

**Analytical method for chlormequat chloride in soil**

**Reports:** ECM: EPA MRID No.: 50747516. Kang, S. 2018. Validation of the Analytical Method for the Determination of Chlormequat Chloride in Soil Matrix by LC-MS/MS. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored and submitted by Eastman Chemical Company, Kingsport, Tennessee; 51 pages. Smithers Viscient Study No.: 14105.6104. Final report issued July 27, 2018.

ILV: EPA MRID No.: 50747519. Cashmore, A. 2018. Chlormequat Chloride Formulation - Independent Laboratory Validation of Analytical Method 14105.6104 for the Determination of Chlormequat Chloride in Soil. Report prepared by Smithers Viscient (ESG) Ltd., North Yorkshire, United Kingdom, and sponsored and submitted by Eastman Chemical Company, Kingsport, Tennessee; 65 pages. Study No.: 3201883. Final report issued November 12, 2018.

**Document No.:** MRIDs 50747516 & 50747519

**Guideline:** 850.6100

**Statements:** **ECM:** The study was conducted in compliance with USEPA FIFRA (40 CFR Part 160) GLP standards, as accepted by OECD GLP (1998; p. 3 of MRID 50747516). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An Authenticity statement was included with the Quality Assurance statement.

**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 3, p. 49 of MRID 50747519). 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 (ECM plus ILV) is classified as supplemental. 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. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method. The method is sufficient for monitoring needs in soil and supports the submitted terrestrial field dissipation studies.

**PC Code:** 018101

**EFED Final Reviewer:** Cheryl Sutton, Ph.D.  
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*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, Smithers Viscient Study No. 14181.6104, is designed for the quantitative determination of chlormequat chloride in soil at the stated LOQ of 0.05 mg/kg using LC/MS/MS. 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 the tested soil matrices (0.05 mg/kg).

The ECM validated the method using characterized sandy loam and loam soil matrices which were sourced from chlormequat chloride terrestrial field dissipation studies (MRIDs 50747530 and 50747532); the ILV validated the method using characterized sandy loam and clay loam soil matrices. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method and if the two ILV soil matrices covered the range of soils used in the four submitted chlormequat chloride terrestrial field dissipation studies. The ILV validated the method for chlormequat chloride in the first trial with insignificant modifications to the analytical parameters including the quantification of the confirmation ion transition. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for chlormequat chloride in tested soil matrices.

The method is sufficient for monitoring needs in soil and supports the submitted terrestrial field dissipation studies.

**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	50747516 <sup>1</sup>	50747519 <sup>2</sup>	04/2021	Soil	27/07/2018	Eastman Chemical Company	LC/MS/MS	0.05 mg/kg

1 In the ECM, loam soil (SMV Lot No. R05-10-17-137; 46% sand, 36% silt, 18% clay; pH 6.0 in 1:1 soil:water ratio; 4.0% organic matter Walkley-Black; cation exchange capacity not applicable) collected from Larned, Kansas, and sandy loam soil (SMV Lot No. R05-03-17-130; 75% sand, 20% silt, 5% clay; pH 7.8 in 1:1 soil:water ratio; 1.07% organic matter Walkley-Black; cation exchange capacity 8.5 meq/100 g) collected from Porterville, California, were used in the study (USDA soil texture classification; p. 10 of MRID 50747516). The soils were characterized by Agvise Laboratories, Northwood, North Dakota. For the KS soil, the the soil texture was verified by the reviewer using USDA-NRCS technical support tools; for the CA soil, the the soil texture was reported based on the results obtained by the reviewer using USDA-NRCS technical support tools. The texture of the CA soil was reported as loamy sand in the study report. The original Certificates of Analysis for each soil were not provided in the study report.

2 In the ILV, clay loam soil (Soil ID: TFD-KS-1; Test System Code: CS72/17; 29% sand, 44% silt, 27% clay; pH 6.6 in 0.01M CaCl<sub>2</sub>; 1.4% organic matter Walkley-Black; cation exchange capacity 27.8 meq/100 g) collected from Kansas (GPS Reference N 38.036709 W 99.100189) and sandy loam soil (Soil ID: TFD-CA-1; Test System Code: CS73/17; 60% sand, 29% silt, 11% clay; pH 7.6 in 0.01M CaCl<sub>2</sub>; 0.6% organic matter Walkley-Black; cation exchange capacity 13.0 meq/100 g) collected from California (GPS Reference 36°00.453'N to 36°00.457'N and 119°04.704"W to 119°04.715"W) were used in the study (USDA soil texture classification; p. 12; Appendix 2, pp. 47-48 of MRID 50747519). The soils were characterized by Agvise Laboratories, Northwood, North Dakota. The soil texture was verified by the reviewer using USDA-NRCS technical support tools.

## I. Principle of the Method

Soil samples (10 g dry wt.) in 50-mL centrifuge tubes were fortified (0.05 mL of 100 or 10.0 mg/L fortification solution) and extracted four times with 30 mL with methanol:1M (pH 7) potassium carbonate (50:50, v:v) via sonication for 15 minutes, shaking on a shaker table for 30 minutes (at 300 rpm), and centrifugation at 1500 rpm for 10 minutes (pp. 15-17 of MRID 50747516). The volume of the combined supernatants was adjusted to 120 mL with methanol:1M (pH 7) potassium carbonate (50:50, v:v). The LOQ and 10×LOQ samples (0.10 mL aliquot) were diluted 1200xs with acetonitrile:purified reagent water:trifluoroacetic acid (80:20:0.1, v:v:v). An aliquot was taken for LC/MS/MS analysis.

Samples were analyzed for chlormequat chloride using a Shimadzu LC-20AD HPLC coupled with a Sciex TripleTOF 5600 MS with a Sciex DuoSpray (ESI and APCI) ion source operated in the positive ion mode with multiple reaction monitoring (MRM; pp. 10, 17-18 of MRID 50747516). The following LC conditions were used: Waters BEH Amide column (2.1 mm x 100 mm, 2.5 μm; column temperature 40°C), mobile phase of (A) 50mM (pH 3) ammonium formate and (B) acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.50 min. 3:97, 2.50-3.00 min. 60:40 (flow rate change during time interval), 3.10-5.00 min. 3:97] and injection volume of 10.0 μL. MS source temperature was 500°C. Two ion pair transitions were monitored for chlormequat chloride (quantitation and confirmation, respectively):  $m/z$  122.07→58.0651 ± 0.0025 and  $m/z$  122.07→62.9996 ± 0.0025. Reported retention time was *ca.* 1.9 minutes for chlormequat chloride.

The ILV performed the ECM methods as written, except for insignificant modifications to the analytical parameters (pp. 13-18 of MRID 50747519). Samples were analyzed for chlormequat chloride using Shimadzu Nexera series HPLC coupled with an AB Sciex 5000 Triple Quadrupole LC/MS/MS. The LC/MS/MS parameters were the same as those of the ECM. Two ion pair transitions were monitored for chlormequat chloride (quantitation and confirmation, respectively):  $m/z$  122.0→58.1 and  $m/z$  122.0→62.9. These ion transitions were similar to those of the ECM. Reported retention time was *ca.* 1.3 minutes for chlormequat chloride. The ILV modifications did not warrant an updated ECM.

The Limit of Quantification (LOQ) for chlormequat chloride in soil was 0.05 mg/kg in the ECM and ILV (pp. 8, 20-24 of MRID 50747516; pp. 10, 19-22 of MRID 50747519). In the ECM, the Limit of Detection (LOD) for chlormequat chloride was calculated as 0.00760 mg/kg for both test soils. In the ILV, the LOD for chlormequat chloride was calculated as 0.00250-0.0150 mg/kg for KS soil and 0.00281-0.0374 mg/kg for CA soil. 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 50747516): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD  $\leq$ 20%) for analysis of chlormequat chloride at fortification levels of 0.05 mg/kg (LOQ) and 0.5 mg/kg (10 $\times$ LOQ) in two soil matrices (Tables 1-2, pp. 27-28). Two ion pair transitions were monitored; however, only the quantitation ion transition recoveries were quantified. The ion ratio response of the confirmation and quantitation ion transitions was quantified to confirm the quantitation ion transition recovery results (p. 22; Table 4, p. 30). A confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data. The loam soil (SMV Lot No. R05-10-17-137; 46% sand, 36% silt, 18% clay; pH 6.0 in 1:1 soil:water ratio; 4.0% organic matter Walkley-Black; cation exchange capacity not applicable) collected from Larned, Kansas, and sandy loam soil (SMV Lot No. R05-03-17-130; 75% sand, 20% silt, 5% clay; pH 7.8 in 1:1 soil:water ratio; 1.07% organic matter Walkley-Black; cation exchange capacity 8.5 meq/100 g) collected from Porterville, California, were used in the study (USDA soil texture classification; p. 10). The soils were characterized by Agvise Laboratories, Northwood, North Dakota. For the KS soil, the the soil texture was verified by the reviewer using USDA-NRCS technical support tools; for the CA soil, the the soil texture was reported based on the results obtained by the reviewer using USDA-NRCS technical support tools. The texture of the CA soil was reported as loamy sand in the study report. The original Certificates of Analysis for each soil were not provided in the study report.

ILV (MRID 50747519): Mean recoveries and RSDs were within guidelines for analysis of chlormequat chloride at fortification levels of 0.05 mg/kg (LOQ) and 0.5 mg/kg (10 $\times$ LOQ) in one soil matrix (Tables 1-4, pp. 26-29). Two ion pair transitions were monitored; performance data was comparable between the quantitation and confirmation analyses. The clay loam soil (Soil ID: TFD-KS-1; Test System Code: CS72/17; 29% sand, 44% silt, 27% clay; pH 6.6 in 0.01M CaCl<sub>2</sub>; 1.4% organic matter Walkley-Black; cation exchange capacity 27.8 meq/100 g) collected from Kansas (GPS Reference N 38.036709 W 99.100189) and sandy loam soil (Soil ID: TFD-CA-1; Test System Code: CS73/17; 60% sand, 29% silt, 11% clay; pH 7.6 in 0.01M CaCl<sub>2</sub>; 0.6% organic matter Walkley-Black; cation exchange capacity 13.0 meq/100 g) collected from California (GPS Reference 36°00.453'N to 36°00.457'N and 119°04.704''W to 119°04.715''W) were used in the study (USDA soil texture classification; p. 12; Appendix 2, pp. 47-48). The soils were characterized by Agvise Laboratories, Northwood, North Dakota. The soil texture was verified by the reviewer using USDA-NRCS technical support tools. The method for chlormequat chloride in soil was validated in the first trial with insignificant modifications to the analytical parameters (pp. 10, 13-18, 21, 23; Appendix 4, p. 50).

The method is sufficient for monitoring needs in soil and supports the submitted terrestrial field dissipation studies.

**Table 2. Initial Validation Method Recoveries for Chlormequat Chloride in Soil<sup>1,2</sup>**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>KS Loam Soil</b>						
Quantitation ion transition						
Chlormequat chloride	0.05 (LOQ)	5	89.8-104	98.0	5.65	5.77
	0.5	5	102-112	106	4.62	4.35
<b>CA Sandy Loam Soil</b>						
Quantitation ion transition						
Chlormequat chloride	0.05 (LOQ)	5	89.0-110	96.0	8.09	8.43
	0.5	5	103-111	107	3.63	3.38

Data (uncorrected recovery results; p. 20) were obtained from Tables 1-2, pp. 27-28 of MRID 50747516.

1 The loam soil (SMV Lot No. R05-10-17-137; 46% sand, 36% silt, 18% clay; pH 6.0 in 1:1 soil:water ratio; 4.0% organic matter Walkley-Black; cation exchange capacity not applicable) collected from Larned, Kansas, and sandy loam soil (SMV Lot No. R05-03-17-130; 75% sand, 20% silt, 5% clay; pH 7.8 in 1:1 soil:water ratio; 1.07% organic matter Walkley-Black; cation exchange capacity 8.5 meq/100 g) collected from Porterville, California, were used in the study (USDA soil texture classification; p. 10). The soils were characterized by Agvise Laboratories, Northwood, North Dakota. For the KS soil, the the soil texture was verified by the reviewer using USDA-NRCS technical support tools; for the CA soil, the the soil texture was reported based on the results obtained by the reviewer using USDA-NRCS technical support tools. The texture of the CA soil was reported as loamy sand in the study report. The original Certificates of Analysis for each soil were not provided in the study report.

2 Two ion pair transitions were monitored for chlormequat chloride (quantitation and confirmation, respectively):  $m/z$  122.07→58.0651 ± 0.0025 and  $m/z$  122.07→62.9996 ± 0.0025; however, only the quantitation ion transition recoveries were quantified. The ion ratio response of the confirmation and quantitation ion transitions was quantified to confirm the quantitation ion transition recovery results (p. 22; Table 4, p. 30). A confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

**Table 3. Independent Validation Method Recoveries for Chlormequat Chloride in Soil<sup>1,2,3</sup>**

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>KS Clay Loam Soil</b>						
Quantitation ion transition						
Chlormequat chloride	0.05 (LOQ)	5	93-115	99	9.3	9.4
	0.5	5	92-98	96	2.9	3.0
Confirmation ion transition						
Chlormequat chloride	0.05 (LOQ)	5	98-115	105	6.2	5.9
	0.5	5	88-99	95	4.3	4.5
<b>CA Sandy Loam Soil</b>						
Quantitation ion transition						
Chlormequat chloride	0.05 (LOQ)	5	96-98	97	0.8	0.9
	0.5	5	94-97	96	1.5	1.6
Confirmation ion transition						
Chlormequat chloride	0.05 (LOQ)	5	96-108	103	5.2	5.1
	0.5	5	91-98	96	3.0	3.2

Data (uncorrected recovery results; p. 19) were obtained from Tables 1-4, pp. 26-29 of MRID 50747519.

1 The clay loam soil (Soil ID: TFD-KS-1; Test System Code: CS72/17; 29% sand, 44% silt, 27% clay; pH 6.6 in 0.01M CaCl<sub>2</sub>; 1.4% organic matter Walkley-Black; cation exchange capacity 27.8 meq/100 g) collected from Kansas (GPS Reference N 38.036709 W 99.100189) and sandy loam soil (Soil ID: TFD-CA-1; Test System Code: CS73/17; 60% sand, 29% silt, 11% clay; pH 7.6 in 0.01M CaCl<sub>2</sub>; 0.6% organic matter Walkley-Black; cation exchange capacity 13.0 meq/100 g) collected from California (GPS Reference 36°00.453'N to 36°00.457'N and 119°04.704'W to 119°04.715'W) were used in the study (USDA soil texture classification; p. 12; Appendix 2, pp. 47-48). The soils were characterized by Agvise Laboratories, Northwood, North Dakota. The soil texture was verified by the reviewer using USDA-NRCS technical support tools.

2 Two ion pair transitions were monitored for chlormequat chloride (quantitation and confirmation, respectively):  $m/z$  122.0→58.1 and  $m/z$  122.0→62.9. These ion transitions were similar to those of the ECM.

### III. Method Characteristics

The LOQ for chlormequat chloride in soil was 0.05 mg/kg in the ECM and ILV (pp. 8, 20-24 of MRID 50747516; pp. 10, 19-22 of MRID 50747519). In the ECM and ILV, the LOQ was defined as the lowest fortification level validated. Also, in the ECM, the LOQ was defined as the level which the blank values did not exceed 30% of the LOQ. In the ECM, the LOD was calculated as 0.00760 mg/kg for both test soils from the signal-to-noise response of each analyte in matrix at the LOQ level using the following equation:

$$\text{LOD} = (3 \times N_{\text{ctl}}) / (\text{Res}_{\text{PLS}}) \times \text{Con}_{\text{CLS}} \times \text{DF}_{\text{CNTL}}$$

Where, LOD is the limit of detection of the analysis,  $N_{\text{ctl}}$  is the mean signal to noise in height of the control samples (or blanks),  $\text{Res}_{\text{PLS}}$  is the mean response in height of the two low calibration standards,  $\text{Con}_{\text{CLS}}$  is the concentration of the low calibration standard, and  $\text{DF}_{\text{CNTL}}$  is the dilution factor of the control samples (1200).

The LOD for chlormequat chloride in soil was estimated in the ILV as 0.00250-0.0150 mg/kg for KS soil and 0.00281-0.0374 mg/kg for CA soil at 3 x baseline noise 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 Soil**

		Chlormequat Chloride
Limit of Quantitation (LOQ)*	ECM	0.05 mg/kg
	ILV	
Limit of Detection (LOD)	ECM (calc)	0.00760 mg/kg (Q, L & SL) <sup>1</sup>
	ILV (calc)	0.00250 mg/kg (Q, CL) 0.0150 mg/kg (C, CL) 0.00281 mg/kg (Q, SL) 0.0374 mg/kg (C, SL) <sup>2</sup>
Linearity (calibration curve r and concentration range)	ECM	$r = 0.9997$ (Q, L & SL) <sup>1,3</sup> $r \geq 0.9985^4$
	ILV	$r = 0.9997$ (Q & C, CL) $r = 0.9999$ (Q, SL) $r = 0.9998$ (C, SL)
	Range	0.0250-2.50 ng/mL
Repeatable	ECM <sup>5</sup>	Yes at LOQ (0.05 mg/kg) and 10×LOQ (0.50 mg/kg) (two characterized soil matrices)
	ILV <sup>6,7</sup>	
Reproducible <sup>6</sup>		Yes for 0.05 mg/kg (LLMV)* and 0.50 mg/kg in soil matrices
Specific	ECM	Yes, matrix interferences were <15% of the LOQ (based on peak height). <sup>8</sup> An insignificant contaminant was observed near analyte peak (RT <i>ca.</i> 2.2 min.).
	ILV	Yes, no matrix interferences were observed in the Q chromatograms. Significant matrix interferences in the LOQ C chromatograms were observed. <sup>9</sup>

Data were obtained from pp. 8, 20-24 (LOQ/LOD); Tables 1-2, pp. 27-28 (recovery results); pp. 21, 23; Figure 9, p. 39 (calibration curves); Figures 1-8, pp. 31-38 (chromatograms) of MRID 50747516; pp. 10, 19-22 (LOQ/LOD); Tables 1-4, pp. 26-29 (recovery results); pp. 21-22; Figures 1-2, p. 33; Figures 13-14, p. 39 (calibration curves); Figures 3-22, pp. 34-43 (chromatograms) of MRID 50747519. Q = quantitation ion transition; C = confirmation ion transition; L = Loam Soil; SL = Sandy Loam Soil; CL = Clay Loam Soil.

\* 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.

- In the ECM, two ion pair transitions were monitored for chlormequat chloride (quantitation and confirmation, respectively):  $m/z$  122.07→58.0651 ± 0.0025 and  $m/z$  122.07→62.9996 ± 0.0025; however, only the quantitation ion transition recoveries were quantified. The ion ratio response of the confirmation and quantitation ion transitions was quantified to confirm the quantitation ion transition recovery results (p. 22; Table 4, p. 30). A confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.
- The calculated LOD was >30% of the LOQ; however, a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.
- ECM correlation coefficients (r) were reviewer-calculated based on  $r^2$  values reported in the study report (pp. 21, 23; Figure 9, p. 39 of MRID 50747516; Excel Attachment). Matrix effects were insignificant and solvent-based calibration standards were used in the ECM (p. 22 of MRID 50747516). Matrix effects were significant for the confirmation transition and matrix-matched calibration standards were used in the ILV (p. 22 of MRID 50747519)
- Generalized value reported in the Validity Criteria table (p. 23 of MRID 50747516).
- In the ECM, loam soil (SMV Lot No. R05-10-17-137; 46% sand, 36% silt, 18% clay; pH 6.0 in 1:1 soil:water ratio; 4.0% organic matter Walkley-Black; cation exchange capacity not applicable) collected from Larned, Kansas, and sandy loam soil (SMV Lot No. R05-03-17-130; 75% sand, 20% silt, 5% clay; pH 7.8 in 1:1 soil:water ratio; 1.07% organic matter Walkley-Black; cation exchange capacity 8.5 meq/100 g) collected from Porterville, California, were used in the study (USDA soil texture classification; p. 10 of MRID 50747516). The soils were characterized by Agvise Laboratories, Northwood, North Dakota. For the KS soil, the the soil texture was verified by the reviewer using USDA-NRCS technical support tools; for the CA soil, the the soil texture was reported based on the results obtained by the reviewer using USDA-NRCS technical support tools. The texture of the CA soil was reported as loamy sand in the study report. The original Certificates of Analysis for each soil were not provided in the study report.

- In the ILV, clay loam soil (Soil ID: TFD-KS-1; Test System Code: CS72/17; 29% sand, 44% silt, 27% clay; pH 6.6 in

- 0.01M CaCl<sub>2</sub>; 1.4% organic matter Walkley-Black; cation exchange capacity 27.8 meq/100 g) collected from Kansas (GPS Reference N 38.036709 W 99.100189) and sandy loam soil (Soil ID: TFD-CA-1; Test System Code: CS73/17; 60% sand, 29% silt, 11% clay; pH 7.6 in 0.01M CaCl<sub>2</sub>; 0.6% organic matter Walkley-Black; cation exchange capacity 13.0 meq/100 g) collected from California (GPS Reference 36°00.453'N to 36°00.457'N and 119°04.704'W to 119°04.715'W) were used in the study (USDA soil texture classification; p. 12; Appendix 2, pp. 47-48 of MRID 50747519). The soils were characterized by Agvise Laboratories, Northwood, North Dakota. The soil texture was verified by the reviewer using USDA-NRCS technical support tools.
- 7 The ILV validated the method for chlormequat chloride in the first trial with insignificant modifications to the analytical parameters (pp. 10, 13-18, 21, 23; Appendix 4, p. 50 of MRID 50747519). The ILV modifications did not warrant an updated ECM.
- 8 Peak integrations and heights were not reported. Matrix interference assessment was determined using reviewer-determined peak heights. The study author reported that recoveries in the reagent blank and controls were <30% of the LOQ (pp. 22-23; Tables 1-2, pp. 27-28 of MRID 50747516).
- 9 Based on Figure 10, p. 37 and Figure 20, p. 42 of MRID 50747519. Deviations in specificity in the C analyses do not affect the validity of the method since a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

#### IV. Method Deficiencies and Reviewer's Comments

1. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than a true LOQ (pp. 8, 20-24 of MRID 50747516; pp. 10, 19-22 of MRID 50747519). 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 method LOQ for chlormequat chloride in the tested soil matrices (0.05 mg/kg).
2. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method. In the ILV, clay loam soil (Soil ID: TFD-KS-1; Test System Code: CS72/17; 27% clay; **1.4%** organic matter Walkley-Black) and sandy loam soil (Soil ID: TFD-CA-1; Test System Code: CS73/17; 11% clay; **0.6%** organic matter Walkley-Black) were used in the study (p. 12; Appendix 2, pp. 47-48 of MRID 50747519). In the ECM loam soil (SMV Lot No. R05-10-17-137; 18% clay; **4.0%** organic matter Walkley-Black) and sandy loam soil (SMV Lot No. R05-03-17-130; 5% clay; **1.07%** organic matter Walkley-Black) were used in the study (p. 10 of MRID 50747516). OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. Additionally, it could not be determined if the two ILV soil matrices covered the range of soils used in the four terrestrial field dissipation studies. Submitted chlormequat chloride terrestrial field dissipation studies included the following: MRID 50747530 (Larned, Kansas; loam soil (Agvise No. 17-880; 18% clay; 4.0% organic matter Walkley-Black, 2.3% organic carbon; p. 13; Appendix 3, p. 81 of MRID 50747530); MRID 50747531 (Grand Forks, North Dakota; sandy loam soil, 17% clay, 2.4% organic matter, 1.4% organic carbon; p. 13; Appendix 3, p. 95 of MRID 50747531); MRID 50747532 (Porterville, California; sandy loam soil (Agvise No. 17-627; 5% clay; 1.07% organic matter Walkley-Black, 0.62% organic carbon; p. 14; Appendix 3, p. 77 of MRID 50747532); and MRID 50747533 (Ephrata, Washington; sand soil, 1% clay, 0.27% organic matter, 0.16% organic carbon; p. 14; Appendix 3, p. 77 of MRID 50747533). The ECM soils were sourced

from TFD MRIDs 50747530 and 50747532.

3. The communications between the ILV study author (Angela Cashmore, Smithers Viscient (ESG) Ltd.) and ILV Study Monitor (Rebecca Curie, Eastman Chemical Company) were summarized (pp. 1, 3, 6, 21; Appendix 5, p. 51 of MRID 50747519). Reported communications included: protocol issue, test material Certificate of Analysis exchange, and exchange of the results of the first attempt of the ILV. The correspondence details also reported that the Sponsor “emailed that TOF-MS/MS was not available and recommended changing to TQ-MS/MS” (Appendix 5, p. 51). The use of TQ-MS/MS versus TOF-MS/MS was based on availability and not method difficulties; therefore, this correspondence does not affect the validity of the independence of the ILV.
4. The reviewer noted that the ECM and ILV laboratories were part of the same company, Smithers Viscient and Smithers Viscient (ESG) Ltd., respectively (pp. 1, 5 of MRID 50747516; pp. 1, 6 of MRID 50747519). The laboratory location, personnel and equipment differed between the two laboratories. The only exchange of information was the ECM Method/Protocol (by the ECM study authors) provided to the ILV via email from the ECM (Smithers Viscient, Wareham, Massachusetts; Appendix 5, p. 51; Appendix 6, p. 65 of MRID 50747519). This protocol transferred via email from the ECM to the ILV does not affect the validity of the independence of the ILV.
5. 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. 8, 20-24 of MRID 50747516; pp. 10, 19-22 of MRID 50747519). In the ECM and ILV, the LOQ was defined as the lowest fortification level validated. Also, in the ECM, the LOQ was defined as the level at which the blank values did not exceed 30% of the LOQ. No further justification of the LOQ was reported in the ECM or ILV. The LOD was calculated in the ECM using the following equation:  $LOD = (3 \times N_{ctl}) / (Res_{PLS}) \times Con_{CLS} \times DF_{CNTL}$ , where, LOD is the limit of detection of the analysis,  $N_{ctl}$  is the mean noise in height of the control samples (or blanks),  $Res_{PLS}$  is the mean response in height of the two low calibration standards,  $Con_{CLS}$  is the concentration of the low calibration standard, and  $DF_{CNTL}$  is the dilution factor of the control samples (1200). The LOD was estimated in the ILV using the following equation: 3 x baseline noise for the primary and confirmatory transitions. 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 ECM and ILV as dependent upon the lowest concentration calibration standard and the dilution factor of the controls (p. 21 of MRID 50747516; p. 22 of MRID 50747519). In the ECM and ILV, the MDL was equivalent to  $0.0025 \mu\text{g/L} \times 1200 = 0.030 \text{ mg/kg}$ . This MDL calculation was not in accordance with the EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2 (2016).

6. The ECM soil from Porterville, California, (SMV Lot No. R05-03-17-130) was reported as loamy sand in the study report, but the the soil texture was reported as sandy loam in the

- DER based on the results obtained by the reviewer using USDA-NRCS technical support tools (p. 10 of MRID 50747516). The original Certificates of Analysis for each soil were not provided in the study report.
7. The list of validation attempts noted that one of the control samples for the KS soil validation was re-injected due to suspected contamination (Appendix 4, p. 50 of MRID 50747519). The re-injection demonstrated that the sample was free of contamination.
  8. The matrix interferences were determined to be insignificant, and solvent-based calibration standards were used in the ECM (p. 22 of MRID 50747516). Matrix effects were significant for the confirmation transition and matrix-matched calibration standards were used in the ILV to cover both transitions (p. 22 of MRID 50747519).
  9. In the ECM, two ion pair transitions were monitored for chlormequat chloride (quantitation and confirmation, respectively):  $m/z$  122.07 $\rightarrow$ 58.0651  $\pm$  0.0025 and  $m/z$  122.07 $\rightarrow$ 62.9996  $\pm$  0.0025; however, only the quantitation ion transition recoveries were quantified (p. 18; Tables 1-2, pp. 26-27 of MRID 50747516). The ion ratio response of the confirmation and quantitation ion transitions was quantified to confirm the quantitation ion transition recovery results (p. 22; Table 4, p. 30 of MRID 50747516). A confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.
  10. The total time required to complete one set of samples was not reported in the ECM or ILV.

## 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:** CICC[N+](C)(C)C.[Cl-]

