Test Material:	Malathion
MRID:	48800202
Title:	Validation of the Residue Analytical Method: "Determination of Malathion and Malaoxon in Soil by LC-MS/MS"
MRID:	48800204
Title:	Independent Laboratory Validation of the Analytical Method for Malathion and Malaoxon in Soil by LC-MS/MS
EPA PC Code:	057701
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 6/30/16

Secondary Reviewer: Kathleen Ferguson **Signature:** 

Date: 6/30/16

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 6/30/16

Analytical method for malathion and malaoxon in soil

Reports:	ECM: EPA MRID No.: 48800202. Brown, S. 2011. Validation of the Residue Analytical Method: "Determination of Malathion and Malaoxon in Soil by LC-MS/MS". Study No.: 66797. Report prepared by Morse Laboratories, LLC, Sacramento, California, sponsored by Cheminova A/S, Lemvig, Denmark, and submitted by Cheminova, Inc., Arlington, Virginia; 169 pages. Final report issued July 12, 2011. ILV: EPA MRID No. 48800204. Cremin, P. 2012. Independent Laboratory Validation of the Analytical Method for Malathion and Malaoxon in Soil by LC-MS/MS. PTRL Study No.: 2220W. Report prepared by PTRL West, Inc., Hercules, California, sponsored by Cheminova A/S, Lemvig, Denmark, and submitted by Cheminova, Inc., Arlington, Virginia; 97 pages. Final report issued January 12, 2012.
Document No.:	MRIDs 48800202 & 48800204
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practices (GLP; p. 3 of MRID 48800202). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5). ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 48800204). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).
Classification:	This analytical method is classified as unacceptable. In the ECM, no samples were prepared at 10×LOQ. ECM and ILV representative chromatograms showed interferences at the LOQ due to contaminants or baseline noise. ECM and ILV procedural recoveries were corrected for residues in the controls.
PC Code:	057701
Reviewer:	Andrew Shelby, Physical Scientist **Signature:** **Date:** August 1, 2016

All page numbers refer to those listed in the upper-most right-hand corner of the MRIDs.

Executive Summary

The analytical method, Morse Laboratories, LLC Analytical Method #Meth-207, Revision #1, is designed for the quantitative determination of malathion and malaoxon in soil matrices at the LOQ of 0.01 ppm using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil for both analytes. Characterized sandy loam and sandy clay loam soil matrices were used in the ECM; characterized sandy clay loam soil matrix was used in the ILV. Three parent-daughter ion transitions were monitored per analyte; all three ion transitions were quantified in the ILV, but only the quantitative ion transition was quantified in the ECM. ILV study report did not specify the number of trials performed to validate the method; the reviewer assumed that the method was validated in the first trial with minor modifications to the analytical instrumentation/equipment. ILV representative chromatograms of malathion showed significant interferences at the LOQ (*ca*. 36-47% of the LOQ); for malaoxon, baseline noise interfered with peak integration at the LOQ. ECM representative chromatograms of malathion showed notable interferences at the LOQ (*ca*. 2-23% of the LOQ). ECM and ILV procedural recoveries were corrected for residues quantified in the controls. In the ECM, no samples were prepared at 10×LOQ. The LOD for both analytes differed in the ECM (0.003 ppm) and in the ILV (0.005 ppm).

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						
Malathion	48800202	48800204		Soil[1,2]	12/07/2011[3]	Cheminova, Inc.	LC/MS/MS	0.01 ppm
Malaoxon					16/06/2011[4]			

1 For the ECM, characterized sandy loam soil (Sample ID 1810W-029; 75% sand, 14% silt, 11% clay; 0.6% organic matter; pH 7.5) from Fresno, California, and sandy clay loam soil (Sample ID 1810W-033; 63% sand, 16% silt, 21% clay; 3.4% organic matter; pH 6.8) from Northwood, North Dakota, were used in the study (pH data based on 1:1, soil:water; USDA soil texture classifications; pp. 23-24; Appendix IV, pp. 168-169 of MRID 48800202).

2 For the ILV, characterized sandy clay loam soil (Sample ID MSL-PF 4-8"; 64% sand, 14% silt, 22% clay; 4.3% organic matter; pH 6.9) from Northwood, North Dakota, was used in the study (pH data based on 1:1, soil:water; USDA soil texture classification; p. 10; Appendix C, p. 82 of MRID 48800204).

3 From MRID 48800202.

4 From Morse Laboratories, LLC Analytical Method #Meth-207, Revision #1 contained in Appendix I of MRID 48800202.

I. Principle of the Method

Samples (10.0 g) of soil in 250-mL HDPE centrifuge bottles were fortified, as necessary (pp. 21-22; Appendix I, pp. 120-122; Appendix I, Appendix I, p. 127; Appendix I, Appendix II, p. 129 of MRID 48800202). The sample was extracted three times with 100 mL of acetonitrile via shaking on a platform shaker at medium speed (*ca*. 180 excursions per minute) for 30 minutes. After centrifugation (*ca*. 3000 rpm for *ca*. 10 minutes), the extract was decanted into a 500-mL glass graduated mixing cylinder. The volume of the combined extracts was adjusted to 500 mL with acetonitrile and thoroughly mixed. The sample was transferred to a *ca*. 30- to 50-mL graduated polypropylene centrifuge tube with a cap (centrifuge, if necessary, to mix well). An aliquot (1.0 mL) was transferred to a fresh 15-mL graduated polypropylene centrifuge tube with a cap containing 9.0 mL of 0.088% formic acid in DI water. This 1-to-10 diluted sample was taken for additional clean-up, while the concentrated stock sample was stored at 1-8°C if needed for reanalysis. The 1-to-10 diluted sample was purified using solid phase extraction (SPE) procedure (Oasis® HLB SPE cartridge, size 3 cc, 60 mg). The SPE column was pre-conditioned with methanol then deionized soil (2 mL each); the column was not allowed to go dry between conditioning solvents, as well as the sample. All cartridge elutions were stopped when the solvent reached the top of the frit unless noted otherwise. The sample was applied to the column. The sample centrifuge tube was rinsed with 1 mL of 5% methanol in deionized soil which was applied to the column. The column was washed with 1 mL of ammonium hydroxide:5% methanol in deionized soil (2:98, v:v) then 1 mL of acetic acid:5% methanol in deionized soil (2:98, v:v). The analytes were eluted with 2.0 mL of HPLC methanol, allowing the cartridge to dry under vacuum after elution. The eluate was concentrated to 1.0 mL using an N-Evap with a water bath set to 35°C. The residue was brought to a final volume of 2.0 mL with 0.088% formic acid in HPLC water. The final extracts were analyzed by liquid chromatography using positive-ion electrospray ionization (ESI) with tandem mass spectrometry. The method noted that the SPE columns must be profiled in the presence of matrix and optimized if necessary.

Samples were analyzed for malathion and malaoxon using an Applied Biosystems/Sciex API 4000 LC/MS/MS with ACQUITY UPLC system (Appendix I, pp. 122-123 of MRID 48800202). The instrumental conditions consisted of a Phenomenex Luna column C18(2)-HST (2.0 x 100 mm, 2.5-µm; column temperature, 40°C), a mobile phase gradient of (A) HPLC soil containing 0.1% formic acid and (B) 100% HPLC acetonitrile [percent A:B (v:v) at 0.0-0.5 min. 75:25, 5.0-7.0 min. 5:95, 7.1-10 min. 75:25], MS/MS detection in positive ionization mode (MRM; temperature, 350°C), and injection volume 10 µL. Three parent-daughter ion transitions were monitored per analyte (quantitation, confirmation 1 and confirmation 2, respectively): *m/z* 331 → 285, *m/z* 331 → 127 and *m/z* 331 → 99 for malathion and *m/z* 315 → 127, *m/z* 315 → 143 and *m/z* 315 → 99 for malaoxon. Retention times were reported as *ca*. 5.75 and 4.30 min. for malathion and malaoxon, respectively.

ILV

In the ILV, the ECM was performed exactly as written, except for three modifications of LC/MS/MS conditions: an Agilent 1100 LC equipped with a Phenomenex Synergi Fusion RP, 100A (100 mm x 2.0 mm I.D.) plus a 4 x 2 mm Phenomenex Fusion security guard pre column cartridge was used, and the mobile phase gradient was modified to percent A:B (v:v) at 0.0-0.5

min. 75:25, 5.0-9.0 min. 5:95, 9.5 -13 min. 75:25 (pp. 14-18 of MRID 48800204). Three parent-daughter ion transitions were monitored per analyte (quantitation, confirmation 1 and confirmation 2, respectively): *m/z* 331.1→ 285.3, *m/z* 331.1 → 127.0 and *m/z* 331.1 → 99.1 for malathion and *m/z* 315.2 → 126.9, *m/z* 315.2 → 99.1 and *m/z* 315.2 → 143.1 for malaoxon (C1 and C2 were switched from that of the ECM). Retention times were reported as 8.6 and 7.1 min. for malathion and malaoxon, respectively.

LOQ/LOD

The LOQ for both analytes was 0.01 ppm in the ECM and ILV (pp. 21, 33 of MRID 48800202; p. 21 of MRID 48800204). The LOD for both analytes was reported as 0.003 ppm in the ECM and 0.005 ppm in the ILV.

II. Recovery Findings

ECM (MRID 48800202): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of malathion and malaoxon in soil matrices at fortification levels of 0.01 mg/kg (LOQ; 0.01 ppm) and 1.0 mg/kg (100×LOQ); quantitative HPLC analysis only; Tables 1-4, pp. 42-45). No samples were prepared at 10×LOQ. The confirmation transitions 1 and 2 were monitored, but only peak areas were provided as results (Tables 5a-8c, pp. 46-63). Percent recoveries were not reported by the study author; no calibration curve was provided for confirmation ion transitions. The ratios of the peak areas of the ion transitions were used to confirm the quantitation ion transition results. Recovery results were corrected for residues found in the controls; residues in the controls measured 0.000478-0.00244 ppm for malathion and 0-0.000308 ppm for malaoxon (pp. 26-29; Tables 1-4, pp. 42-45). The soil matrices were well characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classifications; pp. 23-24; Appendix IV, pp. 168-169). Sandy loam soil (Sample ID 1810W-029; 75% sand, 14% silt, 11% clay; 0.6% organic matter; pH 7.5) from Fresno, California, and sandy clay loam soil (Sample ID 1810W-033; 63% sand, 16% silt, 21% clay; 3.4% organic matter; pH 6.8) from Northwood, North Dakota, were used in the study (pH data based on 1:1, soil:water).

ILV (MRID 48800204): Mean recoveries and RSDs were within guidelines for analysis of malathion and malaoxon in soil matrices at fortification levels of 0.01 mg/kg (LOQ; 0.01 ppm) and 0.1 (10×LOQ; quantitative and confirmation 1 and 2 HPLC analyses; Tables 1-2, pp. 25-26). Performance data (recovery results) of the quantitative HPLC analysis and confirmation 1 and 2 HPLC analysis were comparable. Procedural recoveries of soil samples fortified with malathion were corrected for residues quantified in the controls (*ca*. 30% of the LOQ; <LOD, < 0.005 ppm; pp. 19-20, 22; Appendix D, pp. 84-86, 90-91). No residues were quantified or observed in the control samples for malaoxon. The soil matrix was well characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 10; Appendix C, p. 82). Sandy clay loam soil (Sample ID MSL-PF 4-8"; 64% sand, 14% silt, 22% clay; 4.3% organic matter; pH 6.9) from Northwood, North Dakota, was used in the study (pH data based on 1:1, soil:water). The ILV study report did not specify the number of trials performed to validate the method; the reviewer assumed that the method was validated in the first trial with minor modifications (pp. 10, 23).

Table 2. Initial Validation Method Recoveries for Malathion and Malaoxon in Soil[1,2]

Analyte	Fortification Level (mg/kg or ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
	Quantitation transition					
Malathion	0.01 (LOQ)	5	89-98	94	3.6	3.8
	1.0	5	89-93	91	1.8	2.0
Malaoxon	0.01 (LOQ)	5	94-97	95	1.1	1.2
	1.0	5	84-90	87	2.8	3.2
	Confirmation transitions 1 and 2					
Malathion	0.01 (LOQ)	5	Not reported[3]			
	1.0	5				
Malaoxon	0.01 (LOQ)	5				
	1.0	5				
Sandy Clay Loam Soil						
	Quantitation transition					
Malathion	0.01 (LOQ)	5	108-119	114	4.5	3.9
	1.0	5	76-95	87	7.0	8.0
Malaoxon	0.01 (LOQ)	5	94-110	101	6.6	6.6
	1.0	5	79-97	90	6.9	7.6
	Confirmation transitions 1 and 2					
Malathion	0.01 (LOQ)	5	Not reported[3]			
	1.0	5				
Malaoxon	0.01 (LOQ)	5				
	1.0	5				

Data (procedural recoveries were corrected for residues quantified in the controls; pp. 26-29; Tables 1-4, pp. 42-45) were obtained from Tables 1-4, pp. 42-45 of MRID 48800202.

1 The soil matrices were well characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classifications; pp. 23-24; Appendix IV, pp. 168-169). Sandy loam soil (Sample ID 1810W-029; 75% sand, 14% silt, 11% clay; 0.6% organic matter; pH 7.5) from Fresno, California, and sandy clay loam soil (Sample ID 1810W-033; 63% sand, 16% silt, 21% clay; 3.4% organic matter; pH 6.8) from Northwood, North Dakota, were used in the study (pH data based on 1:1, soil:water).

2 Three parent-daughter ion transitions were monitored per analyte (quantitation, confirmation 1 and confirmation 2, respectively): *m/z* 331 → 285, *m/z* 331 → 127 and *m/z* 331 → 99 for malathion and *m/z* 315 → 127, *m/z* 315 → 143 and *m/z* 315 → 99 for malaoxon (Appendix I, p. 123).

3 The confirmation transitions 1 and 2 were monitored, but only peak areas were provided as results (Tables 5a-8c, pp. 46-63). Percent recoveries were not reported by the study author; no calibration curve was provided for confirmation ion transitions. The ratios of the peak areas of the ion transitions were used to confirm the quantitation ion transition results.

Table 3. Independent Validation Method Recoveries for Malathion and Malaoxon in Soil[1,2]

Analyte	Fortification Level (mg/kg or ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil						
	Quantitation transition					
Malathion	0.01 (LOQ)	5	84-89	87	2.1	2.4
	0.1	5	73-80	75	4.2	5.6
Malaoxon	0.01 (LOQ)	5	96-105	100	3.6	3.6
	0.1	5	91-96	93	1.8	1.9
	Confirmation transition 1					
Malathion	0.01 (LOQ)	5	83-93	87	3.9	4.5
	0.1	5	73-79	76	2.4	3.2
Malaoxon	0.01 (LOQ)	5	94-102	99	3.1	3.1
	0.1	5	89-94	91	2.1	2.3
	Confirmation transition 2					
Malathion	0.01 (LOQ)	5	80-98	88	6.8	7.7
	0.1	5	73-79	76	2.8	3.7
Malaoxon	0.01 (LOQ)	5	88-103	94	6.4	6.8
	0.1	5	90-94	91	1.7	1.9

Data were obtained from Tables 1-2, pp. 25-26 of MRID 48800204. Procedural recoveries of malathion in soil were corrected for residues found in the controls (pp. 19-20; Appendix D, pp. 84-86, 90-91); recoveries of malaoxon were not corrected since no residues were quantified in the controls.

1 The soil matrix was well characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification; p. 10; Appendix C, p. 82). Sandy clay loam soil (Sample ID MSL-PF 4-8"; 64% sand, 14% silt, 22% clay; 4.3% organic matter; pH 6.9) from Northwood, North Dakota, was used in the study (pH data based on 1:1, soil:water).

2 Three parent-daughter ion transitions were monitored per analyte (quantitation, confirmation 1 and confirmation 2, respectively): *m/z* 331.1→ 285.3, *m/z* 331.1 → 127.0 and *m/z* 331.1 → 99.1 for malathion and *m/z* 315.2 → 126.9, *m/z* 315.2 → 99.1 and *m/z* 315.2 → 143.1 for malaoxon (C1 and C2 were switched from that of the ECM; p. 18).

III. Method Characteristics

In the ECM and ILV, the LOQ for both analytes was 0.01 ppm (pp. 21, 33 of MRID 48800202; p. 21 of MRID 48800204). The LOQ was defined as the lowest level of fortification which was validated by the method, i.e. demonstrated to have acceptable recovery and precision, in the ECM and ILV. The LOD for both analytes was reported as 0.003 ppm in the ECM and 0.005 ppm in the ILV. In the ECM, the LOD was defined as 1/3 of the LOQ; in the ILV, the LOD was defined as the concentration of the lowest linearity calibrant injected, 0.05 ng/mL malathion and malaoxon.

Table 4. Method Characteristics

		Malathion	Malaoxon
Limit of Quantitation (LOQ)	ECM	0.01 mg/kg (0.01 ppm)	
	ILV		
Limit of Detection (LOD)	ECM	0.003 ppm	
	ILV	0.005 ppm	
Linearity (Least squares calibration curve r and concentration range)	ECM[1,2]	r^2 = 0.9990 (Q)	r^2 = 0.9992 (Q)
		0.05-2.5 ng/mL	
	ILV[3]	r^2 = 0.999557495671 (Q) r^2 = 0.999750801285 (C1) r^2 = 0.999079844185 (C2)	r^2 = 0.999214065534 (Q) r^2 = 0.999494789891 (C1) r^2 = 0.998793410532 (C2)
		0.05-2.5 ng/mL	
Repeatable	ECM[4]	Yes at LOQ and 100×LOQ (n = 5). No samples were prepared at 10×LOQ.	
	ILV[5]	Yes at LOQ and 10×LOQ (n = 5).	
Reproducible		Yes at the LOQ and 10×LOQ.	
Specific	ECM	Residues (*ca*. 2-23% of the LOQ) were noted in the matrix control in Q, C1 and C2 chromatograms.[6] In C2 chromatograms, the analyte peak was small and not distinct compared to the baseline noise and nearby contaminate peaks at LOQ.	Yes, matrix interferences were <10% of the LOQ in the matrix control.
	ILV	Significant matrix interferences were noted in the Q, C1 and C2 chromatograms (*ca*. 36-47% of the LOQ).[7]	Yes, no interferences were observed in the matrix control; some baseline noise around the analyte peak interfered with peak integration at the LOQ.

Data were obtained from pp. 21, 33; Tables 1-4, pp. 42-45; Figure 6, p. 73; Figure 11, p. 78; Figures 16-21, pp. 83-88 (quantitative ion transition chromatograms); Figures 27-32, pp. 94-99 (confirmation ion 1 chromatograms); Figures 38-43, pp. 105-110 (confirmation ion 2 chromatograms) of MRID 48800202; pp. 14, 21; Tables 1-2, pp. 25-26; Figures 1-3, pp. 28-30; Figures 10-12, pp. 37-39; Appendix D, pp. 90-97 (soil chromatograms) of MRID 48800204. Q = Quantitative HPLC analysis; C1 = Confirmation 1 HPLC analysis; C2 = Confirmation 2 HPLC analysis.

1 ECM standard curves were weighted 1/x. ECM r^2 values are reviewer-generated for both analytes from reported r values of 0.9995-0.9996 (Q; calculated from data in Figure 6, p. 73; Figure 11, p. 78 of MRID 48800202; see DER Attachment 2).

2 The confirmation transitions 1 and 2 were monitored, but only peak areas were provided as results (Tables 5a-8c, pp. 46-63 of MRID 48800202). Percent recoveries were not reported by the study author; no calibration curve was

provided for confirmation ion transitions. The ratios of the peak areas of the ion transitions were used to confirm the quantitation ion transition results.

3 ILV standard curves were weighted 1/x. ILV r^2 values are reviewer-generated for both analytes from reported r values of 0.999606955525-0.999778723354 (Q) and 0.999396523174-0.999875392879 (C; calculated from data in Figures 1-3, pp. 28-30; Figures 10-12, pp. 37-39 of MRID 48800204; see DER Attachment 2).

4 For the ECM, characterized sandy loam soil (Sample ID 1810W-029; 75% sand, 14% silt, 11% clay; 0.6% organic matter; pH 7.5) from Fresno, California, and sandy clay loam soil (Sample ID 1810W-033; 63% sand, 16% silt, 21% clay; 3.4% organic matter; pH 6.8) from Northwood, North Dakota, were used in the study (pH data based on 1:1, soil:water; USDA soil texture classifications; pp. 23-24; Appendix IV, pp. 168-169 of MRID 48800202).

5 For the ILV, characterized sandy clay loam soil (Sample ID MSL-PF 4-8"; 64% sand, 14% silt, 22% clay; 4.3% organic matter; pH 6.9) from Northwood, North Dakota, was used in the study (pH data based on 1:1, soil:water; USDA soil texture classification; p. 10; Appendix C, p. 82 of MRID 48800204).

6 Reviewer-calculated from peak areas reported in Figures 16-17, pp. 83-84; Figures 27-28, pp. 94-95; Figures 38-39, pp. 105-106 of MRID 48800202.

7 Reviewer-calculated from peak areas reported in Appendix D, pp. 90-92 of MRID 48800204.

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM, no samples were prepared at 10×LOQ. OSCPP guidelines recommend a minimum of five samples spiked at each fortification level (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

2. In ILV chromatograms of malathion, significant matrix interferences were observed in the controls for all three monitored ions of malathion in the sandy clay loam soil matrix (*ca*. 36-47 of the LOQ; reviewer-calculated based on peak areas reported in the chromatograms; Appendix D, pp. 90-92 of MRID 48800204). The ILV LOD (0.005 ppm) was 50% of the LOQ, so none of these residues appeared to be >LOD based on the ILV LOD; however, OCSPP guidelines prefer for interferences with the peak areas to be less than 50% at the LOD. Since 50% of the LOD was equivalent to 25% of the LOQ, the residues quantified in the control samples of malathion were >50% of the LOD.

   In the ECM chromatograms of malathion, matrix interferences were observed in the controls for all three monitored ions of malathion in the sandy loam soil matrix (*ca*. 2-23% of the LOQ) and sandy clay loam soil matrix (*ca*. 11-13% of the LOQ; reviewer-calculated based on peak areas reported in the chromatograms; Figures 16-17, pp. 83-84; Figures 27-28, pp. 94-95; Figures 38-39, pp. 105-106 of MRID 48800202). The ECM LOD (0.003 ppm) was 30% of the LOQ, so none of these residues appeared to be >LOD based on the ILV LOD; however, OCSPP guidelines prefer for interferences with the peak areas to be less than 50% at the LOD. Since 50% of the LOD was equivalent to 15% of the LOQ, the quantitative ion analysis for the control samples of malathion in sandy loam soil were >50% of the LOD (*ca*. 23%).

   In ILV chromatograms of malaoxon, baseline noise interfered with peak integration at the LOQ (Appendix D, pp. 94-95 of MRID 48800204). No residues were quantified or observed in the ILV control samples for malaoxon.

3. In the ECM and ILV, procedural recoveries were corrected for residues quantified in the

controls (pp. 26-29; Tables 1-4, pp. 42-45 of MRID 48800202; pp. 19-20, 22; Appendix D, pp. 84-86, 90-91 of MRID 48800204). In the ECM, residues in the controls measured 0.000478-0.00244 ppm for malathion and 0-0.000308 ppm for malaoxon. In the ILV, only recoveries of soil samples fortified with malathion were corrected for residues quantified in the controls (*ca*. 30% of the LOQ; <LOD, < 0.005 ppm); no residues were quantified or observed in the control samples for malaoxon.

4. The ILV study report did not specify the number of trials performed to validate the method; the reviewer assumed that the method was validated in the first trial with minor modifications involving the analytical instrumentation/equipment (pp. 10, 23 of MRID 48800204).

5. The estimations of the LOQ and LOD in the ECM were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (0.01 ppm; pp. 21, 33 of MRID 48800202; p. 21 of MRID 48800204). The LOQ was defined as the lowest level of fortification which was validated by the method, i.e. demonstrated to have acceptable recovery and precision, in the ECM and ILV. In the ECM, the LOD was defined as 1/3 of the LOQ; in the ILV, the LOD was defined as the concentration of the lowest linearity calibrant injected, 0.05 ng/mL malathion and malaoxon.

   The LOD for both analytes differed in the ECM (0.003 ppm) and in the ILV (0.005 ppm).

   Additionally, the toxicological level of concern was not reported for the analytes in soil. A LOQ above toxicological levels of concern results in an unacceptable method classification.

6. The ILV reported communications between the ILV and the sponsor were limited to routine study updates and routine project management; no log of communications was provided (p. 23 of MRID 48800204).

7. A reagent blank was not included in the ECM.

8. In the ECM, confirmation transitions 1 and 2 were monitored, but only peak areas were provided as results (Tables 5a-8c, pp. 46-63 of MRID 48800202). Percent recoveries were not reported by the study author; no calibration curve was provided for confirmation ion transitions. The ratios of the peak areas of the ion transitions were used to confirm the quantitation ion transition results. The reviewer noted that confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

9. In the ECM, matrix effects were studied (pp. 33-34; Tables 9-12, pp. 64-67 of MRID 48800202). Matrix effects were insignificant (±15%) for all matrices/analytes.

10. It was reported for the ILV that the analytical procedure for one set of 13 samples required approximately 14 hours for extraction/clean-up (p. 21 of MRID 48800204). The LC/MS/MS required approximately 13 hours. The overall time to complete a set of samples was 2 working days.

V. References

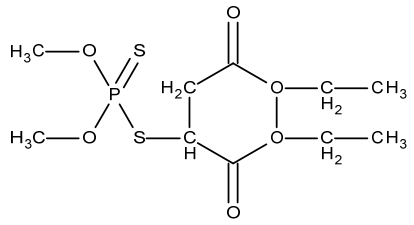
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Malathion

IUPAC Name: Diethyl (dimethoxyphosphinothioylthio)succinate
CAS Name: Diethyl 2-[(dimethoxyphosphinothioyl)thio]butanedioate
CAS Number: 121-75-5
SMILES String: CCOC(=O)CC(SP(=S)(OC)OC)C(=O)OCC



Malaoxon

IUPAC Name: Diethyl 2-dimethoxyphosphorylsulfanylbutanedioate
CAS Name: Butanedioic acid, [(dimethoxyphosphinyl)thio]-diethyl ester
CAS Number: 1634-78-2
SMILES String: CCOC(=O)CC(SP(=O)(OC)OC)C(=O)OCC