Analytical method for thiophanate-methyl and its metabolite carbendazim in water

Reports: ECM: EPA MRID No.: 50084803. Jooβ, S. 2012. Validation of an Analytical Method for the Determination of Thiophanate-Methyl and Carbendazim in Drinking, Ground and Surface Water. Report prepared by PTRL Europe, Ulm, Germany, sponsored by Nippon Soda Co., Ltd., Tokyo, Japan, and submitted by TM-MBC Task Force None-Food Use Technical Committee (no address found) and Nisso America Inc., New York, New York (a wholly owned subsidiary of Nippon Soda Co., Ltd.); 61 pages. PTRL Europe ID: P 2681 G. Final report issued September 24, 2012.

ILV: EPA MRID No.: 50084804. Witte, A. 2014. Independent Laboratory Validation (ILV) of an Analytical Method for Determination of Residues of Thiophanate-methyl and its Metabolite Carbendazim in Drinking Water. Report prepared by CIP Chemisches Institut Pforzheim GmbH, Pforzheim, Germany, sponsored by Nippon Soda Co., Ltd., Tokyo, Japan, and submitted by TM-MBC Task Force None-Food Use Technical Committee (no address found) and Nisso America Inc., New York, New York (a wholly owned subsidiary of Nippon Soda Co., Ltd.); 54 pages. CIP Study Code: 14N07016-01-VMWA. Final report issued July 24, 2014.

Document No.: MRIDs 50084803 & 50084804

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with German (2011) and OECD (2007) Good Laboratory Practice (GLP) standards, which are accepted by Regulatory Authorities in Europe, the United States and Japan, as well as the EC Guidance document on residue analytical methods (SANCO/825/00 rev. 8.1, 2010; pp. 3, 5; Appendix 2, p. 56 of MRID 50084803). The test/reference items were certified according to DIN ISO 9001 or DIN EN ISO/IEC 17025:2005. Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-5; Appendix 2, p. 56). The statement of authenticity was included with the QA statement.

ILV: The study was conducted in compliance with German (2013) and OECD (1998) GLP standards, which are accepted by Regulatory Authorities in Europe, the United States and Japan (pp. 3-4 of MRID 50084804). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4, 8). Statements of authenticity were included with the QA and GLP statement.

Classification: This analytical method and the Independent Laboratory Validation (ILV) are classified as **supplemental**. The communications between the ILV and study developers were not addressed. Without details of these communications the ILV of the ECM cannot be determined to have been independently conducted. In the ECM, the linearity of the method for thiophanate-methyl did not fully meet the $r^2 \geq 0.995$ standard. The linearity was above this standard for MBC in the ECM and for both thiophanate methyl and MBC in the ILV. The ECM used three diverse water matrices to validate the method, while the ILV only used one drinking water matrix.

PC Code: 102001

EFED Final Reviewer:	Dena Barrett, Senior Chemist	Signature: Date:	Dena Barrett 6/3/20
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist	Signature: Date:	Lisa Muto 8/14/17
	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature: Date:	Kathleen P. Ferguson 8/14/17

Executive Summary

The analytical method, PTRL Europe ID P 2681 G, is designed for the quantitative determination of thiophanate-methyl and its metabolite carbendazim (also known as MBC) in water at the stated LOQ of 0.05 µg/L for both compounds using HPLC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water identified in current Environmental Fate and Effects (EFED) risk assessments. The ECM used characterized ground, drinking and surface water matrices for the internal validation; the ILV used one characterized drinking water matrix (different from the ECM water source) for the independent validation. It could not be determined if the ILV was provided with the most difficult matrix or test set-up with which to validate the method. The method was validated for drinking water with insignificant analytical instrument and equipment modifications. The communications between the ILV and study developers were not addressed, consequently, the independence of the ILV cannot be confirmed. All ILV data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for thiophanate-methyl and carbendazim. All ECM data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for carbendazim in all three water matrices. All ECM data regarding repeatability, accuracy and precision were satisfactory for thiophanate-methyl in all three water matrices; the specificity data was fairly satisfactory, and the linearity data was below the $r^2 \geq 0.995$ standard.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Thiophanate-methyl	50084803[2]	50084804[3]		Water	24/09/2012	TM-MBC Task Force Non-Food Use Technical Committee Nisso America Inc.[4]	LC/MS/MS	0.05 µg/L
Carbendazim								

1 Thiophanate-methyl (TM) = Dimethyl 4,4′-(o-phenylene)bis(3-thioallophanate); Carbendazim (MBC) = Methyl benzimidazol-2-ylcarbamate.

2 In the ECM, drinking (tap) water (pH 7.72, total water hardness 2.43 mmol/L), obtained from a PTRL Europe laboratory tap in Ulm, Germany, ground (well) water (pH 7.91, total water hardness 3.04 mmo/L, total organic carbon 2.6 mg/L), obtained from Herbrechtingen, Germany, and surface (river) water (pH 7.96, total water hardness 1.27 mmol/L, total organic carbon 0.82 mg/L), obtained from Brenz River, Herbrechtingen, Germany, were characterized (non-GLP) and used in the study (p. 12 of MRID 50084803).

3 In the ILV, drinking water (pH 8.03, total hardness 0.87 mmol/L, total organic carbon 0.56 mg/L), obtained from the public water supply of the village of Schmberg, Kreiss Calw, Germany, was characterized (non-GLP) and used in the study (p. 15 of MRID 50084804).

4 A wholly owned subsidiary of Nippon Soda Co., Ltd.

I. Principle of the Method

Water (1.0 mL) in an autosampler vial was fortified with 0.010 mL of 5.0 or 50 ng/mL fortification solutions, if necessary (pp. 13, 15 of MRID 50084803). The sample was acidified with 10 µL of water containing 10% formic acid, then vortexed and analyzed by LC/MS/MS.

Samples are analyzed using a Applied Biosystems MDS Sciex API 5500 triple quadrupole mass spectrometer coupled with an Agilent 1200 SL HPLC (pp. 13, 15-16 of MRID 50084803). The following LC conditions were used: Agilent Zorbax column (4.6 mm x 75 mm, 3.5 µ; column temperature 35°C), gradient mobile phase of A) water containing 0.1% formic acid and B) acetonitrile containing 0.1% formic acid [time, percent A:B; 0.00 min. 80:20, 3.00-6.00 min. 0:100, 6.10-10.00 min. 80:20], injection volume of 100 µL, MS/MS with TurboIonspray (ESI) source in positive polarity (source temp. 600°C). Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 343→151 and *m/z* 343→192 for thiophanate-methyl and *m/z* 192→160 and *m/z* 192→132 for carbendazim. Approximate retention times were 4.6 minutes for thiophanate-methyl and 2.3 minutes for carbendazim.

The ILV performed the ECM method for each analyte as written, except for insignificant analytical instrument and equipment modifications (pp. 16-19 of MRID 50084804). The LC/MS/MS instrument was Dionex Ultimate coupled with an AB Sciex API 5500 QTrap mass spectrometer. An Agilent Zorbax Eclipse XDB-C8 column (4.6 mm x 150 mm, 5 µ) was used. All other LC/MS/MS parameters and the ion pair transitions were the same as those of the ECM, Approximate retention times were 5.1 minutes for thiophanate-methyl and 3.8 minutes for carbendazim.

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.05 µg/L for both analytes (pp. 9, 20; Tables 1-3, pp. 21-23 of MRID 50084803; pp. 11, 25 of MRID 50084804). The Limit of Detection (LOD) for both analytes was 0.01 µg/L in the ECM and 0.015 µg/L in the ILV.

II. Recovery Findings

ECM (MRID 50084803): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of thiophanate-methyl and its metabolite carbendazim at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ) in three water matrices (MRID 50084803, Tables 1-3, pp. 21-23). Two ion pair transitions were monitored, one quantitation and one confirmation; quantitation and confirmation recovery results were comparable, except for the LOQ thiophanate-methyl analyses which differed in all three matrices. The first sample of each set was injected twice, and the mean of those two injections was used for the recovery value. Drinking (tap) water (pH 7.72, total water hardness 2.43 mmol/L), obtained from a PTRL Europe laboratory tap in Ulm, Germany, ground (well) water (pH 7.91, total water hardness 3.04 mmo/L, total organic carbon 2.6 mg/L), obtained from Herbrechtingen, Germany, and surface (river) water (pH 7.96, total water hardness 1.27 mmol/L, total organic carbon 0.82 mg/L), obtained from Brenz River, Herbrechtingen, Germany, were characterized (non-GLP) and used in the study (p. 12).

ILV (MRID 50084804): Mean recoveries and RSDs were within guidelines for analysis of thiophanate-methyl and its metabolite carbendazim at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ) in one water matrix (MRID 50084804, p. 24). Two ion pair transitions were monitored, one quantitation and one confirmation; quantitation and confirmation recovery results were comparable. Drinking water (pH 8.03, total hardness 0.87 mmol/L, total organic carbon 0.56 mg/L), obtained from the public water supply of the village of Schmberg, Kreiss Calw, Germany, was characterized (non-GLP) and used in the study (i.e., a different water source was tested than for the analytical method in MRID 50084803). The method was apparently validated for drinking water in the first trial with insignificant analytical instrument and equipment modifications (MRID 50084803, pp. 11-12, 16-19, 24, 29).

Table 2. Initial Validation Method Recoveries for Thiophanate-methyl and Carbendazim in Water[1,2,3]

Analyte	Fortification Level (µg/L)	Number of Tests[4]	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[5]	Relative Standard Deviation (%)
	Drinking (Tap) Water					
	Quantitation Ion Transition					
Thiophanate-methyl	0.05 (LOQ)	5	88-107	98	8	8
	0.5	5	70-113	87	16	19
Carbendazim	0.05 (LOQ)	5	83-99	93	6	7
	0.5	5	94-98	95	2	2
	Confirmation Ion Transition					
Thiophanate-methyl	0.05 (LOQ)	5	74-109	92	13	14
	0.5	5	73-112	92	15	17
Carbendazim	0.05 (LOQ)	5	95-101	98	3	3
	0.5	5	97-102	99	2	2
	Ground (Well) Water					
	Quantitation Ion Transition					
Thiophanate-methyl	0.05 (LOQ)	5	91-111	102	9	9
	0.5	5	72-110	88	15	17
Carbendazim	0.05 (LOQ)	5	96-106	100	4	4
	0.5	5	100-103	102	1	1
	Confirmation Ion Transition					
Thiophanate-methyl	0.05 (LOQ)	5	72-111	90	14	16
	0.5	5	78-116	93	15	16
Carbendazim	0.05 (LOQ)	5	88-96	93	3	3
	0.5	5	99-100	100	1	1
	Surface (River) Water					
	Quantitation Ion Transition					
Thiophanate-methyl	0.05 (LOQ)	5	89-99	94	4	4
	0.5	5	69-110	86	15	18
Carbendazim	0.05 (LOQ)	5	97-106	100	4	3
	0.5	5	96-104	100	3	3
	Confirmation Ion Transition					
Thiophanate-methyl	0.05 (LOQ)	5	83-114	102	14	14
	0.5	5	78-126	96	19	19
Carbendazim	0.05 (LOQ)	5	92-108	98	7	7
	0.5	5	98-104	101	2	2

Data (uncorrected recovery results; pp. 16-17) were obtained from Tables 1-3, pp. 21-23 of MRID 50084803.

1 Thiophanate-methyl (TM) = Dimethyl 4,4′-(o-phenylene)bis(3-thioallophanate); Carbendazim (MBC) = Methyl benzimidazol-2-ylcarbamate.

2 Drinking (tap) water (pH 7.72, total water hardness 2.43 mmol/L), obtained from a PTRL Europe laboratory tap in Ulm, Germany, ground (well) water (pH 7.91, total water hardness 3.04 mmo/L, total organic carbon 2.6 mg/L), obtained from Herbrechtingen, Germany, and surface (river) water (pH 7.96, total water hardness 1.27 mmol/L, total organic carbon 0.82 mg/L), obtained from Brenz River, Herbrechtingen, Germany, were characterized (non-GLP) and used in the study (p. 12).

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 343→151 and *m/z* 343→192 for thiophanate-methyl and *m/z* 192→160 and *m/z* 192→132 for carbendazim.

4 The first sample of each set was injected twice, and the mean of those two injections was used for the recovery value.

5 Standard deviations were reviewer-calculated using the data in the study report since the study author did not report these values

Table 3. Independent Validation Method Recoveries for Thiophanate-methyl and Carbendazim in Water[1,2,3]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[4]	Relative Standard Deviation (%)
	Drinking Water					
	Quantitation ion					
Thiophanate-methyl	0.05 (LOQ)	5	102-106	105	2	1.7
	0.5	5	96-103	99	3	2.9
Carbendazim	0.05 (LOQ)	5	100-108	103	3	2.9
	0.5	5	98-101	99	1	1.2
	Confirmation ion					
Thiophanate-methyl	0.05 (LOQ)	5	106-114	109	3	2.8
	0.5	5	94-106	100	5	4.5
Carbendazim	0.05 (LOQ)	5	100-106	102	2	2.4
	0.5	5	99-102	100	1	1.2

Data (uncorrected recovery results; p. 20) were obtained from p. 24 of MRID 50084804.

1 Thiophanate-methyl (TM) = Dimethyl 4,4′-(o-phenylene)bis(3-thioallophanate); Carbendazim (MBC) = Methyl benzimidazol-2-ylcarbamate.

2 Drinking water (pH 8.03, total hardness 0.87 mmol/L, total organic carbon 0.56 mg/L), obtained from the public water supply of the village of Schmberg, Kreiss Calw, Germany, was characterized (non-GLP) and used in the study (p. 15).

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 343→151 and *m/z* 343→192 for thiophanate-methyl and *m/z* 192→160 and *m/z* 192→132 for carbendazim.

4 Standard deviations were reviewer-calculated using the data in the study report since the study author did not report these values. Rules of significant figures were followed.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.05 µg/L for both analytes (pp. 9, 18, 20; Tables 1-3, pp. 21-23 of MRID 50084803; pp. 11, 25 of MRID 50084804). In the ECM, no justification of the LOQ was reported. In the ILV, the LOQ was defined as the lowest fortification level at which acceptable recovery data was obtained, i.e. mean recoveries ranging 70% to 110% at a relative standard deviation of 20% and control blanks not exceeding 30% of the LOQ. The LOD for both analytes was 0.01 µg/L in the ECM and 0.015 µg/L in the ILV. In the ECM, the LOD was set to 20% of the LOQ. In the ILV, the LOD was 30% of the LOQ, as required by SANCO guideline for residues in control samples. In the ECM and ILV, no calculations based on standard deviations or background levels or further justification was provided for the LOQ or LOD.

Table 4. Method Characteristics Thiophanate-methyl and Carbendazim[1] in Water

		Thiophanate-methyl	Carbendazim
Limit of Quantitation (LOQ)		0.05 µg/L	
Limit of Detection (LOD)	ECM	0.01 µg/L (20% of the LOQ)	
	ILV	0.015 µg/L (30% of the LOQ)	
Linearity (calibration curve r^2 and concentration range)	ECM[2]	$r^2 = 0.9831$ (Q)[10] $r^2 = 0.9841$ (C)[10]	$r^2 = 0.9996$ (Q) $r^2 = 0.9920$ (C)[10]
		0.010-10 µg/mL	
	ILV[3]	$r^2 = 0.99970$ (Q) $r^2 = 0.99972$ (C)	$r^2 = 0.99992$ (Q) $r^2 = 0.99996$ (C)
		0.010-1 µg/L	
Repeatable	ECM[4]	Yes at LOQ and 10×LOQ in characterized ground, surface and drinking water matrices.	
	ILV[5,6]	Yes at LOQ and 10×LOQ in characterized drinking water matrix.	
Reproducible		Yes at LOQ and 10×LOQ.	
Specific	ECM	Q: Yes, matrix interferences were <4% of the LOQ based on peak area. The LOQ peak was well resolved, but high baseline noise (5% of the LOQ) caused interference with peak integration in one of the three matrices. C: Matrix interferences were <12% of the LOQ based on peak area in the surface and drinking water matrices, but *ca*. 22.5% of the LOQ[7] based on peak area in ground water. Additionally, the reviewer noted that the LOQ peak was surrounded by background contaminants which were equal to or greater than the LOQ peak height, interfering with peak integration.[8,9]	Yes, matrix interferences were <3% of the LOQ based on peak area. Some minor non-uniform LOQ integration was noted, mainly in the C chromatograms.
	ILV	Yes, matrix interferences were <5% of the LOQ based on peak height (peak areas not reported); however, the reviewer noted that the LOQ C peak was only 2xs the height of background contaminants.[9]	Yes, matrix interferences were <5% of the LOQ based on peak height (peak areas not reported).

Q = quantitation ion; C = confirmation ion.

ECM data were obtained from MRID 50084803:

Recovery results in Tables 1-3 (pp. 21-23)
Calibration curves in Figure 1 (p. 26) and Figure 3 (p. 28)
Chromatograms in Figures 9-26 (pp. 34-51)

ILV data were obtained from MRID 50084804:

Recovery results on pp. 22, 24, and 28
Calibration curves in Appendix 3 (pp. 34-37)
Chromatograms in Appendix 4 (pp. 40-42).

1 Thiophanate-methyl (TM) = Dimethyl 4,4′-(o-phenylene)bis(3-thioallophanate); Carbendazim (MBC) = Methyl benzimidazol-2-ylcarbamate.

2 Correlation coefficient (r^2) was reviewer-calculated based on the r value (1/x weighted linear regression analysis) reported in the study report; solvent standards were used (p. 14; Figure 1, p. 26; Figure 3, p. 28 of MRID 50084803).

3 Correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis)

reported in the study report; solvent standards were used (pp. 22, 28; Appendix 3, pp. 34-37 of MRID 50084804).

4 In the ECM, drinking (tap) water (pH 7.72, total water hardness 2.43 mmol/L), obtained from a PTRL Europe laboratory tap in Ulm, Germany, ground (well) water (pH 7.91, total water hardness 3.04 mmo/L, total organic carbon 2.6 mg/L), obtained from Herbrechtingen, Germany, and surface (river) water (pH 7.96, total water hardness 1.27 mmol/L, total organic carbon 0.82 mg/L), obtained from Brenz River, Herbrechtingen, Germany, were characterized (non-GLP) and used in the study (p. 12 of MRID 50084803).

5 In the ILV, drinking water (pH 8.03, total hardness 0.87 mmol/L, total organic carbon 0.56 mg/L), obtained from the public water supply of the village of Schmberg, Kreiss Calw, Germany, was characterized (non-GLP) and used in the study (p. 15 of MRID 50084804).

6 Although the number of trials was not specified, the reviewer assumed that the method was validated for drinking water in the first trial with insignificant analytical instrument and equipment modifications (pp. 11-12, 16-19, 24, 29 of MRID 50084804).

7 Based on Figure 17, p. 42 and Figure 19, p. 44 of MRID 50084803.

8 Based on Figure 11, p. 36; Figure 17, p. 42; Figure 23, p. 48 of MRID 50084803.

9 A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

10 Values in **red** represent calibrations with coefficient of determination (r^2) <0.995.

IV. Method Deficiencies and Reviewer's Comments

1. The ILV did not reported if communications between the ILV and study developers occurred during the conduct of the study. The amount, type and timing of communications between the ILV laboratory personnel and the ECM study personnel should be reported to ensure that the ILV was conducted independently of the ECM.

2. In the ECM, the linearity of the method was slightly below the standard of greater than or equal 0.995 for the coefficient of determination for thiophanate-methyl (r^2 = 0.9831-0.9841) and the confirmation ion transition of carbendazim (r^2 = 0.9920); See MRID 50084803, p. 26 (Figure 1) and p. 28 (Figure 3). Linearity is satisfactory when $r^2 \geq 0.995$. A confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data.

3. All water matrices of the ECM and ILV were characterized; however, the ECM used three water matrices (drinking, ground and surface) to validate the method, while the ILV only used one water matrix (drinking, with a different source than the ECM) to validate the method. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. Also, the ILV should validate the method using means which are as or more rigorous than those used by the ECM.

4. In the ILV representative chromatograms of thiophanate-methyl, the LOQ peak was only two times the height of background contaminants (MRID 50084804, Appendix 4, pp. 40-42). A confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data.

5. The determinations of LOD and LOQ in the ECM and ILV were not fully based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 9, 18, 20; Tables 1-3, pp. 21-23 of MRID 50084803; pp. 11, 25 of MRID 50084804). In the ECM, no justification of the LOQ was reported. In the ILV, the LOQ was defined as the lowest fortification level at which acceptable recovery data was obtained, i.e. mean recoveries ranging 70% to 110% at a relative standard deviation of 20% and control blanks not exceeding 30% of the LOQ. The LOD of the ECM and ILV differed. In the ECM, the LOD was set to 20% of the LOQ. In the ILV, the

LOD was 30% of the LOQ, as required by SANCO guideline for residues in control samples. In the ECM and ILV, no calculations based on standard deviations or background levels or further justification was provided for the LOQ or LOD. The performance of the method, however, was generally within guidelines, see Comment # 6.

6. All ILV data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for thiophanate-methyl and carbendazim. All ECM data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for carbendazim in all three water matrices. All ECM data regarding repeatability, accuracy and precision were satisfactory for thiophanate-methyl in all three water matrices; the specificity data was fairly satisfactory, and the linearity data was below the $r^2 \geq 0.995$ standard (as low as 0.9.831).

7. In the representative chromatograms, matrix interferences were <12% of the LOQ based on peak area in the surface and drinking water matrices, but *ca*. 22.5% of the LOQ based on peak area in ground water (Figures 9-26, pp. 34-51 of MRID 50084803). Additionally, this reviewer observed that the LOQ peak was surrounded by background contaminants which were equal to or greater than the LOQ peak height, interfering with peak integration. There was variation in the quantitation and confirmation recovery results: for drinking water the quantitation ion was 98 ± 8 and the confirmation ion was 92 ± 14; for ground water the quantitation ion was 102 ± 9 and the confirmation ion was 90 ± 16; and for surface water the quantitation ion was 94 ± 4 and the confirmation ion was 102 ± 14 (mean ± RSD; Tables 1-3, pp. 21-23). However, a confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data. Therefore, the deficiencies of the LOQ confirmation ion analysis of thiophanate-methyl did not affect the acceptability of the method. The ECM study author reported that no apparent response greater than 20% of the LOQ was observed in the control samples (MRID 50084803, p. 18).

8. Matrix effects were studied in the ECM and ILV and found to be insignificant (<20%; p. 19 of MRID 50084803; pp. 22, 26-28 of MRID 50084804). Solvent-based calibration standards were used for quantification of the residues.

9. The stability of the standard solutions and sample extracts was investigated by the ECM (p. 14; Tables 4-5, pp. 24-25 of MRID 50084803). The standard solutions were found to be stable under refrigerated storage for seven days (deviation <15%). The sample extracts were found to be stable under refrigerated storage for four days (recovery range 69-104% for both analytes in all three matrices).

10. In the ECM, the total time required to perform the method (extraction and analysis) with one sample set was *ca*. 1 calendar day (p. 19 of MRID 50084803). One set of 12 samples (two matrix controls and ten fortified samples) required *ca*. 2 hours (sample processing), *ca*. 4 hours (analysis) and *ca*. 2 hours (data processing). The time requirement for the method was not reported in the ILV.

V. References

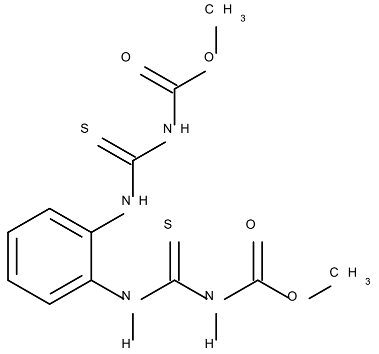
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Attachment 1: Chemical Names and Structures

Thiophanate-methyl

IUPAC Name: Dimethyl 4,4′-(o-phenylene)bis(3-thioallophanate)
Dimethyl N,N′-[1,2-phenylenebis(iminocarbonothioyl)]bis[carbamate]
CAS Name: C,C’-dimethyl ester-N,N’-[1,2-phenylenebis(iminocarbonthioyl)biscarbamic acid
CAS Number: 23564-05-8
SMILES String: COC(=O)NC(=S)Nc1ccccc1NC(=S)NC(=O)OC



Carbendazim (MBC)

IUPAC Name: Methyl benzimidazol-2-ylcarbamate
CAS Name: Methyl 1H-benzimidazol-2-ylcarbamate
Methyl ester 2-benzimidazolecarbamic acid
CAS Number: 10605-21-7
SMILES String: COC(=O)Nc1nc2ccccc2[nH]1