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

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**. Only one set of performance data was submitted: the ECM 49846001 was a method only. The ILV soil matrix was undescribed and uncharacterized. In the ILV, the specificity of the method was not supported by the LOQ representative chromatograms for metribuzin DK. The LOD of the method was not reported in the ECM and ILV.

PC Code: 101101

EPA Primary Reviewer:	Kristy Crews, Chemist	Signature:	KRISTY CREWS Digitally signed by KRISTY CREWS Date: 2017.06.27 08:52:57 -04'00'
		Date:	
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		Date:	
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist	Signature:	Lisa Muto
		Date:	4/26/17
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		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 soil at the stated LOQ of 10 ng/g using HPLC/MS/MS. The LOQ is **less than** the lowest toxicological level of concern in soil (IUPAC PPDB 6/27/17[1]). 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 type and characterization of the soil matrix used for validation were not reported. All ILV data regarding repeatability, accuracy, and precision were satisfactory for all analytes. In the ILV, the specificity of the method was not supported by the LOQ representative chromatograms for metribuzin DK due significant interference of the baseline noise to peak resolution and integration; in the ECM, the specificity of the method was not well supported by the LOQ representative chromatograms for metribuzin DK 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.

[1] http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/469.htm

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]		Soil	05/06/2015	Bayer CropScience	LC/MS/MS	10 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

Soil samples (10 ± 0.10 g) were placed in 125-mL glass jars and fortified, if necessary with the mixed fortification solution (pp. 11-12; Appendix 2A, p. 20 of MRID 49846001). After sitting for *ca*. 10 minutes, the samples were extracted twice with 40 mL of acetonitrile:water (1:1, v:v) via microwave extraction using a magnetic stirrer (0-5 min. ramp temperature from ambient to 70°C, then maintain at 70°C for 10 minutes) and with temperature monitoring of the control sample. Once the samples have cooled, 0.650 mL of the 1 µg/mL mixed internal standard was added to each sample with mixing. An aliquot (*ca*. 1.5 mL) was transferred to a micro centrifuge tube and centrifuged (*ca*. 2 minutes at 12000 rpm). An aliquot (*c*a. 1 mL) of the supernatant was mixed with *ca*. 0.625 mL of water. After vortex mixing, the sample was analyzed by LC/MS/MS.

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→187.1 and *m/z* 215.1→145.1 for metribuzin, *m/z* 200.1→172.1 and *m/z* 200.1→116.0 for metribuzin DA, *m/z* 185.1→155.9 and *m/z* 185.1→110.5 for metribuzin DK and *m/z* 170.1→141.4 and *m/z* 170.1→125.7 for metribuzin DADK. Observed retention times were *ca*. 2.87-2.90, 2.45-2.46, 1.89-1.92 and 2.04-2.06 minutes for metribuzin, metribuzin DA, metribuzin DK and metribuzin DADK, respectively (Appendix 4, pp. 25-28).

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→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. Observed retention times were *ca*. 3.13, 2.6, 2.08 and 2.25 minutes for metribuzin, metribuzin DA, metribuzin DK and metribuzin DADK, respectively (Figures 69-72, pp. 108-111; Figures 77-80, pp. 116-119; Figures 85-88, pp. 124-127; Figures 93-96, pp. 132-135).

In the ECM and ILV, the Limit of Quantification (LOQ) was 10 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 soil 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 ≤20%) for analysis of metribuzin, metribuzin DA, metribuzin DK and metribuzin DADK at fortification levels of 10 ng/g (LOQ) and 100 ng/g (10×LOQ) in one soil matrix (Tables 5-8, pp. 33-36). Two ion pair transitions were monitored for each analyte; quantitation and confirmatory ion analyses were comparable. The soil matrix was provided by the sponsor, Bayer (p. 19). The specific soil type and 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 Soil

Analyte[1]	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Soil					
Metribuzin	10 (LOQ)	5	No data was reported; method only.			
	100	5				
Metribuzin DA (AE B142111)	10 (LOQ)	5				
	100	5				
Metribuzin DK (AE 1344183)	10 (LOQ)	5				
	100	5				
Metribuzin DADK (AE F149970)	10 (LOQ)	5				
	100	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 Soil

Analyte[1]	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Soil[2]					
	Quantitation ion[3]					
Metribuzin	10 (LOQ)	5	93.9-99.5	97.5	2.2	2.2
	100	5	107-109	108	0.7	0.7
Metribuzin DA (AE B142111)	10 (LOQ)	5	94.5-95.7	95.1	0.5	0.5
	100	5	105-106	106	0.4	0.4
Metribuzin DK (AE 1344183)	10 (LOQ)	5	98.2-119	108	9.0	8.3
	100	5	101-106	103	1.9	1.9
Metribuzin DADK (AE F149970)	10 (LOQ)	5	88.6-104	96.6	6.4	6.6
	100	5	103-112	107	3.6	3.3
	Confirmatory ion[3]					
Metribuzin	10 (LOQ)	5	95.2-98.9	97.0	1.6	1.6
	100	5	106-108	107	1.0	0.9
Metribuzin DA (AE B142111)	10 (LOQ)	5	94.5-95.4	95.0	0.4	0.4
	100	5	106-107	107	0.4	0.4
Metribuzin DK (AE 1344183)	10 (LOQ)	5	87.6-1.3	97.3	5.9	6.0
	100	5	101-103	101	0.9	0.9
Metribuzin DADK (AE F149970)	10 (LOQ)	5	90.3-109	100	6.9	6.9
	100	5	103-109	106	2.2	2.0

Data (uncorrected recovery results; Tables 5-8, pp. 33-36; Appendix 2, pp. 153-154) were obtained from Tables 5-8, pp. 33-36 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 soil matrix was provided by the sponsor, Bayer (p. 19). The specific soil type and 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 10 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 Soil

Analyte[1]		**Metribuzin**	**Metribuzin DA (AE B142111)**	**Metribuzin DK (AE 1344183)**	**Metribuzin DADK (AE F149970)**
Limit of Quantitation (LOQ)		10 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 fairly small compared to baseline noise.	No matrix interferences were observed.
		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. Major baseline noise interfered with peak integration at the LOQ.	No matrix interferences were observed. Minor baseline noise interfered with peak integration of the C ion at the LOQ.

Data were obtained from pp. 6, 10; Appendix 3, pp. 21-22 (calibration curves); Appendix 4, pp. 25-28 (chromatograms) of MRID 49846001; pp. 16, 23; Tables 5-8, pp. 33-36 (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 65-96, pp. 104-135 (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 soil matrix was not reported.

5 In the ILV, soil matrix was provided by the sponsor, Bayer (p. 19 of MRID 49647301). The specific soil type and 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 soil matrix. No samples were prepared to validated 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 type and characterization of the ILV test soil matrix were not reported; however, it was reported that the soil 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. In the ILV, the specificity of the method was not supported by the LOQ representative chromatograms for metribuzin DK (Figures 85-86, pp. 124-125 of MRID 49647301). The analyte peak was not well-resolved from the baseline, and the LOQ peak height was small compared to baseline noise. Additionally, the height of multiple nearby peaks of the baseline noise was significant (>75% of the LOQ), interfering with analyte integration at the LOQ.

   In the ECM, the specificity of the method was not well supported by the LOQ representative chromatograms for metribuzin DK (Appendix 4, p. 26 of MRID 49846001). The LOQ peak height was small compared to baseline noise. Additionally, the height of multiple nearby peaks of the baseline noise was significant (>50% of the LOQ), creating a messy chromatogram.

4. 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.

5. In the ECM, no control chromatograms were provided (Appendix 4, pp. 25-28 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

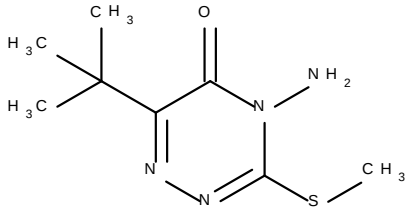
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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
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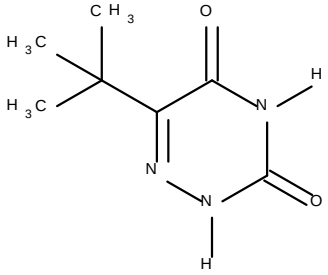

Metribuzin DK (AE 1344183; DK-Metribuzin)

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
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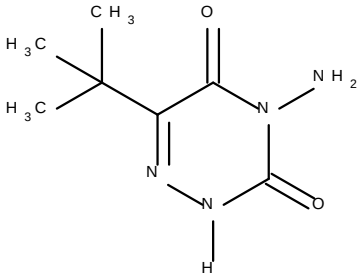

Metribuzin DA (AE B142111; DA-Metribuzin)

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