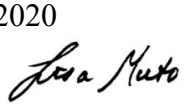
Analytical method for mancozeb and its metabolite ETU in soil

Reports:	ECM: EPA MRID No.: MRID 50452902. Budgeon, Jr., A.D. 2017. Method Development and Validation of Mancozeb and ETU Analysis in Soil. Analytical Method No.: AU-275R0. Laboratory Project ID AU-2017-08. Report prepared by JRF America, Inc., Audubon, Pennsylvania; and sponsored and submitted by Mancozeb Task Force, c/o McDermott, Will and Emery, Washington, D.C.; 189 pages. Final report issued November 28, 2017. ILV: EPA MRID Nos. 50774407/50661202. Khanvilkar, T, 2018. Independent Laboratory Validation of an Analytical Method for the Determination of Mancozeb and ETU in Soil by LC-MS/MS Analysis. Laboratory Project ID: JRF Study No. 228-2-14-18970. Report prepared by Jai Research Foundation, Gujarat, India; and sponsored and submitted by Mancozeb Task Force c/o McDermott, Will and Emery, Washington, D.C; 58 pages. Final report issued July 17, 2018.
Document No.:	MRIDs 50452902 & 50774407 & 50661202 (a reprint of 50774407)
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with the USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 50452902). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). The authenticity statement was included with the Quality Assurance statement (p. 4). ILV: The study was conducted in accordance with OECD and Indian GLP standards, which are considered to be comparable to EPA FIFRA GLP standards (p. 3; Appendix 8, pp. 57-58 of MRID 50774407). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4; Appendix 8, pp. 57-58). The authenticity statement was included with the Quality Assurance statement (p. 4).
Classification:	This analytical method is classified as supplemental. ILV linearity was not satisfactory for ETU. The specificity of the method was not supported by ILV representative chromatograms. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. Communications between the ILV and ECM/Study Monitor were not detailed in the ILV. The number of trials required to validate the method was not reported. The LOD was not reported in the ILV.
PC Code:	014504
EFED Final Reviewer:	Mohammed Ruhman, Ph.D., Senior Scientist — Signature:  Date: 06/15/2020
CDM/CSS-	Lisa Muto, M.S., — Signature: 

Dynamac JV Reviewers:	Environmental Scientist	Date:	12/31/2019
	Mary Samuel, M.S., Environmental Scientist	Signature:	Marysamuel
		Date:	12/31/2019

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

Executive Summary

The analytical methods, JRFA Analytical Method No. AU-275R0, is designed for the quantitative determination of mancozeb and its metabolite ETU in soil at the LOQ of 0.05 mg/kg using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil for mancozeb and less than for ETU. Due to the hydrolytic instability of mancozeb, mancozeb was methylate and quantified as dimethyl-EBDC. The ECM validated the method using characterized loamy sand soil; the ILV soil matrix characterization was poor, and the soil texture could not be determined. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The number of ILV trials required to validate the method was not reported; however, the reviewer assumed that the method was validated for soil in the first trial with insignificant modifications to the analytical instrumentation and parameters. Communications between the ILV (JRFI) and ECM (JRFA) were not detailed. All ILV and ECM data was satisfactory regarding accuracy, precision, reproducibility and linearity for both analytes at the LOQ and 10×LOQ, except that the ILV linearity was unacceptable for ETU. The specificity of the method was not supported by ILV representative chromatograms since chromatograms were provided without fortification labels; ECM representative chromatograms were satisfactory.

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						
Mancozeb	50452902[1]	50774407[2] & 50661202[3]		Soil	28/11/2017	Mancozeb Task Force, c/o McDermott, Will and Emery	LC/MS/MS	0.05 mg/kg
ETU								

1 In the ECM, the loamy sand soil {Sample ID: NY-OC[…]3-6"; sand 86%, silt 9%, clay 5%; pH 6 in soil:water (1:1 ratio); 2.0% organic matter Walkley Black} was used (USDA soil texture classification; Table 11, p. 40; Appendix IX, p. 185 of MRID 50452902). The soil was characterized by Agvise Laboratories, Northwood, North Dakota; the soil source was not reported but appeared to be located in New York. The soil was reportedly selected to representative of a typical soil and collected according to guideline requirements (p. 16). Further information of the soil was kept with the raw data.

2 In the ILV, the soil [coarse sand 35.06%, fine sand 35.11%, silt 3.68%, clay 16.43%; pH 6.87 in distilled water (1:2.5 ratio); pH 5.96 in 0.01M $CaCl_2$ (1:2.5 ratio); 0.76% organic carbon] was collected from the 0-25 cm depth of the Bank of Par River in Nani Vahiyal village in Dharampur Tehsil, Valsad, Gujarat, India, and characterized in JRF Study No. 609-3-15-19504 (p. 11; Appendix 7, p. 56 of MRID 50774407). The USDA soil texture classification was not reported, and it could not be determined by the reviewer using USDA-NRCS technical support tools since the following particle distribution, sand 70.17%, silt 3.68%, clay 16.43%, did not total 100%.

3 MRID 50661202 was also submitted; however, MRID 50661202 was a reprint of MRID 50774407.

Citations for MRID 50774407 also refer to citations in MRID 50661202, since MRID 50661202 was a reprint of MRID 50774407.

I. Principle of the Method

Fortification solutions (10000 and/or 1000 µg/L) were prepared for mancozeb in isopropanol:water (1:1, v:v) and ETU and dimethyl-ethylenebisdithiocarbamate (dimethyl-EBDC) in acetonitrile (p. 18 of MRID 50452902). Calibrations solutions of dimethyl-EBDC (1-10 µg/L) and ETU (1-10 µg/L) were prepared in water:acetonitrile (95:5, v:v) for analyte quantification (p. 19).

For mancozeb analysis, soil samples (2 ± 0.05 g) in a 50-mL centrifuge tube were mixed with 1-2 mL of water then fortified by adding 0.1 g of L-cysteine + 0.5 g EDTA-4Na + the appropriate mancozeb fortification standard solution (p. 19; Appendix II, Figure 47, p. 84 of MRID 50452902). The samples were methylated by adding 0.05M dimethyl sulfate + 0.1M iodomethane. Samples were mixed via vortex for 1 minute and then shaking via wrist action shaker for 15 minutes. One packet of QuEChERS mix of 4 g anhydrous $MgS0_4$ + 1 g NaCl was added to the samples. After shaking for 1 minute, samples were centrifuged for 10 minutes at 3500 rpm. A 3-mL aliquot of the supernatant was transferred to a new 15 mL centrifuge tube containing a QuEChERS mix of 150 mg Supelclean PSA, 150 mg Discovery DSC-18, and 900 mg $MgS0_4$. Samples were mixed via vortex for 1 min and centrifugation for 10 minutes at 3500 rpm. Samples were filtered, diluted, if necessary, and then vialed for LC/MS/MS analysis.

For ETU analysis, soil samples (2 ± 0.05 g) in a 50-mL centrifuge tube were mixed with 1-2 mL of water then fortified by adding the appropriate ETU fortification standard solution (pp. 19-20; Appendix II, Figure 48, p. 85 of MRID 50452902). The sample was extracted via adding 10 mL of methanol:water (50:50, v:v). Samples were mixed via vortex for 1 minute and then shaking via wrist action shaker for 15 minutes After centrifugation for 10 minutes at 3500 rpm, a 3-mL aliquot of the supernatant was transferred to a new 15 mL centrifuge tube containing a QuEChERS mix of 150 mg Supelclean PSA, 150 mg Discovery DSC-18, and 900 mg $MgS0_4$. Samples were mixed via vortex for 1 minute and centrifugation for 10 minutes at 3500 rpm. Samples were filtered, diluted, if necessary, and then vialed for LC/MS/MS analysis.

The method cautioned that mancozeb samples should be analyzed as soon as possible after extraction due to fast hydrolysis of mancozeb (Appendix VIII, pp. 122-123 of MRID 50452902).

Samples were analyzed using an Agilent 1290 series HPLC coupled to a Sciex API 6500 Q Trap mass spectrometer (pp. 21-22 of MRID 50452902). The LC/MS conditions consisted of a Waters HSS T3 column (100 x 2.1 mm, 1.8 µm particle size; oven temperature ambient) with a mobile phase gradient of A) 5mM ammonium formate with 0.1% formic acid in HPLC-grade water and B) 0.1% formic acid in HPLC-grade methanol [percent A:B (v:v) at 0.00-1.00 min. 90:10, 2.00 min. 50:50, 3.00-5.5.0 min. 10:90, 6.00-8.00 min. 90:10] and ESI ionization MS detection in positive ion mode with MRM (TEM 520°C). Injection volume was 8 µL for mancozeb (as dimethyl-EBDC) and 15 µL for ETU. Two ion transitions were monitored for each analyte as follows (quantitative and confirmatory, respectively): *m/z* 240.870→133.900 and *m/z* 240.922→193.000 for mancozeb (as dimethyl-EBDC) and *m/z* 102.887→44.000 and *m/z* 102.887→59.900 for ETU. Retention times were *ca*. 3.31 and 0.5-0.60 minutes for mancozeb (as dimethyl-EBDC) and ETU, respectively.

The ILV performed Analytical Method No. AU-275R0 as written, except for the insignificant modifications to the LC/MS/MS instrument and monitored MS transitions (pp. 12-17 of MRID 50774407). The filter size was specified (0.45 µm). Samples were analyzed using a Shimadzu Nexera X2 coupled with a Qtrap 6500 MS. All LC/MS parameters were the same as the ECM, except for adjustments to the monitored MS transitions. Two ion transitions were monitored for each analyte as follows (quantitative and confirmatory, respectively): *m/z* 241.1→134.1 and *m/z* 241.1→193.2 for mancozeb (as dimethyl-EBDC) and *m/z* 103.4→44.0 and *m/z* 103.4→60.0 for ETU. Retention times were *ca*. 3.43 and 0.57 minutes for mancozeb (as dimethyl-EBDC) and ETU, respectively (Appendix I, pp. 32, 37).

In the ECM and ILV, Limit of Quantification (LOQ) in soil was 0.05 mg/kg for mancozeb and ETU (pp. 24-26 of MRID 50452902; Tables 5-8, pp. 25-28 of MRID 50774407). In the ECM, the Limit of Detection (LOD) for water was calculated as 0.0120 µg/g and 0.0187 µg/g for mancozeb and ETU, respectively. The LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 50452902): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of mancozeb and ETU in one soil matrix at fortification levels of 0.05 mg/kg (LOQ) and 0.5 mg/kg (10×LOQ; Appendix I, Tables 1-5, pp. 30-34). Analytes were identified and quantified using two ion transitions; performance data was comparable between the primary and confirmatory analyses. The loamy sand soil {Sample ID: NY-OC[…]3-6"; sand 86%, silt 9%, clay 5%; pH 6 in soil:water (1:1 ratio); 2.0% organic matter Walkley Black} was used (USDA soil texture classification; Table 11, p. 40; Appendix IX, p. 185 of MRID 50452902). The soil was characterized by Agvise Laboratories, Northwood, North Dakota; the soil source was not reported but appeared to be located in New York. The soil was reportedly selected to representative of a typical soil and collected according to guideline requirements (p. 16). Further information of the soil was kept with the raw data.

ILV (MRID 50774407): Mean recoveries and RSDs were within guidelines for analysis of mancozeb and ETU in one soil matrix at fortification levels of 0.05 mg/kg (LOQ) and 0.5 mg/kg (10×LOQ; Tables 5-8, pp. 25-28). Analytes were identified and quantified using two ion transitions; performance data was comparable between the primary and confirmatory analyses. The soil [coarse sand 35.06%, fine sand 35.11%, silt 3.68%, clay 16.43%; pH 6.87 in distilled water (1:2.5 ratio); pH 5.96 in 0.01M $CaCl_2$ (1:2.5 ratio); 0.76% organic carbon] was collected from the 0-25 cm depth of the Bank of Par River in Nani Vahiyal village in Dharampur Tehsil, Valsad, Gujarat, India, and characterized in JRF Study No. 609-3-15-19504 (p. 11; Appendix 7, p. 56). The USDA soil texture classification was not reported, and it could not be determined by the reviewer using USDA-NRCS technical support tools since the following particle distribution, sand 70.17%, silt 3.68%, clay 16.43%, did not total 100%. The number of trials required to validate the method was not reported; however, the reviewer assumed that the method was validated for soil in the first trial with insignificant modifications to the analytical instrumentation and parameters (pp. 7, 12-17).

Table 2. Initial Validation Method Recoveries for Mancozeb and its Metabolite ETU in Soil[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Loamy Sand Soil					
	Quantitation ion transition					
Mancozeb	0.05 (LOQ)	7	81.0-104	95.6	7.7	8.0
	0.5	5	87.2-98.7	94.9	4.6	4.8
ETU	0.05 (LOQ)	7	80.6-111	98.6	11.9	12.0
	0.5	5	98.7-111	105	5.9	5.6
	Confirmation ion transition					
Mancozeb	0.05 (LOQ)	7	81.6-101	95.2	6.9	7.2
	0.5	5	85.6-98.8	93.6	5.2	5.6
ETU	0.05 (LOQ)	7	66.0-119	95.4	18.2	19.0
	0.5	5	82.8-113	104	12.4	11.9

Data (uncorrected recovery results; pp. 23-26; Appendix I, Tables 2-5, pp. 31-34) were obtained from Appendix I, Tables 1-5, pp. 30-34 of MRID 50452902.

1 The loamy sand soil {Sample ID: NY-OC[…]3-6"; sand 86%, silt 9%, clay 5%; pH 6 in soil:water (1:1 ratio); 2.0% organic matter Walkley Black} was used (USDA soil texture classification; Table 11, p. 40; Appendix IX, p. 185). The soil was characterized by Agvise Laboratories, Northwood, North Dakota; the soil source was not reported but appeared to be located in New York. The soil was reportedly selected to representative of a typical soil and collected according to guideline requirements (p. 16). Further information of the soil was kept with the raw data.

2 Two ion transitions were monitored for each analyte as follows (quantitative and confirmatory, respectively): *m/z* 240.870→133.900 and *m/z* 240.922→193.000 for mancozeb (as dimethyl-EBDC) and *m/z* 102.887→44.000 and *m/z* 102.887→59.900 for ETU.

Table 3. Independent Validation Method Recoveries for Mancozeb and its Metabolite ETU in Soil[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)[4]
	Soil					
	Quantitation ion transition					
Mancozeb	0.05 (LOQ)	7	96.00-104.00	98.57	2.76	2.04
	0.5	5	89.60-96.20	92.84	2.90	3.02
ETU	0.05 (LOQ)	7	114.00-122.00	116.85	2.79	1.72
	0.5	5	102.40-123.20	108.48	8.67	7.93
	Confirmation ion transition					
Mancozeb	0.05 (LOQ)	7	94.00-102.00	98.86	3.02	4.08
	0.5	5	90.80-94.60	92.68	1.59	1.73
ETU	0.05 (LOQ)	7	112.00-120.00	115.43	2.51	1.72
	0.5	5	102.20-120.20	107.32	7.58	7.08

Data (uncorrected recovery results; p. 16) were obtained from Tables 5-8, pp. 25-28 of MRID 50774407 and DER Attachment 2.

1 The soil [coarse sand 35.06%, fine sand 35.11%, silt 3.68%, clay 16.43%; pH 6.87 in distilled water (1:2.5 ratio); pH 5.96 in 0.01M $CaCl_2$ (1:2.5 ratio); 0.76% organic carbon] was collected from the 0-25 cm depth of the Bank of Par River in Nani Vahiyal village in Dharampur Tehsil, Valsad, Gujarat, India, and characterized in JRF Study No. 609-3-15-19504 (p. 11; Appendix 7, p. 56). The USDA soil texture classification was not reported, and it could not be determined by the reviewer using USDA-NRCS technical support tools since the following particle distribution, sand 70.17%, silt 3.68%, clay 16.43%, did not total 100%.

2 Two ion transitions were monitored for each analyte as follows (quantitative and confirmatory, respectively): *m/z* 241.1→134.1 and *m/z* 241.1→193.2 for mancozeb (as dimethyl-EBDC) and *m/z* 103.4→44.0 and *m/z* 103.4→60.0 for ETU. These were similar to those of the ECM.

3 Standard deviations were reviewer-calculated since they were reported in units of concentration, not % applied. Rules of significant figures were followed.

4 RSD values were reported from the study report. Reviewer-generated RSDs varied from those reported in the study report; differences were assumed to be due to calculation methods.

III. Method Characteristics

In the ECM and ILV, LOQ in soil was 0.05 mg/kg for mancozeb and ETU (pp. 25-27 of MRID 50452902; Tables 5-8, pp. 25-28 of MRID 50774407). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated (Appendix VIII, pp. 129-130 of MRID 50452902). Generally, for accurate quantitation, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No LOQ calculations or justifications were reported ILV. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. The LOD was also calculated from the data of the seven LOQ recovery samples, as described in "Assigning Values to Non-detected/Non-quantified Pesticide Residues in Human Health Food Exposure Assessments, Item 6047, U.S. EPA, March 23, 2000" (p. 14). The following equations were used (pp. 24-26):

$$LOD = Stdev(LOQ\ R1: LOQ\ R7) \times t_{0.99}$$

The standard deviation is calculated using the following equation:

$$Stdev(LOQ\ R1: LOQ\ R7) = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

Where, Stdev is the sample standard deviation of the calculated concentrations of the seven LOQ samples; n is number of samples, and x is the average calculated concentration; and $t_{0.99}$ is the one-tailed t-statistic at the 99% confidence level for n-1 replicates and is equal to 3.143 for n=7 samples.

The calculated LODs were 0.0120 µg/g and 0.0187 µg/g for mancozeb and ETU, respectively. (pp. 25-27 of MRID 50452902). The LOD was not reported in the ILV.

Table 4. Method Characteristics for Mancozeb and its Metabolite ETU in Soil

Analyte		Mancozeb (as dimethyl-EBDC)	ETU
Limit of Quantitation (LOQ)		0.05 mg/kg	
Limit of Detection (LOD)	ECM (calc)	0.0120 µg/g	0.0187 µg/g
	ILV	**Not reported**	
Linearity (calibration curve r and concentration range)	ECM	r = 0.99533712858 (Q) r = 0.99502491816 (C)	r = 0.99571780866 (Q) r = 0.99562212221 (C)
		1-10 ng/mL	
	ILV	r = 0.9990 (Q) r = 0.9994 (C)	r = **0.9905** (Q) r = **0.9902** (C)
		1.008-10.085 ng/mL	1.010-10.100 ng/mL
Repeatable	ECM[1]	Yes at LOQ and 10×LOQ (one characterized soil matrix)	
	ILV[2,3]	Yes at LOQ and 10×LOQ (one uncharacterized soil matrix)	
Reproducible		Yes at LOQ and 10×LOQ	
Specific	ECM	Yes, matrix interferences were <5% of the LOQ (based on peak area).	Yes, matrix interferences were <10% of the LOQ (based on peak area). A minor contaminant (RT *ca.* 0.67) shouldered the LOQ analyte peak and inferred with consistent analyte integration.
	ILV	**Could not be determined.**[4] Matrix interferences appeared insignificant, and analyte peaks were well-defined; however, only one representative chromatogram without a fortification label was provided for each analyte.	

Data were obtained from pp. 24-26 (LOQ/LOD); Appendix I, Tables 1-5, pp. 30-34 (recovery results); Appendix II, Figure 1, p. 41; Appendix II, Figure 21, p. 61 (calibration coefficients); Appendix II, Figures 2-40, pp. 42-80; (chromatograms) of MRID 50452902; Tables 5-8, pp. 25-28 (LOQ and recovery results); p. 7; Tables 1-4, pp. 21-24 (calibration coefficients); Appendix I, pp. 29-37 (chromatograms) of MRID 50774407; DER Attachment 2.

1 In the ECM, the loamy sand soil {Sample ID: NY-OC[…]3-6"; sand 86%, silt 9%, clay 5%; pH 6 in soil:water (1:1 ratio); 2.0% organic matter Walkley Black} was used (USDA soil texture classification; Table 11, p. 40; Appendix IX, p. 185 of MRID 50452902). The soil was characterized by Agvise Laboratories, Northwood, North Dakota; the soil source was not reported but appeared to be located in New York. The soil was reportedly selected to representative of a typical soil and collected according to guideline requirements (p. 16). Further information of the soil was kept with the raw data.

2 In the ILV, the soil [coarse sand 35.06%, fine sand 35.11%, silt 3.68%, clay 16.43%; pH 6.87 in distilled water (1:2.5 ratio); pH 5.96 in 0.01M $CaCl_2$ (1:2.5 ratio); 0.76% organic carbon] was collected from the 0-25 cm depth of the Bank of Par River in Nani Vahiyal village in Dharampur Tehsil, Valsad, Gujarat, India, and characterized in JRF Study No. 609-3-15-19504 (p. 11; Appendix 7, p. 56 of MRID 50774407). The USDA soil texture classification was not reported, and it could not be determined by the reviewer using USDA-NRCS technical support tools since the following particle distribution, sand 70.17%, silt 3.68%, clay 16.43%, did not total 100%.

3 The number of ILV trials required to validate the method was not reported; however, the reviewer assumed that the method was validated for soil in the first trial with insignificant modifications to the analytical instrumentation and parameters (pp. 7, 12-17 of MRID 50774407).

4 Based on Appendix I, pp. 32-33, 37 of MRID 50774407.

Linearity is satisfactory when r ≥0.995 [updated DER acceptance criteria (11/2019); Linearity criterion is consistent with Superfund analytical methods for inorganic analytes (National Functional Guidelines for Inorganic Superfund Methods Data Review, EPA-540-R-2017-001, January 2017. **https://www.epa.gov/sites/production/files/201701/documents/national_functional_guidelines_for_inorganic_superfund_methods_data_review_01302017.pdf**)].

IV. Method Deficiencies and Reviewer's Comments

1. ILV linearity (quantitation ion analysis) was not satisfactory for ETU (r = 0.9905; p. 7 of MRID 50774407). Linearity is satisfactory when r ≥0.995 [Linearity criterion is consistent with Superfund analytical methods for inorganic analytes (National Functional Guidelines for Inorganic Superfund Methods Data Review, EPA-540-R-2017-001, January 2017); updated DER acceptance criteria as of 11/2019]. The linearity of the confirmation analysis calibration curve was not satisfactory for ETU; however, deficiencies in the confirmation analysis do not affect the validity of the method since a confirmation method is not usually required when LC/MS or GC/MS is the primary method to generate study data.

2. The specificity of the method was not supported for mancozeb and ETU based on ILV representative chromatograms. Matrix interferences appeared insignificant, and analyte peaks were well-defined; however, only one representative chromatogram without a fortification label was provided for each analyte (Appendix I, pp. 32-33, 37 of MRID 50774407). The representative chromatograms consisted of a quantitation and confirmation chromatogram of "extracted" analyte for each analyte.

3. The ILV soil matrix was inadequately characterized. USDA soil texture classification was not reported, and it could not be determined by the reviewer using USDA-NRCS technical support tools since the following particle distribution, sand 70.17%, silt 3.68%, clay 16.43%, did not total 100% (p. 11; Appendix 7, p. 56 of MRID 50774407). It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method since only one uncharacterized soil matrix was tested. 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. Even though a certain number of soil matrices is not specified in the OCSPP guidelines, more than one soil/soil matrix would need to be included in an ILV in order to cover the range of soils used in the terrestrial field dissipation studies. Additionally, it could not be determined if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies since no mancozeb terrestrial field dissipation studies

4. The communications between the ILV (Jai Research Foundation, Gujarat, India) and ECM (JRF America, Inc.) were not reported, summarized, or detailed in the ILV (pp. 7-8 of MRID 50774407). Communication should be provided to demonstrate that no collusion occurred between the ECM and ILV. The reviewer also believed that communications between the Study Director and Sponsor Monitor during validation should have been provided since both laboratories were part of Jai Research Foundation (JRF).

5. The number of ILV trials required to validate the method was not reported; however, the reviewer assumed that the method was validated for soil in the first trial with insignificant modifications to the LC/MS/MS instrument and monitored MS transitions (pp. 7, 12-17

of MRID 50774407).

6. The reviewer noted that the analytical method (Budgeon, Jr., A.D. and A. Li. 2017. Analytical Method for the Determination of Mancozeb and ETU in Soil. JRFA Method No.: AU-275R0. Report prepared by JRF America, Inc., Audubon, Pennsylvania; and sponsored by Mancozeb Task Force, c/o McDermott, Will and Emery, Washington, D.C.; 75 pages. Final report issued August 28, 2017) provided in Appendix VIII, pp. 110-184, of the ECM MRID 50452902 contained the same recovery data as the main ECM report. Some additional justifications for the LOQ and LOD were found in this second ECM.

7. The ECM reported that mancozeb is a very unstable compound; when ionized by an ion-spray in high voltage, it provides poor reproducibility (p. 14 of MRID 50452902). Hence, it is required to transform the analyte into its methylated form, dimethyl ethylene bisdithiocarbamate (EBDC), for improved solubility, stability, and instrument sensitivity.

8. In the ECM, the mancozeb LOQ final soil extracts were not stable after *ca*. one week of storage; the mancozeb 10×LOQ final soil extracts were fairly stable (storage conditions were not reported; pp. 20-21, 27; Appendix I, Tables 6-9, pp. 35-38 of MRID 50452902). The ETU final soil extracts were stable after *ca*. one week of storage. Similar stability was determined for the stock and fortification solutions. Dimethyl-EBDC and ETU stock solutions were considered stable for up to 6 months in a refrigerator (*ca*. 4°C).

9. Matrix effects were studied in the ECM and determined to be insignificant (<20%) for both analytes; neat solvent standards were used (pp. 20, 27; Appendix I, Table 10, p. 39 of MRID 50452902).

10. The estimations of the LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 25-27 of MRID 50452902; Tables 5-8, pp. 25-28 of MRID 50774407). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated (Appendix VIII, pp. 129-130 of MRID 50452902). Generally, for accurate quantitation, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. No LOQ calculations or justifications were reported ILV. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. The LOD was also calculated from the data of the seven LOQ recovery samples, as described in "Assigning Values to Non-detected/Non-quantified Pesticide Residues in Human Health Food Exposure Assessments, Item 6047, U.S. EPA, March 23, 2000" (p. 14). The following equations were used (pp. 24-26): LOD = Stdev(LOQ R1: LOQ R7) × $t_{0.99}$. See above for equation definitions. The LOD was not reported in the ILV. No calculations were reported to justify the LOQ for the method in the ECM and ILV. Detection limits should not be based on arbitrary values.

11. The time required to complete the method for a validation set of 15 samples was reported as 8 working hours for one chemist in the ECM (Appendix VIII, p. 122 of MRID

50452901). This included the instrument analysis and data processing. The time required to complete the method for a validation set was not reported in the ILV.

V. References

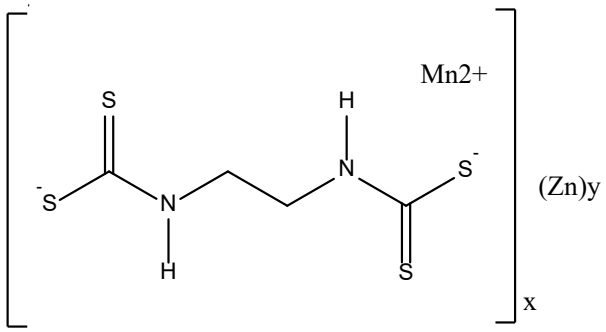
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Attachment 1: Chemical Names and Structures

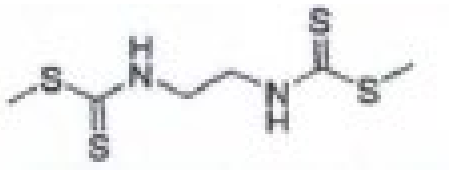
Mancozeb

IUPAC Name: Zinc Manganese ethylenebis(dithiocarbamate)
CAS Name: [[2-[(Dithiocarboxy)amino]ethyl]carbamodithioato(2−)-κ*S*,κ*S*′]manganese mixture with [[2-[(dithiocarboxy)amino]ethyl]carbamodithioato(2−)-κ*S*,κ*S*′]zinc
CAS Number: 8018-01-7
SMILES String: C(CNC(=S)[S-])NC(=S)[S-].C(CNC(=S)[S-])NC(=S)[S-].[Mn+2].[Zn+2]



Dimethyl-EBDC

IUPAC Name: Dimethyl Ethylenebisdithiocarbamate
CAS Name: Not reported
CAS Number: 20721-48-6
SMILES String: Not found



ETU (Ethylenethiourea)

IUPAC Name:	2-Imidazolidinethione
CAS Name:	Not reported
CAS Number:	96-45-7
SMILES String:	S=C1N([H])CCN1[H]