

Analytical method for methiozolin and 2,6-difluorobenzyl alcohol in water

Reports: ECM: EPA MRID No. 50446912. MacGregor, J.A., R. VanHoven. 2017. ANALYTICAL METHOD VALIDATION FOR THE DETERMINATION OF METHIOZOLIN AND 2,6-DIFLUOROBENZYL ALCOHOL (DFB ALCOHOL) IN SURFACE AND GROUND WATER. Wildlife International Project No.: 716C-106. Report prepared by Wildlife International, now doing business as EAG Laboratories, Easton, Maryland, sponsored by Moghu Research Center, Daejeon, Korea, and submitted by toXcel, LLC, Gainesville, Virginia, and Moghu Research Center, Daejeon, Korea; 100 pages. Final report issued July 31, 2017.

ILV: EPA MRID No. 50446918. Mannella, L. 2017. Independent Laboratory Validation of an Environmental Chemistry Method for the Determination of Methiozolin and 2,6-Difluorobenzyl Alcohol (DFB) in Surface and Ground Water. EAG Project No.: 2892W. Report prepared by EAG Laboratories-Hercules, Hercules, California, sponsored and submitted by Moghu Research Center, Daejeon, Korea; 100 pages. Final report issued November 17, 2017.

Document No.: MRIDs 50446912 & 50446918

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 50446912). 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.

ILV: The study was conducted in accordance with USEPA GLP standards, except that the certification of the test materials was not specified whether analyses were conducted under GLP (p. 3 of MRID 50446918). 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 quality assurance statement (p. 4).

Classification: This analytical method is classified as supplemental. One percent recovery in the extraction procedure of 2,6-difluorobenzyl alcohol (DFB alcohol) alcohol in was not within guideline requirements (mean 158%, relative standard deviation 24.2%). Additionally, the environmental chemistry method (ECM) and independent laboratory validation (ILV) used different equipment (gas chromatograph/mass selective detector, GC/MS versus liquid chromatography, LC/MS/MS) and different ions for confirmation of the presence of DFBA alcohol. The number of trials required to validate the method was not reported by the ILV.

PC Code: 090088

EFED Final Reviewer: Taimei Harris, Ph.D.,
Fate Scientist

Signature:
Date:

CDM/CSS-Dynamac JV Reviewers: Lisa Muto, M.S.
Environmental Scientist

Signature:



Date: 7/16/18

Joan Gaidos, Ph.D.,
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Date: 7/16/18

EPA Reviewer: Katrina White, Ph.D., Senior Scientist

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, **Wildlife International Project No. 716C-106**, is designed for the quantitative determination of methiozolin in water at the LOQ of 0.05 µg/L via direct injection of water to HPLC/MS/MS and of 2,6-difluorobenzyl alcohol (DFB alcohol) in water at the LOQ of 5.0 µg/L using a liquid-liquid extraction with DCM followed by GC-MSD. The respective LOQs are less than the lowest toxicological level of concern in water for methiozolin and DFB alcohol. The ECM and ILV was performed using different characterized ground, and surface water matrices. Analytes were identified using two ion transitions or ion fragments. The number of trials was not reported in the ILV. However, communications between the ILV and the Study Sponsor, the reviewer determined that the method for methiozolin and DFB alcohol was validated in the first trial with the modification of the confirmation ion fragment for DFB alcohol to m/z 143 and the use LC/MS/MS in the ILV versus GC/MSD for the ECM. Based on quantitation analysis, all ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory.

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						
Methiozolin	50446912	50446918		Water ^{1,2}	31/07/2017	toXcel, LLC, and Moghu Research Center	LC/MS/MS	0.05 µg/L
2,6-Difluorobenzyl alcohol (DFB alcohol)							GC/MS	5.0 µg/L

1 In the ECM, surface water (pH 7.0, specific conductance 188 µS/cm, hardness 64.0 mg/L as CaCO₃, alkalinity 30.0 mg/L as CaCO₃) and ground water (pH 8.1, specific conductance 341-363 µS/cm, hardness 144-145 mg/L as CaCO₃, alkalinity 181 mg/L as CaCO₃) were used in the study (p. 14; Appendices IV-V, pp. 98-99 of MRID 50446912). The ground water was sourced from a well on the EAG Laboratories' site in Easton, Maryland. The surface water was sourced from Tuckahoe Lake in Ridgely, Maryland.

2 In the ILV, surface water (2706W-085; pH 6.7, conductivity 0.22 mmhos/cm, hardness 66 mg/L as CaCO₃, alkalinity 37 mg/L as CaCO₃, 222 ppm total dissolved solids) and ground water (pH 7.7, conductivity 1.21 mmhos/cm, hardness 691 mg/L as CaCO₃, alkalinity 297 mg/L as CaCO₃, 1016 ppm total dissolved solids) were used in the study and characterized by Agvise Laboratories, Northwood, North Dakota (p. 21; Appendix C, pp. 89-90 of MRID 50446918). The ground water was sourced from a well in Northwood, North Dakota. The surface water was sourced from Brandywine Creek, Chadds Ford, Pennsylvania.

I. Principle of the Method

Methiozolin

Water samples (*ca.* 5 mL) were fortified with 0.05 mL of methiozolin fortification solution in 10.0 mL volumetric flasks (pp. 13, 20; Figure 1, p. 43 of MRID 50446912). The total volume was adjusted to 10 mL using the water matrix. A 2-mL aliquot of the water samples were diluted 2X with 2 mL of acetonitrile into 15-mL tubes then filtered via syringe and Whatman 0.2 µm Puradisk 25 TF (PTFE) syringe filter. An aliquot was transferred to an autosampler vial for analysis by LC/MS/MS.

Water samples were analyzed for methiozolin using an Agilent 1200 Infinity Series HPLC system coupled to an Applied Biosystems/MDS Sciex API 5000 mass spectrometer with a Turbo-Ion Spray source (pp. 20-21; Table 1, p. 33 of MRID 50446912). The LC/MS conditions consisted of a Thermo EC Betasil C18 column (2.1 x 50 mm, 5-µm; column temperature 40°C), Thermo EC Javelin Betasil C18 guard column (10 mm x 2.1 mm), a gradient mobile phase of (A) 0.1% formic acid in HPLC grade water and (B) 0.1% formic acid in acetonitrile [percent A:B (v:v) at 0.00-1.00 min. 50.0:50.0, 4.00-5.00 min. 5.00:95.0, 5.10-8.00 min. 50.0:50.0] and MS/MS detection in positive ion mode (ionization temperature 400.00°C). Injection volume was 25 µL. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 338→127 and *m/z* 338→211. Retention time was *ca.* 4.8 minutes for methiozolin.

2,6-Difluorobenzyl alcohol (DFB alcohol)

Water samples (10.0 mL) were fortified with 0.05 mL of DFB alcohol fortification solution in 50 mL graduated tubes (pp. 13, 21-23; Figure 2, p. 44 of MRID 50446912). The samples were extracted with 5.00 mL of dichloromethane (DCM) via swirling by hand, shaking on a SPEX GenoGrinder sample processor for *ca.* 2 minutes at a setting of 1250 rpm. After centrifugation (*ca.* 5 minutes at *ca.* 400 rpm), the DCM layer was removed via disposable pipet, and an aliquot was transferred to an autosampler vial for analysis by GC/MS.

Water samples were analyzed for DFB alcohol using an Agilent HP6890N gas chromatograph (Agilent DB-624 column, 0.25 mm x 30 m, 1.4 μ m thickness) using a column temperature program (60°C for 1.00 min., 60°C to 250°C at 20.0°C/min.) and helium carrier gas coupled with an Agilent 5975 inert mass selective detector (source temperature 300°C) using electron ionization in Single Ion Monitoring (SIM) mode (Table 2, p. 34 of MRID 50446912). Injection volume was 1.00 μ L (splitless). Two ions were monitored as follows (quantitation and confirmation, respectively): *m/z* 144 and 123. Retention time was *ca.* 7.5 minutes for DFB alcohol.

ILV

In the ILV, the ECM was performed as written, except for the modification of the confirmation ion fragment for DFB alcohol to *m/z* 143 and the use of a different analytical systems and equipment (pp. 20-33 of MRID 50446918). The LC/MS/MS system was an Agilent 1260 Series HPLC system coupled to an AB Sciex API 5500 mass spectrometer equipped with a Phenomenex Gemini C18 column (50 mm x 2 mm, 3 μ m) and Phenomenex AJO-7596 C18 guard column (4 mm x 2 mm). Other LC/MS/MS parameters were the same as the ECM. The GC/MS system was an Agilent 6890 gas chromatograph coupled to an Agilent 5975 inert mass selective detector; other parameters were the same as the ECM. Similar ion transitions and ion fragments were monitored as in the ECM other than previously mentioned. Retention time was *ca.* 4.7 minutes for methiozolin (LC/MS/MS) and *ca.* 7.5 DFB alcohol (GC/MS). No other modifications to the ECM were reported.

In the ECM and ILV, the Limit of Quantification (LOQ) in water was 0.0500 μ g/L for methiozolin and 5.00 μ g/L for DFB alcohol (pp. 13, 23-24 of MRID 50446912; pp. 12-13 of MRID 50446918). In the ECM and ILV, the Limit of Detection (LOD) in water was 0.0200 μ g/L for methiozolin and 1.25 μ g/L for DFB alcohol. In the ECM, LOD for determination of methiozolin and DFB alcohol in water were calculated using the standard deviation from the LOQ recovery results. The calculated values support the LOQ and LOD established for the study.

II. Recovery Findings

ECM (MRID 50446912): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of methiozolin in water matrices at the fortification level of 0.050 μ g/L (LOQ) and 0.500 μ g/L (10 \times LOQ) using LC/MS/MS (Tables 3-10, pp. 35-42). Mean recoveries and RSDs were within guideline requirements for analysis of 2,6-difluorobenzyl alcohol (DFB alcohol) in water matrices at the

fortification level of 5.0 µg/L (LOQ) and 50 µg/L (10×LOQ) using GC/MS, except for the LOQ confirmation analysis in surface water (mean 158%, RSD 24.2%). The high and variable recoveries obtained were attributed to the degree of surface water background interferences observed in the retention time region of the DFB Alcohol analyte at the LOQ and were directly related to the sensitivity and selectivity limitations associated with the secondary confirmation analysis method. Two ion transitions or ion fragments were quantified; performance data (recovery results) from primary and confirmatory analyses were comparable, except for the LOQ analysis of DFB alcohol in surface water. The surface water (pH 7.0, specific conductance 188 µS/cm, hardness 64.0 mg/L as CaCO₃, alkalinity 30.0 mg/L as CaCO₃) and ground water (pH 8.1, specific conductance 341-363 µS/cm, hardness 144-145 mg/L as CaCO₃, alkalinity 181 mg/L as CaCO₃) were used in the study (p. 14; Appendices IV-V, pp. 98-99). The ground water was sourced from a well on the EAG Laboratories' site in Easton, Maryland. The surface water was sourced from Tuckahoe Lake in Ridgely, Maryland.

ILV (MRID 50446918): Mean recoveries and RSDs were within guideline requirements for analysis of methiozolin in water matrices at the fortification level of 0.050 µg/L (LOQ) and 0.500 µg/L (10×LOQ) using LC/MS/MS (Summary Tables I-II, pp. 14-17). Mean recoveries and RSDs were within guideline requirements for analysis of DFB alcohol in water matrices at the fortification level of 5.0 µg/L (LOQ) and 50 µg/L (10×LOQ) using GC/MS. Two ion transitions or ion fragments were quantified; performance data (recovery results) from primary and confirmatory analyses were comparable. The surface water (2706W-085; pH 6.7, conductivity 0.22 mmhos/cm, hardness 66 mg/L as CaCO₃, alkalinity 37 mg/L as CaCO₃, 222 ppm total dissolved solids) and ground water (pH 7.7, conductivity 1.21 mmhos/cm, hardness 691 mg/L as CaCO₃, alkalinity 297 mg/L as CaCO₃, 1016 ppm total dissolved solids) were used in the study and characterized by Agvise Laboratories, Northwood, North Dakota (p. 21; Appendix C, pp. 89-90). The ground water was sourced from a well in Northwood, North Dakota. The surface water was sourced from Brandywine Creek, Chadds Ford, Pennsylvania. The number of trials was not reported in the ILV, but, based on the communications between the ILV and the Sponsor, the reviewer determined that the method for methiozolin and DFB alcohol was validated in the first trial with insignificant modifications of the modification of the confirmation ion fragment for DFB alcohol to *m/z* 143 and the use of a different analytical systems and equipment (pp. 37-38).

Table 2. Initial Validation Method Recoveries for Methiozolin and 2,6-Difluorobenzyl Alcohol (DFB alcohol) in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface (Lake) Water						
Quantitation Ion Transition/Ion Fragment						
Methiozolin	0.050 (LOQ)	5	91.3-98.7	94.4	2.75	2.91
	0.50	5	98.4-103	102	2.23	2.19
DFB alcohol	5.0 (LOQ)	5	95.7-98.4	97.3	1.11	1.14
	50	5	92.8-100	96.8	2.91	3.01
Confirmation Ion Transition/Ion Fragment						
Methiozolin	0.050 (LOQ)	5	98.1-101	99.4	1.09	1.10
	0.50	5	98.3-106	103	3.10	3.01
DFB alcohol	5.0 (LOQ)	5	92.7-187	158	38.2	24.2

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	50	5	70.2-100	91.9	12.4	13.5
Ground (Well) Water						
Quantitation Ion Transition/Ion Fragment						
Methiozolin	0.050 (LOQ)	5	90.7-97.8	92.7	3.19	3.42
	0.50	5	91.6-100	95.7	3.50	3.66
DFB alcohol	5.0 (LOQ)	5	100-105	103	1.92	1.86
	50	5	90.6-96.8	93.5	2.49	2.66
Confirmation Ion Transition/Ion Fragment						
Methiozolin	0.050 (LOQ)	5	93.9-102	97.3	3.12	3.21
	0.50	5	96.0-106	101	3.81	3.77
DFB alcohol	5.0 (LOQ)	5	102-124	112	8.14	7.27
	50	5	85.0-109	102	9.53	9.34

Data (uncorrected recovery results, pp. 24-28) were obtained from Tables 3-10, pp. 35-42 of MRID 50446912.

1 For methiozolin, two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 338→127 and m/z 338→211. For DFB alcohol, two ions were monitored as follows (quantitation and confirmation, respectively): m/z 144 and 123.

2 The surface water (pH 7.0, specific conductance 188 µS/cm, hardness 64.0 mg/L as CaCO₃, alkalinity 30.0 mg/L as CaCO₃) and ground water (pH 8.1, specific conductance 341-363 µS/cm, hardness 144-145 mg/L as CaCO₃, alkalinity 181 mg/L as CaCO₃) were used in the study (p. 14; Appendices IV-V, pp. 98-99). The ground water was sourced from a well on the EAG Laboratories' site in Easton, Maryland. The surface water was sourced from Tuckahoe Lake in Ridgely, Maryland.

Table 3. Independent Validation Method Recoveries for Methiozolin and 2,6-Difluorobenzyl Alcohol (DFB alcohol) in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface (Creek) Water						
Quantitation Ion Transition/Ion Fragment						
Methiozolin	0.050 (LOQ)	5	92-110	101	8	8
	0.50	5	94-102	97	3	3
DFB alcohol	5.0 (LOQ)	5	108-110	109	1	1
	50	5	78-105	97	11	11
Confirmation Ion Transition/Ion Fragment						
Methiozolin	0.050 (LOQ)	5	93-107	100	6	6
	0.50	5	94-102	98	3	3
DFB alcohol	5.0 (LOQ)	5	104-110	107	2	2
	50	5	77-105	96	11	11
Ground (Well) Water						
Quantitation Ion Transition/Ion Fragment						
Methiozolin	0.050 (LOQ)	5	93-97	94	2	2
	0.50	5	87-96	92	4	4
DFB alcohol	5.0 (LOQ)	5	96-108	102	5	5
	50	5	85-95	90	5	6
Confirmation Ion Transition/Ion Fragment						
Methiozolin	0.050 (LOQ)	5	93-98	95	2	2
	0.50	5	87-96	92	4	4

Analyte	Fortification Level ($\mu\text{g/L}$)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
DFB alcohol	5.0 (LOQ)	5	98-114	106	7	7
	50	5	87-95	90	3	3

Data (uncorrected recovery results, pp. 33-35, Appendices D-F, pp. 91-100) were obtained from Summary Tables I-II, pp. 14-17 of MRID 50446918.

- For methiozolin, two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: m/z 338 \rightarrow 127 and m/z 338 \rightarrow 211. For DFB alcohol, two ions were monitored as follows (quantitation and confirmation, respectively): m/z 144 and 143.
- The surface water (2706W-085; pH 6.7, conductivity 0.22 mmhos/cm, hardness 66 mg/L as CaCO_3 , alkalinity 37 mg/L as CaCO_3 , 222 ppm total dissolved solids) and ground water (pH 7.7, conductivity 1.21 mmhos/cm, hardness 691 mg/L as CaCO_3 , alkalinity 297 mg/L as CaCO_3 , 1016 ppm total dissolved solids) were used in the study and characterized by Agvise Laboratories, Northwood, North Dakota (p. 21; Appendix C, pp. 89-90). The ground water was sourced from a well in Northwood, North Dakota. The surface water was sourced from Brandywine Creek, Chadds Ford, Pennsylvania.

III. Method Characteristics

In the ECM and ILV, the LOQ in water was 0.0500 $\mu\text{g/L}$ for methiozolin and 5.00 $\mu\text{g/L}$ for DFB alcohol (pp. 13, 23-24 of MRID 50446912; pp. 12-13, 36 of MRID 50446918). In the ECM, the LOQ was defined as the lowest level fortified and analyzed during each validation set. No justifications or calculations were provided to support the LOQ in the ILV. In the ECM and ILV, the LOD in water was 0.0200 $\mu\text{g/L}$ for methiozolin and 1.25 $\mu\text{g/L}$ for DFB alcohol. In the ECM, the theoretical LOD was defined as the product of the lowest calibration standard analyzed and the dilution factor of the blank and LOQ sample. In the ECM, LOD for determination of methiozolin and DFB alcohol in water were calculated using the standard deviation from the respective LOQ recovery results. The LOD was calculated as the standard deviation multiplied by the t-statistic (3.747). In the ECM, the LOD was calculated as 0.0013725-0.0016002 $\mu\text{g/L}$ and 0.211-0.333 $\mu\text{g/L}$, respectively, for methiozolin and DFB alcohol (matrices combined). In the ILV, the LOD was defined as 40% of the LOQ for methiozolin and 25% of the LOQ for DFB alcohol. The calculated values support the LOQ and LOD established for the study.

Table 4. Method Characteristics

Analyte		Methiozolin		2,6-Difluorobenzyl Alcohol (DFB alcohol)
Analysis		LC/MS/MS		GC/MS
Limit of Quantitation (LOQ)	ECM	0.050 µg/L		5.00 µg/L
	ILV			
Limit of Detection (LOD)	ECM	Method	0.0200 µg/L	1.25 µg/L
		Calculated ¹	0.0013725-0.0016002 µg/L	0.211-0.333 µg/L
	ILV	Method	0.0200 µg/L	1.25 µg/L
		Calculated	Not calculated	
Linearity (calibration curve r^2 and concentration range) ²	ECM	Surface	$r^2 = 0.9987$ (Q) ²	$r^2 = 0.9942$ (Q)
		Ground	$r^2 = 0.9991$ (C) ²	$r^2 = 0.9972$ (C)
	ILV	Surface	$r^2 = 0.9998$ (Q & C)	$r^2 = 0.9974$ (Q)
		Ground	$r^2 = 0.9986$ (Q)	$r^2 = 0.9976$ (C)
			$r^2 = 0.9992$ (C)	$r^2 = 0.9986$ (Q)
	Concentration Range		0.0100-0.500 µg/L	2.50-50.0 µg/L
Repeatable	ECM ³	Yes at LOQ and 10×LOQ in two characterized water matrices.		Yes at LOQ and 10×LOQ in two characterized water matrices, but LOQ C mean 158% and RSD 24.2% in one water matrix. ⁴
	ILV ^{5,6}	Yes at LOQ and 10×LOQ in two characterized water matrices.		
Reproducible		Yes at LOQ and 10×LOQ.		
Specific	ECM	Yes, no matrix interferences were observed.		Yes, no matrix interferences were observed. C matrix interferences were <i>ca.</i> 36-50% of the LOQ (based on peak area). ⁴
	ILV	Yes, matrix interferences were <3% of the LOQ (based on peak area). Minor baseline noise (<10% of the LOQ, based on peak height) interfered with peak attenuation and integration in surface water.		Yes, no matrix interferences were observed. Some minor baseline noise was observed.

Data were obtained from pp. 13, 23-24, 28-29; Tables 3-10, pp. 35-42 (recovery data); Figures 3 p. 45 and Appendix III, pp. 94-97 (calibration curves); Figures 11-30, pp. 53-72 (chromatograms) of MRID 50446912; pp. 12-13, 36; Tables 2-5, pp. 19-20 (correlation coefficients); Summary Tables I-II, pp. 14-17 (recovery data); Figure 5, pp. 57-60 and Figure 18, pp. 72-73 (calibration curves); Figures 1-4, pp. 53-56 ; Figures 7-12, pp. 61-76; Figures 14-17, pp. 68-71 and Figures 19-23, pp. 74-78 (chromatograms) of MRID 50446918; DER Attachment 2. Q = Quantitation ion transition/fragment; C = Confirmatory ion transition/fragment.

1 Matrices combined.

2 Reported ECM correlation coefficients (r^2) for methiozolin were reviewer-calculated based on the reported r values in the study report (Figure 3, p. 45 of MRID 50446912; DER Attachment 2). Solvent calibration standards were used. The reviewer only reported values up to 4 significant figures even though 7 significant figures were reported in the study report.

3 In the ECM, surface water (pH 7.0, specific conductance 188 µS/cm, hardness 64.0 mg/L as CaCO₃, alkalinity 30.0 mg/L as CaCO₃) and ground water (pH 8.1, specific conductance 341-363 µS/cm, hardness 144-145 mg/L as CaCO₃, alkalinity 181 mg/L as CaCO₃) were used in the study (p. 14; Appendices IV-V, pp. 98-99 of MRID 50446912). The ground water was sourced from a well on the EAG Laboratories' site in Easton, Maryland. The surface water was sourced from Tuckahoe Lake in Ridgely, Maryland.

- 4 A confirmatory method is not usually required when LC/MS or GC/MS is the primary method(s) to generate study data.
- 5 In the ILV, surface water (2706W-085; pH 6.7, conductivity 0.22 mmhos/cm, hardness 66 mg/L as CaCO₃, alkalinity 37 mg/L as CaCO₃, 222 ppm total dissolved solids) and ground water (pH 7.7, conductivity 1.21 mmhos/cm, hardness 691 mg/L as CaCO₃, alkalinity 297 mg/L as CaCO₃, 1016 ppm total dissolved solids) were used in the study and characterized by Agvise Laboratories, Northwood, North Dakota (p. 21; Appendix C, pp. 89-90 of MRID 50446918). The ground water was sourced from a well in Northwood, North Dakota. The surface water was sourced from Brandywine Creek, Chadds Ford, Pennsylvania.
- 6 The number of trials was not reported in the ILV, but, based on the communications between the ILV and the Sponsor, the reviewer determined that the method for methiozolin and DFB alcohol was validated in the first trial with insignificant modifications of the modification of the confirmation ion fragment for DFB alcohol to *m/z* 143 and the use of a different analytical systems and equipment (pp. 37-38 of MRID 50446918).

IV. Method Deficiencies and Reviewer's Comments

1. The number of trials required to validate the method were not reported in the ILV. Based on the communications between the ILV and the Sponsor, the reviewer determined that the method for methiozolin and DFB alcohol was validated in the first trial with the modification of the confirmation ion fragment for DFB alcohol to *m/z* 143 and the use of LC/MS/MS for the ILV versus GC/MSD for the ECM (pp. 37-38 of MRID 50446918). In the communications, the sample extracts were re-injected for DFB alcohol analysis after the modified monitored ion fragment. Re-injections of the same sample extracts are generally considered to be within the same trial. Since the communications did not indicate any additional trials, the reviewer assumed that only one trial was performed.
2. ECM performance data was not satisfactory for the LOQ confirmation analysis of DFB alcohol in surface water (mean 158%, RSD 24.2%). Tables 3-10, pp. 35-42 of MRID 50446918). OCSPP guideline requirements state that the mean recovery is 70-120% and the RSD is $\leq 20\%$.

ECM representative chromatograms for the confirmation ion analysis of DFB alcohol did not support the specificity of the method since C matrix interferences were *ca.* 36-50% of the LOQ (based on peak area; Figures 11-30, pp. 53-72 of MRID 50446912).

The reviewer did not consider these guideline deviations to be significant since 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. In the ILV, the matrix effects were found to be insignificant ($<20\%$) for methiozolin and DFB alcohol in surface water, but significant in ground water (pp. 39-40, 42; Tables I-II, pp. 45-46; Tables V-VI, pp. 49-50 of MRID 50446918). The matrix interferences were $<3\%$ of the LOQ (based on peak area). Minor baseline noise ($<10\%$ of the LOQ, based on peak height) interfered with peak attenuation and integration in surface water. However, the matrix effects were found to be insignificant ($<20\%$) for DFB alcohol in both matrices.

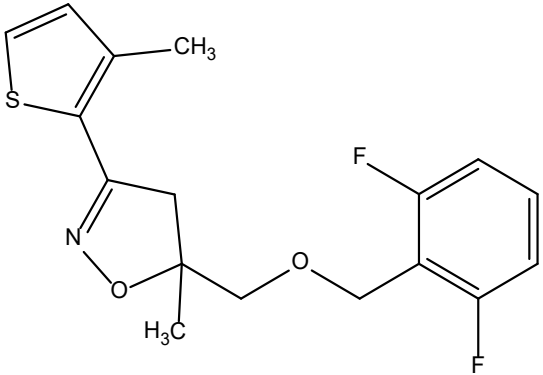
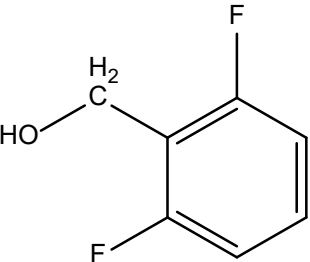
4. The ILV study author summarized communications between the ILV and Study Sponsor (Moghu Research Center; pp. 37-38 of MRID 50446918). Communications involved the Study Sponsor's acceptance of the ILV modifications which were initiated by the ILV.
5. The determinations of LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 13, 23-24 of MRID 50446912; pp. 12-13, 36 of MRID 50446918). In the ECM, the LOQ was defined as the lowest level fortified and analyzed during each validation set. No justifications or calculations were provided to support the LOQ in the ILV. In the ECM, the theoretical LOD was defined as the product of the lowest calibration standard analyzed and the dilution factor of the blank and LOQ sample. In the ECM, LOD for determination of methiozolin and DFB alcohol in water were calculated using the standard deviation from the respective LOQ recovery results. The LOD was calculated as the standard deviation multiplied by the t-statistic (3.747). In the ILV, the LOD was defined as 40% of the LOQ for methiozolin and 25% of the LOQ for DFB alcohol. The calculated values support the LOQ and LOD established for the study. Detection limits should not be based on arbitrary values.
6. The time required to perform the method was not reported in the ECM or 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.

DER ATTACHMENT 1.

Table 1. Methiozolin and Its Environmental Transformation Products. ^A

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
PARENT						
Methiozolin (MRC-01)	<p>IUPAC: (5RS)-5-[(2,6-difluorobenzyloxy)methyl]-4,5-dihydro-5-methyl-3-(3-methyl-2-thienyl)-1,2-oxazole</p> <p>CAS: 5-[[[2,6-difluorophenyl)methoxy]methyl]-4,5-dihydro-5-methyl-3-(3-methyl-2-thienyl)isoxazole</p> <p>CAS No.: 403640-27-7</p> <p>Formula: C₁₇H₁₇F₂NO₂S</p> <p>MW: 337.38 g/mol</p> <p>SMILES: Cc1ccsc1C2=NOC(C)(C)COCc3c(cccc3F)F</p>		850.6100	50446912 +	PRT	PRT
TRANSFORMATION PRODUCTS						
2,6-Difluorobenzyl alcohol	<p>IUPAC: (2,6-Difluorophenyl)methanol</p> <p>CAS No.: 19064-18-7</p> <p>Formula: C₇H₆F₂O</p> <p>MW: 144.1 g/mol</p> <p>SMILES: OCC1=C(C=CC=C1F)F</p>		850.6100	50446912 +	NA	NA

^A AR means “applied radioactivity”. MW means “molecular weight”. PRT means “parent”. NA means “not applicable”. ND means “not detected”.