Test Material:	Thiodicarb
MRID:	49145602
Title:	Independent Laboratory Validation (ILV) of the BAYER Method TD-002-W13-01: The Determination of Thiodicarb and its Metabolite Methomyl in Water using LC/MS/MS
EPA PC Code:	114501
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** 

Date: 8/18/14

Secondary Reviewer: Lisa Muto **Signature:** 

Date: 8/18/14

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

Date: 8/18/14

Analytical method for thiodicarb and its transformation product methomyl in water

Reports:	ECM: EPA MRID No.: 49145602 (Appendix 1, pp. 53-70). Kormos, T. 2013. Bayer Method TD-002-W13-01: An Analytical Method for the Determination of Residues of Thiodicarb and its Metabolite Methomyl in Water Using LC/MS/MS. Study No.: METDL004. Report prepared, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 18 pages. Final report issued April 4, 2013. ILV: EPA MRID No.: 49145602. Patel, D. and S. Perez. 2013. Independent Laboratory Validation (ILV) of the BAYER Method TD-002-W13-001: The Determination of Thiodicarb and its Metabolite Methomyl in Water using LC/MS/MS. ADPEN Study No.: 2K13-982-0415. Bayer CropScience Study No.: METDL004. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 94 pages. Final report issued June 5, 2013.
Document No.:	MRID 49145602
Guideline:	850.6100
Statements:	ECM: The study was not conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (Appendix 1, p. 55). Signed and dated No Data Confidentiality, GLP non-compliance, and Authenticity Certification statements were provided (Appendix 1, pp. 54-56). A Quality Assurance statement was not provided. ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statement were provided (pp. 2-4). An Authenticity Certification statement was not provided.
Classification:	This analytical method is classified as Supplemental. No originating ECM performance data were reported. The LOD of each analyte was not reported. ILV performance data for 0.1 µg/L (LOQ) fortified methomyl using the confirmatory ion method did not meet OCSPP Guideline 850.6100 criteria. Thiodicarb recoveries were corrected in the ILV.
PC Code:	114501
Reviewer:	Jessica L. O. Joyce, Fate Scientist **Signature:** Jessica L. O. Joyce Date: 2018.03.28 08:13:37 -04'00' **Date:** 3/28/2018

All page citations refer to the page numbers located in the top right corner of the MRID.

Executive Summary

The analytical method Bayer Analytical Method TD-002-W13-01, is designed for the quantitative determination of thiodicarb and its product methomyl in water using LC/MS/MS. Thiodicarb recovery is determined as total residues of thiodicarb and methomyl in a sample. However, the recovery of just thiodicarb was also acceptable. The method is quantitative for both analytes at the stated LOQ of 0.1 µg/L. The LOQ is less than the lowest toxicological level of concern in water. ILV thiodicarb recoveries were corrected for residues in the matrix control samples; no residues were detected in the methomyl matrix control samples to be used for recovery correction. The independent laboratory validated the method after two trials, with the exception of 0.1 µg/L (LOQ) methomyl confirmatory ion analysis (mean 63%). No major modifications were made by the independent laboratory.

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						
Thiodicarb	49145602	49145602	Supplemental	Water	04/04/2013	Bayer CropScience	LC/MS/MS	0.1 µg/L
Methomyl								0.1 µg/L

I. Principle of the Method

Water (10 mL) was centrifuged (*ca*. 12,500g, *ca*. 3 minutes), then a 5-mL aliquot was loaded onto a reverse-phase solid-phase extraction (SPE) cartridge (Waters Oasis HLB 1cc Vac RC Cartridge, 60 mg), preconditioned with methanol (2 mL) and water (2 mL; Appendix 1, pp. 59, 62). The loaded sample was dried under vacuum (*ca*. 20" Hg, *ca*. 15 minutes; Appendix 1, p. 62). Residues were sequentially eluted by gravity flow with methanol (2 mL), followed by methanol:methylene chloride (3:1, v:v, 1 mL) and again with methanol:methylene chloride (3:1, v:v, 1 mL); the cartridge was dried under vacuum (*ca*. 5" HG, *ca*. 1 minute) between each elution step. The eluate was taken to dryness under nitrogen (Turbo-Vap, 45°C, *ca*. 20 psi, *ca*. 30 minutes) and the residues reconstituted in 2.5 mL L-cysteine HCl in methanol. Reconstituted samples were transferred to LC vials for LC/MS/MS analysis.

Samples were analyzed for thiodicarb and its product methomyl by HPLC (Supelco Ascentis Express C18, 2.1 mm x 100 mm, 2.7 µm column) using a mobile phase of (A) water:methanol (90:10, v:v) in 10mM ammonium formate and 120 µL/L formic acid and (B) methanol:water (90:10, v:v) in 10mM ammonium formate and 120 µL/L formic acid [percent A:B at 0.0-0.5 min. 95:5, 0.51 min. 30:70, 1.5-2.5 min. 0:100, 2.51-5.0 min. 95:5] with MS/MS-ESI (electrospray ionization, positive) detection and multiple reaction monitoring (MRM; Appendix 1, pp. 59, 62-66). Injection volume was 5 µL. Analytes were identified using two ion transitions; one for

quantitation (Q) and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 355→88 (Q) and *m/z* 355→108 (C) for thiodicarb and *m/z* 163→88 (Q) and *m/z* 163→106 (C) for methomyl.

The study author noted rapid degradation of thiodicarb, thus fortifications for each analyte must be performed separately, and thiodicarb recovery is determined as the total residues of thiodicarb and methomyl in a sample (Appendix 1, pp. 61, 64). However, thiodicarb recovery was acceptable without being combined with methomyl residues. Due to the potential for thiodicarb degradation from the time of sample collection to laboratory analysis, it is sufficient for thiodicarb recovery to be analyzed with a total residue approach (thiodicarb and methomyl).

In the ILV, the water samples were centrifuged at 4,000 rpm for 10 minutes prior to SPE clean-up (p. 12; Table 1, p. 18). The ILV also implemented minor modifications to the instrument conditions to optimize chromatography (p. 13; Appendix 1, pp. 65-66). In addition, final evaporation of the sample to dryness was considered a critical step. The independent laboratory recommended that the sample be taken to near dryness, then use the technique of rolling the concentration tube and allowing the final volume of solvent to evaporate on its own.

LOQs for both analytes were the same in the ECM and ILV at 0.1 µg/L (ppb; p. 11; Appendix 1, p. 59). LODs for the analytes were not specified in either the ECM or ILV.

II. Recovery Findings

ECM (Appendix 1, pp. 53-70 of MRID 49145602): Originating ECM performance data were not reported.

ILV (MRID 49145602): Mean recoveries and RSDs were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of thiodicarb (as total residues of thiodicarb plus methomyl) and its product methomyl in surface water, with the exception of 0.1 µg/L (LOQ) methomyl confirmatory ion analysis (mean 63%; pp. 11, 14). Recovery values of thiodicarb were corrected for residues in the matrix control samples; no residues were detected in the methomyl matrix control samples (Appendix 4, pp. 82-83; Appendix 5, pp. 89-92). The method was validated after two trials, with minor method and instrument parameter modifications (pp. 12, 14). The water matrix was well characterized (p. 11; Appendix 3, p. 81).

Table 2. Initial Validation Method Recoveries for Analytes in Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Thiodicarb	LOQ					
	10x LOQ	No originating ECM performance data were reported.				
Methomyl	LOQ					
	10x LOQ					

1 Water characterized with a pH of 5.5, Hardness: 2 mg eq $CaCO_3$/L, Total Dissolved Solids: 76 ppm, Turbidity: 1.88 NTU, Appendix 3, p. 80.

Table 3. Independent Validation Method Recoveries for Analytes in Surface Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Thiodicarb[2]	Quantitation ion					
	0.1 (LOQ)	4[3]	83-106	99	10.7	10.7
	1.0	5	90-100	96	3.4	3.6
	Confirmatory ion					
	0.1 (LOQ)	4[3]	80-89	84	3.8	4.5
	1.0	5	87-105	94	7.5	8.0
Methomyl	Quantitation ion					
	0.1 (LOQ)	5	72-81	75	4.2	5.6
	1.0	5	78-86	81	3.7	4.6
	Confirmatory ion					
	0.1 (LOQ)	5	55-75	**63**	7.4	11.7
	1.0	5	86-94	90	3.3	3.6

Data were obtained from Table 4, p. 21 and p. 89; Table 6, p. 23 and p. 90; Table 8, p. 25 and p. 91; Table 10, p. 27 and p. 92.

1 This table represents validation data from the second trial, only.

2 Due to rapid degradation of thiodicarb, its recovery is determined as the total residues of thiodicarb and methomyl recovered in the sample (Table 4, p. 21).

3 Five replicate fortifications were performed; however, low recovery from one replicate (6% quantitation ion, 5% confirmatory ion) was considered an outlier as per the Grubbs and Dixon test and was not used in the statistics (Table 4, p. 21; Table 6, p. 23).

III. Method Characteristics

In the ECM and ILV, the LOQ value for thiodicarb (as total residues of thiodicarb plus methomyl) and its product methomyl in water was 0.1 µg/L (p. 11; Appendix 1, p. 59). No justification for the selected LOQ was provided. The LOD for each analyte was not specified in either the ECM or ILV.

Table 4. Method Characteristics

Analyte		Thiodicarb*	Methomyl
Limit of Quantitation (LOQ)		0.1 µg/L	0.1 µg/L
Limit of Detection (LOD)		Not reported.	Not reported.
Linearity (calibration curve r^2 and concentration range)[1]	ECM	No linearity data provided in the ECM.	
	ILV	Quantitation (Q) ion	
		r^2 = 0.9986 (0.1-20 ng/mL)	r^2 = 0.9982 (0.1-20 ng/mL)
		Confirmatory (C) ion	
		r^2 = 0.9971 (0.1-20 ng/mL)	r^2 = 0.9992 (0.1-20 ng/mL)
Repeatable	ECM	No data provided in the ECM.	
	ILV	Q and C ion analyses: Yes	Q ion analysis: Yes C ion analysis: No (mean recovery 63% at 0.1ppb)
Reproducible[2]		Yes	Yes
Specific	ECM	No data provided in ECM.	

	ILV	Yes	Yes; however, baseline noise was fairly significant at the retention time of the analyte.

Data were obtained from p. 11; Figures 1-4, pp. 29-32; Figures 13-14, pp. 41-42; Figure 16, p. 44; Figure 18, p. 46; Figure 20, p. 48; Appendix 6, p. 94.

* Thiodicarb (as total residues of thiodicarb plus methomyl)

1 Reviewer-calculated calibration curves verified linearity for the ILV (r^2 = 0.9996-1; see DER Attachment 2).

2 The ILV validated the method at the LOQ, with the exception of 0.1 µg/L (LOQ) methomyl confirmatory ion analysis. Performance data establishing the LOQ at 0.1 µg/L were not provided with the ECM.

IV. Method Deficiencies and Reviewer's Comments

1. No originating ECM performance data were reported, including mean recovery at each spiking level, the relative standard deviation, and linearity (calibration curve r^2 and concentration range). The only results presented in the ECM report were MS spectra of thiodicarb and methomyl and chromatograms of 0.1 and 10 µg/L mixed calibration standards for the two analytes (Appendix 1, pp. 68-70).

2. It is preferred that an ILV is performed and documented in a report separate from the ECM report; not as an appendix.

3. The determination of the LOQ and LOD were not based on scientifically acceptable procedures. No justification for the selected LOQ (0.1 µg/L) for the two analytes was provided, and LODs were not reported (p. 11; Appendix 1, p. 59). Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

4. For the ILV, the following fortifications using the confirmatory ion method did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) ≤20%): confirmatory ion analysis of 0.1 µg/L (LOQ) fortified methomyl in surface water (mean 63%, p. 14).

5. For the ILV, reported recoveries of thiodicarb (as thiodicarb and methomyl) were corrected for residues in the matrix control samples; no residues were detected in the methomyl matrix control samples to be used for recovery correction (Appendix 4, pp. 82-83; Appendix 5, pp. 89-92). ECM residue calculations did not specify the correction of sample recoveries for any residues found in the matrix control samples (Appendix 1, pp. 63-64).

6. Five replicate fortifications at LOQ and 10x LOQ were performed for each analyte in the water matrix; however, low recovery from one 0.1 µg/L (LOQ) thiodicarb replicate (6% quantitation ion, 5% confirmatory ion) was considered an outlier as per the Grubbs and Dixon test and was not used in the statistics (Table 4, p. 21; Table 6, p. 23).

7. For the ILV, baseline noise during methomyl analyses was fairly significant at the retention time of the analyte (Figure 16, p. 44; Figure 18, p. 46; Figure 20, p. 48; Appendix 6, p. 94). For thiodicarb, interferences, based on peak area, in the reagent blank and matrix control samples at the retention time of the analyte were <10% of the LOQ (Figure 15, p. 43; Figure 17, p. 45; Figure 19, p. 47).

8. The independent laboratory concluded that the method was demonstrated to be suitable as written (pp. 9, 15). However, an amendment was added with clarification to the method because the first validation trial set did not pass.

9. Communication prior to running the samples between the laboratory and the developers was not documented. However, all communication during validation was documented.

10. It was reported for the ILV that a single analyst completed a sample set consisting of 13 samples in one working day with LC/MS/MS analysis performed overnight (p. 15).

V. References

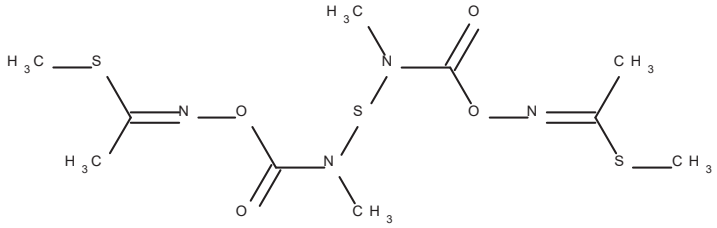
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Thiodicarb

IUPAC Name: (3EZ,12EZ)-3,7,9,13-tetramethyl-5,11-dioxa-2,8,14-trithia-4,7,9,12-tetraazapentadeca-3,12-diene-6,10-dione
CAS Name: Dimethyl N,N′-[thiobis[(methylimino)carbonyloxy]]bis[ethanimidothioate]
CAS Number: 59669-26-0
SMILES String: CC(SC)=NOC(=O)N(C)SN(C)C(=O)ON=C(SC)C



Methomyl

IUPAC Name: S-methyl (EZ)-N-(methylcarbamoyloxy)thioacetimidate
CAS Name: Methyl N-[[(methylamino)carbonyl]oxy]ethanimidothioate
CAS Number: 16752-77-5
SMILES String: CNC(=O)ON=C(C)SC