Test Material: Telone

MRID: 44536504

Title: Method Validation Report for GRM 94.14 and GRM 94.17 - Determination of Residues of *cis*- and *trans*-3-Chloroacrylic Acid in Water and Soil by Capillary Gas Chromatography with Mass Selective Detection

MRID: 44536506

Title: Independent Laboratory Validation of Method GRM 94.14 - Determination of Residues of *cis*- and *trans*-3-Chloroacrylic Acid in Water by Capillary Gas Chromatography with Mass Selective Detection

MRID: 44536503

Title: Independent Laboratory Validation of Method GRM 94.17 - Determination of Residues of *cis*- and *trans*-3-Chloroacrylic Acid in Soil by Capillary Gas Chromatography with Mass Selective Detection

EPA PC Code: 029001

OCSPP Guideline: 850.6100

For CDM Smith

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Analytical methods for telone transformation products cis- and trans-3-chloroacrylic acid in water and soil

Reports: ECM: EPA MRID No.: 44536504. Duebelbeis, D.O., and S.E. Fisher. 1997. Method Validation Report for GRM 94.14 and GRM 94.17 - Determination of Residues of *cis*- and *trans*-3-Chloroacrylic Acid in Water and Soil by Capillary Gas Chromatography with Mass Selective Detection. Report prepared, sponsored, and submitted by Global Environmental Chemistry Laboratory - Indianapolis Lab, DowElanco, Indianapolis, Indiana; 85 pages. DowElanco Study ID: RES94071. Final report issued December 10, 1997.

ILV: EPA MRID No. 44536506. Duebelbeis, D.O., and B.A. Sorenson. 1997. Independent Laboratory Validation of Method GRM 94.14 - Determination of Residues of *cis*- and *trans*-3-Chloroacrylic Acid in Water by Capillary Gas Chromatography with Mass Selective Detection. Report prepared by Global Environmental Chemistry Laboratory - Indianapolis Lab, DowElanco, Indianapolis, Indiana, and Quality Management & Analytical Services, Inc. (QMAS), Walhalla, North Dakota, sponsored and submitted by Global Environmental Chemistry Laboratory - Indianapolis Lab, DowElanco, Indianapolis, Indiana; 33 pages. QMAS Study No.: QMAS95013. DowElanco Study No.: RES95080. Final report issued January 23, 1997.

ILV: EPA MRID No. 44536503. Duebelbeis, D.O., K. Hostetler, and B.J. Markley. 1996. Independent Laboratory Validation of Method GRM 94.17 - Determination of Residues of *cis*- and *trans*-3-Chloroacrylic Acid in Soil by Capillary Gas Chromatography with Mass Selective Detection. Report prepared by Global Environmental Chemistry Laboratory - Indianapolis Lab, DowElanco, Indianapolis, Indiana, and Wildlife International Ltd., Easton, Maryland, sponsored and submitted by Global Environmental Chemistry Laboratory - Indianapolis Lab, DowElanco, Indianapolis, Indiana; 34 pages. Wildlife International Project No.: 379C-116. DowElanco Study No.: RES95156. Final report issued August 2, 1996.

Document No.: MRIDs 44536504, 44536506 & 44536503

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with USEPA Good Laboratory Practice (GLP) standards, with minor exceptions (p. 3 of MRID 44536504). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). The certification of the authenticity of the study report is included in the Quality Assurance Statement (p. 4).

ILV (MRID 44536506): The study was conducted in compliance with USEPA GLP standards, with minor exceptions (p. 3). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). The certification of the authenticity of the study report is included in the Quality Assurance Statement (p. 4).

ILV (MRID 44536503): The study was conducted in compliance with USEPA GLP standards (p. 3). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). The certification of the authenticity of the study report is included in the Quality Assurance Statement (p. 4).

Classification: Analytical method GRM 94.14 (water) is classified as unacceptable.

Analytical method GRM 94.17 (soil) is classified as unacceptable. The ILVs were not conducted independently of the ECM originating laboratory. Though the ILV was conducted at a different laboratory, the ECMs and ILV are conducted by the same study director. The experimental design of the ILVs was inadequate to produce a successful validation of the respective ECM, with insufficient performance data at the LOQ (n = 2) and no performance data at 10x LOQ. The registrant failed to verify that the independent laboratories performed the ILV with a matrix (water/soil) of the most difficult analytical sample condition to analyze to demonstrate how well the method performs. The ILVs did not verify the LODs for the analytes. For Method GRM 94.14, the water matrices used in the ECM validation and ILV were not characterized, and the total number of ILV trials conducted was not reported. For the ILV of Method GRM 94.17, the soil matrix was not adequately characterized.

PC Code: 029001

Reviewer:

Andrew Shelby, Physical Scientist **Signature:**

Date: May 10, 2016

For all three MRIDs, page citations in this review refer to the uppermost set of page numbers located in the top right corner of each page of the MRID.

Executive Summary

DowElanco Analytical Methods GRM 94.14 and GRM 94.17 are designed for the quantitative determination of telone transformation products cis- and trans-3-chloroacrylic acid (CAAC, derivatized to the respective cis- and trans-3-chloroacrylic acid t-butyldimethylsilyl ester) in water (GRM 94.14) and soil (GRM 94.17) using GC/MS. The methods are quantitative for the analytes at the stated LOQ of 0.05 ng/mL (µg/L) in water and 0.20 ng/g (µg/kg) in soil. The respective LOQ is less than the lowest toxicological level of concern in water from submitted guideline studies. The respective LOQ is less than the lowest toxicological level of concern in soil (Earthworm 14-day LC_{50} = 55.6mg/kg for 1,3-D from PPDB). No major modifications were made by the independent laboratories. The independent laboratories failed to validate the methods for analysis of cis- and trans-3-CAAC in pond water (uncharacterized) and soil (uncharacterized) because insufficient performance data were reported at the LOQ (n = 2) and no performance data were reported at 10x LOQ. The ILVs did not verify the LODs for the analytes. The registrant failed to verify that the independent laboratories performed the ILVs with a matrix (water/soil) of the most difficult analytical sample condition to analyze to demonstrate how well the method performs. The ILVs were not conducted independently of the ECM originating laboratory. For Method GRM 94.14, the water matrices used in the ECM validation and ILV were not characterized, and the total number of ILV trials conducted was not reported. For the ILV of Method GRM 94.17, the soil matrix was not adequately characterized.

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						
cis-3-CAAC[1]	44536504: GRM 94.14	44536506		Water[2]	23/03/1995[3]	DowElanco	GC/MS	0.05 ng/mL (µg/L)
trans-3-CAAC								
cis-3-CAAC	44536504: GRM 94.17	44536503		Soil[4]	26/07/1995[5]			0.20 ng/g (µg/kg)
trans-3-CAAC								

[1] CAAC = chloroacrylic acid; transformation product of telone.

[2] Uncharacterized surface (ECM validation) and pond (ILV) water matrices were used (p. 9 of MRID 44536504; p. 8 of MRID 44536506). Surface water for ECM validation obtained near Immokalee, Florida. Source location for pond water used in ILV was not reported.

[3] Appendix A, p. 18 of MRID 44536504.

[4] Sand soil used for ECM validation (Appendix C, pp. 83-85 of MRID 44536504). Soil matrix used for ILV was not characterized and source location was not reported. However, the ILV soil was identified as M465B, which was characterized as a sand soil in the ECM validation (Appendix A, p. 31 of MRID 44536503; Appendix C, pp. 83, 85 of MRID 44536504).

[5] Appendix B, p. 49 of MRID 44536504.

I. Principle of the Method

Water: Water (40.0 mL) was fortified with cis- and trans-3-chloroacrylic acid (CAAC) in acetone for procedural recoveries (p. 11; Appendix A, pp. 22, 24 of MRID 44536504). Uncharacterized surface water from a site near Immokalee, Florida, was used for the validation (p. 9). Water samples (40.0 mL) are applied to an ion-exchange (quaternary amine) solid phase extraction (SPE) column pre-conditioned with methanol and deionized water (Appendix A, pp. 20, 24-25). The sample is pulled through the column by vacuum (*ca*. 2 mL/minute). CAAC is eluted with 0.1N hydrochloric acid (5.0 mL). The eluate is amended with 100 µL of 2.0N HCl and *ca*. 2-3 g of sodium chloride, then partitioned with 2.5 mL of methyl-t-butyl ether (MTBE) by vortexing for 15 seconds, then shaking (reciprocating shaker, *ca*. 180 excursion/minute) for 10 minutes. Phases are separated by centrifugation (2,500 rpm, 5 minutes). The organic (MTBE) phase is applied, under gravity flow, to a silica gel SPE column preconditioned with MTBE and isooctane. The remaining aqueous phase is again partitioned with 2.5 mL of MTBE, but without shaking procedure, and the resulting organic phase applied to the silica gel SPE column. The sample is pulled through the column by vacuum (*ca*. 2 mL/minute). CAAC is eluted with 10.0 mL of 0.025% acetic acid in MTBE (Appendix A, p. 26). The eluate is combined with isooctane (0.5 mL) and concentrated to *ca*. 0.25 mL under a gentle nitrogen stream (N-Evap, ambient temperature) to remove the MTBE (Appendix A, pp. 18-19, 26). The sample is amended with 25 µL of acetone, brought to 0.5 mL with isooctane, amended with anhydrous sodium sulfate (*ca*. 0.1 g), then derivatized to the respective cis- and trans-3-chloroacrylic acid t-butyldimethylsilyl esters (CAAC TBDMSE) with N-methyl-N-(t-butyl-dimethylsilyl)trifluoroacetamide (MTBSTFA, 25 µL). The derivatized sample is vortexed (15 seconds), centrifuged (2,500 rpm, 5 minutes), and transferred to an autosampler vial for GC/MS analysis.

Soil: Soil (10.0 g) was fortified with cis- and trans-3-CAAC in acetone for procedural recoveries (p. 11; Appendix B, pp. 53, 56 of MRID 44536504). Sand soil (Sample No.: M465A, M465B, M465E) from a site near Immokalee, Florida, was used for the validation (p. 9; Appendix C, pp. 83-85). Soil samples (10.0 g) are extracted twice with 15.0 mL of acetone:0.1N hydrochloric acid (90:10, v:v) by shaking (reciprocating shaker, *ca*. 180 excursion/minute) for ≥30 minutes for each extraction

(Appendix B, p. 57). Soil and extract are separated by centrifugation (2,500 rpm, 10 minutes), with the extracts decanted and combined. The extract is concentrated to *ca*. 4 mL under nitrogen (N-Evap, 40°C) to remove the acetone. The aqueous sample is diluted with deionized water (20 mL), brought to pH 6.5-8.0 with 0.1N sodium hydroxide, then the sample is purified by ion-exchange and silica gel SPE, and derivatized with MTBSTFA as described above for the water samples (Appendix B, pp. 57-59).

GC/MS: Samples are analyzed using an Hewlett-Packard 5971A Mass Selective Detector (MSD) equipped with an Hewlett-Packard 5890 (II) GC system (Appendix A, pp. 23-24; Appendix B, pp. 55-56 of MRID 44536504). The following GC conditions were used: J&W Scientific fused silica capillary, Durabond-5 (DB-5) liquid phase column (0.25 mm x 30 m, 0.25 µm film thickness, injector temperature 230°C), temperature program [45°C (hold for 1 min.), 10°C/min. to 220°C]. Injection volume was 1 µL. The following MSD conditions were used: electron impact ionization and selected ion monitoring (SIM). Analytes were identified using two ions; one for quantitation (Q) and one for confirmation (C). Ions monitored were *m/z* 163 (Q) and *m/z* 165 (C) for both cis- and trans-3-CAAC TBDMSE. Identification is considered positive when the confirmation ion ratio [(peak area at *m/z* 165)/(peak area at *m/z* 163)] of the sample is ±20% of the average confirmation ion ratio found for the calibration standards (Appendix A, pp. 27-28; Appendix B, p. 60). Retention times for cis- and trans-3-CAAC (as CAAC TBDMSE) were 10.79-10.82 and 10.01-10.04 minutes, respectively (Appendix A, Figure 5, p. 42; Figures 7-8, pp. 44-45; Figure 10, p. 47; Appendix B, Figure 5, p. 76; Figures 7-8, pp. 78-79; Figure 10, p. 81).

ILV - water: Sources of the test compounds and water matrix used for the ILV were not reported (p. 8; Appendix A, pp. 30-31 of MRID 44536506). The water matrix was described as pond water, but characterization was not provided. The independent laboratory performed the method as written, except the make/model of the MSD was not specified (pp. 9-11; Figure 3, p. 20; Figures 5-7, pp. 22-24; Figures 9-10, pp. 26-27; Appendix A, p. 32). Retention times for cis- and trans-3-CAAC (as CAAC TBDMSE) were 10.19-10.20 and 9.45-9.47 minutes, respectively.

ILV - soil: Sources of the test compounds and soil matrix used for the ILV were not reported (p. 9; Appendix A, p. 31 of MRID 44536503). The soil matrix was not characterized. The independent laboratory performed the method as written (pp. 10-15). Retention times for cis- and trans-3-CAAC (as CAAC TBDMSE) were 10.03-10.04 and 9.29 minutes, respectively (Figure 3, p. 22; Figures 5-6, pp. 24-25; Figure 8, p. 27).

LOQ and LOD - water: In the ECM and ILV, the LOQ for both cis- and trans-3-CAAC in water was 0.05 ng/mL (µg/L; Appendix A, p. 18 of MRID 44536504; p. 8 of MRID 44536506). In the ECM, the LODs for cis- and trans-3-CAAC were calculated as 0.009 ng/mL for both analytes (pp. 12-13; Appendix A, p. 30; Tables III-IV, pp. 36-37 of MRID 44536504). In the ILV, the LODs were not specified.

LOQ and LOD - soil: In the ECM and ILV, the LOQ for both cis- and trans-3-CAAC in soil was 0.20 ng/g (µg/kg; Appendix B, p. 49 of MRID 44536504; p. 9 of MRID 44536503). In the ECM, the LODs for cis- and trans-3-CAAC were calculated as 0.065 ng/g and 0.056 ng/g, respectively (pp. 12-13; Appendix B, pp. 63-64; Tables III-IV, pp. 70-71 of MRID 44536504). In the ILV, the LODs were not specified.

II. Recovery Findings

ECM - water (MRID 44536504): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of cis- and trans-3-CAAC in Florida surface water (uncharacterized) at a fortification level of 0.05 ng/mL (µg/L, LOQ, n = 8; Appendix A, Tables I-II, pp. 34-35 and DER Attachment 2). At fortification levels of 0.25 ng/mL (5x LOQ), 0.50 ng/mL (10x LOQ), 1.25 ng/mL (25x LOQ), 2.50 ng/mL (50x LOQ), and 5.00 ng/mL (100x LOQ), only two recoveries were reported for each fortification level; therefore, meaningful statistics (mean, standard deviation, RSD) for the recovery results per analyte/fortification level/water matrix could not be generated. At the fortification levels of 5x to 100x LOQ, individual recovery results were within 70-120%. Confirmation ion ratio results were only provided for one LOQ (0.050 ng/mL) fortified sample for both cis- and trans-3-CAAC and were ± 6% of the average standard confirmation ratio (Appendix A, Figure 7, p. 44; Figure 10, p. 47).

ECM - soil (MRID 44536504): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of cis- and trans-3-CAAC in a sand soil at a fortification level of 0.20 ng/g (µg/kg, LOQ, n = 9; Appendix B, Tables I-II, pp. 68-69 and DER Attachment 2). At fortification levels of 1.00 ng/g (5x LOQ), 2.00 ng/g (10x LOQ), 5.00 ng/g (25x LOQ), 10.0 ng/g (50x LOQ), and 20.0 ng/g (100x LOQ), 100 ng/g (500x LOQ), 500 ng/g (2,500x LOQ), and 2,000 ng/g (10,000x LOQ), only two recoveries were reported for each fortification level; therefore, meaningful statistics (mean, standard deviation, RSD) for the recovery results per analyte/fortification level/soil matrix could not be generated. At the fortification levels of 5x to 10,000x LOQ, individual recovery results were within 70-120%. Confirmation ion ratio results were only provided for one LOQ (0.20 ng/g) fortified sample for both cis- and trans-3-CAAC and were ± 1% of the average standard confirmation ratio (Appendix B, Figure 7, p. 78; Figure 10, p. 81). The sand soil matrix was characterized by A & L Great Lakes Laboratories, Inc., Fort Wayne, Indiana (Appendix C, pp. 83-85).

ILV - water (MRID 44536506): Only two recoveries were reported for each fortification level; therefore, meaningful statistics (mean, standard deviation, RSD) for the recovery results per analyte/fortification level/water matrix could not be generated. Individual recovery results were within 70-120% for both cis- and trans-3-CAAC in pond water (uncharacterized) at fortification levels of 0.05 ng/mL (LOQ) and 0.25 ng/mL (5x LOQ; p. 8; Tables 1-2, pp. 16-17). Performance data at 10x LOQ were not reported. Confirmation ion ratio results were only provided for one LOQ (0.05 ng/mL) and one 5x LOQ (0.25 ng/mL) fortified sample for both cis- and trans-3-CAAC and were ± 5% of the average standard confirmation ratio (Figures 5-6, pp. 22-23; Figures 9-10, pp. 26-27). The total number of trials conducted was not reported.

ILV - soil (MRID 44536503): Only two recoveries were reported for each fortification level; therefore, meaningful statistics (mean, standard deviation, RSD) for the recovery results per analyte/fortification level/soil matrix could not be generated. Individual recovery results were within 70-120% for both cis- and trans-3-CAAC in soil (uncharacterized) at fortification levels of 0.20 ng/g (LOQ) and 1.0 ng/g (5x LOQ; p. 8; Tables I-II, pp. 17-18). Performance data at 10x LOQ were not reported. Confirmation ion ratio results were only provided for one LOQ (0.20 ng/g) fortified sample for both cis- and trans-3-CAAC and were ± 6% of the average standard confirmation ratio (Figure 5, p. 24; Figure 8, p. 27). One validation run was conducted and considered successful (p. 28).

Table 2a. Initial Validation Method Recoveries for Telone Transformation Products cis- and trans-3-Chloroacrylic Acid (CAAC) in Surface Water[1]

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
cis-3-CAAC	0.050 (LOQ)[2]	8	82-100	89	6	7
	0.25	2	89, 93	--[3]	--	--
	0.50	2	75, 93	--	--	--
	1.25	2	103, 91	--	--	--
	2.50	2	89, 96	--	--	--
	5.00	2	102, 84	--	--	--
trans-3-CAAC	0.050 (LOQ)[4]	8	84-100	90	6	7
	0.25	2	91, 94	--	--	--
	0.50	2	76, 96	--	--	--
	1.25	2	105, 94	--	--	--
	2.50	2	91, 98	--	--	--
	5.00	2	104, 85	--	--	--

Data (recovery results, corrected for residues detected in matrix control samples) were obtained from Appendix A, Tables I-II, pp. 34-35 of MRID 44536504 and DER Attachment 2 (mean, SD, RSD).

1 Uncharacterized surface water collected from a site near Immokalee, Florida (p. 9).

2 Two fortifications made at 0.009 ng/mL (LOD) had recoveries of 93% (0.0084 ng/mL) and 88% (0.0079 ng/mL; Appendix A, Table I, p. 34).

3 Not applicable; meaningful statistics could not be generated because n = 2.

4 Two fortifications made at 0.009 ng/mL (LOD) had recoveries of 81% (0.0073 ng/mL) and 89% (0.0080 ng/mL; Appendix A, Table II, p. 35).

Table 2b. Initial Validation Method Recoveries for Telone Transformation Products cis- and trans-3-Chloroacrylic Acid (CAAC) in Sand Soil[1]

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
cis-3-CAAC	0.200 (LOQ)[2]	9	62-95	83	11	13
	1.000	2	75, 74	--[3]	--	--
	2.000	2	81, 73	--	--	--
	5.000	2	82, 86	--	--	--
	10.00	2	79, 78	--	--	--
	20.00	2	77, 77	--	--	--
	100.00	2	81, 84	--	--	--
	500.00	2	80, 73	--	--	--
	2,000.0	2	74, 80	--	--	--
trans-3-CAAC	0.200 (LOQ)[4]	9	65-98	87	9	11
	1.000	2	81, 81	--	--	--
	2.000	2	85, 75	--	--	--
	5.000	2	83, 87	--	--	--
	10.00	2	81, 82	--	--	--
	20.00	2	80, 81	--	--	--
	100.00	2	83, 86	--	--	--
	500.00	2	82, 79	--	--	--
	2,000.0	2	78, 83	--	--	--

Data (recovery results, corrected for residues detected in matrix control samples) were obtained from Appendix B, Tables I-II, pp. 68-69 of MRID 44536504 and DER Attachment 2 (mean, SD, RSD).

1 USDA classification; soil obtained from site near Immokalee, Florida (p. 9; Appendix C, pp. 83-85).

2 Two fortifications made at 0.060 ng/g (LOD) had recoveries of 85% (0.051 ng/g) and 87% (0.052 ng/g; Appendix B, Table I, p. 68).

3 Not applicable; meaningful statistics could not be generated because n = 2.

4 Two fortifications made at 0.060 ng/g (LOD) had recoveries of 85% (0.051 ng/g) and 97% (0.058 ng/g; Appendix B, Table II, p. 69).

Table 3a. Independent Validation Method Recoveries for Telone Transformation Products cis- and trans-3-Chloroacrylic Acid (CAAC) in Pond Water[1]

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
cis-3-CAAC	0.05 (LOQ)	2	101, 108	--[2]	--	--
	0.25	2	88, 86	--	--	--
trans-3-CAAC	0.05 (LOQ)	2	106, 109	--	--	--
	0.25	2	94, 93	--	--	--

Data (uncorrected recovery results) were obtained from Tables 1-2, pp. 16-17 of MRID 44536506.

1 Uncharacterized; source location was not reported (p. 8).

2 Not applicable; meaningful statistics could not be generated because n = 2.

Table 3b. Independent Validation Method Recoveries for Telone Transformation Products cis- and trans-3-Chloroacrylic Acid (CAAC) in Soil[1]

Analyte	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
cis-3-CAAC	0.200 (LOQ)	2	91, 96	--[2]	--	--
	1.0	2	115, 102	--	--	--
trans-3-CAAC	0.200 (LOQ)	2	82, 87	--	--	--
	1.0	2	112, 97	--	--	--

Data (uncorrected recovery results) were obtained from Tables I-II, pp. 17-18 of MRID 44536503.

1 Uncharacterized; source location was not reported (p. 8).

2 Not applicable; meaningful statistics could not be generated because n = 2.

III. Method Characteristics

Water: In the ECM and ILV, the LOQ for both cis- and trans-3-CAAC was 0.05 ng/mL (µg/L; Appendix A, p. 18 of MRID 44536504; p. 8 of MRID 44536506). In the ECM, the LODs for cis- and trans-3-CAAC were calculated as 0.009 ng/mL for both analytes (pp. 12-13; Appendix A, p. 30; Tables III-IV, pp. 36-37 of MRID 44536504). In the ILV, the LODs were not specified. Following the method of Keith, L.H., *et al*. (see section **V. References** below), the LOD and LOQ for determination of telone transformation products cis- and trans-3-chloroacrylic acid in water were calculated using the standard deviation from the 0.05 ng/mL (LOQ) recovery results (Appendix A, p. 30; Tables III-IV, pp. 36-37 of MRID 44536504). The LOD was calculated as three times the standard deviation (3*s*), and the LOQ was calculated as ten times the standard deviation (10*s*) of the recovery results. Mean matrix blank values (n = 3) were 0.0043 ng/mL and 0.0035 ng/mL for the cis- and tran-CAAC analyses, respectively, and were not included in the calculations (Appendix A, Tables I-IV, pp. 34-37 of MRID 44536504; DER Attachment 2). Calculated LOQ values were 0.030 ng/mL and 0.029 ng/mL for cis- and trans-CAAC, respectively.

Soil: In the ECM and ILV, the LOQ for both cis- and trans-3-CAAC was 0.20 ng/g (µg/kg; Appendix B, p. 49 of MRID 44536504; p. 9 of MRID 44536503). In the ECM, the LODs for cis- and trans-3-CAAC were calculated as 0.065 ng/g and 0.056 ng/g, respectively (pp. 12-13; Appendix B, pp. 63-64; Tables III-IV, pp. 70-71 of MRID 44536504). In the ILV, the LODs were not specified. Following the method of Keith, L.H., *et al*., as described above, the LOD and LOQ for determination of telone transformation products cis- and trans-3-chloroacrylic acid in soil were calculated using the standard deviation from the 0.20 ng/g (LOQ) recovery results (Appendix B, pp. 63-64; Tables III-IV, pp. 70-71 of MRID 44536504). Mean matrix blank values (n = 8) were 0.0221 ng/g and 0.0168 ng/g for the cis- and tran-CAAC analyses, respectively, and were not included in the LOD and LOQ calculations (Appendix B, Tables I-IV, pp. 68-71 of MRID 44536504; DER Attachment 2). Calculated LOQ values were 0.22 ng/g and 0.19 ng/g for cis- and trans-CAAC, respectively.

Table 4a. Method Characteristics for Telone Transformation Products cis- and trans-3-Chloroacrylic Acid (CAAC)[1] in Surface Water

		cis-3-CAAC	**trans-3-CAAC**
Limit of Quantitation (LOQ)		0.05 ng/mL (µg/L)	
Limit of Detection (LOD)		0.009 ng/mL	
Linearity (calibration curve r^2 and concentration range)[2]	ECM:	Q ion: $r^2 = 0.9996$ (0.025-6.25 ng/mL in water)	Q ion: $r^2 = 0.9996$ (0.025-6.25 ng/mL in water)
	ILV:[3]	Q ion: $r^2 = 0.9997$ (0.025-2.50 ng/mL)	Q ion: $r^2 = 0.9995$ (0.025-2.5 ng/mL)
Repeatable	ECM:	Yes at LOQ (n = 8). Fortifications at 5x, 10x, 25x, 50x, and 100x LOQ performed at n = 2.	
	ILV:	Undetermined. Fortifications at LOQ and 5x LOQ performed at n = 2. No performance data at 10x LOQ.	
Reproducible		Undetermined. ILV performed at n = 2 at LOQ and 5x LOQ.	
Specific[4]	ECM:	Interferences of 44-54% of LOD were detected at analyte retention time.	Interferences of 23-56% of LOD were detected at analyte retention time.
		Matrix was uncharacterized Florida surface water.	
	ILV:	No interferences were detected at analyte retention times. Matrix was uncharacterized pond water; source location was not reported.	

Data were obtained from Appendix A, p. 18; Tables I-IV, pp. 34-37; Figures 3-4, pp. 40-41; Figure 6, p. 43; Figure 9, p. 46 of MRID 44536504; p. 8; Figures 1-2, pp. 18-19; Figure 4, p. 21; Figure 8, p. 25 of MRID 44536506.

Linearity is satisfactory when $r^2 \geq 0.995$.

1 Derivatized as cis- and trans-3-chloroacrylic acid t-butyldimethylsilyl ester (CAAC TBDMSE; Appendix A, p. 18 of MRID 44536504).

2 Calibration curve r^2 values determined using power regression equation (Appendix A, Figures 3-4, pp. 40-41 of MRID 44536504; Figures 1-2, pp. 18-19 of MRID 44536506).

3 Ion *m/z* 136 reported in the calibration curve figures appears to be a typographical error and should be *m/z* 163. Ion *m/z* 136 was the quantitation ion for cis- and trans-3-CAAL (MRIDs 44536505 & 44536507).

4 For the ECM validation, confirmation ion ratio results were only provided for one LOQ (0.05 ng/mL) fortified sample for both cis- and trans-3-CAAC and were ± 6% of the average standard confirmation ratio (Appendix A, Figure 7, p. 44; Figure 10, p. 47 of MRID 44536504). For the ILV, confirmation ion ratio results were only provided for one LOQ (0.05 ng/mL) and one 5x LOQ (0.25 ng/mL) fortified sample for both cis- and trans-3-CAAC and were ± 5% of the average standard confirmation ratio (Figures 5-6, pp. 22-23; Figures 9-10, pp. 26-27 of MRID 44536506). A confirmatory method is typically not required where GC/MS and LC/MS methods are used as the primary method.

Table 4b. Method Characteristics for Telone Transformation Products cis- and trans-3-Chloroacrylic Acid (CAAC)[1] in Soil

		cis-3-CAAC	**trans-3-CAAC**
Limit of Quantitation (LOQ)		0.20 ng/g (µg/kg)	
Limit of Detection (LOD)		0.065 ng/g	0.056 ng/g
Linearity (calibration curve r^2 and concentration range)[2]	ECM:	Q ion: $r^2 = 0.9997$ (0.10-25.0 ng/g in soil)	Q ion: $r^2 = 0.9999$ (0.10-25.0 ng/g in soil)
	ILV:	Q ion: $r^2 = 0.9996$ (0.10-10.0 ng/g in soil)	Q ion: $r^2 = 0.9988$ (0.10-10.0 ng/g in soil)
Repeatable	ECM:	Yes at LOQ (n = 9). Fortifications at 5x, 10x, 25x, 50x, 100x, 500x, 2,500x, and 10,000x LOQ performed at n = 2.	
	ILV:	Undetermined. Fortifications at LOQ and 5x LOQ performed at n = 2. No performance data at 10x LOQ.	
Reproducible		Undetermined. ILV performed at n = 2 at LOQ and 5x LOQ.	
Specific[3]	ECM:	Interferences of 27-44% of LOD were detected at analyte retention time.	Interferences of 22-36% of LOD were detected at analyte retention time.
		Matrix was sand soil.	
	ILV:	No interferences were detected at analyte retention time.	Interferences were <50% of LOD at analyte retention time.
		Matrix was uncharacterized soil; source location was not reported.[4]	

Data were obtained from Appendix B, p. 49; Tables I-IV, pp. 68-71; Figures 3-4, pp. 74-75; Figure 6, p. 77; Figure 9, p. 80; Appendix C, pp. 83-85 of MRID 44536504; p. 9; Figures 1-2, pp. 20-21; Figure 4, p. 23; Figure 8, p. 27 of MRID 44536503.

Linearity is satisfactory when $r^2 \geq 0.995$.

1 Derivatized as cis- and trans-3-chloroacrylic acid t-butyldimethylsilyl ester (CAAC TBDMSE; Appendix B, p. 49 of MRID 44536504).

2 Calibration curve r^2 values determined using power regression equation (Appendix A, Figures 3-4, pp. 40-41 of MRID 44536504; Figures 1-2, pp. 18-19 of MRID 44536506).

3 For the ECM validation, confirmation ion ratio results were only provided for one LOQ (0.20 ng/g) fortified sample for both cis- and trans-3-CAAC and were ± 1% of the average standard confirmation ratio (Appendix B, Figure 7, p. 78; Figure 10, p. 81 of MRID 44536504). For the ILV, confirmation ion ratio results were only provided for one LOQ (0.20 ng/g) fortified sample for both cis- and trans-3-CAAC and were ± 6% of the average standard confirmation ratio (Figure 5, p. 24; Figure 8, p. 27 of MRID 44536503). A confirmatory method is typically not required where GC/MS and LC/MS methods are used as the primary method.

4 Soil was only identified as M465B, which was characterized as a sand soil in the ECM validation (Appendix A, p. 31 of MRID 44536503; Appendix C, pp. 83, 85 of MRID 44536504).

IV. Method Deficiencies and Reviewer's Comments

Methods GRM 94.14 (water) and GRM 94.17 (soil):

1. The ILVs were not conducted independently of the ECM originating laboratory. Global Environmental Chemistry Laboratory - Indianapolis Lab, DowElanco was the originating laboratory for the ECMs. Quality Management & Analytical Services, Inc. performed the ILV for GRM 94.14 (water) and Wildlife International Ltd. performed the ILV for GRM 94.17 (soil). However, the study director of the ECM validations, and co-author of the methods and ECM validation study reports, D.O. Duebelbeis, was also the study director of the ILVs and co-author of the ILV study reports (pp. 1, 3, 5; Appendix A, p. 18; Appendix B, p. 49 of MRID 44536504; pp. 1, 3, 5 of MRID 44536506; pp. 1, 3, 5 of MRID 44536503).

2. The experimental design of the ILVs was inadequate to produce a successful validation of the respective ECM. For the ILV, only two recoveries were reported for both the LOQ and 5x LOQ fortification levels, with no performance data at 10x LOQ (Tables 1-2, pp. 16-17 of MRID 44536506; Tables I-II, pp. 17-18 of MRID 44536503). A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10x LOQ for each analyte and matrix.

3. The ILVs did not verify the LODs for the analytes as determined by the ECM validations.

4. For the ECM validations, sample recoveries were corrected for residues found in the matrix control samples (Appendix A, Tables I-II, pp. 34-35; Appendix B, Tables I-II, pp. 68-69 of MRID 44536504).

5. For the ECM validations at all fortification levels >LOQ, only two recoveries were reported for each fortification level (Appendix A, Tables I-II, pp. 34-35; Appendix B, Tables I-II, pp. 68-69 of MRID 44536504).

6. For the ECM validations, chromatograms for reagent blank and spiked samples, other than LOQ, were not provided (Appendix A, Figures 6-7, pp. 43-44; Figures 9-10, pp. 46-47; Appendix B, Figures 6-7, pp. 77-78; Figures 9-10, pp. 80-81 of MRID 44536504). For the calibration standards, only chromatograms of 0.050 ng/mL in water matrix and 0.20 ng/g in soil matrix (both equivalent to 4.0 ng/mL standard) cis- and trans-3-CAAC standards were provided (calibration standard ranges 0.025-6.25 ng/mL in water matrix and 0.10-25.0 ng/g in soil matrix; Appendix A, Figures 3-5, pp. 40-42; Figure 8, p. 45; Appendix B, Figures 3-5, pp. 74-76; Figure 8, p. 79).

   In the ECM study reports, the study authors described r^2 as both correlation coefficient and coefficient of determination (p. 11; Appendix A, p. 30; Figures 3-4, pp. 40-41; Appendix B, p. 63; Figures 3-4, pp. 74-75). The reviewer verified that the r^2 values reported in the ECM and ILV study reports are coefficients of determination (r^2; DER Attachment 2).

7. For the ECM validation of method GRM 94.14 water matrix blank samples, interferences of 44-54% and 23-56% of the LOD were detected at the analyte retentions times for cis- and

trans-3-CAAC, respectively (Appendix A, Tables I-IV, pp. 34-37; Figure 6, p. 43; Figure 9, p. 46 of MRID 44536504). For method GRM 94.17 soil matrix blank samples, interferences of 27-44% and 22-36% of the LOD were detected at the analyte retentions times for cis- and trans-3-CAAC, respectively (Appendix B, Tables I-IV, pp. 68-71; Figure 6, p. 77; Figure 9, p. 80). The study authors reported that the interference peaks were also observed in the reagent blanks (p. 13); however, quantitative results and chromatograms for the reagent blanks were not provided. The study authors concluded that the "interference amounting to approximately one-tenth of the LOQ" did not "adversely affect the performance of the methods."

Method GRM 94.14 (water):

8. For the ILV, the total number of trials conducted to yield acceptable recovery results was not reported.

9. For both the ECM validation and ILV, the surface (ECM) and pond (ILV) water matrices were not characterized. The registrant failed to verify that the independent laboratory performed the ILV with a water matrix of the most difficult analytical sample condition to analyze to demonstrate how well the method performs.

10. For the ILV, chromatograms for reagent blank samples were not provided (Figures 4-6, pp. 21-23; Figures 8-10, pp. 25-27 of MRID 44536506). For the calibration standards, only chromatograms of 0.05 ng/mL (in water matrix, 4.0 ng/mL standard) cis- and trans-3-CAAC standards were provided (calibration standard range 0.025-2.5 ng/mL in water matrix; Figures 1-3, pp. 18-20; Figure 7, p. 24).

11. It was reported for the ILV that a set of seven samples (one reagent blank, two matrix controls, and four fortified samples) required *ca.* 16 person hours (2 calendar days) to complete (Appendix A, p. 33 of MRID 44536506).

Method GRM 94.17 (soil):

12. For the ILV, the soil matrix was not characterized and the source was not reported, but the soil sample was identified as M465B (p. 8; Appendix A, p. 31 of MRID 44536503). In the ECM study report, soil M465B was characterized as a sand soil (Appendix C, pp. 83, 85 of MRID 44536504). The registrant failed to verify that the independent laboratory performed the ILV with a soil of the most difficult analytical sample condition to analyze to demonstrate how well the method performs.

13. For the ILV, chromatograms for reagent blank samples and 1.0 ng/g (5x LOQ) spiked samples were not provided (Figures 4-5, pp. 23-24; Figures 7-8, pp. 26-27 of MRID 44536503). For the calibration standards, only chromatograms of 0.10 ng/g (in soil matrix, 2.0 ng/mL standard) cis- and trans-3-CAAC standards were provided (calibration standard range 0.100-10.0 ng/g in soil matrix; Figures 1-3, pp. 20-21; Figure 6, p. 25).

14. The independent laboratory made no modifications to the soil method. However, regarding standardization of the SPE elution profiles, the independent laboratory noted a typographical error in Section M.4.b.(8) of the ECM that was corrected (p. 16; Appendix A, p. 33 of

MRID 44536503; Appendix B, p. 66 of MRID 44536504). Also in Section M.4., the independent laboratory added 1 µL of acetic acid prior to the derivatization with MTBSTFA and recommended that illustration of SPE elution profile data would be useful. These modifications are not considered substantial changes to the ECM.

15. It was reported for the ILV that a set of seven samples (one reagent blank, two matrix controls, and four fortified samples) required *ca.* 12 person hours (1.5 calendar days) to complete (p. 16; Appendix A, p. 33 of MRID 44536503).

V. References

Keith, L.H.; Crummett, W.B.; Deegan, J.; Libby, R.A.; Taylor, J.T.; Wentler, G., "Principles of Environmental Analysis", *Anal. Chem.*, 1983, **55**, pp. 2210-2218 (Appendix A, p. 33; Appendix B, p. 67 of MRID 44536504).

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.

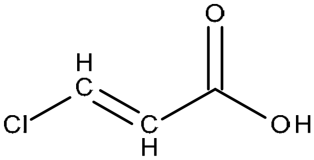
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

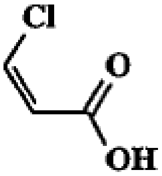
cis- and trans-3-Chloroacrylic acid (CAAC)

IUPAC Name:	(EZ)-3-Chloro-2-propenoic acid cis-: Z-3-Chloro-2-propenoic acid trans-: E-3-Chloro-2-propenoic acid
CAS Name:	Not found
CAS Number:	cis- : 1609-93-4 trans- : 2345-61-1
SMILES String:	OC(=O)C=CCl

3-CAAC



cis-3-CAAC



trans-3-CAAC