Analytical method for etridiazole and its metabolites etridiazole acid and 3-DCMT (DCE) in water

Reports: ECM: EPA MRID No. 50534503. DeVellis, S.R. 2017. Validation of the Analytical Method for the Determination of Etridiazole and its Metabolites in Surface and Ground Water by LC-MS/MS and GC-MS. Smithers Viscient Study No.: 14088.6157. Report prepared by Smithers Viscient, Wareham, Massachusetts; sponsored and submitted by MacDermid Agricultural Solutions, Inc. c/o Arysta LifeScience North America, LLC, Cary, North Carolina; 124 pages. Final report issued December 21, 2017.

ILV: EPA MRID No. 50584601. Cashmore, A. 2018. Independent Laboratory Validation of: Etridiazole – Independent Laboratory Validation of Analytical Method 14088.6157 for the Determination of Etridiazole and its metabolites Etridiazole acid and DCE in Water. Smithers Viscient (ESG) Ltd. Study No.: 3202058 and Document No.: 2017-356. Report prepared by Smithers Viscient (ESG) Ltd., North Yorkshire, United Kingdom; sponsored and submitted by MacDermid Agricultural Solutions, Inc. c/o Arysta LifeScience North America, LLC, Cary, North Carolina; 100 pages. Final report issued April 24, 2018 (pp. 2-5).

Document No.: MRIDs 50534503 & 50584601

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with the USEPA FIFRA (40 CFR Part 160) and OECD Good Laboratory Practices (GLP; p. 3 of MRID 50534503). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A certification of the authenticity of the report was included with the QA statement.

ILV: The study was conducted in accordance with the UK GLP and OECD GLP and was in compliance with the GLP regulation and are suitable for submission to the US FDA/EPA/Japanese regulatory authorities (p. 3 of MRID 50584601). Signed and dated No Data Confidentiality, GLP, Authenticity, and Quality Assurance statements were provided (pp. 2-5).

Classification: This analytical method is classified as supplemental. ECM and ILV linearity were not satisfactory for etridiazole analysis. ILV linearity was not satisfactory for 3-Carb-T (etridiazole acid) in surface water. The specificity of the method was not supported for 3-DCMT and 3-Carb-T based on ILV representative chromatograms. The ECM representative chromatogram did not support the specificity for 3-Carb-T in surface water at the LOQ. ECM matrices were not well-characterized.

PC Code: 084701

EFED Final Reviewer:	Cheryl Sutton, Ph.D. Environmental Scientist	Signature: CHERYL SUTTON Digitally signed by CHERYL SUTTON Date: 2018.10.30 09:38:14 -04'00' Date: October 24, 2018
CDM/CSS-Dynamac JV Reviewers:	Teresa Nelis, M.S., Environmental Scientist	Signature: Teresa Nelis Date: 9/28/18
	Lisa Muto, M.S., Environmental Scientist	Signature: Lisa Muto Date: 9/28/18

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

Executive Summary

This analytical method, Smithers Viscient Study No. 14088.6157, is designed for the quantitative determination of etridiazole and its metabolite 3-DCMT (DCE) in water at the LOQ of 0.100 µg/L using GC-MS/EI, and metabolite 3-Carb-T (etridiazole acid) in water at the LOQ of 0.100 µg/L using LC-MS/MS. The ECM and ILV validated the method using ground and surface water matrices; however, the ECM matrices were not well-characterized. In the ECM and ILV, three ions were monitored for etridiazole and 3-DCMT, and two ion transitions were monitored for 3-Carb-T analysis. The ILV validated the ECM method for the quantitation and confirmation analyses of etridiazole, 3-DCMT, and 3-Carb-T in ground water and surface water with insignificant modifications to the analytical instruments. The ILV validation was completed in the first trial for all analyses, except for etridiazole and 3-DCMT in ground water at the LOQ which passed in the second trial without additional modification of the ECM. All ECM and ILV data were satisfactory regarding repeatability, accuracy, and precision for all analytes. All ECM and ILV data were satisfactory regarding specificity for etridiazole. The specificity of the method was not supported for 3-DCMT and 3-Carb-T based on ILV representative chromatograms due to significant baseline noise which interfered with accurate identification and integration of the quantitation analysis LOQ peak in both matrices. Matrix interference was also observed in the ECM chromatogram for 3-Carb-T in surface water at the LOQ. The reviewer believed that additional clean-up of the matrix or matrix-matched standards may be required. ECM and ILV linearity was satisfactory for 3-DCMT analyses, but was not satisfactory for etridiazole analysis. ECM and ILV linearity was satisfactory for 3-Carb-T analyses, except for ILV analysis in surface water.

Table 1. Analytical Method Summary.

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Etridiazole	50534503	50584601		Water[1,2]	12/21/2017	MacDermid Agricultural Solutions, Inc. c/o Arysta LifeScience North America, LLC	GC/MS	0.100 µg/L
3-DCMT (DCE)								
3-Carb-T							LC/MS/MS	

1 In the ECM, ground water was obtained from a 100-meter bedrock well, location not reported, and filtered (method not specified) prior to use; and surface water (pH 6.18, 5.92 mg/L dissolved oxygen content) was obtained from Weweantic River, Wareham, Massachusetts. Ground water characterization was absent; surface water characterization was performed by Smithers Viscient, Wareham, Massachusetts (pp. 16-17 of MRID 50534503).

2 In the ILV, ground water (pH 7.6, 176 mg/L total hardness (as $CaCO_3$), 4 mg/L suspended solids, 0.0 mg/L dissolved organic carbon, and 467 µS/cm conductivity) was obtained from AgroChemex, well location not reported; and surface water (pH 7.9, 200 mg/L total hardness (as $CaCO_3$), 15 mg/L suspended solids, 4.94 mg/L dissolved organic carbon, and 49 µS/cm conductivity] was obtained from Fountains Abbey, Ripon, UK (Appendix 2, pp. 96-97). Water characterization was performed by Smithers Viscient, Wareham, Massachusetts (p. 15, Appendix 2, pp. 96-97 of MRID 50584601).

I. Principle of the Method

Samples (*ca.* 60.0 mL) were fortified with 0.0600 mL or 0.600 mL of the fortification solutions (0.100 mg/L) of etridiazole and 3-DCMT in acetonitrile to prepare LOQ (0.100 µg/L) and 10×LOQ (1.00 µg/L) fortified samples, respectively; samples (*ca.* 25.0 or 8.00 mL) were fortified with 0.0250 mL or 0.0800 mL, respectively, of the fortification solutions (0.100 mg/L) of 3-Carb-T in acetonitrile to prepare LOQ and 10×LOQ fortified samples (pp. 18, 21-23 of MRID 50534503). The water samples fortified with etridiazole and 3-DCMT were extracted with 2.00 mL of iso-octane using 60 mL glass vials with PTFE lined caps, placed on a shaker table for 30 minutes at 250 rpm, settled for 10 minutes, and the entire extraction layer, including residual water and any emulsion, was transferred using a transfer pipet into disposable conical glass vials (pp. 23-25 of MRID 50534503). The etridiazole and 3-DCMT samples were centrifuged at 1200 rpm for 20 minutes to complete sufficient phase separation. The 10×LOQ-level extracts were further diluted into the calibration range with the prepared matrix matched blank before analysis via GC-MS/EI.

The water samples fortified with 3-Carb-T received ammonium hydroxide (5 µL to the 25.0 mL sample and 1 µL to the 8.00 mL sample), were loaded onto Oasis Mixed-Mode Strong Anion Exchange (MAX) SPE columns (60 mg, 3 mL, pre-conditioned with two column volumes of methanol and two column volumes of purified reagent water), and allowed to flow through under vacuum (1 drop/sec. flow) (pp. 25-27 of MRID 50534503). Each sample vessel and column were rinsed with 5.00 mL of purified reagent water, the rinsate loaded on the column, and allowed to flow under vacuum at 1 drop/sec., and then rinsed in a similar manner with 5.00 mL of methanol, and the rinsates discarded. The columns were quickly dried under full vacuum, and the test substance was eluted from the SPE columns with 3.00 mL of 2% trifluoroacetic acid in methanol under vacuum at 1 drop/sec. and collected into glass conical vials. The samples were concentrated to incipient dryness under a gentle stream of nitrogen at 50.0°C. The residue was reconstituted to 5.00 mL with 20:80:0.1 acetonitrile:purified reagent water:trifluoroacetic acid (v:v:v) via mixing and sonication for five minutes before analysis via LC-MS/MS.

Etridiazole and 3-DCMT sample extracts were analyzed via GC-MS/EI using an Agilent 6890 series GC and Agilent DB-5MS column (15 m × 0.25 mm × 0.25 µm) using a column temperature program (initial 65°C and held for 2.00 minutes to post 150°C held for 0.00 minutes); injection volume of 2.00 µL; and helium carrier gas coupled with MS detection (300°C transfer line and 230°C source temperature) (pp. 16, 27-28 of MRID 50534503). Three ions were monitored as follows (quantitation, confirmation 1, and confirmation 2, respectively): *m/z* 211.00, 185.00, and 183.00 for etridiazole, and *m/z* 149.00, 184.00, and 186.00 for 3-DCMT. Reported retention times for etridiazole and 3-DCMT were *ca*. 12.9 and 11.0 minutes, respectively.

3-Carb-T sample extracts were analyzed via LC-MS/MS using an Agilent column and MDS Sciex API 5000 mass spectrometer (pp. 28-29 of MRID 50534503). The LC conditions consisted of a Agilent Poroshell 120 EC-C8 column (2.7 µm × 3.0 mm × 50 mm column, column temperature 35°C), a mobile phase of (A) 0.1% trifluoroacetic acid in water and (B) 0.1% trifluoroacetic acid in acetonitrile [percent A:B (v:v) at 0.01 min. 98.0:2.0, 0.50 min. 98.0:2.0, 3.00 min. 0:100, 4.00 min. 0:100, 4.10 min. 98.0:2.0, and 5.00 min. 98.0:2.0] and MS/MS detection in positive ion mode (ionization temperature 550°C). Injection volume was 50 µL. Two ion transitions were monitored (quantitation and confirmation, respectively) as follows: *m/z* 175.16→147.10 and *m/z* 175.16→129.00. Retention time was *ca.* 2.2 minutes.

In the ILV, the ECM was performed as written, except for insignificant modifications of the analytical instrumentation and parameters, and differences in calibration standards used and matrix effect assessments. The ILV reported that a Thermo Trace 1300 Gas Chromatograph with ISQ LT single quadrupole mass spectrometer detector was used for etridiazole and 3-DCMT (similar GC-MS parameters; retention times for etridiazole and 3-DCMT were 11.8 and 9.9 min., respectively) and a Shimadzu Nexera UPLC system coupled with an AB Sciex API 5000 MS/MS detector (similar LC-MS/MS parameters; same ionization temperature, 550°C) was used for 3-Carb-T (pp. 15, 22-23 of MRID 50584601). A 10 µL injection volume was used for the first attempt of the matrix assessment for 3-Carb-T by LC-MS/MS, but sensitivity and precision were insufficient at this volume and the ILV determined a 50 µL injection volume was necessary for the matrix assessment and validation; the ECM also used a 50 µL injection volume (pp. 23, 28 of MRID 50584601).

In the ECM and ILV, the method LOQs in water were 0.100 µg/L for etridiazole, 3-DCMT, and 3-Carb-T (pp. 32-33, 35-40 of MRID 50534503; pp. 12, 28 of MRID 50584601). In the ECM, the method LODs were 0.01 µg/L (Q) and 0.03-0.05 µg/L (C1/2) in ground water and 0.03 µg/L (Q, C1/2) in surface water for etridiazole; 0.04 µg/L (Q) and 0.03-0.05 µg/L (C1/2) in ground water and 0.03 µg/L (Q) and 0.02-0.03 µg/L (C1/2) in surface water for 3-DCMT; and 0.009 µg/L (Q) and 0.002 µg/L (C) in ground water and 0.05 µg/L (Q) and 0.01 µg/L (C) in surface water for 3-Carb-T (pp. 35-40 of MRID 50534503). In the ILV, the method LODs were 0.0252 µg/L (Q) and 0.0379-0.0599 µg/L (C1/2) in ground water and 0.0123 µg/L (Q) and 0.0218-0.0383 µg/L (C1/2) in surface water for etridiazole; 0.0636 µg/L (Q) and 0.0133-0.0134 µg/L (C1/2) in ground water and 0.0152 µg/L (Q) and 0.009-0.0106 µg/L (C1/2) in surface water for 3-DCMT; and 0.0304 µg/L (Q) and

0.0268 µg/L (C) in ground water and 0.962 µg/L (Q) and 0.0506 µg/L (C) in surface water for 3-Carb-T (pp. 26-27 of MRID 50584601).

II. Recovery Findings

ECM (MRID 50534503): Mean recoveries and relative standard deviations (RSD) from GC-MS/EI analysis were within guideline requirements (mean 70-120%; RSD ≤20%) for etridiazole and its metabolite 3-DCMT at 0.100 µg/L (LOQ) and 1.00 µg/L (10×LOQ) in two water matrices (Tables 1-12, pp. 43-54). Mean recoveries and relative standard deviations from LC-MS/MS analysis were within guideline requirements for 3-Carb-T at 0.100 µg/L (LOQ) and 1.00 µg/L (10×LOQ) in two water matrices (Tables 13-16, pp. 55-58). Performance data (recovery results) from primary and confirmatory analyses were comparable, except for etridiazole at the LOQ in both matrices and 3-DCMT at the LOQ in ground water (based on RSD values). The ground water was obtained from a 100-meter bedrock well, location not reported, and filtered (method not specified) prior to use; and surface water (pH 6.18, 5.92 mg/L dissolved oxygen content) was obtained from Weweantic River, Wareham, Massachusetts (pp. 16-17). Ground water characterization was absent; surface water was poorly characterized and was performed by Smithers Viscient, Wareham, Massachusetts.

ILV (MRID 50584601): Mean recoveries and RSDs from GC-MS analysis were within guideline requirements for etridiazole and its metabolite 3-DCMT at 0.10 µg/L (LOQ) and 1.0 µg/L (10×LOQ) in two water matrices (Tables 1-6, pp. 33-38, Tables 9-14, pp. 41-46 of MRID 50584601). Mean recoveries and relative standard deviations from LC-MS/MS analysis were within guideline requirements for 3-Carb-T at 0.10 µg/L (LOQ) and 1.0 µg/L (10×LOQ) in two water matrices (Tables 7-8, pp. 39-40, Tables 15-16, pp. 47-48 of MRID 50584601). Performance data (recovery results) from primary and confirmatory analyses were comparable. Ground water (pH 7.6, 176 mg/L total hardness (as $CaCO_3$), 4 mg/L suspended solids, 0.0 mg/L dissolved organic carbon, and 467 µS/cm conductivity) was obtained from AgroChemex, well location not reported; and surface water (pH 7.9, 200 mg/L total hardness (as $CaCO_3$), 15 mg/L suspended solids, 4.94 mg/L dissolved organic carbon, and 49 µS/cm conductivity] was obtained from Fountains Abbey, Ripon, UK (Appendix 2, pp. 96-97). Water characterization was performed by Smithers Viscient, Wareham, Massachusetts (p. 15, Appendix 2, pp. 96-97). The ILV validated the ECM method for the quantitation and confirmation analyses of etridiazole, 3-DCMT, and 3-Carb-T in ground water and surface water with insignificant modifications to the analytical instruments (pp. 15, 22-23). The ILV validation was completed in the first trial for all analyses, except for etridiazole and 3-DCMT in ground water at the LOQ which passed in the second trial without additional modification of the ECM (pp. 28-29).

Table 2. Initial Validation Method Recoveries for Etridiazole and Metabolites 3- DCMT and 3-Carb-T in Water.[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Ground (Well) Water					
	Quantitation ion					
Etridiazole	0.100 (LOQ)	5	88.0-109	92.9	11.3	12.2
	1.00	5	90.8-99.8	95.1	3.65	3.83
3-DCMT	0.100 (LOQ)	5	88.9-107	98.5	7.99	8.11
	1.00	5	97.3-99.3	98.1	0.751	0.766
3-Carb-T	0.100 (LOQ)	5	102-113	108	4.08	3.78
	1.00	5	84.8-91.7	88.1	2.95	3.35
	Confirmation ion 1					
Etridiazole	0.100 (LOQ)	5	90.5-105	99.1	6.33	6.39
	1.00	5	90.6-93.7	92.1	1.15	1.24
3-DCMT	0.100 (LOQ)	5	98.6-104	100	2.65	2.64
	1.00	5	90.9-103	96.8	4.37	4.51
3-Carb-T	0.100 (LOQ)	5	105-112	109	3.25	2.99
	1.00	5	83.4-93.7	88.7	4.35	4.90
	Confirmation ion 2					
Etridiazole	0.100 (LOQ)	5	98.0-104	101	2.59	2.55
	1.00	5	93.7-101	96.9	2.65	2.74
3-DCMT	0.100 (LOQ)	5[3]	68.1-104	95	15	16
	1.00	5	91.4-101	96.2	4.78	4.97
	Surface (River) Water					
	Quantitation ion					
Etridiazole	0.100 (LOQ)	5	93.2-102	97.0	3.40	3.51
	1.00	5	95.2-102	98.8	3.31	3.35
3-DCMT	0.100 (LOQ)	5	95.0-104	98.2	3.52	3.59
	1.00	5	95.3-102	98.0	2.61	2.66
3-Carb-T	0.100 (LOQ)	5	95.9-109	101	5.67	5.60
	1.00	5	85.0-102	93.0	5.85	6.30
	Confirmation ion 1					
Etridiazole	0.100 (LOQ)	5	75.5-106	97.3	12.7	13.1
	1.00	5	90.9-99.8	97.4	3.74	3.84
3-DCMT	0.100 (LOQ)	5	92.3-97.8	93.9	2.30	2.45
	1.00	5	94.0-103	98.0	3.34	3.41
3-Carb-T	0.100 (LOQ)	5	101-115	106	5.48	5.19
	1.00	5	93.3-98.2	96.4	1.94	2.02
	Confirmation ion 2					
Etridiazole	0.100 (LOQ)	5	86.5-104	94.0	6.27	6.67
	1.00	5	93.1-98.7	95.7	2.00	2.09
3-DCMT	0.100 (LOQ)	5	88.0-111	102	8.60	8.42
	1.00	5	93.0-101	96.8	3.02	3.12

Data (uncorrected recovery results) were obtained from Tables 1-16, pp. 43-58, of MRID 50534503; DER Attachment 2.

1 The ground water was obtained from a 100-meter bedrock well, location not reported, and filtered (method not specified) prior to use; and surface water (pH 6.18, 5.92 mg/L dissolved oxygen content) was obtained from Weweantic River, Wareham, Massachusetts (pp. 16-17). Ground water characterization was absent; surface water characterization was performed by Smithers Viscient, Wareham, Massachusetts.

2 For GC-MS/EI: three ions were monitored as follows (quantitation, confirmation 1, and confirmation 2, respectively): *m/z* 211.00, 185.00, and 183.00 for etridiazole, and *m/z* 149.00, 184.00, and 186.00 for 3-DCMT. For LC-MS/MS: two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 175.16→147.10 and m/z 175.16→129.00 for 3-Carb-T.

3 Mean, standard deviation, and relative standard deviation were reviewer-calculated based on all five recovery values in the study report; the study author omitted one recovery value from the statistics since it was deemed to be an outlier (DER Attachment 2). Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Etridiazole and Metabolites 3-DCMT and 3-Carb-T in Water.[1,2,3]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Ground (Well) Water					
	Quantitation ion					
Etridiazole	0.100 (LOQ)	5	91-114	105	8.9	8.5
	1.00	5	108-116	112	3.6	3.2
3-DCMT	0.100 (LOQ)	5	91-95	93	1.5	1.6
	1.00	5	91-98	95	2.9	3.0
3-Carb-T	0.100 (LOQ)	5	71-98	83	12.0	14.5
	1.00	5	85-93	88	3.2	3.7
	Confirmation ion 1					
Etridiazole	0.100 (LOQ)	5	103-121	111	8.8	7.9
	1.00	5	106-120	111	5.8	5.2
3-DCMT	0.100 (LOQ)	5	102-115	109	5.3	4.9
	1.00	5	93-102	98	3.5	3.6
3-Carb-T	0.100 (LOQ)	5	87-102	97	6.1	6.3
	1.00	5	85-99	92	6.1	6.7
	Confirmation ion 2					
Etridiazole	0.100 (LOQ)	5	94-109	103	5.7	5.5
	1.00	5	110-118	113	3.4	3.0
3-DCMT	0.100 (LOQ)	5	95-106	101	4.8	4.7
	1.00	5	96-101	98	2.2	2.2
	Surface (River) Water					
	Quantitation ion					
Etridiazole	0.100 (LOQ)	5	81-113	94	14.2	15.0
	1.00	5	90-104	99	5.9	6.0
3-DCMT	0.100 (LOQ)	5	80-89	86	4.3	5.0
	1.00	5	76-93	87	6.5	7.5
3-Carb-T	0.100 (LOQ)	5	96-123	110	11.0	10.0
	1.00	5	79-92	85	4.8	5.6
	Confirmation ion 1					
Etridiazole	0.100 (LOQ)	5	85-115	100	14.7	14.7
	1.00	5	95-105	101	4.1	4.0
3-DCMT	0.100 (LOQ)	5	67-100	82	15.3	18.7
	1.00	5	78-90	86	4.8	5.6
3-Carb-T	0.100 (LOQ)	5	77-117	92	15.0	16.4
	1.00	5	81-97	87	6.3	7.2
	Confirmation ion 2					
Etridiazole	0.100 (LOQ)	5	85-116	97	13.4	13.8
	1.00	5	92-107	101	6.0	5.9
3-DCMT	0.100 (LOQ)	5	67-100	83	14.9	17.9
	1.00	5	78-91	86	4.9	5.7

Data (uncorrected recovery results) were obtained from Tables 1-16, pp. 33-48, of MRID 50584601.

1 3-DCMT synonym DCE and 3-Carb-T synonym etridiazole acid used in ILV.

2 The ground water (pH 7.6, 176 mg/L total hardness (as $CaCO_3$), 4 mg/L suspended solids, 0.0 mg/L dissolved organic carbon, and 467 µS/cm conductivity) was obtained from AgroChemex, well location not reported; and surface water (pH 7.9, 200 mg/L total hardness (as $CaCO_3$), 15 mg/L suspended solids, 4.94 mg/L dissolved organic carbon, and 49 µS/cm conductivity] was obtained from The Lake, Ripon, UK (Fountains Abbey). Water characterization was

performed by Smithers Viscient, Wareham, Massachusetts.

[3] For GC-MS: three ions were monitored as follows (quantitation, confirmation 1, and confirmation 2, respectively): *m/z* 211, 185, and 183 for etridiazole, and *m/z* 149, 184, and 186 for 3-DCMT (DCE). For LC-MS/MS: two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 174.9→146.9 and m/z 174.9→129 for 3-Carb-T (etridiazole acid).

III. Method Characteristics

In the ECM and ILV, the method LOQs in water were 0.100 µg/L for etridiazole, 3-DCMT, and 3-Carb-T (pp. 32-33, 35-40 of MRID 50534503; pp. 12, 28 of MRID 50584601). In the ECM and ILV, the LOQ was defined as the lowest fortification level, and it was noted that blank values should not exceed 30% of the LOQ (p. 30 of MRID 50534503; p. 24 of MRID 50584601). For the ECM, all blank values were < 30% for all analytes in ground water and surface water (pp. 35-40 of MRID 50534503). For the ILV, the ILV criteria for specificity of ≤ 50% of the analytes in the blanks at their respective LOD and ≤ 30% of the analytes in their blanks at their respective LOQ were met for all blanks, except one blank for etridiazole at *m/z* 183 (confirmation ion 2) in ground water, which showed an uncharacteristic noisy baseline (p. 27, Figure 13, p. 62 of MRID 50584601). No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM. In the ILV, the LOQ was reported from the ECM without justification.

In the ECM, the method LODs were 0.01 µg/L (Q) and 0.03-0.05 µg/L (C1/2) in ground water and 0.03 µg/L (Q, C1/2) in surface water for etridiazole; 0.04 µg/L (Q) and 0.03-0.05 µg/L (C1/2) in ground water and 0.03 µg/L (Q) and 0.02-0.03 µg/L (C1/2) in surface water for 3-DCMT; and 0.009 µg/L (Q) and 0.002 µg/L (C) in ground water and 0.05 µg/L (Q) and 0.01 µg/L (C) in surface water for 3-Carb-T (pp. 35-40 of MRID 50534503). In the ILV, the method LODs were 0.0252 µg/L (Q) and 0.0379-0.0599 µg/L (C1/2) in ground water and 0.0123 µg/L (Q) and 0.0218-0.0383 µg/L (C1/2) in surface water for etridiazole; 0.0636 µg/L (Q) and 0.0133-0.0134 µg/L (C1/2) in ground water and 0.0152 µg/L (Q) and 0.009-0.0106 µg/L (C1/2) in surface water for 3-DCMT; and 0.0304 µg/L (Q) and 0.0268 µg/L (C) in ground water and 0.962 µg/L (Q) and 0.0506 µg/L (C) in surface water for 3-Carb-T (pp. 26-27 of MRID 50584601).
The LOD was calculated in the ECM using the following equation:

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

Where, LOD is the limit of detection of the analysis, SN_{ctl} is the mean signal to noise in height of the control samples (or blanks), Resp_{LS} is the mean response in height of the two low calibration standards, and Conc_{LS} is the concentration of the low calibration standard (p. 31 of MRID 50534503). The ILV LOD is calculated from 3 × height of control baseline noise × control dilution factor × calibration standard concentration (µg/mL) / height of calibration standard peak (p. 24 of MRID 50584601).

Table 4. Method Characteristics for Etridiazole, 3-DCMT, and 3-Carb-T in Water.

Analyte[1]			**Etridiazole**	**3-DCMT**	**3-Carb-T**
Analysis			**GC/MS**		**LC/MS/MS**
Limit of Quantitation (LOQ)	ECM		0.100 µg/L	0.100 µg/L	0.100 µg/L
	ILV				
Limit of Detection (LOD)	ECM (Calc)	Ground	0.01 µg/L (Q) 0.05 µg/L (C1) 0.03 µg/L (C2)	0.04 µg/L (Q) 0.03 µg/L (C1) 0.05 µg/L (C2)	0.009 µg/L (Q) 0.002 µg/L (C)
		Surface	0.03 µg/L (Q/C1/C2)	0.03 µg/L (Q) 0.02 µg/L (C1) 0.03 µg/L (C2)	0.05 µg/L (Q) 0.01 µg/L (C)
	ILV (Calc)	Ground	0.0252 µg/L (Q) 0.0379 µg/L (C1) 0.0599 µg/L (C2)	0.0636 µg/L (Q) 0.0134 µg/L (C1) 0.0133 µg/L (C2)	0.0304 µg/L (Q) 0.0268 µg/L (C)
		Surface	0.0123 µg/L (Q) 0.0218 µg/L (C1) 0.0383 µg/L (C2)	0.0152 µg/L (Q) 0.009 µg/L (C1) 0.0106 µg/L (C2)	0.962 µg/L (Q) 0.0506 µg/L (C)
Linearity (calibration curve r^2 and concentration range)	ECM	Ground	r^2 = 0.99760 (Q) r^2 = 0.99565 (C1) r^2 = 0.99769 (C2)	r^2 = 0.99768 (Q) r^2 = 0.99687 (C1) r^2 = 0.99773 (C2)	r^2 = 0.99582 (Q) r^2 = 0.99761 (C)
		Surface	r^2 = **0.99385** (Q) r^2 = **0.99353** (C1) r^2 = **0.99262** (C2)	r^2 = 0.99642 (Q) r^2 = **0.99493** (C1) r^2 = 0.99605 (C2)	r^2 = 0.99836 (Q) r^2 = 0.99639 (C)
	ILV[2]	Ground	r^2 = **0.9936**/0.9981 (Q) r^2 = **0.9935**/0.9977 (C1) r^2 = **0.9936**/0.9967 (C2)	r^2 = 0.9964 (Q) r^2 = 0.9992 (C1) r^2 = 0.9986 (C2)	r^2 = 0.9970 (Q) r^2 = **0.9944** (C)
		Surface	r^2 = **0.9926** (Q) r^2 = **0.9888** (C1) r^2 = **0.9882** (C2)	r^2 = 0.9989/0.9968 (Q) r^2 = 0.9976 (C1) r^2 = 0.9986 (C2)	r^2 = **0.9930** (Q) r^2 = **0.9851** (C)
	Range		2-20 µg/L		0.250-2.50 µg/L
Repeatable	ECM[3]		Yes at LOQ and 10×LOQ in one poorly characterized surface water and one uncharacterized ground water matrices.		
	ILV[4]		Yes at LOQ and 10×LOQ in one well-characterized surface water and one well-characterized ground water matrices.		
Reproducible			Yes at LOQ and 10×LOQ		
Specific	ECM[5]		Yes, matrix interferences were <20% of the LOQ (based on peak area).	Yes, no matrix interferences were detected in the controls.	Yes, matrix interferences were <14% of the LOQ (based on peak area); however, Q LOQ chromatogram for surface water showed significant baseline noise.[6]
	ILV		Yes, matrix effects were insignificant for ground water and surface water, but minor baseline noise interfered with Q/C1 LOQ peak attenuation.	**No**, matrix effects at the analyte retention time were insignificant; however, the Q LOQ peak integration and attenuation was significantly altered by baseline noise in ground water and the Q LOQ peak co-eluted with overwhelming baseline	**No**, matrix effects at the analyte retention time were insignificant; however, the Q LOQ peak was only distinguishable from the baseline noise by retention time, and the Q LOQ peak was very small compared to contaminant peaks (*ca*. 80% of the

Analyte[1]		**Etridiazole**	**3-DCMT**	**3-Carb-T**
Analysis		**GC/MS**		**LC/MS/MS**
			noise in surface water.[7]	LOQ peak ht.).[8]

Data were obtained from pp. 32-33, 35-40 (LOQ/LOD); Tables 1-16, pp. 43-58 (recovery results); Figures 1-56, pp. 59-114 (chromatograms and calibration curves) of MRID 50534503; pp. 12, 28 (LOQ/LOD); Tables 1-16, pp. 33-48 (recovery results); pp. 26-27, Figures 1-72, pp. 56-91 (chromatograms and calibration curves); Tables 17-22, pp. 49-54 (matrix effects) of MRID 50584601; DER Attachment 2. Analytes were identified using one quantitation ion and two confirmation ions for etridiazole and 3-DCMT and one quantitation and one confirmatory ion transition for 3-Carb-T. Q = Quantitation ion/ion transition; C1 = Confirmation ion 1; C2 = Confirmation ion 2; C = Confirmation ion transition.

1 3-DCMT synonym DCE and 3-Carb-T synonym etridiazole acid used in ILV.

2 Two correlation coefficients (r^2) values for etridiazole in ground water and 3-DCMT in surface water include the repeat of the validation at the LOQ (0.10 µg/L) for etridiazole and the re-injection of 3-DCMT at the LOQ (0.10 µg/L) to achieve acceptable precision and/or accuracy (pp. 26-27; Appendix 4, p. 99 of MRID 50584601). 3-Carb-T correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (pp. 26-27; DER Attachment 2).

3 In the ECM, ground water was obtained from a 100-meter bedrock well, location not reported, and filtered (method not specified) prior to use; and surface water (pH 6.18, 5.92 mg/L dissolved oxygen content) was obtained from Weweantic River, Wareham, Massachusetts (pp. 16-17 of MRID 50534503). Ground water characterization was absent; surface water characterization was performed by Smithers Viscient, Wareham, Massachusetts.

4 In the ILV, ground water (pH 7.6, 176 mg/L total hardness (as $CaCO_3$), 4 mg/L suspended solids, 0.0 mg/L dissolved organic carbon, and 467 µS/cm conductivity) was obtained from AgroChemex, well location not reported; and surface water (pH 7.9, 200 mg/L total hardness (as $CaCO_3$), 15 mg/L suspended solids, 4.94 mg/L dissolved organic carbon, and 49 µS/cm conductivity] was obtained from Fountains Abbey, Ripon, UK (Appendix 2, pp. 96-97). Water characterization was performed by Smithers Viscient, Wareham, Massachusetts (p. 15, Appendix 2, pp. 96-97 of MRID 50584601)..

5 In the ECM, matrix interferences based on representative peak areas of the control and the LOQ were <20%, 0%, and <14% for etridiazole, 3-DCMT, and 3-Carbon-T, respectively. Matrix effects assessment performed by the study author determined matrix effects were minimal for etridiazole and 3-Carb-T ground water and surface water but were significant for 3-DCMT in ground water and surface water (pp. 35-40 of MRID 50534503); results of the matrix assessment were not provided; the study author used matrix-matched standards for the method validation.

6 The chromatogram for 3-Carb-T for surface water primary quantitation for at the LOQ (0.100 µg/L) showed significant baseline noise the LOQ (0.100 µg/L) (Figure 47, p.105 of MRID 50534503). Additional clean-up may be required to reduce the baseline noise or matrix-matched calibration standards should be used.

7 Based on Figure 32, p. 71 and Figure 35, p. 73 of MRID 50584601. Similar issues were not observed in the confirmation ion chromatograms.

8 Based on Figure 59, p. 85 and Figure 62, p. 86 of MRID 50584601. Similar issues were noted in the confirmation ion chromatograms.

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

IV. Method Deficiencies and Reviewer's Comments

1. For the quantitation analysis, ILV linearity was not satisfactory for etridiazole in ground water for the analyses at 0.10 µg/L (the LOQ), $r^2 = 0.9936$, or in surface water $r^2 = 0.9926$ and for 3-Carb-T in surface water ($r^2 = 0.9930$; pp. 26-27, Figure 1, p. 56 of MRID 50584601; DER Attachment 2).

   For the quantitation analysis, ECM linearity was not satisfactory for etridiazole in surface water, $r^2 = 0.99385$ (p. 36, Figure 34, p. 92 of MRID 50534503).

   For the confirmation analysis, ILV linearity was not satisfactory for etridiazole in ground water for the analyses at 0.10 µg/L (the LOQ), $r^2 = 0.9935$ (C1) and 0.9936 (C2), or in surface water $r^2 = 0.98888$ (C1) and 0.9882 (C2) and for 3-Carb-T [$r^2 = 0.9944$ (ground) and 0.9851 (surface); pp. 26-27 of MRID 50584601]. For the confirmation analysis, ECM linearity was not satisfactory for etridiazole in surface water, $r^2 = 0.99353$ (C1) and 0.99262 (C2) and for 3-DCMT in surface water [$r^2 = 0.99493$ (C1); pp. 36, 38 of MRID 50534503]. In the case of the confirmation analyses, the reviewer did not consider this guideline deviation to be significant since a confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data.

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

2. The specificity of the method was not supported for 3-DCMT and 3-Carb-T based on ILV representative chromatograms. For both analytes, matrix effects at the analyte retention time were insignificant; however, significant baseline noise interfered with accurate identification and integration of the quantitation analysis LOQ peak in both matrices. For 3-DCMT, the quantitation LOQ peak integration and attenuation was significantly altered by baseline noise in ground water and the quantitation LOQ peak co-eluted with overwhelming baseline noise in surface water (Figure 32, p. 71 and Figure 35, p. 73 of MRID 50584601). Similar issues were not observed in the confirmation ion chromatograms. For 3-Carb-T, the quantitation LOQ peak was only distinguishable from the baseline noise by retention time, and the quantitation LOQ peak was very small compared to contaminant peaks (*ca*. 80% of the LOQ peak ht.; Figure 59, p. 85 and Figure 62, p. 86). Similar issues were noted in the confirmation ion chromatograms.

3. In the ECM, the chromatograms for etridiazole and 3-DCMT analysis by GC/MS in ground water and surface water were acceptable, even though the LOQ peak was broad (Figures 1-5, 9-13, 29-33, 37-41, pp. 59-63, 67-71, 87-91, 95-99 of MRID 50534503). The chromatogram for 3-Carb-T analysis by LC-MS/MS for surface water primary quantitation at the LOQ (0.100 µg/L) showed significant baseline noise, which indicates additional clean-up of the matrix or matrix-matched standards may be required (Figure 47, p. 105 of MRID 50534503). The reviewer did not fault the support of the specificity of the method since representative ground water chromatograms were acceptable.

4. In the ECM, ground water characterization was absent; and surface water was poorly characterized (p. 16 of MRID 50534503).

5. For the ILV, the first validation attempt for etridiazole and 3-DCMT in ground water required re-injection due to precision and accuracy failure for etridiazole at the LOQ (0.10 µg/L). Etridiazole at 10 × LOQ and 3-DCMT at the LOQ and 10 × LOQ passed on the first attempt. Appendix 4, p. 99 of MRID 50584601 indicates that the validation was repeated, without modification to the method, and was successful on the fourth attempt; however, no explanation was given why the two re-injections failed.

6. In the ILV, matrix-matched standards were not used for calibration; matrix effects data indicated there were no matrix interferences (Tables 17-22, pp. 49-54 of MRID 50584601). The chromatogram for the etridiazole control in ground water for confirmation ion two showed appreciable noise, indicating clean-up of the matrix or matrix-matched standards may be required (Figure 13, p. 62 of MRID 50584601).

   In the ECM, matrix-matched standards were used for the calibration of etridiazole and 3-DCMT; matrix-matched standards were not used for 3-Carb-T calibration. The ECM reported matrix effects were significant for 3-DCMT in ground water and surface water and were insignificant for etridiazole and 3-Carb-T in both waters (pp. 35-40 of MRID 50534503); however, no matrix effects data were provided.

The ILV reported matrix effects as: (mean measured concentration with solvent – mean measured concentration with matrix)/(measured concentration with solvent) x 100 (p. 17, Tables 17-22, pp. 49-54 of MRID 50584601). The ILV matrix assessment differed from the ECM in concentration of matrix matched standards [10 µg/L etridiazole and 3-DCMT and 1.0 µg/L for 3-Carb-T in the ILV (p. 18 of MRID 50584601); and 3.0 µg/L for etridiazole and 3-DCMT and 0.5 µg/L for 3-Carb-T in the ECM (pp. 20-21 of MRID 50534503)]. All monitored ion transitions were the same as those of the ECM.

7. The ECM laboratory is Smithers Viscient Laboratory in Wareham, Massachusetts, and the ILV laboratory is also a Smithers Viscient Laboratory, but located in Harrogate, North Yorkshire, United Kingdom. One communication was documented between the two laboratories, and that was to confirm the SPE cartridge particle size. The other documented communications were between the Harrogate laboratory and the sponsor MacDermid Agricultural Solutions, Inc., concerning schedule and approval to repeat of etridiazole at 0.10 µg/L in ground water (Appendix 5, p. 100 of MRID 50584601).

   Note, if the laboratory that conducted the validation belonged to the same organization as the originating laboratory, the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion. The analysts, study director, equipment, instruments, and supplies of the two laboratories were different and operated separately. Furthermore, the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies; the unfamiliarity of the ILV with the method and its development was not stated in the ILV, but the report stated the method was independently validated (p. 30 of MRID 50584601).

8. The reviewer noted that the ILV reported that a single calibration standard run after the sample analysis for etridiazole and 3-DCMT on ground water was used to verify that the instrument had not drifted significantly after analysis of samples because the GC-MS stopped after the first calibration standard run post sample analysis (p. 17 of MRID 50584601). For 3-Carb-T, the LC-MS/MS stopped before the last three standards were run after the samples were analyzed; the first three calibration standards were accepted and any re-analyzed samples were analyzed with the initial full set of calibration standards so the calibration and sample extracts were equally aged (quality of aged calibration standards was verified by acceptable correlation coefficients.

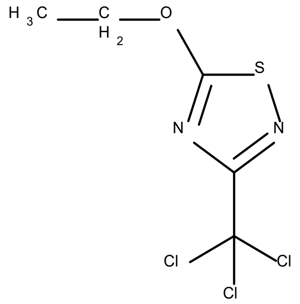
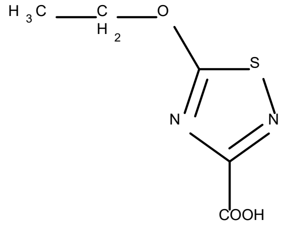
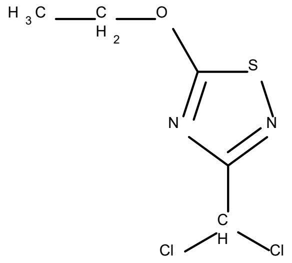
9. The time required to complete the method for a sample set was not reported in the ECM or ILV.

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.

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

DER ATTACHMENT 1. Etridiazole and Its Environmental Transformation Products. [A]

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
PARENT						
Etridiazole	IUPAC: Ethyl 3-trichloromethyl-1,2,4-thiadiazol-5-yl ether CAS: 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole CAS No.: 2593-15-9 Formula: $C_5H_5Cl_3N_2OS$ MW: 247.5 g/mol SMILES: CCOc1nc(ns1)C(Cl)(Cl)Cl		850.6100 ECM water	50534503	NA	NA
			850.6100 ILV water	50584601		
Etridiazole acid (3-Carb-T)	IUPAC: 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid CAS No.: 67472-43-9 Formula: $C_5H_6N_2O_3S$ MW: 174.17 g/mol SMILES: CCOc1nc(ns1)C(=O)O		850.6100 ECM water	50534503	NA	NA
			850.6100 ILV water	50584601		
DCE (3-DCMT; T-03)	IUPAC: 5-Ethoxy-3-dichloromethyl-1,2,4-thiadiazole Formula: $C_5H_6Cl_2N_2OS$ MW: 213.08 g/mol SMILES: CCOc1nc(ns1)C(Cl)Cl		850.6100 ECM water	50534503	NA	NA
			850.6100 ILV water	50584601		

[A] AR means “applied radioactivity”. MW means “molecular weight”. NA means “not applicable”. ECM means "environmental chemical methods". ILV means "independent laboratory validation".