² = 0.9920 (C) ²
		Surface	r ² = 0.9978 (Q) r ² = 0.9948 (C) ²	r ² = 0.9904 (Q) r ² = 0.9972 (C)	r ² = 0.9988 (Q) r ² = 0.9944 (C) ²
		0.003-0.050 µg/L			
Repeatable	ECM ⁴	Yes at LOQ and 10×LOQ. (one characterized and one uncharacterized water matrices used)			
	ILV ^{5,6}	Yes at LOQ and 10×LOQ. (two characterized water matrices used)			
Reproducible		Yes at LOQ and 10×LOQ.			
Specific	ECM	Q	Yes, no matrix interferences were observed, but some minor baseline noise was observed at LOQ.	Yes, no matrix interferences were observed, but some minor baseline noise was observed at LOQ.	
		C			
	ILV	Ground	Yes, matrix interferences were <i>ca.</i> 9-14% of the LOQ (based on peak area), but minor baseline noise was observed in C ion chromatograms.	No, no matrix interferences were observed, but LOQ peak was very small to small compared to surrounding baseline noise (25-30% of LOQ peak height). ⁷	No, matrix interferences were <i>ca.</i> 20-23% of the LOQ (based on peak area). ⁸ Minor baseline noise was observed at LOQ.
		Surface	Yes, matrix interferences were <i>ca.</i> 6% of the LOQ (based on peak area), but minor baseline noise was observed in C ion chromatograms.		Yes, matrix interferences were <i>ca.</i> 4% of the LOQ (based on peak area), but some minor baseline noise was observed at LOQ.

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); Tables 1-12, pp. 30-41 (recovery results); p. 24 (calibration data); Figures 1-58, pp. 49-81 (calibration curves & chromatograms) of MRID 50691103; DER Attachment 2. Q = quantitation ion; C = confirmation ion. All results reported for Q and C ions unless specified otherwise.

¹ Ranges include Q and C LODs unless separated.

- 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^2 values were reviewer-calculated from r values provided in the study report (p. 24 of MRID 50691103; DER Attachment 2). Reported ranges include LOQ and 10×LOQ sample set correlation data; LOQ and 10×LOQ samples were run separately for novaluron and CPU in ground water (second injection). Solvent-based standards were used for all samples, except for CLA in surface water (p. 23).
 - 4 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.
 - 5 In the ILV, the samples of water were sourced by Smithers Viscient (ESG; pp. 14-15; Appendix 2, pp. 88-89 of MRID 50691103). The waters used were CS 14/18 Fountains Abbey surface water (pH 7.44, hardness 86 mg/L CaCO_3 , dissolved organic carbon 11.2 mg/L) and CS 13/18 Borehole ground water (pH 8.0, hardness 349 mg/L CaCO_3 , dissolved organic carbon 0.00 mg/L).
 - 6 The ILV validated the ECM in the first trial for all three analytes in both water matrices with the further dilution of a majority of the 10×LOQ samples and insignificant modifications to the analytical equipment and parameters; however, a few samples [novaluron (10×LOQ ground water) and CPU (10×LOQ ground water and LOQ/10×LOQ surface water)] passed in the second injection (pp. 19-21, 25-26 of MRID 50691103). No modification was noted for ground water re-injection; for surface water CPU samples, the integration smoothing points were changed from 3 to 1 for the CPU primary transition.
 - 7 Based on Figure 49, p. 77 and Figure 55, p. 80 of MRID 50691103.
 - 8 Based on Figures 23-28, pp. 62-64 of MRID 50691103.
- Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The specificity of the method for CPU was not supported by ILV representative chromatograms since the LOQ peak was very small to small compared to surrounding baseline noise (25-30% of LOQ peak height; Figure 49, p. 77 and Figure 55, p. 80 of MRID 50691103).

The specificity of the method for CLA in ground water was not supported by ILV representative chromatograms since matrix interferences were *ca.* 20% (Q) and *ca.* 23% (C) of the LOQ (based on peak area; Figures 23-28, pp. 62-64 of MRID 50691103). Since the calculated LODs were 0.0371 $\mu\text{g/L}$ (Q) and 0.0443 $\mu\text{g/L}$ (C) for CLA in ground water, these matrix interferences are >50% of the LOD.

ECM representative chromatograms did not support the specificity of the method for the confirmation ion of CPU since the LOQ peak was very small and surrounded by significant matrix interferences (3xLOQ peak height); however, 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 (Figures 1-30, pp. 61-90 of MRID 50610215).

Based on the ILV chromatograms, additional clean-up steps may need to be applied to the method.

2. The LOQ (0.10 $\mu\text{g/L}$) is greater than the lowest toxicological level of concern (0.03 $\mu\text{g/L}$; USEPA, 2014) in water for all three analytes.
3. The ECM ground water matrix was not characterized (p. 16 of MRID 50610215).

4. ILV linearity was not satisfactory for the quantitation ion analysis of CPU in ground water ($r^2 = 0.9904-0.9926$, LOQ and $10\times\text{LOQ}$) and surface water ($r^2 = 0.9904$; p. 24 of MRID 50691103; DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$.

ILV linearity was not satisfactory for the confirmation ion analysis of novaluron in ground water ($r^2 = 0.9910-0.9912$, LOQ and $10\times\text{LOQ}$) and surface water ($r^2 = 0.9948$), CPU in ground water ($r^2 = 0.9948$, LOQ), and CLA in ground water ($r^2 = 0.9920$) and surface water ($r^2 = 0.9944$; p. 24 of MRID 50691103; DER Attachment 2). ECM linearity was not satisfactory for the confirmation ion analysis of CPU ($r^2 = 0.994$) and CLA ($r^2 = 0.993$; Figures 31-36, pp. 91-96 of MRID 50610215). The deviations do not affect the validity of the linearity of the method since a confirmation method is not usually required when LC/MS is the primary method used to generate study data.

5. The ILV noted that, after the first validation attempt for surface water failed for the CPU primary transition, the precision also looked poor for the re-injection (p. 26 of MRID 50691103). On examination of the data, there were insufficient data points per peak using 3-point smoothing for the CPU primary transition. The smoothing was removed for all samples for CPU primary for the surface water validation batch, allowing more accurate integration of the peaks. The recovery data was then acceptable.
6. The ECM was performed by Smithers Viscient, Wareham, Massachusetts, and the ILV was performed by Smithers Viscient (ESG) Ltd., North Yorkshire, United Kingdom (pp. 1, 5 of MRID 50610215; pp. 1, 6 of MRID 50691103). No personnel overlapped between the ECM and ILV.

The communications between the ECM and ILV were reportedly limited to study progress and trial results (Appendix 5, p. 92 of MRID 50691103). Detailed communication records were not provided.

7. The reported limit of quantification (LOQ) was determined as the lowest level of method validation (LLMV). Further work could have been done to explore the actual LOQ. This means that concentrations may be reliably quantified at the LOQ (i.e., LLMV), but whether lower concentrations may also be reliably quantified is uncertain. (pp. 12, 23-26 of MRID 50610215; pp. 22-25 of MRID 50691103). 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.
8. The matrix effects were assessed in the ILV (pp. 23, 25, 27; Tables 13-18, pp. 42-47 of MRID 50691103). Solvent-based standards were used for all samples, except for CLA in surface water for which matrix effects were found to be significant. In the ECM, it was determined that matrix effects were not significant for any analyte in either matrix (pp. 27, 29-34; Tables 13-24, pp. 49-60 of MRID 50610215).
9. 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.

10. The time required to complete the method was not reported in the ECM or ILV.

V. References

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

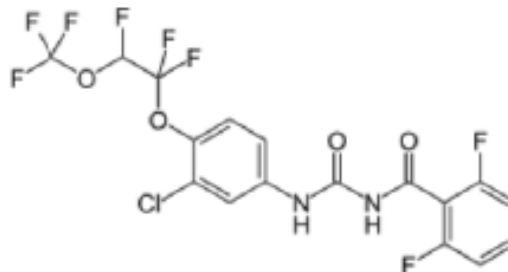
USEPA. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

USEPA. 2014. Aquatic Life Benchmarks and Ecological Risk Assessments for Registered Pesticides. http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm.

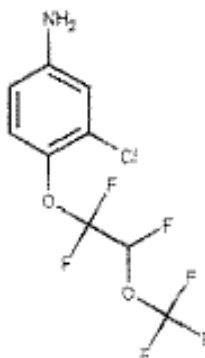
USEPA 2015. Registration Review Problem Formulation for Novaluron. DP barcode 447941, 449151. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Environmental Fate and Effects Division. Memorandum to the Pesticide Re-Evaluation Division. Feb. 26, 2015.

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

IUPAC Name: Not reported
CAS Name: Not reported
CAS Number: 116714-46-6
SMILES String: Not found

**CLA**

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



CPU

IUPAC Name: Not reported
CAS Name: 1-[3-Chloro-4-(1,1,2-trifluoro-2 trifluoromethoxyethoxy)phenyl]urea
CAS Number: 554-187-04
SMILES String: Not found

