

Analytical method for metribuzin and its transformation products, metribuzin DADK (AE F149970), metribuzin DK (AE 1344183) and metribuzin DA (AE B142111) in water

Reports: ECM: EPA MRID No.: 49846001. Williams, J. 2015. Bayer Method SE-001-S15-02: An Analytical Method for the Determination of Residues of Metribuzin and its metabolites Metribuzin DADK (AE F149970), Metribuzin DK (AE 1344183) and Metribuzin DA (AE B142111) in Soil and Water Using LC/MS/MS. Report prepared, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 41 pages. Bayer Method SE-001-S15-02. Final report issued June 5, 2015.

ILV: EPA MRID No.: 49647301. Rutt, D. and Li, F. 2015. Independent Laboratory Validation of Bayer Method SE-001-S15-01: An Analytical Method for the Determination of Residues of Metribuzin and its metabolites Metribuzin DADK (AE F149970), Metribuzin DK (AE 1344183) and Metribuzin DA (AE B142111) in Soil and Water Using LC/MS/MS. Report prepared by Critical Path Services, LLC (CPS), Garnet Valley, Pennsylvania, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 212 pages. Bayer Study No: MESEN024. CPS Study No.: 15-CPS-009. Final report issued June 4, 2015.

Document No.: MRIDs 49846001 & 49647301


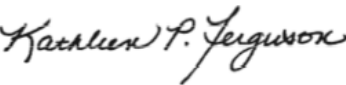
Guideline: 850.6100

Statements: ECM: The study was not conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards, since it was not an experimental study (p. 3 of MRID 49846001). Signed and dated Data Confidentiality and GLP and statements were provided (pp. 2-3). The Quality Assurance and Authenticity statements were not included.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR 160; p. 3 of MRID 49647301). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). The statement of authenticity was not included.

Classification: This analytical method is classified as **unacceptable**. The LOQ is greater than the lowest toxicological level of concern in water. Only one set of performance data was submitted: the ECM 49846001 was a method only. The ILV water matrix was undescribed and uncharacterized. The LOD of the method was not reported in the ECM and ILV.

PC Code: 101101

EPA Primary Reviewer:	Kristy Crews, Chemist	Signature:	
		Date:	
EPA Secondary Reviewer:	Andrew Shelby, Physical Scientist	Signature:	
		Date:	
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist	Signature:	
		Date:	4/26/17
	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature:	
		Date:	4/26/17

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Executive Summary

The analytical method, Bayer Method SE-001-S15-01/SE-001-S15-02, is designed for the quantitative determination of metribuzin and its transformation products, metribuzin DADK (AE F149970), metribuzin DK (AE 1344183) and metribuzin DA (AE B142111), in water at the stated LOQ of 0.5 ng/g using HPLC/MS/MS. The LOQ is **greater than** the lowest toxicological level of concern in water (MRID 49006501). Only one set of performance data was submitted: the ECM 49846001 was a method only. Two ion pair transitions were monitored for each analyte. The ILV validated the method with first trial with insignificant modifications to the analytical methods and instrumentation; however, specific source type and characterization of the water matrix used for validation were not reported. All ILV data regarding repeatability, accuracy, and precision were satisfactory for all analytes. In the ILV and ECM, the specificity of the method was not well supported by the LOQ representative chromatograms for metribuzin DK and metribuzin DADK due to the size of the analyte peak relative to the baseline noise. The LOD of the method was not reported in the ECM and ILV.

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						
Metribuzin	49846001 ²	49647301 ³		Water	05/06/2015	Bayer CropScience	LC/MS/MS	0.5 ng/g
Metribuzin DADK (AE F149970)								
Metribuzin DK (AE 1344183)								
Metribuzin DA (AE B142111)								

¹ Metribuzin = 4-Amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one; Metribuzin DA (AE B142111) = 6-tert-Butyl-3-methylsulfanyl-2H-1,2,4-triazin-5-one; Metribuzin DK (AE 1344183) = 4-Amino-6-tert-butyl-2H-1,2,4-triazine-3,5-dione; Metribuzin DADK (AE F149970) = 6-tert-Butyl-2H-1,2,4-triazine-3,5-dione.

² Method only; no water matrix was described, and no samples were prepared.

³ In the ILV, the water matrix was provided by the sponsor, Bayer (p. 19 of MRID 49647301). The specific water source type and water characterization were not reported.

I. Principle of the Method

Water (10 ± 0.10 g) in a 50-mL conical tube was fortified, if necessary, with the mixed fortification solution and 0.100 mL of the 0.10 $\mu\text{g/mL}$ mixed internal standard (p. 12; Appendix 2B, p. 20 of MRID 49846001). The sample was extracted with methylene chloride (*ca.* 10 mL) via mixing. After the phases separated, an aliquot of the bottom layer (*ca.* 10 mL) was transferred via pasture pipet to a culture tube. The solvent was evaporated to dryness using a TurboVap set at 50°C. The residue was reconstituted in *ca.* 1 mL of water:acetonitrile (4:1, v:v) and mixed thoroughly before being transferred to an autosampler vial for LC/MS/MS analysis.

Samples were analyzed for the analytes using an AB Sciex 6500 Chromatograph/Mass Spectrometer equipped with electrospray ionization (ESI) interface (pp. 7, 12-14 of MRID 49846001). The following LC conditions were used: Phenomenex Kinetex C8 column (3.0 mm x 100 mm, 2.6 μ ; column temperature not reported), Security Guard ULTRA UHPLC C8 optional guard column (dimensions not reported), mobile phase of (A) water:acetonitrile (9:1, v:v) with 0.2% acetic acid and (B) acetonitrile with 0.2% acetic acid [mobile gradient phase of percent A:B (v:v) at 0.0-0.20 min. 80:20, 6.0-7.0 min. 10:90, 7.1-8.0 min. 80:20], injection volume of 50 μL , and MRM with positive ESI (400°C). Two ion pair transitions were monitored for each analyte (quantitation and confirmatory, respectively): m/z 215.1 \rightarrow 187.1 and m/z 215.1 \rightarrow 145.1 for metribuzin, m/z 200.1 \rightarrow 172.1 and m/z 200.1 \rightarrow 116.0 for metribuzin DA, m/z 185.1 \rightarrow 155.9 and m/z 185.1 \rightarrow 110.5 for metribuzin DK and m/z 170.1 \rightarrow 141.4 and m/z 170.1 \rightarrow 125.7 for metribuzin DADK. Observed retention times were *ca.* 2.92, 2.48, 1.93 and 2.07 minutes for metribuzin, metribuzin DA, metribuzin DK and metribuzin DADK, respectively (Appendix 4, pp. 29-32).

The ILV performed the ECM methods for each analyte as written, except for insignificant modifications of the analytical methods and instrumentation (pp. 21, 23, 26; Table 10, p. 38 of MRID 49647301). Two ion pair transitions were monitored for each analyte (quantitation and confirmatory, respectively): m/z 215.1 \rightarrow 187.1 and m/z 215.1 \rightarrow 145.0 for metribuzin, m/z 200.1 \rightarrow 172.1 and m/z 200.1 \rightarrow 116.0 for metribuzin DA, m/z 185.1 \rightarrow 156.1 and m/z 185.1 \rightarrow 110.0 for metribuzin DK and m/z 170.1 \rightarrow 142.0 and m/z 170.1 \rightarrow 126.0 for metribuzin DADK. Observed retention times were *ca.* 3.15, 2.65, 2.14 and 2.29 minutes for metribuzin, metribuzin DA, metribuzin DK and metribuzin DADK, respectively (Figures 37-40, pp. 76-79; Figures 45-48, pp. 84-87; Figures 53-56, pp. 92-95; Figures 61-64, pp. 100-103).

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.50 ng/g for metribuzin, metribuzin DADK, metribuzin DK and metribuzin DA (p. 6 of MRID 49846001; p. 16 of MRID 49647301). In the ECM and ILV, the Limit of Detection (LOD) was not reported.

II. Recovery Findings

ECM (MRID 49846001): Mean recoveries and relative standard deviations (RSDs) were not reported. No water matrix was described. No samples were prepared; only the method was described.

ILV (MRID 49647301): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of metribuzin, metribuzin DA, metribuzin DK and metribuzin DADK at fortification levels of 0.50 ng/g (LOQ) and 5.0 ng/g (10 \times LOQ) in one water matrix (Tables 1-4, pp.

29-32). Two ion pair transitions were monitored for each analyte; quantitation and confirmatory ion analyses were comparable. The water matrix was provided by the sponsor, Bayer (p. 19). The specific water source type and water characterization were not reported. The method was validated with first trial with insignificant modifications to the analytical methods and instrumentation (pp. 21, 23, 25-26).

Table 2. Initial Validation Method Recoveries for Metribuzin and Its Transformation Products, Metribuzin DA, Metribuzin DK and Metribuzin DADK, in Water

Analyte ¹	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Water						
Metribuzin	0.50 (LOQ)	5	No data was reported; method only.			
	5.0	5				
Metribuzin DA (AE B142111)	0.50 (LOQ)	5				
	5.0	5				
Metribuzin DK (AE 1344183)	0.50 (LOQ)	5				
	5.0	5				
Metribuzin DADK (AE F149970)	0.50 (LOQ)	5				
	5.0	5				

Data were obtained from MRID 49846001.

Table 3. Independent Validation Method Recoveries for Metribuzin and Its Transformation Products, Metribuzin DA, Metribuzin DK and Metribuzin DADK, in Water

Analyte ¹	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Water²						
Quantitation ion ³						
Metribuzin	0.50 (LOQ)	5	105-108	107	1.3	1.2
	5.0	5	105-108	107	1.1	1.0
Metribuzin DA (AE B142111)	0.50 (LOQ)	5	106-110	109	1.7	1.5
	5.0	5	109-113	110	1.7	1.5
Metribuzin DK (AE 1344183)	0.50 (LOQ)	5	98.6-113	103	6.0	5.9
	5.0	5	100-108	105	3.0	2.8
Metribuzin DADK (AE F149970)	0.50 (LOQ)	5	101-130	119	11.3	9.5
	5.0	5	101-126	118	10.4	8.8
Confirmatory ion ³						
Metribuzin	0.50 (LOQ)	5	103-110	105	2.8	2.7
	5.0	5	104-110	108	2.3	2.1
Metribuzin DA (AE B142111)	0.50 (LOQ)	5	106-113	110	2.5	2.3
	5.0	5	109-113	111	1.5	1.4
Metribuzin DK (AE 1344183)	0.50 (LOQ)	5	84.8-101	93.0	6.0	6.4
	5.0	5	100-108	103	3.1	3.0
Metribuzin DADK (AE F149970)	0.50 (LOQ)	5	102-126	118	9.3	7.9
	5.0	5	93.2-129	120	15.1	12.6

Data (uncorrected recovery results; Tables 1-4, pp. 29-32; Appendix 2, pp. 153-154) were obtained from Tables 1-4, pp. 29-32 of MRID 49647301.

1 Metribuzin = 4-Amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one; Metribuzin DA (AE B142111) = 6-tert-Butyl-3-methylsulfanyl-2H-1,2,4-triazin-5-one; Metribuzin DK (AE 1344183) = 4-Amino-6-tert-butyl-2H-1,2,4-triazine-3,5-dione; Metribuzin DADK (AE F149970) = 6-tert-Butyl-2H-1,2,4-triazine-3,5-dione.

2 The water matrix was provided by the sponsor, Bayer (p. 19). The specific water source type and water characterization were not reported.

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmatory, respectively): m/z 215.1→187.1 and m/z 215.1→145.0 for metribuzin, m/z 200.1→172.1 and m/z 200.1→116.0 for metribuzin DA, m/z 185.1→156.1 and m/z 185.1→110.0 for metribuzin DK and m/z 170.1→142.0 and m/z 170.1→126.0 for metribuzin DADK.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.50 ng/g for metribuzin, metribuzin DADK, metribuzin DK and metribuzin DA (p. 6 of MRID 49846001; p. 16 of MRID 49647301). No justifications, calculations or comparisons to background levels were reported to support the method LOQ. In the ECM and ILV, the LOD for the method was not reported.

Table 4. Method Characteristics for Metribuzin and Its Transformation Products, Metribuzin DA, Metribuzin DK and Metribuzin DADK, in Water

Analyte ¹	Metribuzin	Metribuzin DA (AE B142111)	Metribuzin DK (AE 1344183)	Metribuzin DADK (AE F149970)	
Limit of Quantitation (LOQ)	0.50 ng/g				
Limit of Detection (LOD)	Not reported				
Linearity (calibration curve r^2 and concentration range)	ECM ²	$r^2 = 0.9992$	$r^2 = 0.9992$	$r^2 = 0.9996$	$r^2 = 0.9994$
	ILV ³	$r^2 = 0.9990$ (Q) $r^2 = 0.9986$ (C)	$r^2 = 0.9990$ (Q & C)	$r^2 = 0.9996$ (Q) $r^2 = 0.9994$ (C)	$r^2 = 0.9984$ (Q & C)
	Range:	1-100 ppb			
Repeatable	ECM ⁴	Could not be determined ; only method was reported.			
	ILV ⁵	Yes at LOQ and 10×LOQ.			
Reproducible	Could not be determined ; only one set of performance data was submitted.				
Specific	ECM	Minor baseline noise interfered with peak integration at the LOQ.	No matrix interferences were observed.	LOQ peak height was small compared to baseline noise.	LOQ peak height was fairly small compared to baseline noise.
		No control chromatograms were provided. Only quantitation ion chromatograms were provided.			
	ILV	No matrix interferences were observed.		LOQ peak height was small compared to baseline noise. Minor baseline noise interfered with peak integration at the LOQ.	LOQ peak height was fairly small compared to baseline noise. Minor baseline noise interfered with peak integration at the LOQ.

Data were obtained from pp. 6, 10; Appendix 3, pp. 21-22 (calibration curves); Appendix 4, pp. 29-32 (chromatograms) of MRID 49846001; pp. 16, 23; Tables 1-4, pp. 29-32 (recovery results); Figures 1-2, pp. 40-41; Figures 9-10, pp. 48-49; Figures 17-18, pp. 56-57; Figures 25-26, pp. 64-65 (calibration curves); Figures 33-64, pp. 72-103 (chromatograms) of MRID 49647301. Q = quantitation ion transition; C = confirmation ion transition.

1 Metribuzin = 4-Amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one; Metribuzin DA (AE B142111) = 6-tert-Butyl-3-methylsulfanyl-2H-1,2,4-triazin-5-one; Metribuzin DK (AE 1344183) = 4-Amino-6-tert-butyl-2H-1,2,4-triazine-3,5-dione; Metribuzin DADK (AE F149970) = 6-tert-Butyl-2H-1,2,4-triazine-3,5-dione.

2 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. 6, 10; Appendix 3, pp. 21-22 of MRID 49846001; DER Attachment 2). Only one set of calibration curves was presented; the reviewer assumed that it was the quantitation ion transition calibration curve which applied to the water and soil methods.

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 (Figures 1-2, pp. 40-41; Figures 9-10, pp. 48-49; Figures 17-18, pp. 56-57; Figures 25-26, pp. 64-65 of MRID 49647301; DER Attachment 2). Only one set of calibration curves was presented; the reviewer assumed that they applied to the water and soil methods.

4 In the ECM, only the method was reported. Calibration curves and chromatograms were included, but the description of the water matrix was not reported.

5 In the ILV, water matrix was provided by the sponsor, Bayer (p. 19 of MRID 49647301). The specific water source type and water characterization were not reported.

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

IV. Method Deficiencies and Reviewer's Comments

1. Only one set of performance data was submitted. ECM 49846001 was a method only, including representative calibration curves and chromatograms of an undescribed water matrix. No samples were prepared to validate the method with an internal validation. OCSPP guidelines state that sets of performance data should be submitted, one for the initial or other internal validation and one for the ILV, with the following exception: if the initial validation was performed by a governmental agency, a reference to the agency's documentation of the ECM will serve as the ECM report.
2. The specific source type and characterization of the ILV test water matrix were not reported; however, it was reported that the water matrix was provided by the sponsor, Bayer (p. 19 of MRID 49647301). It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.
3. The determination of the LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 6 of MRID 49846001; p. 16 of MRID 49647301). No justifications, calculations or comparisons to background levels were reported to support the method LOQ. No method LOD was reported in the ECM or ILV.
4. In the ILV and ECM, the specificity of the method was not well supported by the LOQ representative chromatograms for metribuzin DK and metribuzin DADK (Appendix 4, p. 30 of MRID 49846001; Figures ~~9253-9354~~, pp. 92-93; Figures 61-62, pp. 100-101 of MRID 49647301). The analyte peaks were well-resolved from the baseline, but the LOQ peak height was small compared to baseline noise. Additionally, the height of multiple nearby peaks of the baseline noise was significant (>30% of the LOQ), creating a messy chromatogram.
5. In the ECM, no control chromatograms were provided (Appendix 4, pp. 29-32 of MRID 49846001). The review of control chromatograms is necessary to determine the level of matrix interferences. Additionally, only quantitation ion chromatograms were provided; the reviewer noted that a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.
6. The ILV was provided Bayer Method SE-001-S15-01 (method date May 4, 2015) as the ECM (Appendix 4, pp. 164-204 of MRID 49647301). The ILV submitted two typographical error corrections to Bayer regarding Bayer Method SE-001-S15-01 (p. 26). Bayer corrected these typographical errors in Bayer Method SE-001-S15-02 (MRID 49846001; method date June 5, 2015; Appendix 6, p. 41 of MRID 49846001).
7. The communications between the ILV and validation laboratory Study Director and Study Monitor were briefly described (p. 26 of MRID 49647301). The ILV reported that emails were exchanged regarding study progress only; no technical or procedural aspects of the analytical method were discussed.

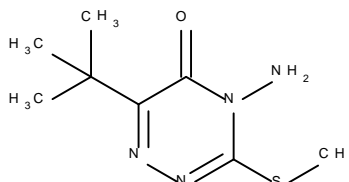
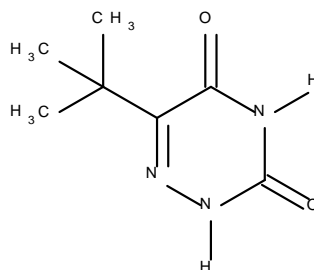
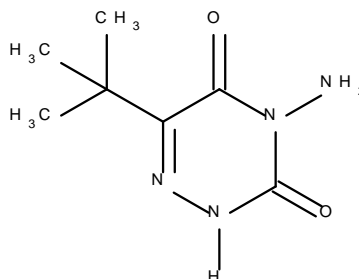
8. In the ILV, the total time required to complete one set of 13 samples (one reagent blank, two matrix controls and ten fortified samples) was reported as *ca.* 1 day to complete, where sample preparation required *ca.* 3 hours and LC/MS/MS analysis and data processing required *ca.* 5 hours (p. 26 of MRID 49647301).

V. References

Bruns, E. (2013) Lemna gibba G3 - Growth Inhibition Test with AE F055208 (Metribuzin) under Semi-Static Conditions: Final Report. Project Number: M/455636/01/1/OCR, EBSEN001, E/412/3823/3. Unpublished study prepared by Bayer CropScience. 107p.

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**Metribuzin (DIC 1468)****IUPAC Name:** 4-Amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one**CAS Name:** 4-Amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one**CAS Number:** 21087-64-9**SMILES String:** O=C1N(N)C(SC)=NN=C1C(C)(C)C**Metribuzin DADK (AE F149970; DADK-Metribuzin)****IUPAC Name:** 6-tert-Butyl-2H-1,2,4-triazine-3,5-dione**CAS Name:** 6-(1,1-Dimethylethyl)-1,2,4-triazin-3,5-(2H, 4H)-dione**CAS Number:** 52236-30-3**SMILES String:** [H]n1c(=O)c(nn(c1=O)[H])C(C)(C)C**Metribuzin DK (AE 1344183; DK-Metribuzin)****IUPAC Name:** 4-Amino-6-tert-butyl-2H-1,2,4-triazine-3,5-dione**CAS Name:** 4-Amino-6-(1,1-dimethylethyl)-1,2,4-triazin-3,5-(2H, 4H)-dione**CAS Number:** 56507-37-0**SMILES String:** [H]n1c(=O)n(c(=O)c(n1)C(C)(C)C)N

Metribuzin DA (AE B142111; DA-Metribuzin)**IUPAC Name:** 6-tert-Butyl-3-methylsulfanyl-2H-1,2,4-triazin-5-one**CAS Name:** 6-(1,1-Dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(2H)-one**CAS Number:** 35045-02-4**SMILES String:** [H]n1c(nc(=O)c(n1)C(C)(C)C)SC