

Analytical method for 1,2,4-triazole in water


- Reports:** ECM: EPA MRID No.: 49762553. Gooding R. 2017. Methods of Analysis of 1,2,4-Triazole in Water with Limit of Determination (LOD) Calculation. BASF Study No.s: 428292 and 835160_3. BASF Registration Document No.: 2017/7008348. Report prepared by BASF Corporation, Crop Protection, Ecology and Environmental Analytics, Limburgerhof, Germany, and BASF Corporation, Research Triangle Park, North Carolina, and sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 111 pages. Final report issued May 22, 2017.
- ILV: EPA MRID No. 50289802. Soddu, R. 2016. Independent Laboratory Validation (ILV) for the Determination of 1,2,4-Triazole in Surface and Groundwater by LC-MS/MS. Analytical Phase Report No.: RA.16.06. BASF Registration Document No.: 2017/1066522. Report prepared and sponsored by ISAGRO SpA, Centro Recherche, Novara, Italy, and submitted by U.S. Triazole Task Force; 159 pages. Final report issued June 10, 2016.
- Document No.:** MRIDs 49762553 & 50289802
- Guideline:** 850.6100
- Statements:** ECM: The study was not conducted in accordance with Good Laboratory Practice (GLP) standards since it was not a study (p. 3 of MRID 49762553). Signed and dated No Data Confidentiality and GLP statements were provided; Quality Assurance and Authenticity statements were not provided (pp. 2-3). The ECM was a compilation of the study reports for an analytical method and LOD determination (p. 5). BASF Study # 428292 was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160, as well as OECD and German GLP (Appendix A, pp. 9-10); BASF Study # 835160_3 was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160 (Appendix B, p. 67). For BASF Study # 428292, signed and dated, GLP, and Quality Assurance statements were provided (Appendix A, pp. 9-11). No Data Confidentiality and Authenticity statements were not provided. For BASF Study # 835160_3, signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (Appendix B, pp. 66-69).
- ILV: The study was conducted in accordance with USEPA FIFRA GLP standards, 40 CFR, Part 160, as well as OECD German GLP (pp. 3, 8 of MRID 50289802). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 8, 10). A second unsigned/undated No Data Confidentiality statement was provided (p. 7). A GLP certificate of the ISAGRO facility was provided (Enclosure I, p. 158). An authenticity statement was not provided.

Classification: This analytical method is classified as supplemental. No documentation of the communication between the ECM developers and the laboratory performing the ILV was provided for review. The specificity of the method using the Hypercarb column was not supported by ECM study data and chromatograms. ILV linearity was not satisfactory for one of the calibration curves for the Hypercarb column. Procedural recoveries were corrected for residues found in the controls.

PC Code: 122000


EFED Final

Reviewer: Dena Barrett
Senior Chemist

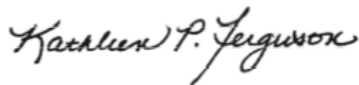
Signature: 
Date: 4/18/19

**CDM/CSS-
Dynamac JV**

Lisa Muto,
Environmental Scientist

Signature: 
Date: 3/6/18

Reviewers: Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature: 
Date: 3/6/18

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

This analytical method, BASF Method L0199/01, is designed for the quantitative determination of 1,2,4-triazole (Reg.No. 87084) in water at the LOQ of 0.05 µg/kg using LC/MS/MS. The LOQ for these methods should be less than the lowest toxicological level of concern in water for mefentrifluconazole and any metabolites such as 1,2,4-triazole that might be included in the Residues of Concern for drinking water exposure. This cannot be assessed at this time since no risk assessment has yet been completed for this new chemical petition for registration. Both the ECM and ILV used characterized surface and ground water matrices; matrices differed between the studies. One ion transition was monitored; two analytical columns, Aquasil C18 and Hypercarb, were used as primary and confirmatory methods, respectively. The method was validated by the ILV in the first trial with insignificant modifications to the analytical instrumentation and equipment for all matrices/fortifications/columns, except for the LOQ fortification in ground water analyzed with the Hypercarb column. The second trial was successful for that analysis. However, no documentation of the communication between the ECM and ILV entities was provided for review. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were generally satisfactory, but the specificity of the method using the Hypercarb column was not supported by ECM study data and chromatograms. Procedural recoveries were corrected for residues found in the controls, mainly in the ECM but also in the ILV. Also, ILV linearity was not fully satisfactory for one of the calibration curves for the Hypercarb column. The ILV reported that solvent injections were interspersed often to purge the column and detector; this was not suggested as a method

modification, but possibly should be incorporated into the method due to the prevalence of matrix interferences at the analyte retention time.

The LOD for Method L0199/01 (0.013 µg/L) was validated in an accompanying study using well (drinking) water according to the methodology in 40 CFR Ch. 1 Part 136 Appendix B.

Table 1. Analytical Method Summary – BASF Analytical Method L0199/01

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						
1,2,4-Triazole (Reg.No. 87084)	49762553 ¹	50289802		Water ^{2,3}	22/05/2017 ⁴	BASF Corporation	LC/MS/MS	0.05 µg/kg

1 A compilation of BASF Study #s 428292 and 835160_3.

2 In the ECM, ground water (pH 6.85, 3.5 mg/L total organic carbon, 43.7 mg/L calcium) obtained from Schifferstadt and surface water (pH 6.26, 16.7 mg/L total organic carbon, 38.8 mg/L calcium) obtained from Kelmetschweiher were used. Waters were characterized by BioChem agrar.

3 In the ILV, ground water (pH 7.9 ±0.05, 0.928 ±0.093 mg/L dissolved organic carbon, 9.82 ±0.2 °F hardness) obtained from a private well and surface (river) water (pH 7.73 ±0.05, 1.71 ±0.17 mg/L dissolved organic carbon, 5.78 ±0.12 °F hardness) obtained from Ticino River at Cameri, Italy, were used (°F hardness = French Degreed hardness measurement). Waters were characterized by Theolab, Italy.

4 Method dates were February 19, 2013 for BASF Study # 428292, and May 22, 2017 for BASF Study # 835160_3.

All referenced page numbers refer to those reported on the right-handed margin of the document pages.

I. Principle of the Method

Method L0199/01

Samples (2 g) of water were fortified, as necessary, at the LOQ and 10×LOQ (pp. 20-21, 23-24 of MRID 49762553). A Phenomenex Strata-X-CW solid phase extraction (SPE) cartridge (60 mg, 3 mL, 33 µ) was prepared by conditioning with 1 x 2 mL of methanol followed by 1 x 2 mL of water. The fortified sample (2 mL) and 0.5 mL of internal standard (IS; 1 ng [C¹³-N¹⁵]1,2,4-triazole/mL) were applied to the column simultaneously and pulled through at 1 drop per 2 seconds via gravity or weak vacuum. The filtrate was collected in a 12-mL tube and concentrated to dryness using a nitrogen evaporator set at 45°C. The volume of the residue was adjusted to 0.5 mL using water and analyzed by LC/MS/MS. The SPE column was washed 3 x 0.5 mL water; this wash was considered a blank value which was <LOD.

A Flowchart was provided (Appendix A, Figure A.3, p. 35 of MRID 49762553).

LC/MS/MS Analysis

Samples were analyzed for analytes by Agilent 1100 HPLC [Thermo Aquasil C18 column, 3 mm x 150 mm, 3 µm column (primary) or Thermo Hypercarb column, 4.6 mm x 50 mm, 3 µm column (secondary); column temperature 20-25°C (RT)] using a mobile phase of (A) water:formic acid (100:1, v:v) and (B) methanol:formic acid (100:1, v:v) [percent A:B at 0.0-2.1 min. 95:5, 2.5-5.0 min. 15:85, 5.1-7.0 min. 95:5] with PE Sciex API 4000 MS using electrospray ionization (ESI) in positive polarity (p. 25 of MRID 49762553). Injection volume was 50 µL. 1,2,4-Triazole and the IS were identified using one ion transition: m/z 70→43 and m/z 75→46, respectively. Expected retention time was *ca.* 1.6 minutes for 1,2,4-triazole and the IS.

ILV:

In the ILV, the ECM was performed as written, except for insignificant modifications to the analytical instrumentation/equipment and the fact that SPE column washes were combined with the filtrate prior to evaporation (pp. 19, 23-27, 42 of MRID 50289802). A Shimadzu LC30AD UPLC [Thermo Aquasil C18 column, 3 mm x 150 mm, 3 µm column (primary) or Thermo Hypercarb column, 2.1 mm x 100 mm, 5 µm column (secondary); column temperature 20-25°C (RT)] coupled with AB Sciex QTRAP 6500 MS (Linear Ion Trap Quadrupole) using TurboIonSpray (ESI, positive) was used. All other analytical parameters were the same as the ECM. Analytes were identified using the same ion transitions as the ECM. Expected retention times for 1,2,4-triazole and the IS were *ca.* 1.4 minutes using the Aquasil column and *ca.* 0.45 minutes using the Hypercarb column. No other modifications to the ECM were reported; however, the ILV noted that solvent injections were interspersed often to purge the column and detector (p. 29).

LOQ/LOD

The Limit of Quantification (LOQ) and Limit of Detection (LOD) were reported as 0.05 µg/kg and 0.013 µg/kg (25% of the LOQ), respectively, for 1,2,4-triazole in the ECM and ILV (pp. 12, 28, 31-32 of MRID 49762553; pp. 19, 29 of MRID 50289802). The LOQ corresponded to a concentration of 0.2 ng/mL of the extract.

The LOD for Method L0199/01 was evaluated in another study (BASF Study # 835160_3) using well (drinking) water from Bahama, North Carolina (CM15-030; pH 7.5, 0.8 ppm total organic carbon, hardness 116 mg equivalent CaCO₃/L; characterized by Agvise Laboratories, Northwood, North Dakota; Appendix B, pp. 70, 74; Appendix B, Appendix A, pp. 82 of MRID 49762553). The MDL as calculated based on the statistical analysis of seven samples spiked at using the Aquasil column (the least sensitive chromatography), according to the methodology in 40 CFR Ch. 1 Part 136 Appendix B. The MDL was determined as 1.91 pg on-column. From the MDL, the LOD was determined to be 2.5 pg on-column, taking into account factors which would increase variability of results. This LOD was equivalent to 50 pg/mL (0.05 ng/mL) on-column injected at 0.05 mL and a method LOD of 13 ng/L or 0.013 µg/L.

II. Recovery Findings

Method L0199/01

ECM (MRID 49762553): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of 1,2,4-triazole in two water matrices at fortification levels of 0.05 $\mu\text{g}/\text{kg}$ (LOQ) and 0.5 $\mu\text{g}/\text{L}$ ($10\times$ LOQ; See Reviewer's Comment #4; Appendix A, Table 2, p. 29 and Tables A.5-A.8, pp. 53-56). 1,2,4-Triazole was identified using one ion transition with two HPLC columns; performance data (recovery results) from primary and confirmatory analyses were comparable. Recovery results were corrected since residues were found in the controls, except for results in ground water using the Aquasil column. Ground water (pH 6.85, 3.5 mg/L total organic carbon, 43.7 mg/L calcium) obtained from Schifferstadt and surface water (pH 6.26, 16.7 mg/L total organic carbon, 38.8 mg/L calcium) obtained from Kelmetschweiher were used (Appendix A, p. 19; Appendix A, Figures A.26-27, pp. 58-61). Waters were characterized by BioChem agrar.

ILV (MRID 50289802): Mean recoveries and RSDs were within guideline requirements for analysis of 1,2,4-triazole in two water matrices at fortification levels of 0.05 $\mu\text{g}/\text{kg}$ (LOQ) and 0.5 $\mu\text{g}/\text{L}$ ($10\times$ LOQ; Tables 3-6, pp. 37-40). 1,2,4-Triazole was identified using one ion transition with two HPLC columns; performance data (recovery results) from primary and confirmatory analyses were comparable. Recovery results in surface water using the Aquasil column were corrected since residues were found in the controls. Ground water (pH 7.9 ± 0.05 , 0.928 ± 0.093 mg/L dissolved organic carbon, 9.82 ± 0.2 °F hardness) obtained from a private well and surface (river) water (pH 7.73 ± 0.05 , 1.71 ± 0.17 mg/L dissolved organic carbon, 5.78 ± 0.12 °F hardness) obtained from Ticino River at Cameri, Italy, were used (°F hardness = French Degreed hardness measurement; p. 20; Table 1, p. 35; Enclosures B-D, pp. 77-79). Waters were characterized by Theolab, Italy. The method was validated in the first trial with insignificant modifications to the analytical instrumentation and equipment for all matrices/fortifications/columns, except for the LOQ fortification in ground water analyzed with the Hypercarb column (pp. 18, 23-27, 31-33). The second trial was successful for that analysis.

Table 2. Initial Validation Method Recoveries for 1,2,4-Triazole in Water^{1,2}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Surface Water						
Aquasil Column (Primary) ³						
1,2,4-Triazole (Reg.No. 87084)	0.05 (LOQ)	5	91.8-98.8	94.1	2.8	3.0
	0.5	5	94.0-97.0	95.5	1.1	1.1
Hypercarb Column (Confirmatory) ³						
1,2,4-Triazole (Reg.No. 87084)	0.05 (LOQ)	5	84.8-119.3	97.2	13.6	14.0
	0.5	5	86.1-93.1	90.7	2.9	3.2
Ground Water						
Aquasil Column (Primary)						
1,2,4-Triazole (Reg.No. 87084)	0.05 (LOQ)	5	94.5-98.5	97.2	1.6	1.7
	0.5	5	95.0-101.0	97.2	2.5	2.6
Hypercarb Column (Confirmatory) ³						
1,2,4-Triazole (Reg.No. 87084)	0.05 (LOQ)	5	76.0-105.0	87.2	11.4	13.0
	0.5	5	89.6-99.6	95.0	3.8	4.0

Data (recovery results were corrected when residues were quantified in the controls, Appendix A, pp. 26-27) were obtained from Appendix A, Table 2, p. 29 and Tables A.5-A.8, pp. 53-56 of MRID 49762553.

1 Ground water (pH 6.85, 3.5 mg/L total organic carbon, 43.7 mg/L calcium) obtained from Schifferstadt and surface water (pH 6.26, 16.7 mg/L total organic carbon, 38.8 mg/L calcium) obtained from Kelmetschweiher were used (Appendix A, p. 19; Appendix A, Figures A.26-27, pp. 58-61). Waters were characterized by BioChem agrar.

2 1,2,4-Triazole was identified using one ion transition: m/z 70→43.

3 Recovery results were corrected; residues were quantified in the controls (Appendix A, Tables A.5-A.7, pp. 53-55).

Table 3. Independent Validation Method Recoveries for 1,2,4-Triazole in Water^{1,2}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ³	Relative Standard Deviation (%)
Surface Water						
Aquasil Column (Primary) ³						
1,2,4-Triazole (Reg.No. 87084)	0.05 (LOQ)	5	75.0-97.0	86.6	8.6	10.0
	0.5	5	90.8-109.6	99.7	7.8	7.8
Hypercarb Column (Confirmatory)						
1,2,4-Triazole (Reg.No. 87084)	0.05 (LOQ)	5	77.0-103.0	85.0	10.5	12.3
	0.5	5	98.5-118.6	111.5	8.3	7.4
Ground Water						
Aquasil Column (Primary)						
1,2,4-Triazole (Reg.No. 87084)	0.05 (LOQ)	5	104.0-116.0	109.6	5.5	5.1
	0.5	5	87.8-100.6	94.2	4.6	4.8
Hypercarb Column (Confirmatory)						
1,2,4-Triazole (Reg.No. 87084)	0.05 (LOQ)	5	76.0-114.0	89.6	16.7	18.6
	0.5	5	110.5-119.6	115.9	3.8	3.3

Data (recovery results were corrected when residues were quantified in the controls, pp. 27-28) were obtained from Tables 3-6, pp. 37-40 of MRID 50289802

1 Ground water (pH 7.9 ±0.05, 0.928 ±0.093 mg/L dissolved organic carbon, 9.82 ±0.2 °F hardness) obtained from a private well and surface (river) water (pH 7.73 ±0.05, 1.71 ±0.17 mg/L dissolved organic carbon, 5.78 ±0.12 °F hardness) obtained from Ticino River at Cameri, Italy, were used (°F hardness = French Degreed hardness measurement; p. 20; Table 1, p. 35; Enclosures B-D, pp. 77-79). Waters were characterized by Theolab, Italy.

2 1,2,4-Triazole was identified using one ion transition: m/z 70→43.

3 Recovery results were corrected; residues were quantified in the controls (Table 5, p. 39)

III. Method Characteristics

The LOQ and LOD were reported as 0.05 µg/kg and 0.013 µg/kg (25% of the LOQ), respectively, for 1,2,4-triazole in the ECM and ILV (pp. 12, 28, 31-32 of MRID 49762553; pp. 19, 29 of MRID 50289802). The LOQ corresponded to a concentration of 0.2 ng/mL of the extract. The LOQ was defined as the lowest fortification level successfully tested. The method LOD was defined as the lowest analyte concentration injected as a calibration solution (0.05 ng/mL in the extract). The ECM also noted that the signal-to-noise ratio at the LOD was >3:1 (p. 36 of MRID 49762553).

The LOD for Method L0199/01 was evaluated in another study (BASF Study # 835160_3) using well (drinking) water according to the methodology in 40 CFR Ch. 1 Part 136 Appendix B (Appendix B, pp. 70, 74; Appendix B, Appendix A, pp. 82 of MRID 49762553). The MDL was determined as 1.91 pg on-column. From the MDL, the LOD was determined to be 2.5 pg on-column, taking into account factors which would increase variability of results. This LOD was equivalent to 50 pg/mL (0.05 ng/mL) on-column injected at 0.05 mL and a method LOD of 13 ng/L or 0.013 µg/L. Results of BASF Study # 835160_3 are shown in Table 4 below.

Table 4: Calculation of MDL for 1,2,4-Triazole (Aquasil C-18 Column), the Least Sensitive Method, for Method L0199/01, using the Methodology Set Forth in 40 CFR Ch 1 Part 136 Appendix B

0.1 ng/mL Matrix-Spike Replicate	Measured Concentration (ng/mL)
1	0.0843
2	0.0765
3	0.0461
4	0.0773
5	0.0751
6	0.0735
7	0.0748
Standard Deviation (S) =	0.0122
N-1 =	6
Critical t value (t) =	3.143
Injection Volume (µL)	50
MDL (ng/mL)=	0.03824
MDL (ng on-column)=	0.001912
MDL (pg on-column)=	1.912

Data obtained from Appendix B, p. 78 of MRID 49762553.

Table 5. Method Characteristics - Analytical Method L0199/01

Analyte		1,2,4-Triazole (Reg.No. 87084)	
		Aquasil C18 Column	Hypercarb Column
Limit of Quantitation (LOQ)	ECM	0.05 µg/kg	
	ILV		
Limit of Detection (LOD)	ECM	0.013 µg/kg (25% of the LOQ)	
	ILV		
Linearity (calibration curve r^2 and concentration range) ¹	ECM ²	$r^2 = 0.9996$	
	ILV ³	$r^2 = 0.9988$	$r^2 = 0.9908-0.9958$
	Concentration Range	0.05-5 ng/mL	
Repeatable	ECM ^{4,5}	Yes at LOQ and 10×LOQ (two characterized water matrices)	
	ILV ^{6,7,8}	Yes at LOQ and 10×LOQ (two characterized water matrices)	
Reproducible		Yes at LOQ and 10×LOQ	
Specific	ECM/ILV	Baseline noise interfered with LOQ peak attenuation and integration.	
	ECM	Yes, matrix interferences were <i>ca.</i> 19-23% (based on measured residues) in surface water. Yes, no matrix interferences were observed in ground water.	No , matrix interferences were <i>ca.</i> 37-50% (surface water) and <i>ca.</i> 34-58% (ground water) based on measured residues and peak height. ⁹
		No 10×LOQ chromatograms were provided for review.	
	ILV	Yes, matrix interferences were <20% (based on measured residues and peak height).	

Data were obtained from Appendix A, 12, 28, 31-32; Appendix A, Table 2, p. 29 and Tables A.5-A.8, pp. 53-56 (recovery results); Appendix A, Figure A.2, p. 34 (calibration curves); Appendix A, Figures A.6-23, pp. 38-46 (chromatograms) of MRID 49762553; pp. 19, 29, 31; Tables 3-6, pp. 37-40 (recovery results); 13.2-13.4, pp. 47-49 (calibration curves); Panel 6-11, pp. 57-62 and Panel 16-21, pp. 67-72 (chromatograms) of MRID 50289802.

1 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Appendix A, Figure A.2, p. 34 of MRID 49762553; 13.2-13.4, pp. 47-49 of MRID 50289802; DER Attachment 2). Solvent-based calibration standards were used in the ECM and ILV.

2 Only one calibration curve was provided; the calibration curve was not identified as originating from an Aquasil or Hypercarb column analysis.

3 For the Hypercarb column, two calibration curves were reported; only one calibration curve was reported for the Aquasil column.

4 In the ECM, ground water (pH 6.85, 3.5 mg/L total organic carbon, 43.7 mg/L calcium) obtained from Schifferstadt and surface water (pH 6.26, 16.7 mg/L total organic carbon, 38.8 mg/L calcium) obtained from Kelmetschweiher were used (Appendix A, p. 19; Appendix A, Figures A.26-27, pp. 58-61 of MRID 49762553). Waters were characterized by BioChem agrar.

5 Most of the recoveries were corrected since residues were quantified in the controls (Appendix A, Tables A.5-A.7, pp. 53-55 of MRID 49762553).

6 In the ILV, ground water (pH 7.9 ±0.05, 0.928 ±0.093 mg/L dissolved organic carbon, 9.82 ±0.2 °F hardness) obtained from a private well and surface (river) water (pH 7.73 ±0.05, 1.71 ±0.17 mg/L dissolved organic carbon, 5.78 ±0.12 °F hardness) obtained from Ticino River at Cameri, Italy, were used (°F hardness = French Degreed hardness measurement; p. 20; Table 1, p. 35; Enclosures B-D, pp. 77-79 of MRID 50289802). Waters were characterized by Theolab, Italy.

7 The method was validated in the first trial with insignificant modifications to the analytical instrumentation and equipment for all matrices/fortifications/columns, except for the LOQ fortification in ground water analyzed with the Hypercarb column (pp. 18, 23-27, 31-33 of MRID 50289802). The second trial was successful for that analysis.

8 Recoveries in surface water analyzed by the Aquasil column were corrected since residues were quantified in the controls (Table 5, p. 39 of MRID 50289802).

9 Based on Appendix A, Figures A.6-12, pp. 38-41 of MRID 49762553.
Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. No documentation of the communication between the ECM developers and the ILV laboratory was provided for review. The interactions of the ECM personnel and ILV personnel were not discussed.
2. In the ECM, the specificity of the method using the Hypercarb column was not supported because significant matrix interferences were quantified in the control samples and observed in representative chromatograms of control samples (Appendix A, Tables A.5-A.8, pp. 53-56; Appendix A, Figures A.6-23, pp. 38-46 of MRID 49762553). Matrix interferences were observed as *ca.* 37-50% for surface water and *ca.* 34-58% for ground water. Additionally, no 10×LOQ chromatograms were provided for review. Representative chromatograms of all fortifications/matrices/ions should be provided for review.

The reviewer noted that the ILV reported that solvent injections were interspersed often to purge the column and detector (p. 29 of MRID 50289802). Based on the prevalence of matrix interferences at the analyte retention time, the reviewer believed that the practice of purging the column and detector should be incorporated into the method.

3. In the ILV, linearity was not fully satisfactory for one of the 1,2,4-triazole calibration curves for the Hypercarb column ($r^2 = 0.9908$; 13.2-13.4, pp. 47-49 of MRID 50289802; DER Attachment 2). Linearity is generally satisfactory when $r^2 \geq 0.995$.
4. Procedural recoveries were corrected for residues found in the controls, mainly in the ECM (3 of 4 analyses) but also in the ILV (1 of 4 analyses; Appendix A, Tables A.5-A.7, pp. 53-55 of MRID 49762553; Table 5, p. 39 of MRID 50289802).
5. The reviewer noted a significant typographical error in the ECM: the units of the recovery table were reported as **mg/kg** instead of **µg/kg** (Table 2, p. 29 of MRID 49762553). Perhaps due to this error, the Tier II summary for the ECM reported the LOQ as **0.05 ppm** (0.05 mg/kg) instead of **0.05 µg/kg** (0.05 ppt), throughout the entire report (Tier II Summary of MRID 49762553). Also, the fortifications were reported as 0.05 and 0.5 ppm, instead of 0.05 and 0.5 ppt.
6. In the ECM, the standard solutions were shown to be stable in water for at least 30 days (less than 10% decline) under refrigerated storage, and that the 1,2,4-triazole SPE-filtrates of ground water were shown to be stable for 7 days (temperatures and storage conditions were not further specified; pp. 29-30; Tables 3-4, pp. 29-30 for MRID 49762553).

7. The ECM determined that matrix effects did not influence the analytical results (p. 24 of MRID 49762553).
8. It was reported for the ILV that one sample set of 6 specimens and 25 procedural recoveries required *ca.* 12.5 person/hours for preparation and evaluation (p. 28 of MRID 50289802).

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**1,2,4-Triazole (Reg.No. 87084)**

IUPAC Name: 4H-[1,2,4]Triazole.

CAS Name: 4H-1,2,4-Triazole.

CAS Number: 288-88-0.

SMILES String: n1ncnc1 (EPI Suite, v3.12 SMILES).