

Test Material: Methiocarb

MRID: 49502502

Title: Development and Validation of a Method for the Determination of Methiocarb, Methiocarb Sulfoxide and Methiocarb Sulfone in Soil

MRID: 49519605

Title: INDEPENDENT LABORATORY VALIDATION OF METHODS FOR THE DETERMINATION OF METHIOCARB AND ITS METABOLITES METHIOCARB SULFOXIDE AND METHIOCARB SULFONE IN SOIL BY LC/MS/MS

EPA PC Code: 100501

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto

Signature:



Date: 7/14/15

Secondary Reviewer: Lynne Binari

Signature:



Date: 7/14/15

QC/QA Manager: Joan Gaidos

Signature:




Date: 7/14/15

Analytical method for methiocarb sulfoxide and its transformation products methiocarb sulfoxide and methiocarb sulfone in soil

- Reports:** ECM: EPA MRID No.: 49502502. Shen, H. 2014. Development and Validation of a Method for the Determination of Methiocarb, Methiocarb Sulfoxide and Methiocarb Sulfone in Soil. PTRL Study No.: 2630W. Report prepared by PTRL West (a division of EAG, Inc.), Hercules, California; sponsored and submitted by Gowan Company, Yuma, Arizona; 75 pages. Final report issued August 21, 2014.
ILV: EPA MRID No. 49519605. MacGregor, J.A, and E.S. Bodle. 2014. INDEPENDENT LABORATORY VALIDATION OF METHODS FOR THE DETERMINATION OF METHIOCARB AND ITS METABOLITES METHIOCARB SULFOXIDE AND METHIOCARB SULFONE IN SOIL BY LC/MS/MS. Wildlife International Project No.: 334C-126. Report prepared by Wildlife International, Evans Analytical Group, Easton, Maryland, sponsored and submitted by Gowan Company, Yuma, Arizona; 166 pages. Final report issued November 25, 2014.
- Document No.:** MRIDs 49502502 & 49519605
- Guideline:** 850.6100
- Statements:** ECM: EPA MRID No.: 49502502. The study was conducted in accordance with USEPA GLP standards (40 CFR 160), with the exception that the certificates of analysis of the methiocarb sulfoxide and methiocarb sulfone reference substances may or may not have been GLP certified (p. 3). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the QA statement.
ILV: EPA MRID No. 49519605. The study was conducted in accordance with USEPA FIFRA, OECD and Japanese GLP standards, with the exception of the storage stability characterization and testing (p. 3). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was not included.
- Classification:** This analytical method is classified as Unacceptable/Upgradable. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. The LOD was not reported in the ILV. The registrant failed to verify that the independent laboratory was provided with a soil of the most difficult analytical sample condition to analyze to demonstrate how well the method performs. Linearity was not always ≥ 0.995 in the ECM. A reagent blank was not included in the ILV.

PC Code: 100501

EPA Primary Reviewer: James C. Hook III
Ecologist

Signature: 
Date: March 7, 2016

EPA Secondary Reviewer: William P. Eckel, Ph.D
Senior Science Advisor

Signature: 
Date: March 7, 2016

Executive Summary

This analytical method, PTRL Study No. 2630W, is designed for the quantitative determination of methiocarb and its transformation products methiocarb sulfoxide and methiocarb sulfone in soil at 1 ppb using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil. The ILV validated the method in the second trial, which was successful using matrix-matched standards; the first trial was unsuccessful due to matrix effects. Both the ECM and ILV validated the method using sandy loam soils; therefore, the registrant failed to verify that the independent laboratory was provided with a soil of the most difficult analytical sample condition to analyze to demonstrate how well the method performs.

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						
Methiocarb	49502502	49519605		Soil*	21/08/2014	Gowan Company	LC/MS/MS	1 ppb (1.00 µg/kg)
Methiocarb sulfoxide								
Methiocarb sulfone								

* The ECM validated the method using sandy loam (17% clay, 4.4% organic matter). The soil matrix of the ILV was also sandy loam (9% clay, 5.6% organic matter).

I. Principle of the Method

Samples (25 g) of soil were fortified (25 µL of 1 µg/mL or 10 µg/mL mixed fortification solution), as necessary, then extracted with 40 mL of acetone and 10 mL of de-ionized water by shaking via wrist action shaker (speed not reported) for 30 minutes (pp. 19-21; Figure 1, p. 34 of MRID 49502502). After filtration (Whatman No. 4) with water aspiration, the sample vessel and filter cake were rinsed with 10 mL acetone. The extract and rinse were transferred to a separatory funnel. The filter vessel was rinsed with 50 mL of hexane:methylene chloride (1:1, v:v) then sonicated and transferred to the separatory funnel. The funnel was shaken vigorously by hand for 2 minutes. The upper organic layer was isolated and transferred to a concentration flask. The aqueous layer was returned to the separatory flask, and the steps were repeated, beginning with the rinsing of the filter vessel with hexane:methylene chloride (1:1, v:v). The combined extracts and rinses were concentrated to dryness using a rotary evaporator at 30°C. The residue was

reconstituted with 3 mL of ethyl acetate:hexane (1:4, v:v) via sonication. The sample was loaded onto a SPE cartridge (20 mL/5 g FL, Agilent Mega Bond Elut), preconditioned with 10 mL of ethyl acetate:hexane (1:4, v:v), collecting the eluate. The concentration flask was rinsed with 30 mL of ethyl acetate:hexane (1:4, v:v) then 10 mL of acetone; each rinse was applied to the SPE column with eluate collection. The combined eluates were concentrated to dryness via rotary evaporator at 30°C. The residue was reconstituted with 2.5 mL methanol via sonication prior to LC/MS/MS analysis.

Samples were analyzed for methiocarb, methiocarb sulfoxide and methiocarb sulfone using a Thermo Scientific Dionex Ultimate 3000 Liquid Chromatograph coupled to an AB Sciex API 4000 Triple Quad Mass Spectrometer (MRM; pp. 17, 22-23 of MRID 49502502). The instrumental conditions consisted of an Agilent Zorbax® SB-CN column (4.6 x 75 mm, 3.5- μ m; column temperature unreported), a mobile phase gradient of (A) water containing 0.05% formic acid and (B) methanol containing 0.05% formic acid [percent A:B (v:v) at 0.0-0.5 min. 80:20, 4.0-6.5 min. 10:90, 7.0-12.5 min. 80:20] and MS/MS detection in APCI positive mode (temperature, 300°C). Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: m/z 226 \rightarrow 169 (Q) and m/z 226 \rightarrow 121 (C) for methiocarb; m/z 242.5 \rightarrow 185 (Q) and m/z 242.5 \rightarrow 170 (C) for methiocarb sulfoxide; and m/z 258 \rightarrow 107 (Q) and m/z 258 \rightarrow 202 (C) for methiocarb sulfone (p. 23; Appendix D, pp. 73-75). Approximate retention times were *ca.* 6.19 min., *ca.* 5.06 min. and *ca.* 5.38 min. for methiocarb, methiocarb sulfoxide and methiocarb sulfone, respectively (Figures 13-14, pp. 53-58). Injection volume was 10 μ L (p. 22)

ILV

In the ILV, the ECM extraction method was performed exactly as written, with the exceptions that a gyratory shaker table (*ca.* 300 rpm) was used in place of the wrist action shaker (p. 17 of MRID 49519605). The analytical method was performed as described in the ECM method using an Agilent Technologies 1260 HPLC coupled to an AB Sciex Triple QUAD 5500 Mass Spectrometer (Turbo-V Ion Spray Source; p. 18; Table 1, p. 24). Approximate retention times for the analytes were *ca.* 6.9 min., *ca.* 5.8 min. and *ca.* 6.1 min. for methiocarb, methiocarb sulfoxide and methiocarb sulfone, respectively. For methiocarb sulfoxide, the monitored ion transitions differed slightly from those reported in the ECM: m/z 242 \rightarrow 185 (Q) and m/z 242 \rightarrow 170 (C). For methiocarb sulfone, the monitored confirmation ion transitions differed slightly from that reported in the ECM: m/z 258 \rightarrow 201 (C). For quantification, matrix-matched standards were used instead of solvent standards for the second trial (pp. 12, 16, 21).

LOQ/LOD

The Limit of Quantification (LOQ) and Limit of Detection (LOD) for all analytes were reported as 1 ppb and 0.2 ppb (20% of the LOQ), respectively, in the ECM (p. 24 of MRID 49502502). The LOQ and LOD represented 10 ng/mL and 2 ng/mL in solution, respectively. In the ILV, only the LOQ (1.00 μ g/kg) was reported (p. 14 of MRID 49519605).

II. Recovery Findings

ECM (MRID 49502502): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD \leq 20%) for analysis of methiocarb, methiocarb sulfoxide and methiocarb sulfone in sandy loam soil at the LOQ (1 ppb) and 10 \times LOQ (10 ppb; Table I, pp. 31-32). Analytes were identified and quantified using two ion transitions; quantification and confirmation ion results were comparable. The sandy loam soil (60% sand, 23% silt, 17% clay; 4.4% organic matter) was fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; soil source not reported; p. 18; Appendix C, p. 72).

ILV (MRID 49519605): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD \leq 20%) for analysis of methiocarb, methiocarb sulfoxide and methiocarb sulfone in sandy loam soil at the LOQ (1 ppb) and 10 \times LOQ (10 ppb; Tables 2-7, pp. 25-30). Analytes were identified and quantified using two ion transitions; quantification and confirmation ion results were comparable. The sandy loam soil (70% sand, 21% silt, 9% clay; 5.6% organic matter) was fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; soil source not reported; pp. 14-15; Appendix III, p. 155). The method was validated in the second trial which was successful using matrix-matched standards; the first trial was unsuccessful due to matrix suppression effects (pp. 12, 16, 21).

Table 2. Initial Validation Method Recoveries for Methiocarb, Methiocarb Sulfoxide and Methiocarb Sulfone in Sandy Loam Soil^{1,2}

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Quantitation ion						
Methiocarb	1 (LOQ)	5	99-122	112	8	8
	10	5	104-122	111	7	6
Methiocarb sulfoxide	1 (LOQ)	5	69-104	88	13	15
	10	5	60-95	82	13	16
Methiocarb sulfone	1 (LOQ)	5	89-122	108	14	13
	10	5	76-104	93	11	12
Confirmation ion						
Methiocarb	1 (LOQ)	5	101-121	113	8	7
	10	5	103-121	111	7	6
Methiocarb sulfoxide	1 (LOQ)	5	69-100	86	11	13
	10	5	61-97	83	13	16
Methiocarb sulfone	1 (LOQ)	5	97-126	113	12	11
	10	5	76-104	92	11	12

Data (uncorrected recovery results, Appendix D, pp. 73-75) were obtained from Table I, pp. 31-32 of MRID 49502502.

1 The soil matrix was fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; p. 18; Appendix C, p. 72).

2 Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: m/z 226 \rightarrow 169 (Q) and m/z 226 \rightarrow 121 (C) for methiocarb; m/z 242.5 \rightarrow 185 (Q) and m/z 242.5 \rightarrow 170 (C) for methiocarb sulfoxide; and m/z 258 \rightarrow 107 (Q) and m/z 258 \rightarrow 202 (C) for methiocarb sulfone (p. 23; Appendix D, pp. 73-75).

Table 3. Independent Validation Method Recoveries for Methiocarb sulfoxide and Methiocarb sulfoxide Sulfone in Loamy Sand Soil¹

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Quantitation ion						
Methiocarb	1 (LOQ)	5	88.2-90.0	88.8	0.736	0.829
	10	5	87.2-91.5	89.4	1.87	2.09
Methiocarb sulfoxide	1 (LOQ)	5	73.0-81.6	76.9	3.30	4.29
	10	5	76.7-81.0	78.2	1.70	2.17
Methiocarb sulfone	1 (LOQ)	5	78.0-83.9	81.6	2.35	2.88
	10	5	76.7-84.1	79.1	3.23	4.08
Confirmation ion						
Methiocarb	1 (LOQ)	5	86.6-89.1	88.1	0.980	1.11
	10	5	87.3-91.3	89.4	1.66	1.86
Methiocarb sulfoxide	1 (LOQ)	5	72.1-82.5	77.5	4.15	5.35
	10	5	76.2-81.2	77.9	1.99	2.55
Methiocarb sulfone	1 (LOQ)	5	78.9-83.5	81.0	1.89	2.33
	10	5	77.4-84.6	80.0	3.11	3.89

Data (uncorrected recovery results, pp. 19-20) were obtained from Tables 2-7, pp. 25-30 of MRID 49519605.

1 The soil matrix was fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; pp. 14-15; Appendix III, p. 155).

2 Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: m/z 226 → 169 (Q) and m/z 226 → 121 (C) for methiocarb; m/z 242 → 185 (Q) and m/z 242 → 170 (C) for methiocarb sulfoxide; and m/z 258 → 107 (Q) and m/z 258 → 201 (C) for methiocarb sulfone (p. 18).

III. Method Characteristics

The LOQ and LOD for all analytes were reported in the ECM as 1 ppb and 0.2 ppb, respectively (pp. 24, 28-29 of MRID 49502502). The LOQ represented 10 ng/mL in solution, and the LOD was 20% of the LOQ and represented 2 ng/mL in solution. The LOQ was justified by the acceptable linearity and recovery in the validation study; the LOD was justified by the good sensitivity and linearity in the calibration solution analysis. In the ILV, only the LOQ (1.00 µg/kg) was reported; the LOD was not reported (p. 14 of MRID 49519605). No calculations or comparisons to noise level were reported in the ECM or ILV.

Table 4. Method Characteristics

		Methiocarb	Methiocarb sulfoxide	Methiocarb sulfone
Limit of Quantitation (LOQ)		1 ppb (1 µg/kg)		
Limit of Detection (LOD)		0.2 ppb (0.2 µg/kg)		
Linearity (calibration curve r^2 and concentration range)	ECM ¹	$r^2 = \mathbf{0.9945}$ (Q) $r^2 = 0.9957$ (C)	$r^2 = 0.9980$ (Q) $r^2 = 0.9970$ (C)	$r^2 = \mathbf{0.9933}$ (Q) $r^2 = 0.9971$ (C)
	ILV ²	$r^2 = 0.9969$ (Q) $r^2 = 0.9976$ (C)	$r^2 = 0.9967$ (Q) $r^2 = 0.9966$ (C)	$r^2 = 0.9983$ (Q) $r^2 = 0.9970$ (C)
	Concentration range	(2.00-100 ng/mL)		
Repeatable	ECM	Yes at LOQ and 10×LOQ [sandy loam (17% clay, 4.4% organic matter)]		
	ILV	Yes at LOQ and 10×LOQ [sandy loam (9% clay, 5.6% organic matter)]		
Reproducible	ECM	Yes at LOQ and 10×LOQ.		
	ILV			
Specific	ECM	Yes, matrix interferences at analyte retention times were <LOD.		
	ILV			

Data were obtained from pp. 18, 24, 28-29; Table I, pp. 31-32; Figures 2-3, pp. 35-40; Figures 7-9, pp. 47-49; Figures 13-15, pp. 53-61; Appendix C, p. 72 of MRID 49502502; and pp. 14-16; Tables 2-7, pp. 25-30; Figures 1-36, pp. 31-66; Appendix III, p. 155 of MRID 49519605; and DER Attachment 2.

1 Solvent-based standards were used. ECM r^2 values are reviewer-generated from reported r values of 0.996648414620 to 0.999001659519 for the quantitative and confirmatory ions of methiocarb, methiocarb sulfoxide and methiocarb sulfone (Figures 7-9, pp. 47-49 of MRID 49502502; DER Attachment 2).

2 Matrix-matched standards were used. ILV r^2 values are reviewer-generated from reported r values of 0.9983233 to 0.9991672 for the quantitative and confirmatory ions of methiocarb, methiocarb sulfoxide and methiocarb sulfone (Figures 1-3, pp. 31-33; Figures 19-21, pp. 49-51 of MRID 49519605; DER Attachment 2).

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

IV. Method Deficiencies and Reviewer's Comments

1. The determinations of the LOQ and LOD in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. The LOQ and LOD were not adequately supported by calculations or comparison to background levels. The LOQ was justified by the acceptable linearity and recovery in the validation study; the LOD was justified by the good sensitivity and linearity in the calibration solution analysis. In the ILV, the LOQ was reported from the ECM, and the LOD was not reported.

Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification

2. The registrant failed to verify that the independent laboratory was provided with a soil of the most difficult analytical sample condition to analyze to demonstrate how well the method performs. The ECM validated the method using sandy loam (17% clay, 4.4% organic matter; p. 18; Appendix C, p. 72 of MRID 49502502). The soil matrix of the ILV was also sandy loam (9% clay, 5.6% organic matter), and the clay content was less (pp. 14-15; Appendix III, p. 155 of MRID 49519605).
3. Linearity was not ≥ 0.995 for the quantitative ions of methiocarb and methiocarb sulfone in the ECM (Figures 7-9, pp. 47-49 of MRID 49502502; DER Attachment 2).
4. The ILV did not include a reagent blank.
5. Modifications to the ECM by the ILV included: 1) the use of a gyratory shaker table in place of the wrist action shaker; 2) different, but comparable LC/MS/MS equipment; and 3) the use of m/z 242 \rightarrow 185 (Q) and m/z 242 \rightarrow 170 (C) as the monitored ion transitions for methiocarb sulfoxide (pp. 17-18; Table 1, p. 24 of MRID 49519605). None of these modifications were considered significant.
6. The ILV study authors noted the following problems which were encountered: 1) m/z 201 was determined to be the dominant structural isomer of methiocarb sulfone for ion identification, instead of m/z 202 which was reported by the ECM; and 2) matrix-matched standards were necessary for the success of the method with the test soil matrix which was chosen (Appendix V, pp. 164-165 of MRID 49519605). None of these modifications were considered significant.

The ECM study author assessed matrix effects (pp. 26-27; Table II, p. 33 of MRID 49502502). No significant matrix effects were found, so solvent-based calibrants were used.

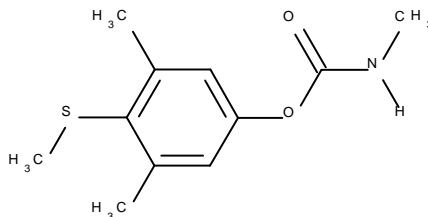
7. The ECM study author noted that calibration solutions should be freshly prepared for analysis since methiocarb sulfone is unstable (p. 20 of MRID 49502502). The ILV study authors concurred with this critical step (Appendix V, p. 165 of MRID 49519605).
8. The communications between the ILV study personnel and the ECM study personnel were reported in the ILV (Appendix V, pp. 165-166 of MRID 49519605).
9. In the ILV, it was reported that that one set of twelve samples required *ca.* 2 calendar days to complete, including LC/MS/MS analysis (Appendix V, p. 165 of MRID 49502505). It was reported in the ECM that two subsample sets of eight samples (sixteen samples total) required 24 person hours to complete (pp. 24-25 of MRID 49502502). The preparation and extraction required *ca.* 6 hours per subsample set; subsequent LC/MS analysis and evaluation also required *ca.* 6 hours per subsample set.

V. References

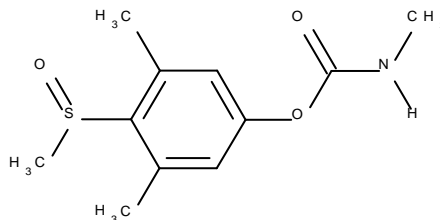
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures**Methiocarb (BAY 37344, Mercaptodimethur)**

IUPAC Name: 4-Methylthio-3,5-xylol methylcarbamate.
 3,5-Dimethyl-4-(methylthio)phenyl methylcarbamate.
CAS Name: 3,5-Dimethyl-4-(methylthio)phenyl N-methylcarbamate.
CAS Number: 2032-65-7
SMILES String: CNC(=O)Oc1cc(C)c(SC)c(C)c1

**Methiocarb sulfoxide (MOX)**

IUPAC Name: (3,5-Dimethyl-4-methylsulfinyl-phenyl) N-methylcarbamate.
 3,5-Dimethyl-4-(methylsulfinyl)phenyl methylcarbamate.
CAS Name: Phenol, 3,5-Dimethyl-4-(methylsulfinyl)-, methylcarbamate.
CAS Number: 2635-10-1
SMILES String: S(=O)(C)c(c(cc1OC(=O)NC)C)c(c1)C

**Methiocarb sulfone (MON)**

IUPAC Name: (3,5-Dimethyl-4-methylsulfonyl-phenyl) N-methylcarbamate.
 3,5-Dimethyl-4-(methylsulfonyl)phenyl methylcarbamate.
CAS Name: Phenol, 3,5-Dimethyl-4-(methylsulfonyl)-, methylcarbamate.
CAS Number: 2179-25-1
SMILES String: S(=O)(=O)(C)c(c(cc1OC(=O)NC)C)c(c1)C

