GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
Doug Rutt	Research Scientist, Study Director
Derek Netzband	Sponsor Representative/Study Monitor (Bayer CropScience)

Study Dates

Study initiation date: 12 May 2015
Experimental start date: 14 May 2015
Experimental termination date: 26 May 2015
Study completion date: See title page

Protocol Amendments and Deviations

None

Deviations from the Guidelines

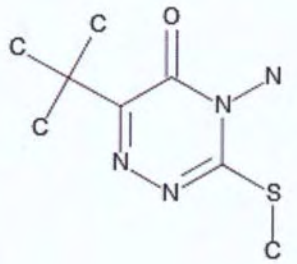
None

Retention of Samples

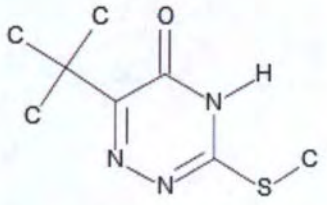
None necessary

Analytical Reference Standards

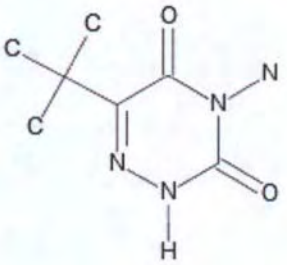
Standard name:	Metribuzin
Standard no.:	K-2094
CAS name:	4-Amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one
CAS number:	21087-64-9
GLP purity:	93.8%
Expiration date:	01 November 2018
Storage conditions:	Frozen
Molecular structure:	



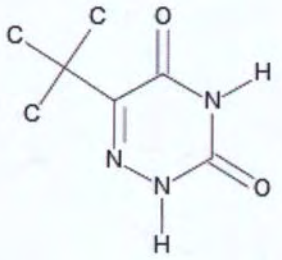
Standard name:	DA-Metribuzin (DA Sencor, Desamino-Metribuzin)
Standard no.:	K-2095
CAS name:	6-(1,1-Dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one
CAS number:	35045-02-4
Ref. substance lot:	K-2095
GLP purity:	99.3%
Expiration date:	01 March 2017
Storage conditions:	Frozen
Molecular structure:	



Standard name:	DK-Metribuzin (DK Sencor, Diketo-Metribuzin)
Standard no.:	K-2096
CAS name:	4-Amino-6-(1,1-dimethylethyl)-1,2,4-triazine-3,5(2H,4H)-dione
CAS number:	56507-37-0
GLP purity:	99.2%
Expiration date:	05 September 2020
Storage conditions:	
Molecular structure:	

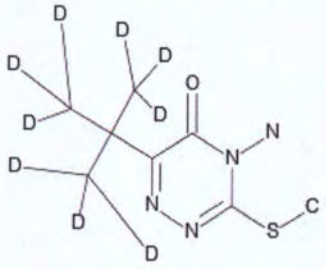


Standard name:	DADK-Metribuzin (DADK Sencor, Desamino-diketo-Metribuzin)
Standard no.:	K-2097
CAS name:	6-{1,1-Dimethylethyl)-1,2,4-triazine-3,5(2H,4H)-dione
CAS number:	52236-30-3
GLP purity:	99.9%
Expiration date:	26 November 2017
Storage conditions:	Frozen
Molecular structure:	

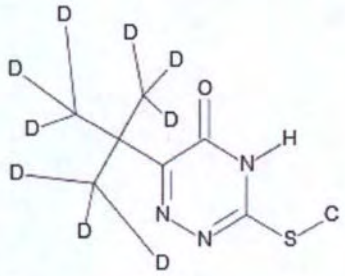


Internal Standard (IS)

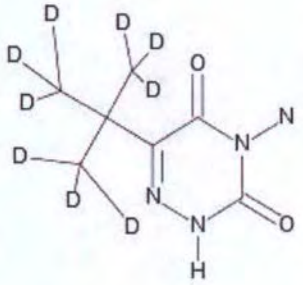
Standard name:	D_9-Metribuzin
Standard no.:	K-682
CAS name:	4-Amino-6-[1, 1-di(methyl-d_3)ethyl-2,2,2-d_3]-3-(methylthio)-1 ,2,4-triazin-5(4H)-one
CAS number:	Unavailable
GLP purity:	100%
Expiration date:	08 January 2020
Storage conditions:	Frozen
Molecular structure:	



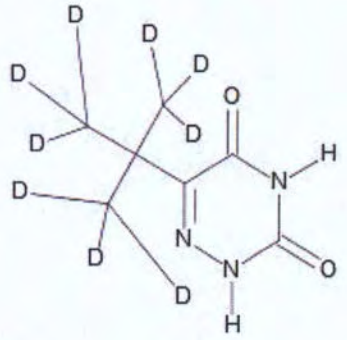
Standard name: D_9-DA-Metribuzin (D_9-DA Sencor)
Standard no.: K-685
CAS name: 6-[1,1-Di(methyl-d_3)ethyl-2,*2,2-d_3*]-3-(methylthio)-1,2,4-triazin-5(2H)-one
CAS number: Unavailable
GLP purity: 99.9%
Expiration date: 08 January 2020
Storage conditions: Frozen
Molecular structure:



Standard name: D_9-DK-Metribuzin (D_9-DK Sencor)
Standard no.: K-683
CAS name: 4-Amino-6-[1,1-di(methyl-d_3)ethyl-2,*2,2-d_3*]-1,2,4-triazin-3,5(2H,4H)-dione
CAS number: Unavailable
GLP purity: 100%
Expiration date: 08 January 2020
Storage conditions: Frozen
Molecular structure:



Standard name:	D_9-DADK-Metribuzin (D_9-DADK-Sencor)
Standard no.:	K-686
CAS name:	6-[1,1-Di(methyl-d_3)ethyl-2, *2, 2-d_3*]-1,2,4-triazin-3,5(2H,4H)-dione
CAS number:	Unavailable
GLP purity:	100%
Expiration date:	07 November 2021
Storage conditions:	Frozen
Molecular structure:	



Other

Upon completion of the study, a copy of the protocol and the final report will be archived at CPS. The original protocol, final report, raw data, correspondence, and other documentation will be transferred to the Bayer CropScience Archives, Bayer CropScience, 2 T.W. Alexander Drive, RTP, NC 27709.

2.0 INTRODUCTION

The objective of this study was to validate 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 [1].

This study was designed to fulfill the requirements of the US EPA Test Guidelines OCSPP 850.6100 [2]. In addition, this study was conducted in compliance with US EPA FIFRA (40 CFR Part 160) GLP standards [3].

3.0 MATERIALS AND METHODS

3.1 Test Substance and Internal Standard

Test Substance

Standard name:	Metribuzin
Standard no.:	K-2094
CAS name:	4-Amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one
CAS no.:	21087-64-9
GLP purity:	93.8%
Expiration date:	01 November 2018
Storage conditions:	Freezer

Standard name:	DA-metribuzin (DA Sencor®, Desamino-metribuzin)
Standard no.:	K-2095
CAS name:	6-(1,1-Dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one
CAS no.:	35045-02-4
GLP purity:	99.3%
Expiration date:	01 March 2017
Storage conditions:	Freezer

Standard name:	DK-metribuzin (DK Sencor®, Diketo-metribuzin)
Standard no.:	K-2096
CAS name:	4-Amino-6-(1,1-dimethylethyl)-1,2,4-triazine-3,5(2H,4H)-dione
CAS no.:	56507-37-0
GLP purity:	99.2%
Expiration date:	05 September 2020
Storage conditions:	Freezer

Standard name:	DADK-metribuzin (DADK Sencor®, Desamino-diketo-metribuzin)
Standard no.:	K-2097
CAS name:	6-{1,1-Dimethylethyl)-1,2,4-triazine-3,5(2H,4H)-dione
CAS no.:	52236-30-3
GLP purity:	99.9%
Expiration date:	26 November 2017
Storage conditions:	Freezer

Internal Standard (IS)

Standard name:	D_9-metribuzin
Standard no.:	K-682
CAS name:	4-Amino-6-[1, 1-di(methyl-d_3)ethyl-2,2,2-d_3]-3-(methylthio)-1,2,4-triazin-5(4H)-one
CAS no.:	Unavailable
Ref. substance lot:	95B086-190
GLP purity:	100%
Expiration date:	08 January 2020
Storage conditions:	Freezer

Standard name:	D_9-DA-metribuzin (D_9-DA Sencor®)
Standard no.:	K-685
IUPAC name:	6-[1,1-Di(methyl-d_3)ethyl-2,*2,2*-d_3]-3-(methylthio)-1,2,4-triazin-5(2H)-one
CAS no.:	Unavailable
Ref. substance lot:	95B086-205
GLP purity:	99.9%
Expiration date:	08 January 2020
Storage conditions:	Freezer

Standard name:	D_9-DK-metribuzin (D_9-DK Sencor®)
Standard no.:	K-683
CAS name:	4-Amino-6-[1,1-di(methyl-d_3)ethyl-2,2,2-d_3]-1,2,4-triazin-3,5(2H,4H)-dione
CAS no.:	Unavailable
Ref. substance lot:	95B086-197
GLP purity:	100%
Expiration date:	08 January 2020
Storage conditions:	Frozen

Standard name:	D_9-DADK-metribuzin (D_9-DADK-Sencor®)
Standard no.:	K-686
CAS name:	6-[1,1-Di(methyl-d_3)ethyl-2, 2,2-d_3]-1,2,4-triazin-3,5(2H,4H) dione
CAS no.:	Unavailable
Ref. substance lot:	95B085-214
GLP purity:	100%
Expiration date:	07 November 2021
Storage conditions:	Frozen

3.2 Test System

The test systems used for the validation were a water sample and a soil sample provided by Bayer. The samples were held in a refrigerator (CPS Equip# REF.1.14) until needed for analysis.

3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in Bayer Method SE-001-S15-01 (Appendix 4, Section 3.0: Apparatus and Section 4.0: Reagents and Consumables). Identical or equivalent apparatus and materials were used.

3.3.1 Equipment and Apparatus

- Falcon 50-mL conical centrifuge tubes (VWR part no. 21008-940)
- Eppendorf 2-mL microcentrifuge tubes (VWR part no. 20170-708)
- Volumetric flasks, glass class A (assorted volumes)
- Eppendorf electronic Pipettors, various volumes
- Glass culture tubes, 16 mm × 100 mm (Fisher part no. 14-962-10D)
- Pasture pipetts (VWR part no. 414004-007)
- Fisherbrand 125-mL glass jars (part no. 02-911-455)
- HPLC vials and caps (2-mL, VWR part no. 46610-722)
- Organomation Associates N-EVAP Analytical Evaporator
- Vortex mixer

- Milestone Ethos EX Microwave Extraction System, model MA074
- Ultrasonic Cleaner (Branson)
- Beckman Coulter microcentrifuge, model Microfuge 16
- Phenomenex Kinetex C8 column, 100 mm × 3.0 mm, 2.6 µm particle size, (part no. 00D-4497-Y0)
- LC-MS/MS – Shimadzu Nexera X2 Modular UHPLC system with a PAL autosampler (CTC Analytics) coupled to API 6500™ Tandem Mass Spectrometer with an electrospray ionization interface and Analyst 1.6.2 data collection software (ABSciex™)
- Various general laboratory glassware and utensils

3.3.2 Reagents

- Water (Ultrapure)
- Acetonitrile (ACN, EMD, LC-MS grade, part no. AX0156-1)
- Methylene Chloride (EMD, HPLC grade, part no. DX0831-1)
- Acetic Acid Glacial (Fisher Scientific, ACS grade, part no. A38-500)
- Water/ACN (50:50, v:v) solution: combined 1000 mL ACN and 1000 mL water using a graduated cylinder to a suitable container and mixed
- Water/ACN (4:1, v:v) solution: combined 200 mL ACN and 800 mL water using a graduated cylinder a suitable container and mixed
- Mobile phase A (Water/Acetonitrile (9:1, v:v) with 0.2% acetic acid): combined 900 mL water and 100 mL ACN using a graduated cylinder to a suitable container, added 2.00 mL acetic acid using a pipette and mixed well
- Mobile phase B (0.2% acetic acid in acetonitrile): measured 1000 mL ACN using a graduated cylinder to a suitable container, added 2.00 mL acetic acid using a pipette and mixed well

3.4 Experimental Design

3.4.1 Establishment of the Method

Prior to performing the ILV, the analyte retention times, instrument detection sensitivity, and linearity of instrument responses to a range of analyte concentrations were determined, and the test system was verified as free of interferences at appropriate retention times.

3.4.2 Sample Validation Sets, Fortification, and Extraction Procedure

Sample Validation Sets

Each analytical set consisted of 13 samples: one reagent blank, two untreated controls, five untreated controls fortified with test substances at the LOQ, and five untreated controls fortified with test substances at 10× LOQ.

Data are summarized in Table 1 to Table 8 for water and soil samples. Residue data sheets are included in Appendix 1.

Calibration standard solutions (1.00 to 100 ng/mL for test substances and 10.0 ng/mL for internal standard) and a solvent blank were also included in each sample analysis batch.

Fortification

The water control LOQ and 10× LOQ samples were fortified with 0.0500 mL of the fortification standard solutions, A3 (0.100 µg/mL) for LOQ, and A2 (1.00 µg/mL) for 10× LOQ. The soil control LOQ and 10× LOQ samples were fortified with 0.100 mL of the fortification standard solutions, A2 (1.00 µg/mL) for LOQ, and A1 (10.0 µg/mL) for 10× LOQ.

Extraction and Workup for Water Samples

The following extraction steps were followed for each water sample.

1. Weigh 10 ± 0.10 g of water into 50-mL conical tube.
2. Fortify the recovery samples at the desired fortification level with the appropriate mixed standard solution (see Section 3.4.4 Fortification and Calibration Standard Solutions Preparation).
3. Add 0.100 mL of the 0.100 µg/mL metribuzin internal standard solution B3 (mixture of four internal standards) to each sample and mix well.
4. Add approximately 10 mL of methylene chloride to the tube and stopper the tube.
5. Mix sample tube thoroughly and allow phases to separate.
6. Transfer approximately 10 mL of the bottom organic layer to a culture tube with a pasture pipette.
7. Dry sample to completeness on an N-EVAP with a water bath set at 50°C.
8. Reconstitute the extract with 1.00 mL water/acetonitrile (4:1, v:v) solution.
9. Mix thoroughly and transfer sample to HPLC autosampler vial for analysis by LC-MS/MS.

Extraction and Workup for Soil Samples

The following extraction steps were followed for each soil sample.

1. Weigh 10 ± 0.10 g of soil into 125-mL glass bottle.
2. Fortify the recovery samples at the desired fortification level with the appropriate mixed standard solution (see Section 3.4.4 Fortification and Calibration Standard Solutions Preparation).
3. Add 40.0 mL of water/acetonitrile (1:1, v:v) solution to each sample.
4. Add a magnetic stirrer to each sample and loosely attach the lid.
5. Load the samples onto the Microwave Extraction System and insert the temperature control probe into the UTC sample.
6. Extract samples using the following method:

Step No.	Time Duration	Temperature Set Point at End of Step	Power Limit to Control Temperature	Comments
1	5 min.	70°C	≤350 W	Ramp from ambient to 70°C
2	10 min.	70°C	≤250 W	Maintain at 70°C

7. Remove samples from the microwave once samples have cooled.
8. Add 0.650 mL of the 0.100 µg/mL metribuzin internal standard solution B3 (mixture of four internal standards) to each sample and mix well.
9. Transfer approximately 1.5 mL of the aliquot into a micro centrifuge tube and centrifuge at 12,000 rpm for approximately 2 minutes.
10. Combine 1.00 mL supernatant and 0.625 mL water into a HPLC vial, vortex vial for analysis by LC-MS/MS.

3.4.2 Sample Validation Sets, Fortification, and Extraction Procedure

The samples were processed and analyzed as described by Bayer Method SE-001-S15-01 with no modifications except a few mass spectrometer conditions which were generated from the instrument optimization.

3.4.4 Fortification and Calibration Standard Solutions Preparation

Fortification and calibration standard solutions were prepared following the methods below.

Primary stock standard solutions of metribuzin, DA-metribuzin, DK-metribuzin, and DADK-metribuzin

Prepare individual stock solutions of approximately 100 µg/mL of metribuzin, DA-metribuzin, DK-metribuzin, and DADK-metribuzin. Prepare the primary stock solution for the each reference standard separately in a 100-mL volumetric flask by dissolving each pre-weighed standard (approximately 0.01 g) with acetonitrile.

Mixed secondary standard solutions (A1 10.0 µg/mL) of metribuzin, DA-metribuzin, DK-metribuzin, and DADK-metribuzin

Prepare a mixed stock solution containing 10 µg/mL of metribuzin, DA-metribuzin, DK-metribuzin, and DADK-metribuzin by taking an appropriate volume (approximately 5.00 mL) of each of the primary stock solutions and diluting to 50.0 mL with acetonitrile.

Mixed secondary standard solutions (A2 1.00 µg/mL) of metribuzin, DA-metribuzin, DK-metribuzin, and DADK-metribuzin

Transfer 5 mL of the 10.0 µg/mL mixed standard solution into a 50-mL volumetric flask. Dilute to volume with acetonitrile. Mix well.

Mixed secondary standard solutions (A3 0.100 µg/mL) of metribuzin, DA-metribuzin, DK-metribuzin, and DADK-metribuzin

Transfer 5 mL of the 1.00 µg/mL mixed standard solution into a 50-mL volumetric flask. Dilute to volume with acetonitrile. Mix well.

Primary stock IS solutions of D_9-metribuzin, D_9-DA-metribuzin, D_9-DK-metribuzin, and D_9-DADK-metribuzin

Prepare individual stock solutions of approximately 100 µg/mL of D_9-metribuzin, D_9-DA-metribuzin, D_9-DK-metribuzin, and D_9-DADK-metribuzin. Prepare the primary stock solution for the IS separately in a 50-mL volumetric flask by dissolving each pre-weighed standard (approximately 0.005 g) with acetonitrile.

Mixed secondary IS solutions (B1 10.0 µg/mL) of D_9-metribuzin, D_9-DA-metribuzin, D_9-DK-metribuzin, and D_9-DADK-metribuzin

Prepare a mixed solution containing 10.0 µg/mL of D_9-metribuzin, D_9-DA-metribuzin, D_9-DK-metribuzin, and D_9-DADK-metribuzin by taking an appropriate volume (approximately 5 mL) of each of the primary stock solutions and diluting to 50 mL with acetonitrile.

Mixed secondary IS solutions (B2 1.00 µg/mL) of D_9-metribuzin, D_9-DA-metribuzin, D_9-DK-metribuzin, and D_9-DADK-metribuzin

Transfer 5 mL of the 10.0 µg/mL mixed IS standard solution into a 50-mL volumetric flask. Dilute to volume with acetonitrile. Mix well.

Mixed secondary IS solutions (B3 0.100 µg/mL) of D_9-metribuzin, D_9-DA-metribuzin, D_9-DK-metribuzin, and D_9-DADK-metribuzin

Transfer 5 mL of the 1.00 µg/mL mixed IS standard solution into a 50-mL volumetric flask. Dilute to volume with acetonitrile. Mix well.

Calibration standard solutions

Prepare working calibration solutions consisting of 1.00, 2.00, 5.00, 10.0, 20.0, 50.0, and 100 ppb of metribuzin, DA-metribuzin, DK-metribuzin, and DADK-metribuzin by diluting to 50.0 mL with 4:1 water/ACN solution. Before bringing the calibration solutions to volume, add by pipet 50.0 µL of the 10.0 µg/mL mixed IS solution to each of the calibration solutions.

Concentration of Standard Solution Used for Dilution (µg/mL)	Concentration of IS Solution Used for Dilution (µg/mL)	Aliquot Native Mix Taken (mL)	Aliquot IS Taken (mL)	Concentration of Calibration Solution (ppb)	Concentration of IS (ppb)
10.0	10.0	0.500	0.0500	100	10.0
10.0	10.0	0.250	0.0500	50.0	10.0
10.0	10.0	0.100	0.0500	20.0	10.0
10.0	10.0	0.0500	0.0500	10.0	10.0
1.00	10.0	0.250	0.0500	5.00	10.0
1.00	10.0	0.100	0.0500	2.00	10.0
1.00	10.0	0.0500	0.0500	1.00	10.0

All standard solutions were stored in a refrigerator (4–8°C) when not in use.

3.5 LC-MS/MS Instrumentation

Shimadzu Nexera X2 Modular UHPLC system with a PAL autosampler (CTC Analytics)
ABSciex API 6500 LC-MS/MS
Software: ABSciex Analyst Software, Analyst® 1.6.2
HPLC column: Phenomenex Kinetex C8, 100 mm × 3.0 mm, 2.6 µm particle size, (part no: 00D-4497-Y0)

3.6 Data Acquisition and Reporting

Peak integration was performed by Analyst® software version 1.6.2. The MS detector responses (peak area) for various injected standard concentrations were used to generate an external calibration curve for the analytes of interest. The overall purpose for the external calibration curve was to display acceptable linearity ($r^2 \geq 0.99$) of the assigned calibration range. The recoveries of the analyte from the fortified samples were calculated by multi-point calibration.

Recovery results were computed for each sample. The equation used for quantification is presented in Appendix 2. A statistical treatment of the data includes the calculation of means, standard deviations (SD), relative standard deviations (RSD) as percentages (%), and the 95% confidence intervals. All statistics were calculated using Microsoft® Excel® 2010.

5.0 CONCLUSIONS

CPS successfully completed and independently validated Bayer Method SE-001-S15-01 (see Appendix 4), entitled "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/". Bayer Method SE-001-S15-01 was demonstrated to be suitable for the determination of metribuzin and its metabolites DADK-metribuzin, DK-metribuzin, and DA-metribuzin in water studied at both LOQ (0.500 ppb) and 10× LOQ (5.00 ppb) levels; and in soil studied at both LOQ (10.0 ppb) and 10× LOQ (100 ppb) levels.

The method was performed as written with no modifications.

It took one person approximately 3 hours to complete the preparation of one set of 13 samples (one reagent blank, two unfortified matrix control samples, and 10 fortified samples). Time of analysis was approximately 5 hours. Completing one set, including sample preparation and analysis, took approximately 1 day.

6.0 REFERENCES

1. Williams, J., 2015. 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.
2. US Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention. 2012. Ecological Effects Test Guidelines OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation.
3. US Environmental Protection Agency, Office of Compliance Monitoring. 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule, 40 CFR, Part 160. Federal Register, Vol. 54, No. 158: pp. 34052–34074.

Table 9 HPLC System Operating Parameters for Bayer Method SE-001-S15-01 for Metribuzin and Its Metabolites DADK-Metribuzin (AE F149970), DK-Metribuzin (AE 1344183), and DA-Metribuzin (AE B142111)

HPLC System:	Shimadzu Nexera X2 Modular UHPLC system
Autosampler:	CTC Analytics PAL system
Software:	ABSciex™, Analyst® 1.6.2
Analytical Column:	Phenomenex Kinetex C8, 100 mm × 3.0 mm, 2.6 µm particle size, (part no.: 00D-4497-Y0)
Mobile Phase:	(A): Water/Acetonitrile (9:1, v:v) with 0.2% acetic acid (B): 0.2% acetic acid in acetonitrile
Column Temperature:	40°C
Injection Volume:	50.0 µL
Injection Speed:	5.00 µL/second
Run Time:	8.0 minutes
Gradient:	

Time (min)	A (%)	B (%)	Flow (µL/min)
0.01	80.0	20.0	500
0.20	80.0	20.0	500
6.00	10.0	90.0	500
7.00	10.0	90.0	500
7.10	80.0	20.0	500
8.00	80.0	20.0	500

Table 10 MS/MS Operating Parameters for Bayer Method SE-001-S15-01 for Metribuzin and Its Metabolites DADK-Metribuzin (AE F149970), DK-Metribuzin (AE 1344183), and DA-Metribuzin (AE B142111)

Tandem Mass Spectrometry System, ABSciex™, API 6500™
Software: ABSciex™, Analyst® 1.6.2

The following parameters were used for operation of the mass spectrometer:

Parameter	Setting
Ion Source:	Turbo Spray (electrospray ionization)
Scan Type:	MRM
Polarity:	Positive
Collision Gas (CAD):	9
Curtain Gas (CUR):	20
Ion Source Gas 1 (GS1):	80
Ion Source Gas 2 (GS2):	80
Ion Spray Voltage (IS):	5000
Temperature (TEM):	450
Interface Heater (IHE):	ON

Analyte Name	Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)	DP	CE	EP	CXP
Metribuzin	215.1	187.1	50	96	25	10	12
Metribuzin IS	224.1	196.2	50	111	25	10	14
DA-Metribuzin	200.1	172.1	50	106	25	10	10
DA-Metribuzin IS	209.1	181.1	50	111	25	10	18
DK-Metribuzin	185.1	156.1	50	121	23	10	18
DK-Metribuzin IS	194.1	166.1	50	96	21	10	12
DADK-Metribuzin	170.1	142.0	50	100	23	10	24
DADK-Metribuzin IS	179.1	151.1	50	106	25	10	14
Metribuzin Confirmatory	215.1	145.0	50	96	25	10	12
DA-Metribuzin Confirmatory	200.1	116.0	50	106	31	10	12
DK-Metribuzin Confirmatory	185.1	110.0	50	121	27	10	12
DADK-Metribuzin Confirmatory	170.1	126.0	50	96	35	10	10

APPENDIX 2 CALCULATIONS

Residue concentrations were determined using calibration curves which were generated after each analysis using ABSciex Analyst software (version 1.6.2) using linear regression with 1/x weighting. The results were exported to Microsoft® Excel® 2010 and further processed. Data summary documents were prepared with Microsoft® Excel® 2010.

The standards were fit to the linear equation:

$$Y = MX + B \text{ with } 1/x \text{ weighting.}$$

where: X is the concentration of the reference standard in ppb
M is the calibration line slope
B is the calibration line intercept
Y is the native peak area: isotopic peak area ratio

After regression coefficients were calculated, the residue found in ppb was determined using the following equation,

$$\text{Residue found (ppb)} = \frac{(Y-B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Initial volume (V1)}}{\text{Initial sample wt. (W)}} \times \frac{\text{Final dilution volume (V3)}}{\text{Aliquot taken (V2)}}$$

For Soil Where:		For Water Where:	
W =	10 g	W =	10 g
V1 =	40 mL	V1 =	10 mL
V2 =	1 mL	V2 =	10 mL
V3 =	1.625 mL	V3 =	1 mL

Samples were fortified prior to extraction at the LOQ of 10 ng/g (ppb) for soil and at the LOQ of 0.5 ng/g (ppb) for water. Theoretical ppb in fortified sample can be calculated as following:

$$\text{Theoretical ppb in fortified sample (ppb)} = \frac{C_{sp} \times V_{sp}}{W}$$

For Soil Where: C_{sp} = Spiking solution concentration (1000 ng/mL and 10000 ng/mL for LOQ and 10 × LOQ, respectively)
V_{sp} = Spiking solution volume (0.100 mL)
W = Initial sample weight (10.00 g)

For Water Where: C_{sp} = Spiking solution concentration (100 ng/mL and 1000 ng/mL for LOQ and 10 × LOQ, respectively)
V_{sp} = Spiking solution volume (0.0500 mL)
W = Initial sample weight (10.00 g)

Recoveries were calculated using the following equation:

$$\text{Recovery (\%)} = \frac{\text{ppb found in fortified sample} - \text{ppb found in control sample}}{\text{Theoretical ppb in fortified sample}} \times 100$$