Test Material:	Malathion
MRID:	48800201
Title:	Validation of the Residue Analytical Method: "Determination of Malathion and Malaoxon in Water by LC-MS/MS"
MRID:	48800203
Title:	Independent Laboratory Validation of the Analytical Method for Malathion and Malaoxon in Water by LC-MS/MS
EPA PC Code:	057701
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 6/30/15

Secondary Reviewer: Kathleen Ferguson **Signature:** 

Date: 6/30/15

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

Date: 6/30/15

Analytical method for malathion and malaoxon in surface and ground water

Reports:	ECM: EPA MRID No.: 48800201. Brown, S. 2011. Validation of the Residue Analytical Method: "Determination of Malathion and Malaoxon in Water by LC-MS/MS". Study No.: 66799. Report prepared by Morse Laboratories, LLC, Sacramento, California, sponsored by Cheminova A/S, Lemvig, Denmark, and submitted by Cheminova, Inc., Arlington, Virginia; 173 pages. Final report issued July 1, 2011. ILV: EPA MRID No. 48800203. Cremin, P. 2012. Independent Laboratory Validation of the Analytical Method for Malathion and Malaoxon in Water by LC-MS/MS. PTRL Study No.: 2221W. Report prepared by PTRL West, Inc., Hercules, California, sponsored by Cheminova A/S, Lemvig, Denmark, and submitted by Cheminova, Inc., Arlington, Virginia; 119 pages. Final report issued April 6, 2012.
Document No.:	MRIDs 48800201 & 48800203
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practices (GLP; p. 3 of MRID 48800201). 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 48800203). 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. An updated ECM should be provided to incorporate the ILV modification of the extraction procedure to include centrifuging during extraction. In the ECM, no samples were prepared at 10×LOQ. ILV representative chromatograms showed interferences at the LOQ due to contaminants or baseline noise. ILV procedural recoveries were corrected for malathion in surface water due to residues in the controls.
PC Code:	057701
Reviewer:	Andrew Shelby, Physical Scientist **Signature:** **Date:** July 28, 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-206, Revision #1, is designed for the quantitative determination of malaoxon and malathion in ground and surface water matrices at the LOQ of 0.018 µg/L (18 ppt) using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for both analytes. Characterized ground and surface water matrices were used in the ECM. The ground and surface water matrices were not characterized in the ILV; therefore, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. 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 second trial after the incorporation of the centrifugation step (2000 rpm for 5 min.) to completely separate the aqueous and organic phases during extraction. An updated ECM should be provided to incorporate the ILV modification of the extraction procedure since this modification was necessary for the successful validation of the method. ILV representative chromatograms of malathion showed significant interferences at the LOQ (*ca*. 26-45% of the LOQ) in both matrices; for malaoxon, baseline noise interfered with peak integration at the LOQ. ILV procedural recoveries were corrected for malathion in surface water due to residues quantified in the controls. In the ECM, no samples were prepared at 10×LOQ. The LOD for both analytes differed in the ECM (6 ppt) and in the ILV (10 ppt).

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	48800201	48800203		Water[1,2]	01/07/2011[3]	Cheminova, Inc.	LC/MS/MS	0.018 µg/L 18 ppt 18 ng/kg
Malaoxon					16/06/2011[4]			

1 For the ECM, characterized surface water (Sample ID 66799B; pH 7.7, total dissolved solids 14 ppm) and ground water (Sample ID 66799A; pH 7.7, total dissolved solids 330 ppm) were used in the study. The surface water was obtained from the American River near Sunrise Boulevard in Sacramento, California. The well water was obtained from a well from a residence in Sacramento, California (pp. 23-24; Appendix IV, pp. 172-173 of MRID 48800201).

2 For the ILV, uncharacterized surface water (Sample ID 2221W-004A) obtained from Refugio Park Pond, Hercules, California, and ground water (Sample ID 2221W-0005A) obtained from a well from North Gate Road, Walnut Creek, California, were used in the study (pp. 11-12 of MRID 48800203).

3 From MRID 48800201.

4 From Morse Laboratories, LLC Analytical Method #Meth-206, Revision #1 contained in Appendix I of MRID 48800201.

I. Principle of the Method

Samples (250 mL) of water in 500-mL separatory funnels were fortified, as necessary, then mixed with 88 g of sodium chloride (pp. 21-22; Appendix I, pp. 120-123; Appendix I, Appendix I, p. 128; Appendix I, Appendix II, p. 130 of MRID 48800201). The sample was extracted three times with 50 mL of dichloromethane with vigorous shaking for 2 minutes (the separatory funnel should be frequently vented during extraction). After layer separation (*ca*. 5 minutes), the lower dichloromethane layer was drained into a 200-mL Zymark tube through a glass funnel containing *ca*. 20 g of sodium sulfate and a glass wool plug. After all extractions, the aqueous layer was discarded. The sodium sulfate was rinsed with 10 mL of dichloromethane which was added to the extracts. The combined extracts and rinse were reduced to *ca.* 0.2 mL using a Turbo-Vap evaporator set to 40°C then evaporated to dryness manually under nitrogen. The residue was reconstituted in 10 mL of methanol:0.088% formic acid (50:50, v:v) via sonication. The sample was transferred to a 15-mL graduated polypropylene centrifuge tube with a cap. An aliquot (1.0 mL) was transferred to another 15-mL graduated polypropylene centrifuge tube with a cap containing 4.0 mL of HPLC methanol:0.088% formic acid (50:50, v:v). This 1-to-5 diluted sample was taken for HPLC analysis, while the concentrated stock sample was stored at 1-8°C if needed for reanalysis or additional clean-up. If additional clean-up was required, an aliquot (1.0 mL) of the concentrated stock sample was transferred to a fresh 15-mL graduated polypropylene centrifuge tube containing 9.0 mL of 0.088% formic acid in de-ionized water. This 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 water (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 water which was applied to the column. The column was washed with 1 mL of ammonium hydroxide:5% methanol in deionized water (2:98, v:v) then 1 mL of acetic acid:5% methanol in deionized water (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 mixed with 0.5 mL HPLC methanol, and the final volume was adjusted to 5.0 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. 123-124 of MRID 48800201). 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 water 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 one modification of the extraction procedure to include centrifuging (2000 rpm for 5 min.) to completely separate the aqueous and organic phases during extraction, and 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. 16-19 of MRID 48800203). 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 0.018 µg/L (18 ppt) in the ECM and ILV (pp. 19, 21, 33 of MRID 48800201; p. 22 of MRID 48800203). The LOD for both analytes was reported as 0.006 µg/L (6 ppt) in the ECM and 10 ppt in the ILV.

II. Recovery Findings

ECM (MRID 48800201): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of malathion and malaoxon in surface and ground water matrices at fortification levels of 0.018 µg/L (LOQ; 18 ppt) and 18,000 µg/L (1000000×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. Calculations allowed for recovery results to be corrected for residues found in the controls; however, no residues were observed or quantified in the controls (pp. 20, 28-29; Figure 16, p. 83; Figure 19, p. 86). The water matrices were well characterized by Agvise Laboratories, Northwood, North Dakota (pp. 23-24; Appendix IV, pp. 172-173). Surface water (Sample ID 66799B; pH 7.7, total dissolved solids 14 ppm) and ground water (Sample ID 66799A; pH 7.7, total dissolved solids 330 ppm) were used in the study. The surface water was obtained from the American River near Sunrise Boulevard in Sacramento, California. The well water was obtained from a well from a residence in Sacramento, California.

ILV (MRID 48800203): Mean recoveries and RSDs were within guidelines for analysis of malathion and malaoxon in surface and ground water matrices at fortification levels of 0.018 µg/L (LOQ; 18 ppt) and 180 (10×LOQ; quantitative and confirmation 1 and 2 HPLC analyses; Tables 2-5, pp. 29-32). Performance data (recovery results) of the quantitative HPLC analysis and confirmation 1 and 2 HPLC analysis were comparable, except for those of the confirmation

transition 2 results in surface water which were slightly lower than those of the quantitative and confirmation 1 transitions. Procedural recoveries of surface water samples fortified with malathion were corrected for residues quantified in the controls [pp. 20-22; Appendix C, pp. 88-90, 95-97; Appendix D, pp. 103-105, 110-112; Appendix C, pp. 91-94, 98-101 (ground water chromatograms); Appendix D, pp. 106-109, 113-116 (surface water chromatograms)]. Residues were only quantified in one of the control samples of the ground water for malathion (<LOD, confirmation 2 ion only); residues were quantified in both of the controls samples of the surface water for all three monitored ions of malathion (7.52-9.52 ppt). No residues were quantified or observed in the control samples for malaoxon. The water matrices were not characterized (pp. 11-12). Surface water (Sample ID 2221W-004A) obtained from Refugio Park Pond, Hercules, California, and ground water (Sample ID 2221W-0005A) obtained from a well from North Gate Road, Walnut Creek, California, were used in the study. 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 second trial after the incorporation of the centrifugation step to completely separate phases after extraction (pp. 23, 25-26; Appendix E, pp. 117-119).

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

Analyte	Fortification Level (µg/L or ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface (River) Water						
	Quantitation transition					
Malathion	0.018 (LOQ)	5	88-95	91	2.5	2.8
	18,000	5	71-76	73	1.8	2.5
Malaoxon	0.018 (LOQ)	5	88-93	90	2.6	2.9
	18,000	5	97-105	101	2.9	2.9
	Confirmation transitions 1 and 2					
Malathion	0.018 (LOQ)	5	Not reported[3]			
	18,000	5				
Malaoxon	0.018 (LOQ)	5				
	18,000	5				
Ground (Well) Water						
	Quantitation transition					
Malathion	0.018 (LOQ)	5	96-106	102	3.8	3.7
	18,000	5	96-100	99	1.7	1.7
Malaoxon	0.018 (LOQ)	5	95-116	106	8.0	7.5
	18,000	5	95-107	99	4.9	5.0
	Confirmation transitions 1 and 2					
Malathion	0.018 (LOQ)	5	Not reported[3]			
	18,000	5				
Malaoxon	0.018 (LOQ)	5				
	18,000	5				

Data (uncorrected results; pp. 20, 28-29; Figure 16, p. 83; Figure 19, p. 86) were obtained from Tables 1-4, pp. 42-45 of MRID 48800201.

1 The water matrices were well characterized by Agvise Laboratories, Northwood, North Dakota (pp. 23-24; Appendix IV, pp. 172-173). Surface water (Sample ID 66799B; pH 7.7, total dissolved solids 14 ppm) and ground water (Sample ID 66799A; pH 7.7, total dissolved solids 330 ppm) were used in the study. The surface water was obtained from the American River near Sunrise Boulevard in Sacramento, California. The well water was obtained from a well from a residence in Sacramento, California.

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. 124).

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 Ground, Drinking and Surface Water[1,2]

Analyte	Fortification Level (µg/L or ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface (Pond) Water						
	Quantitation transition					
Malathion	0.018 (LOQ)	5	81-127	96	17.8	18.5
	0.18	5	75-86	82	4.8	5.9
Malaoxon	0.018 (LOQ)	5	87-95	91	3.0	3.3
	0.18	5	78-85	82	2.9	3.5
	Confirmation transition 1					
Malathion	0.018 (LOQ)	5	80-127	95	18.9	19.9
	0.18	5	79-90	85	4.5	5.3
Malaoxon	0.018 (LOQ)	5	89-101	93	4.9	5.3
	0.18	5	77-84	83	4.3	5.2
	Confirmation transition 2					
Malathion	0.018 (LOQ)	5	74-100	83	11.2	13.4
	0.18	5	77-88	82	4.8	5.9
Malaoxon	0.018 (LOQ)	5	74-88	78	5.7	7.3
	0.18	5	71-82	78	4.8	6.2
Ground (Well) Water						
	Quantitation transition					
Malathion	0.018 (LOQ)	5	81-95	87	5.5	6.3
	0.18	5	65-91	84	10.6	12.6
Malaoxon	0.018 (LOQ)	5	72-92	82	7.6	9.3
	0.18	5	94-110	98	6.7	6.8
	Confirmation transition 1					
Malathion	0.018 (LOQ)	5	74-96	86	8.1	9.4
	0.18	5	68-90	84	9.1	10.8
Malaoxon	0.018 (LOQ)	5	77-88	83	4.8	5.8
	0.18	5	95-98	96	1.7	1.8
	Confirmation transition 2					
Malathion	0.018 (LOQ)	5	81-91	86	4.3	5.0
	0.18	5	65-90	83	10.4	12.5
Malaoxon	0.018 (LOQ)	5	70-81	78	6.0	7.7
	0.18	5	90-118	98	11.2	11.5

Data were obtained from Tables 2-5, pp. 29-32 of MRID 48800203. Procedural recoveries of malathion in surface water were corrected for residues found in the controls (pp. 20-22; Appendix C, pp. 88-90, 95-97; Appendix D, pp. 103-105, 110-112); all other recoveries were not corrected since no residues were quantified in the controls.

1 The water matrices were not characterized (pp. 11-12). Surface water (Sample ID 2221W-004A) obtained from Refugio Park Pond, Hercules, California, and ground water (Sample ID 2221W-0005A) obtained from a well from North Gate Road, Walnut Creek, California, were used in the study.

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. 19).

III. Method Characteristics

In the ECM and ILV, the LOQ for both analytes was 0.018 µg/L (18 ppt; pp. 19, 21, 33 of MRID 48800201; p. 22 of MRID 48800203). 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 0.006 µg/L (6 ppt) in the ECM and 10 ppt 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		0.018 µg/L (18 ppt)	
	ILV			
Limit of Detection (LOD)	ECM		0.006 µg/L (6 ppt)	
	ILV		0.010 µg/L (10 ppt)	
Linearity (Least squares calibration curve r and concentration range)	ECM[1,2]		r^2 = 0.9998 (Q)	r^2 = 0.9994 (Q)
			0.05-2.5 ng/mL	
	ILV[3]		r^2 = 0.996733278911 (Q) r^2 = 0.999703762710 (C1) r^2 = 0.998557231101 (C2)	r^2 = 0.996675923461 (Q) r^2 = 0.999816922536 (C1) r^2 = 0.997726669531 (C2)
			0.05-2.5 ng/mL	
Repeatable	ECM[4]		Yes at LOQ and 1000000×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	Surface	Yes, no matrix interferences were observed in the matrix control in Q chromatograms. Yes, only minor residues (<5% of the LOQ) in the matrix control in C1 chromatograms. In C2 chromatograms, the analyte peak was small and not distinct compared to the baseline noise and nearby contaminate peaks at LOQ.	Yes, no matrix interferences were observed in the matrix control.
		Ground		
	ILV	Surface	Significant matrix interferences were noted in the Q, C1 and C2 chromatograms (*ca*. 35-45% of the LOQ).[6]	Yes, no interferences were observed in the matrix control. Some abnormal peak integration was noted in the C1 chromatogram at the LOQ. Baseline noise height was *ca*. 50% of peak height and baseline noise significantly interfered with peak integration at LOQ in C2 chromatogram.[7]
		Ground	Significant matrix interferences were noted in the Q, C1 and C2 chromatograms (*ca*. 26-32% of the LOQ).[8]	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. 19, 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 48800201; pp. 15, 22; Tables 2-5, pp. 29-32; Figures 1-3, pp. 34-36; Figures 10-12, pp. 43-45; Appendix C, pp. 91-94, 98-101 (ground water chromatograms); Appendix D, pp. 106-109, 113-116 (surface water chromatograms) of MRID 48800203. 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 0.9997-0.9999 (Q; calculated from data in Figure 6, p. 73; Figure 11, p. 78 of MRID 48800201; 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 48800201). 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 0.998341586563-0.998365303339 (Q) and 0.998862688026-0.999908457078 (C; calculated from data in Figures 1-3, pp. 34-36; Figures 10-12, pp. 43-45 of MRID 48800203; see DER Attachment 2).

4 For the ECM, characterized surface water (Sample ID 66799B; pH 7.7, total dissolved solids 14 ppm) and ground water (Sample ID 66799A; pH 7.7, total dissolved solids 330 ppm) were used in the study. The surface water was obtained from the American River near Sunrise Boulevard in Sacramento, California. The well water was obtained from a well from a residence in Sacramento, California (pp. 23-24; Appendix IV, pp. 172-173 of MRID 48800201).

5 For the ILV, uncharacterized surface water (Sample ID 2221W-004A) obtained from Refugio Park Pond, Hercules, California, and ground water (Sample ID 2221W-0005A) obtained from a well from North Gate Road, Walnut Creek, California, were used in the study (pp. 11-12 of MRID 48800203).

6 Reviewer-calculated from peak areas reported in Appendix D, pp. 107-108 of MRID 48800203.

7 Appendix D, p. 115 (bottom-most chromatogram on page).

8 Reviewer-calculated from peak areas reported in Appendix C, pp. 92-93 of MRID 48800203.

IV. Method Deficiencies and Reviewer's Comments

1. An updated ECM should be provided to incorporate the ILV modification of the extraction procedure to include centrifuging (2000 rpm for 5 min.) to completely separate the aqueous and organic phases during extraction (Section 2.11, Step 4; p. 16 of MRID 48800203). Although this was a minor modification, it was necessary for the successful validation of the method by the ILV since the first ILV trial failed and the second ILV trial was successful after the incorporation of the centrifugation step to completely separate phases after extraction (pp. 25-26; Appendix E, pp. 117-119 of MRID 48800203).

2. 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.

3. In ILV chromatograms of malathion, significant matrix interferences were observed in the controls for all three monitored ions of malathion in the surface water matrix (*ca*. 35-45% of the LOQ) and the ground water matrix [*ca*. 26-32% of the LOQ; reviewer-calculated based on peak areas provided in Appendix C, pp. 91-94, 98-101 (ground water chromatograms); Appendix D, pp. 106-109, 113-116 (surface water chromatograms) of MRID 48800203]. The ILV LOD (10 ppt) was *ca*. 55% 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 *ca*. 27% of the LOQ, the quantitative ion analysis for both control samples of malathion were >50% of the LOD: *ca*. 35% for surface water and *ca*. 27% for ground water.

   In ILV chromatograms of malaoxon, baseline noise interfered with peak integration at the LOQ. In the surface water, some abnormal peak integration was also noted in the

confirmation 1 chromatogram at the LOQ (Appendix D, p. 115 of MRID 48800203). Also, at the LOQ in the confirmation 2 chromatogram, baseline noise height was *ca*. 50% of peak height and baseline noise significantly interfered with peak integration. In the ground water, some baseline noise around the analyte peak interfered with peak integration at the LOQ (Appendix C, p. 100). No residues were quantified or observed in the ILV control samples for malaoxon of either matrix.

4. In the ILV, procedural recoveries were corrected for residues quantified in the controls (pp. 20-22; Appendix C, pp. 88-90, 95-97; Appendix D, pp. 103-105, 110-112 of MRID 48800203). Residues were only quantified in one of the control samples of the ground water for malathion (<LOD, confirmation 2 ion only); residues were quantified in both of the controls samples of the surface water for all three monitored ions of malathion (7.52-9.52 ppt). The reviewer noted that matrix interferences (*ca*. 26-45% of the LOQ) were observed in the controls for all three monitored ions of malathion in both water matrices in representative chromatograms (see above), but corrections were only performed for the surface water samples fortified with malathion based on the data spreadsheets.

   Recoveries were not corrected in the ECM, although calculations allowed for corrections, since no residues were quantified in the controls (pp. 20, 28-29; Figure 16, p. 83; Figure 19, p. 86 of MRID 48800201).

5. 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 second trial after the incorporation of the centrifugation step to completely separate phases after extraction (pp. 25-26; Appendix E, pp. 117-119 of MRID 48800203).

6. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. The ILV surface and ground water matrices were not characterized (pp. 11-12 of MRID 48800203). The reviewer noted that the characteristics of the pond water were discussed between the sponsor and study director when trying to determine the source of the failed first trial (Appendix E, pp. 117-119).

7. The estimations of the LOQ and LOD in the ECM were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (18 ppt; pp. 19, 21, 33 of MRID 48800201; p. 22 of MRID 48800203). 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 (0.006 µg/L, 6 ppt) and in the ILV (10 ppt).

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

8. The ILV reported communications between the ILV and the sponsor were summarized (pp. 25-26; Appendix E, pp. 117-119 of MRID 48800203).

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

10. 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 48800201). 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.

11. In the ECM, matrix effects were studied (p. 33; Tables 9-12, pp. 64-67 of MRID 48800201). Matrix effects were insignificant (±10%) for all matrices/analytes.

12. 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. 22 of MRID 48800203). The LC/MS/MS required approximately 13 hours. The overall time to complete a set of samples was 2 working days.

V. References

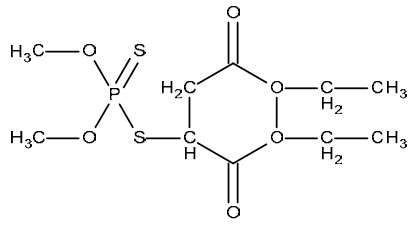
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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