Analytical method for chlormequat chloride in water

Reports: ECM: EPA MRID No.: 51293001. Gassen, M. (Amendment No. 1); Janusch, F. (Final Report). 2016. Report Amendment Number 1: Chlormequat chloride: Validation of a Residue Analytical Method for the Determination of Chlormequat Chloride in Drinking and Surface Water. Report prepared by Envigo CRS (Switzerland) Limited (formerly Harlan Laboratories Ltd.), Itingen, Switzerland, sponsored by Taminco BVBA (a subsidiary of Eastman Chemical Company), Ghent, Belgium, and submitted by Eastman Chemical Company, Kingsport, Tennessee (p. 2 of Amendment No. 1); 73 pages (including an unacknowledged second p. 2 in the Amendment No. 1). Study Identification/Harlan Study No.: Study Number D96103. Final report issued November 6, 2014 (p. 1 of Final Report); Amendment dated March 1, 2016 (p. 1 of Amendment No. 1).

ILV: EPA MRID No.: 51121209. Cashmore, A., and O. Idialu. 2019. Chlormequat Chloride - Independent Laboratory Validation of Analytical Method D96103 for the Determination of Chlormequat Chloride in Water. Report prepared by Smithers ERS Limited, North Yorkshire, United Kingdom, and sponsored and submitted by Eastman Chemical Company, Kingsport, Tennessee; 77 pages. Study No.: 3202533. Final report issued December 18, 2019.

Document No.: MRIDs 51293001 & 51121209

Guideline: 850.6100

Statements: ECM Final Report: The study was conducted in compliance with Swiss GLP standards, which are based on OECD GLP (2005) and accepted by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF, and METI), except for the method development pre-tests (p. 5; Appendix 3, p. 64 of MRID 51293001). Signed and dated GLP and Quality Assurance statements were provided (pp. 5-6; Appendix 3, p. 64). An Authenticity statement was included with the Quality Assurance statement (p. 6). A Data Confidentiality statement was not included.

ECM Amendment No. 1: The report contained signed and dated Data Confidentiality, Authenticity, and Quality Assurance statements were provided (pp. 2-4 of MRID 51293001 Amendment No. 1; unacknowledged second p. 2 included in citation). A signed and dated GLP statement was included, but no compliance guidelines were listed (unacknowledged second p. 2 in the Amendment No. 1).

ILV: The study was conducted in compliance with United Kingdom (1999) GLP standards, as amended by GLP (2004) and OECD GLP (1998), as well as the United Kingdom Department of Health (p. 3; Appendix 6, p. 77 of MRID 51121209). The study was suitable for submission to US FDA, USEPA, and Japanese regulatory authorities. Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5). An Authenticity statement was also included with the GLP and Quality Assurance

statements (pp. 3-4).

Classification: This analytical method is classified as supplemental. The pagination of MRID 51293001 was not uniform or complete. 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 LOQ. Performance data were slightly above the recommended recovery range for the ILV validation in groundwater at the LOQ. The ECM method should be updated to include the use of matrix-matched calibration standards for all analyses, regardless of observed matrix effects.

PC Code: 018101

EFED Final Reviewer:	Cheryl Sutton, Ph.D. Environmental Scientist	Signature: CHERYL SUTTON *Digitally signed by CHERYL SUTTON Date: 2021.06.30 14:44:25 -04'00'* Date: 06/30/2021
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: *Lisa Muto* Date: 05/24/2021
	Mary Samuel, M.S., Environmental Scientist	Signature: *Mary Samuel* Date: 05/24/2021
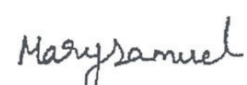
This Data Evaluation Record has been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Harlan Laboratories Ltd./Envigo CRS (Switzerland) Limited Study No. D96103, is designed for the quantitative determination of chlormequat chloride in water at the stated LOQ of 0.10 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for chlormequat chloride. 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. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the method LOQ for chlormequat chloride in water matrices (0.10 µg/L).

The ECM validated the method using characterized drinking water and surface water matrices while the ILV validated the method using characterized groundwater and surface water matrices. The ILV validated the method for chlormequat chloride in surface water at the LOQ and 10×LOQ in the first trial with insignificant modifications to the analytical parameters/instruments. The validation was repeated with matrix-matched calibration standards and was also successful. The method for chlormequat chloride in groundwater at 10×LOQ was validated in the first trial with insignificant modifications to the analytical parameters/instruments; however, the method was not successfully validated in groundwater at the LOQ using all five recoveries. The ILV modifications did not warrant an updated ECM; however, the ECM method should be updated to include the use of matrix-matched calibration standards for all analyses, regardless of matrix effects, since that was how the ECM validation was conducted.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for chlormequat chloride in test water matrices, except for the ILV performance data for groundwater at the LOQ using all five recovery results.

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						
Chlormequat chloride	51293001[1]	51121209[2]		Water	06/11/2014 (Final Report) 01/03/2016 (Amendment No. 1)	Eastman Chemical Company	LC/MS/MS	0.10 µg/L

1 In the ECM, drinking water (pH 6.87, dissolved organic carbon 0.86 mg C/L, total hardness 38.4 °fr.H) obtained as fresh tap water from Harlan Laboratories in Switzerland and surface water (pH 8.23, total organic carbon 56.37 g/L, total hardness 39.6 °fr.H) obtained from a river in Lausen, Switzerland, were used in the study (p. 12 of MRID 51293001). The water characterization laboratory was not specified.

2 In the ILV, groundwater (CS 13/18 Borehole; pH 8.0, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L; hardness 349 mg/L $CaCO_3$) and surface water (CS 14/18 Fountains Abbey; pH 7.44, conductivity 154 µS/cm, dissolved organic carbon 11.2 mg/L; hardness 86 mg/L $CaCO_3$) were collected by Smithers Viscient ERS (p. 13; Appendix 2, pp. 62-63 of MRID 51121209). The surface water was collected from The Lake, Studley Royal, Ripon, United Kingdom. Water characterization was performed at the ILV.

Cited page numbers for "MRID 51293001 Amendment No. 1" refer to the page numbers reported in the Amendment No. 1 of MRID 51293001 authored by M. Gassen and dated March 1, 2016 (PDF file pages 1-9).

Cited page numbers for "MRID 51293001" refer to the page numbers reported in the Final Report of MRID 51293001 authored by F. Janusch and dated November 6, 2014 (PDF file pages 10-73).

I. Principle of the Method

Water samples (5 or 9 mL) were fortified [0.01 or 0.001 µg/mL fortification solution of methanol:water with 100mM heptafluorobutyric acid (50:50, v:v)] and diluted to 10 mL with test water (pp. 13-15 of MRID 51293001). An aliquot was taken for LC/MS/MS analysis.

A schematic representation of the sample preparation was provided (p. 8 of MRID 51293001 Amendment No. 1).

Samples were analyzed for chlormequat chloride using two Shimadzu LC-10AD HPLC coupled with an MDS Sciex API 5000 MS with a Sciex Turbo-V (ESI) ion source operated in the positive ion mode with multiple reaction monitoring (MRM; pp. 16-17 of MRID 51293001). The following LC conditions were used: Inertsil ODS-3 column (2.1 mm x 50 mm, 3.0 µm; column temperature not reported), mobile phase of (A) water with 10mM heptafluorobutyric acid:methanol (95:5, v:v) and (B) water with 10mM heptafluorobutyric acid:methanol (5:95, v:v) [mobile gradient phase of percent A:B (v:v) at 0 min. 100:0, 0.5-2.0 min. 0:100, 2.1-4.0 min. 100:0] and injection volume of 50 µL. MS source temperature was 550°C. Two ion pair transitions were monitored for chlormequat chloride (quantitation and confirmation, respectively): m/z 124.1→58.0 and m/z 122.1→58.0. Observed retention time was *ca*. 1.2 minutes for chlormequat chloride (Figures 2-10, pp. 41-49 of MRID 51293001).

The ILV performed the ECM methods as written, except for insignificant modifications to the analytical parameters/instruments (pp. 14-18; Appendix 3, pp. 66-70 of MRID 51121209). Samples were analyzed for chlormequat chloride using Shimadzu Nexera series HPLC coupled with an AB Sciex API 5000 Triple Quadrupole LC/MS/MS. The LC/MS/MS parameters were the same as those of the ECM, except that the column temperature specified as 40°C and flow rate was specified as 0.3 mL/min. Two ion pair transitions were monitored for chlormequat chloride (quantitation and confirmation, respectively): m/z 124.0→58.0 and m/z 122.0→58.0. These ion transitions were similar to those of the ECM. Reported retention time was *ca*. 1.2 minutes for chlormequat chloride. The ILV noted that the LC column and LC mobile phases could not be modified. The ILV modifications did not warrant an updated ECM.

The Limit of Quantification (LOQ) for chlormequat chloride in water was 0.10 µg/L in the ECM and ILV (pp. 10, 20-21; Appendix 2, p. 62 of MRID 51293001; pp. 11, 19-22 of MRID 51121209). In the ECM, the Limit of Detection (LOD) for chlormequat chloride was calculated as 0.03 µg/L for both test waters based on the lowest calibration standard. In the ILV, the LOD for chlormequat chloride was calculated as 0.03094-0.03271 µg/L for groundwater and 0.01138-0.01179 µg/L (first validation) and 0.00573-0.00634 µg/L (second validation) for 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.

II. Recovery Findings

ECM (MRID 51293001): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of chlormequat chloride at fortification levels of 0.10 µg/L (LOQ) and 1.00 µg/L (10×LOQ) in two water matrices (Tables 1-4, pp. 29-32 of MRID 51293001). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. The reviewer noted that recoveries in the drinking water were lower at 10×LOQ fortification than the LOQ fortification level. The drinking water (pH 6.87, dissolved organic carbon 0.86 mg C/L, total hardness 38.4 °fr.H) obtained as fresh tap water from Harlan Laboratories in Switzerland and surface water (pH 8.23, total organic carbon 56.37 g/L, total hardness 39.6 °fr.H) obtained from a river in Lausen, Switzerland, were used in the study (p. 12 of MRID 51293001). The water characterization laboratory was not specified.

Note: The portion of MRID 51293001 which is deemed MRID 51293001 Amendment No. 1 did not contain any performance data or information about the test matrices.

ILV (MRID 51121209): Mean recoveries and RSDs were within guidelines for analysis of chlormequat chloride at fortification levels of 0.100 µg/L (LOQ) and 1.00 µg/L (10×LOQ) in two water matrices, except for the LOQ fortification in groundwater (means 124-128%, RSDs 51-53%; Tables 1-6, pp. 27-32 of MRID 51121209 and DER Excel Attachment). The study author excluded one value as a statistical outlier (Grubb's test) and reported means, standard deviations, and RSDs for n = 4: 98.1%, 6.45%, and 6.57%, respectively, for the quantitation ion transition and 95.5%, 5.52%, and 5.78%, respectively, for the confirmation ion transition. The surface water validation was repeated due to several low (<70%) recoveries in the first validation, even though overall results of the first validation were acceptable. Matrix-matched calibration standards were used in the second surface water validation while solvent-based calibration standards were used in the first surface water validation since matrix effects were insignificant (<20%; p. 24). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. The groundwater (CS 13/18 Borehole; pH 8.0, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L; hardness 349 mg/L $CaCO_3$) and surface water (CS 14/18 Fountains Abbey; pH 7.44, conductivity 154 µS/cm, dissolved organic carbon 11.2 mg/L; hardness 86 mg/L $CaCO_3$) were collected by Smithers Viscient ERS (p. 13; Appendix 2, pp. 62-63 of MRID 51121209). The surface water was collected from The Lake, Studley Royal, Ripon, United Kingdom. Water characterization was performed at the ILV. The method for chlormequat chloride in surface water at the LOQ and 10×LOQ was validated in the first trial with insignificant modifications to the analytical parameters/instruments (pp. 14-18, 24; Appendix 3, pp. 66-70). The validation was repeated with matrix-matched calibration standards and was also successful. The method for chlormequat chloride in groundwater at 10×LOQ was validated in the first trial with insignificant modifications to the analytical parameters/instruments; however, the method was not successfully validated in groundwater at the LOQ using all five recoveries. The ILV modifications did not warrant an updated ECM.

Table 2. Initial Validation Method Recoveries for Chlormequat chloride in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
Drinking Water						
Quantitation ion transition						
Chlormequat chloride	0.10 (LOQ)	5	96-106	100	4	4
	1.00	5	76-103	87	12	13
Confirmation ion transition						
Chlormequat chloride	0.10 (LOQ)	5	93-111	101	7	7
	1.00	5	74-100	85	11	13
Surface Water						
Quantitation ion transition						
Chlormequat chloride	0.10 (LOQ)	5	83-124	111	16	15
	1.00	5	87-113	103	10	10
Confirmation ion transition						
Chlormequat chloride	0.10 (LOQ)	5	87-133	116	17	15
	1.00	5	92-114	103	10	10

Data (uncorrected recovery results; pp. 17-19) were obtained from Tables 1-4, pp. 24-27 of MRID 51293001 and DER Excel Attachment.

1 The drinking water (pH 6.87, dissolved organic carbon 0.86 mg C/L, total hardness 38.4 °fr.H) obtained as fresh tap water from Harlan Laboratories in Switzerland and surface water (pH 8.23, total organic carbon 56.37 g/L, total hardness 39.6 °fr.H) obtained from a river in Lausen, Switzerland, were used in the study (p. 12 of MRID 51293001). The water characterization laboratory was not specified.

2 Two ion pair transitions were monitored for chlormequat chloride (quantitation and confirmation, respectively): *m/z* 124.1→58.0 and *m/z* 122.1→58.0.

3 Standard deviations were reviewer-calculated since these values were not reported in the study report (see DER Excel Attachment). Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Chlormequat chloride in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
			Borehole Groundwater			
			Quantitation ion transition			
Chlormequat chloride	0.100 (LOQ)	5[3]	93.3-248	**128**	67	**53**
	1.00	5	101-107	103	2.51	2.43
			Confirmation ion transition			
Chlormequat chloride	0.100 (LOQ)	5[3]	91.2-237	**124**	63	**51**
	1.00	5	95.0-105	101	3.91	3.86
			Fountains Abbey Surface Water – First Validation			
			Quantitation ion transition			
Chlormequat chloride	0.100 (LOQ)	5	67.0-79.1	71.2	4.5	6.67
	1.00	5	82.6-86.7	85.2	1.66	1.95
			Confirmation ion transition			
Chlormequat chloride	0.100 (LOQ)	5	67.0-81.8	71.0	6.07	8.54
	1.00	5	85.1-86.4	85.8	0.615	0.717
			Fountains Abbey Surface Water – Second Validation[4]			
			Quantitation ion transition			
Chlormequat chloride	0.100 (LOQ)	5	96.3-100	98.1	1.44	1.47
	1.00	5	102-103	103	0.548	0.534
			Confirmation ion transition			
Chlormequat chloride	0.100 (LOQ)	5	98.1-101	99.7	1.05	1.05
	1.00	5	102-105	103	1.14	1.10

Data (uncorrected recovery results; p. 19) were obtained from Tables 1-6, pp. 27-32 of MRID 51121209 and DER Excel Attachment.

1 The groundwater (CS 13/18 Borehole; pH 8.0, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L; hardness 349 mg/L $CaCO_3$) and surface water (CS 14/18 Fountains Abbey; pH 7.44, conductivity 154 µS/cm, dissolved organic carbon 11.2 mg/L; hardness 86 mg/L $CaCO_3$) were collected by Smithers Viscient ERS (p. 13; Appendix 2, pp. 62-63 of MRID 51121209). The surface water was collected from The Lake, Studley Royal, Ripon, United Kingdom. Water characterization was performed at the ILV.

2 Two ion pair transitions were monitored for chlormequat chloride (quantitation and confirmation, respectively): m/z 124.0→58.0 and m/z 122.0→58.0. These ion transitions were similar to those of the ECM.

3 Means, standard deviations, and RSDs were reviewer-calculated using all five recoveries values (see DER Excel Attachment). Rules of significant figures were followed. The study author excluded one value as a statistical outlier (Grubb's test) and reported means, standard deviations, and RSDs for n = 4; reported means, standard deviations, and RSDs were 98.1%, 6.45%, and 6.57%, respectively, for the quantitation ion transition and 95.5%, 5.52%, and 5.78%, respectively, for the confirmation ion transition (Tables 1-2, pp. 27-28 of MRID 51121209).

4 Surface water validation was repeated due to several low (<70%) recoveries in the first validation, even though overall results were acceptable in the first validation. Matrix-matched calibration standards were used in the second validation while solvent-based calibration standards were used in the first validation since matrix effects were insignificant (<20%; p. 24 of MRID 51121209).

III. Method Characteristics

The LOQ for chlormequat chloride in water was 0.10 µg/L in the ECM and ILV (pp. 10, 17-18, 20-21; Appendix 2, p. 62 of MRID 51293001; pp. 11, 19-22 of MRID 51121209). In the ECM, the LOQ was defined as the lowest fortification level with mean recoveries ranging 70-120% at a RSD of ≤20%. In the ILV, the LOQ was defined as the lowest fortification level validated. In the ECM, the LOD was calculated as 0.03 µg/L for both test waters based on the lowest calibration standard using the following equation:

R (µg/L) = {[final concentration of extract (ng/mL)] × [final sample volume (mL)]} / [volume of sample (mL)],

Where R is the recovered residue analyte, which presumably corresponds to LOD, although this was not specified in the study report.

In the ILV, the LOD was calculated using the following equation:

LOD (µg/L) = 3 × height of control baseline noise × calibration standard concentration (µg/L) / height of calibration peak.

The LOD for chlormequat chloride in water was calculated as 0.03094-0.03271 µg/L for groundwater and 0.01138-0.01179 µg/L (first validation) and 0.00573-0.00634 µg/L (second validation) for surface water for the primary and confirmatory transitions.

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 a true LOQ.

Table 4. Method Characteristics in Water

		Chlormequat chloride
Limit of Quantitation (LOQ)*	ECM	0.10 µg/L
	ILV	
Limit of Detection (LOD)	ECM (calc)	0.03 µg/L (DW & SW)
	ILV (calc)	0.03094-0.03271 µg/L (GW) 0.01138-0.01179 µg/L (SW 1) 0.00573-0.00634 µg/L (SW 2)
Linearity (calibration curve r and concentration range)	ECM[1]	r = 0.9976-0.9989 (Q, DW) r = 0.9990-0.9992 (C, DW) r = 0.9990-0.9991 (Q, SW) r = 0.9989-0.9993 (C, SW)
	ILV[2]	r = 0.9977 (Q, GW) r = 0.9968 (C, GW) r = 0.9981 (Q, SW 1) r = 0.9978 (C, SW 1) r = 0.9981 (Q, SW 2) r = 0.9977 (C, SW 2)
	Range	0.03-1.5 µg/L
Repeatable	ECM[3]	Yes at LOQ (0.10 µg/L) and 10×LOQ (1.00 µg/L) (one characterized drinking water and one characterized surface water)
	ILV[4,5]	Yes at LOQ (0.100 µg/L) and 10×LOQ (1.00 µg/L) (one characterized surface water; first and second validation)[6] **No** at LOQ (0.100 µg/L; means **124-128**%, RSDs **51-53**%)[7] and 10×LOQ (1.00 µg/L) (one characterized groundwater)
Reproducible[6]		Yes for 0.10 µg/L (LLMV)* and 1.00 µg/L in selected water matrices
Specific	ECM	Yes, no matrix interferences were observed. Minor nearby baseline noise was observed.
	ILV	Yes, matrix interferences were <30% for GW, <15% SW 1, and <10% for SW 2. Minor nearby baseline noise was observed which interfered with peak attenuation and integration.

Data were obtained from pp. 10, 17-18, 20-21; Appendix 2, p. 62 (LOQ/LOD); Tables 1-4, pp. 24-27 (recovery results); p. 21; Table 5, p. 28 (correlation coefficients); Tables 6-9, pp. 29-32 (calibration curves); Figures 1-10, pp. 40-49 (chromatograms) of MRID 51293001; pp. 11, 19-22 (LOQ/LOD); Tables 1-6, pp. 27-32 (recovery results); p. 21 (correlation coefficients); Figures 1-2, pp. 36-37; Figures 15-16, pp. 44-45; Figures 29-30, pp. 52-53 (calibration curves); Figures 3-42, pp. 38-59 (chromatograms) of MRID 51121209; DER Excel Attachment. Q = quantitation ion transition; C = confirmation ion transition; DW = Drinking water; SW = Surface Water; GW = Groundwater; SW 1 = Surface Water First Validation; SW 2 = Surface Water Second Validation.

* 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 Matrix-matched calibration standards were used in the ECM for all analyses even though significant matrix influence (> ±20%) was only observed for the quantitation ion transition for the 10×LOQ fortification in surface water (p. 22; Tables 16-17, p. 39 of MRID 51293001).

2 Solvent-based calibration standards were used in the ILV for the groundwater and first surface water validation since insignificant matrix effects (< ±20%) were observed for both transitions in both water matrices (pp. 22-24; Tables 7-8, pp. 33-34 of MRID 51121209). Matrix-matched calibration standards were used in the ILV for the second surface water validation.

3 In the ECM, drinking water (pH 6.87, dissolved organic carbon 0.86 mg C/L, total hardness 38.4 °fr.H) obtained as fresh tap water from Harlan Laboratories in Switzerland and surface water (pH 8.23, total organic carbon 56.37 g/L, total hardness 39.6 °fr.H) obtained from a river in Lausen, Switzerland, were used in the study (p. 12 of MRID 51293001). The water characterization laboratory was not specified.

4 In the ILV, groundwater (CS 13/18 Borehole; pH 8.0, conductivity 436 µS/cm, dissolved organic carbon 0.00 mg/L;

hardness 349 mg/L CaCO$_3$) and surface water (CS 14/18 Fountains Abbey; pH 7.44, conductivity 154 µS/cm, dissolved organic carbon 11.2 mg/L; hardness 86 mg/L CaCO$_3$) were collected by Smithers Viscient ERS (p. 13; Appendix 2, pp. 62-63 of MRID 51121209). The surface water was collected from The Lake, Studley Royal, Ripon, United Kingdom. Water characterization was performed at the ILV.

5 The ILV validated the method for chlormequat chloride in surface water at the LOQ and 10×LOQ in the first trial with insignificant modifications to the analytical parameters/instruments (pp. 14-18, 24; Appendix 3, pp. 66-70 of MRID 51121209). The validation was repeated with matrix-matched calibration standards and was also successful. The method for chlormequat chloride in groundwater at 10×LOQ was validated in the first trial with insignificant modifications to the analytical parameters/instruments; however, the method was not successfully validated in groundwater at the LOQ using all five recoveries. The ILV modifications did not warrant an updated ECM.

6 The surface water validation was repeated due to several low (<70%) recoveries in the first validation, even though overall results of the first validation were acceptable. Matrix-matched calibration standards were used in the second surface water validation while solvent-based calibration standards were used in the first surface water validation since matrix effects were insignificant (<20%; p. 24 of MRID 51121209).

7 Means, standard deviations, and RSDs were reviewer-calculated using all five recoveries values (see DER Excel Attachment). Rules of significant figures were followed. The study author excluded one value as a statistical outlier (Grubb's test) and reported means, standard deviations, and RSDs for n = 4; reported means, standard deviations, and RSDs were 98.1%, 6.45%, and 6.57%, respectively, for the quantitation ion transition and 95.5%, 5.52%, and 5.78%, respectively, for the confirmation ion transition (Tables 1-2, pp. 27-28 of MRID 51121209).

IV. Method Deficiencies and Reviewer's Comments

1.   The pagination of MRID 51293001 was not uniform or complete. Amendment No. 1 of the study report was placed in front of the original report and was paginated independently from the final report. Amendment No. 1 also contained an unacknowledged second p. 2, so the document only reported 8 pages (*i.e,* "1 of 8"), when it had 9 pages total. The study report should have a single page numbering scheme which accounts for all pages.

2.   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 a true LOQ (pp. 10, 17-18, 20-21; Appendix 2, p. 62 of MRID 51293001; pp. 11, 19-22 of MRID 51121209). 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 chlormequat chloride in the tested water matrices (0.10 µg/L).

3.   Performance data were not within recommended ranges for the ILV validation in groundwater at the LOQ. Means and RSDs were 124-128% and 51-53%, respectively, for the quantitation and confirmation ion transition (Tables 1-2, pp. 27-28 of MRID 51121209 and DER Excel Attachment). The study author excluded one value as a statistical outlier (Grubb's test) and reported means, standard deviations, and RSDs for n = 4: 95.5-98.1%, 5.52-6.45%, and 5.78-6.57%, respectively, for both transitions.

4.   Matrix-matched calibrants were used in the ECM for all analyses even though significant matrix influence (> ±20%) was only observed for the quantitation ion transition for the 10×LOQ fortification in surface water (p. 22; Tables 16-17, p. 39 of MRID 51293001). No reasoning was provided.

      The ILV surface water validation was repeated due to several low (<70%) recoveries in the first validation, even though overall results of the first validation were acceptable (p. 24;

Tables 3-6, pp. 29-32 of MRID 51121209). Matrix-matched calibration standards were used in the second surface water validation while solvent-based calibration standards were used in the first surface water validation since matrix effects were insignificant (<20%). The ILV noted that analyte peak shape was better in the matrix-matched surface water calibration standards (p. 23).

Overall, the use of matrix-matched calibration standards appeared to be important for the ECM method even if matrix effects were not observed, but the critical use of matrix-matched calibration standards for all analyses was not specified in the ECM method. **The reviewer recommends updating the ECM method to include the use of matrix-matched calibration standards.**

5.   The test matrices of the ECM were drinking water and surface water while the test matrices of the ILV were groundwater and surface water. The groundwater ILV validation was slightly higher than the recommended range of recoveries. OCSPP guideline 850.6100 does not specify the number of matrices required for method validation studies; however, usually the ILV should be conducted with equally or more difficult matrices than the ECM.

6.   The communications between the ILV study authors (Angela Cashmore and Ofure Idialu, Smithers ERS Limited) and ILV Study Monitor (Senthilkumar Kuppusamy, Eastman Chemical Company) were summarized (pp. 1, 21; Appendix 5, p. 76 of MRID 51121209). Reported communications included**:** protocol issue, the results of the ILV validations, and decision to perform a second validation for the surface water. No technical communication was reported.

7.   In MRID 51293001 Amendment No. 1, the No Data Confidentiality statement contained the header "Smithers Viscient Study No.: 14105.6124" which corresponded to MRID 50747517 (p. 2 of MRID 51293001 Amendment No. 1). Additionally, the signed and dated GLP statement of MRID 51293001 Amendment No. 1 was an unacknowledged second p. 2 and did not list any compliance guidelines (unacknowledged second p. 2 of MRID 51293001 Amendment No. 1).

8.   MRID 51293001 Amendment No. 1 contained the following information: 1) a schematic representation of the analytical method; 2) a statement that the calibration curve covers the range between 30% of the LOQ to 20% above the highest fortification level; 3) the time required for the analysis (i.e., chromatographic run time); and 4) the change of the study director (pp. 5-8 of MRID 51293001 Amendment No. 1).

9.   The reviewer noted some variability in the integration window for the analyte peak in the ILV chromatograms (Figures 3-42, pp. 38-59 of MRID 51121209).

10.   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, 17-18, 20-21; Appendix 2, p. 62 of MRID 51293001; pp. 11, 19-22 of MRID 51121209). In the ECM, the LOQ was defined as the lowest fortification level with mean recoveries ranging 70-120% at a RSD of ≤20%. In the ILV, the LOQ was defined as the lowest fortification level validated. In the ECM, the LOD was calculated as 0.03 µg/L for both test waters based on the lowest calibration standard using the following equation: R (µg/L) = {[final concentration of extract

(ng/mL)] × [final sample volume (mL)]} / [volume of sample (mL)], where R is the recovered residue analyte, which presumably corresponds to LOD, although this was not specified in the study report. In the ILV, the LOD was calculated using the following equation: LOD (μg/L) = 3 × height of control baseline noise × calibration standard concentration (μg/L) / height of calibration 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 a true LOQ.

The method detection limit (MDL) was calculated in the ILV as equivalent to the lowest concentration calibration standard (pp. 19-20, 22 of MRID 51121209). In the ILV, the MDL was equivalent to 0.03 μg/L for chlormequat chloride. This MDL calculation was not in accordance with the EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (2016).

11.   The stability of the sample extract and intermediate calibration solutions were determined to be 6-8 days and 7 days, respectively, when stored in a refrigerator at 5 ± 3°C (pp. 21-22; Tables 10-15, pp. 33-38 of MRID 51293001).

12.   The total time required to complete one set of 13 samples was reported as one working day (8 hours) in the ILV (p. 12 of MRID 51121209).

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. 317-319, and Revision 2; 1994 and 2016.

Attachment 1: Chemical Names and Structures

Chlormequat chloride

IUPAC Name: (2-Chloroethyl)trimethylammonium chloride
CAS Name: 2-Chloro-N,N,N-trimethylethanaminium chloride
CAS Number: 999-81-5
SMILES String: ClCC[N+](C)(C)C.[Cl-]