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|---|---|---|
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## STUDY REPORTS:

MRID No. 46801806 Gould, T. Timberlake, B. and Brungardt, J. 2006. Validation of Bayer CropScience Method AI-001-P04-01 An Analytical Method for the Determination of Residues of AE 0317309, AE 1073910, and AE B197555 in Wheat, Corn, and Soybean Matrices Using LC/MS/MS. Unpublished Bayer CropScience Report No.: RAAIX005. 206 pages.

MRID No. 46801807 Billian, B. 2005. Independent Laboratory Validation of the Analytical Method Al-001-P04-01 for the Determination of Residues of AE 0317309, AE 1073910 and AE B197555 in Plant Material. Unpublished Bayer CropScience, Monheim, Germany Study No.: P612050574. Bayer CropScience Report No.: MR-097/05. 72 pages.

MRID No. 46801808 Gould, T. and Brungardt J. 2006. Extraction Efficiency of AE B197555, AE 1073910, and AE 0317309 by Method AI-0001-P04-01. Unpublished Bayer CropScience Report No.: RAAIX011. 60 pages.

## EXECUTIVE SUMMARY:

Bayer CropScience has developed a high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS), Method AI-004-A05-01, as the data collection and enforcement method for residues of pyrasulfotole (AE 0317309), pyrasulfotole-desmethyl (AE 1073910), and pyrasulfotole-benzoic acid (AE B197555) in crop matrices.

Briefly, the crop matrices are extracted with a mixture of acetonitrile (ACN)/water ($H_2O$)/concentrated hydrochloric acid (HCl) (30:15:3, v/v). The sample extract is heated to 60°C for at least 30 minutes then cooled and a mixture of isotopic internal standards is added to the sample extract. A small aliquot is purified by C18 solid phase extraction (SPE). The solvent is removed from the sample and the residue is reconstituted for analysis by HPLC-MS/MS.

The limit of quantitation (LOQ) is 0.010 ppm for each analyte in each matrix. The proposed enforcement method was adequately validated in soybean grain, corn grain, corn stover, wheat forage, barley hay, and barley grain. A successful independent laboratory validation (ILV) was completed with samples of wheat grain and soybean grain. Extraction efficiency data demonstrated that the enforcement method can account for incurred residues of pyrasulfotole, pyrasulfotole-desmethyl, and pyrasulfotole-benzoic acid in plant matrices.

## STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method test data are classified as scientifically acceptable.

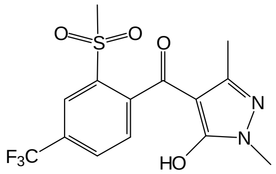
The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP# 333412], in Canada's Regulatory Decision Document, and in Australia's Residues Evaluation Report.

## COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided.

## A. BACKGROUND INFORMATION

Pyrasulfotole, ((5-hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl) phenyl]methanone), is a postemergence dicot herbicide for use in cereal crops. Pyrasulfotole is an effective inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPDase), and consequently blocks the pathway of prenylquinone biosynthesis in plants. The end-use products are applied to the target weeds and act primarily through leaf uptake and translocation to the target site. The first symptoms appear three to five days after application. Bleaching and discoloration appear initially and symptoms progress to tissue necrosis and plant death within two weeks.

| **TABLE A.1. Test Compound Nomenclature** | |
|---|---|
| Compound | Chemical Structure<br><br> |
| Common name | Pyrasulfotole |
| Company Experimental name | AE 0317309 |
| IUPAC name | (5-hydroxy-1,3-dimethylpyrazol-4-yl)(*α*,*α*,*α*-trifluoro-2-mesyl-*p*-tolyl)methanone |
| CAS name | (5-hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl] methanone |
| CAS # | 365400-11-9 |
| End-use product/(EP) | Herbicide; AE 0317309 02 SE06; AE 0317309 03 EC 23 A8 |

## TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound

| Parameter | Value | | Reference |
|---|---|---|---|
| Melting point | Pure: 201°C<br>No boiling point, decomposition starts at 245°C | | 1 |
| pH at 22.9°C | 3.03 | | 2 |
| Density (g/cm$^3$) | 1.53 | | 3 |
| Water solubility (g/L at 20°C) | 2.3<br>4.2<br>69.1<br>49.0 | pH 3.0 (distilled water)<br>pH 3.9 (buffer pH 4.0)<br>pH 5.4 (buffer pH 7.0)*<br>pH 5.2 (buffer pH 9.0)*<br>* exceeded buffer capacity | 4 |
| Solvent solubility (g/L at 20°C) | Ethanol<br>n-Hexane<br>Toluene<br>Dichloromethane<br>Acetone<br>Ethyl acetate<br>Dimethyl sulfoxide | 21.6<br>0.038<br>6.86<br>120-150<br>89.2<br>37.2<br>≥ 600 | 5 |
| Vapour pressure at 20°C | 2.7 X 10$^{-7}$ Pa | | 6 |
| Dissociation constant (pK$_a$) | 4.2 | | 7 |
| *n*-Octanol-water partition coefficient Log(K$_{OW}$) at 23°C | 0.276<br>-1.362<br>-1.580 | pH 4.0<br>pH 7.0<br>pH 9.0 | 8 |
| UV/visible absorption spectrum | $\lambda_{max}$= 264, 241, 216 nm in water, 0.1M HCl, 0.1M NaOH respectively. | | 9 |

## B. MATERIALS AND METHODS

### B.1. Data-Gathering Method

### B.1.1. Principle of the Method:

A 2.0 g aliquot of the crop matrix was weighed into a 60-mL vial and a mixture of ACN/$H_2O$/concentrated HCl (30:15:3, v/v) was added. The sample extract was heated to 60°C for at least 30 minutes. The samples were cooled and a mixture of isotopically labeled internal standards (IS) was added to the sample extract and mixed (0.10 ppm of each IS). A small aliquot (about 1.25 mL) was purified by C18 SPE. The solvent was removed from the sample and the residue was reconstituted for analysis by HPLC-MS/MS.

**TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of AE 0317309 Derived Residues in Plants.**

| | | | |
|---|---|---|---|
| Method ID | AI-001-P04-01 | | |
| Analyte(s) | Pyrasulfotole (AE 0317309), pyrasulfotole-desmethyl (AE 1073910) and pyrasulfotole-benzoic acid (AE B197555) | | |
| Extraction solvent/technique | Acetonitrile: deionized water: HCL (30:15:3) at 60°C for at least 30 minutes | | |
| Cleanup strategies | Solid phase extraction cartridge ( C-18) | | |
| Instrument/Detector | Phenomenex Gemini C-18, 50 x 2.0 mm, 5 µm<br>Thermo Finnigan Quantum Ultra LC-MS/MS with aqueous 0.01 M $NH_4OAc$ and methanol as mobile phases | | |
| Standardization method | Multi-point calibration curve with isotopically labeled internal standards | | |
| Stability of std solutions | Up to 274 days (~ 9 months) in acetonitrile:water (2:1 v/v) | | |
| Retention times (approximate) | AE B197555 2.2 min<br>AE 1073910 2.7 min<br>AE 0317309 3.0 min | | |
| Mass Spectrometer Data - Major daughter ion transitions monitored. AE B197555 was not detected in positive ion mode. | Analyte | Parent Ion | Daughter Ion |
| | AE B19755 | 266.95 | 158.95 |
| | AE 1073910 | 346.95 | 266.95 |
| | AE 0317309 | 360.95 | 78.95 |

### B.2. Enforcement Method

The enforcement method is the same as the data-gathering method.

# C. RESULTS AND DISCUSSION

## C.1. Data-Gathering Method

**TABLE C.1.1. Recovery Results from Method Validation of Soybean, Corn, Wheat, and Barley using the Data-Gathering Analytical Method.**

| Matrix | Analyte | Spiking Level (ppm) | Recoveries (%) | Mean Recovery ± SD (%) |
|---|---|---|---|---|
| Soybean Grain | AE B197555 | 0.010 | 96, 91, 93, 87, 99, 96, 94 | 94 ± 4.0 |
| | | 0.250 | 94, 96, 97, 98, 97, 102, 98 | 97 ± 2.5 |
| | AE 1073910 | 0.010 | 92, 91, 93, 88, 93, 98, 90 | 92 ± 3.1 |
| | | 0.250 | 100, 98, 101, 100, 101, 101, 100 | 100 ± 1.0 |
| | AE 0317309 | 0.010 | 88, 86, 80, 82, 75, 85, 89 | 84 ± 5.8 |
| | | 0.250 | 99,104, 99, 98, 100, 100, 98 | 100 ± 2.0 |
| Corn Grain | AE B197555 | 0.010 | 90, 92, 85, 91, 95, 91, 86 | 90 ± 4.0 |
| | | 0.250 | 99, 97, 98, 96, 97, 96, 94 | 97 ± 1.4 |
| | AE 1073910 | 0.010 | 89, 91, 86, 87, 86, 86, 90 | 88 ± 2.2 |
| | | 0.250 | 100, 100, 100, 101, 102, 99, 97 | 100 ± 1.8 |
| | AE 0317309 | 0.010 | 75, 81, 76, 85, 76, 88, 80 | 80 ± 6.1 |
| | | 0.250 | 95, 95, 97, 95, 95, 95, 92 | 95 ± 1.5 |
| Corn Stover | AE B197555 | 0.010 | 90, 98, 81, 97, 90, 93, 88 | 91 ± 6.3 |
| | | 0.250 | 98, 98, 100, 97, 96, 97, 98 | 98 ± 1.3 |
| | AE 1073910 | 0.010 | 89, 92, 90, 98, 85, 91, 93 | 91 ± 4.3 |
| | | 0.250 | 98, 99, 100, 99, 96, 96, 99 | 98 ± 1.6 |
| | AE 0317309 | 0.010 | 84, 86, 84, 90, 79, 84, 83 | 84 ± 4.0 |
| | | 0.250 | 100, 101, 102, 99, 104, 100, 100 | 101 ± 1.7 |
| Wheat Forage | AE B197555 | 0.010 | 93, 98, 94, 96, 93, 91, 90 | 94 ± 2.8 |
| | | 0.250 | 99, 99, 97, 99, 99, 101, 94 | 98 ± 2.2 |
| | AE 1073910 | 0.010 | 89, 95, 92, 94, 95, 93, 92 | 93 ± 2.3 |
| | | 0.250 | 101, 100, 102, 101, 104, 105, 102 | 102 ± 1.6 |
| | AE 0317309 | 0.010 | 80, 87, 89, 86, 85, 84, 88 | 86 ± 3.3 |
| | | 0.250 | 97, 96, 97, 100, 98, 99, 99 | 98 ± 1.4 |
| Barley Grain | AE B197555 | 0.010 | 100, 88, 96, 96, 89, 91, 99 | 94 ± 5.2 |
| | | 0.250 | 96, 95, 99, 97, 99, 98, 99 | 98 ± 1.9 |
| | AE 1073910 | 0.010 | 94, 92, 91, 95, 92, 96, 93 | 93 ± 1.8 |
| | | 0.250 | 102, 104, 104, 101, 102, 104, 103 | 103 ± 1.2 |
| | AE 0317309 | 0.010 | 87, 91, 88, 86, 91, 90, 92 | 89 ± 2.6 |
| | | 0.250 | 97, 97, 100, 96, 99, 97, 98 | 98 ± 1.2 |
| Barley Hay | AE B197555 | 0.010 | 104, 90, 93, 101, 96, 85, 104 | 96 ± 7.7 |
| | | 0.250 | 94, 98, 94, 99, 94, 94, 95 | 96 ± 2.0 |
| | AE 1073910 | 0.010 | 95, 91, 93, 93, 93, 93, 92 | 93 ± 1.3 |
| | | 0.250 | 99, 101, 98, 101, 99, 100, 101 | 100 ± 1.1 |
| | AE 0317309 | 0.010 | 90, 88, 86, 89, 89, 86, 92 | 89 ± 2.6 |
| | | 0.250 | 99, 100, 100, 101, 101, 99, 100 | 100 ± 0.9 |

**TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of AE 0317309, AE 1073910, and AE B197555 in Soybean, Corn, Wheat, and Barley.**

| Analyte | Soybean Grain | Corn Grain | Corn Stover | Wheat Forage | Barley Grain | Barley Hay |
|---|---|---|---|---|---|---|
| AE 0317309 (Pyrasulfotole) | | | | | | |
| LOQ (ppm) | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 |
| LOD (ppm) | 0.0015 | 0.0020 | 0.0011 | 0.0010 | 0.0009 | 0.0009 |
| Accuracy/ Precision | 75% to 104% | 75% to 97% | 79% to 104% | 80% to 100% | 86% to 100% | 86% to 101% |
| AE 1073910 (Pyrasulfotole-desmethyl) | | | | | | |
| LOQ (ppm) | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 |
| LOD (ppm) | 0.0014 | 0.0012 | 0.0017 | 0.0008 | 0.0008 | 0.0008 |
| Accuracy/ Precision | 88% to 101% | 86% to 102% | 85% to 100% | 89% to 105% | 91% to 104% | 91% to 101% |
| AE B197555 (Pyrasulfotole-benzoic acid) | | | | | | |
| LOQ (ppm) | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 |
| LOD (ppm) | 0.0012 | 0.0014 | 0.0020 | 0.0009 | 0.0019 | 0.0029 |
| Accuracy/ Precision | 87% to 102% | 85% to 99% | 81% to 100% | 90% to 101% | 88% to 100% | 85% to 104% |
| Equipment ID | ThermoFinnigan Surveyor HPLC with Quantum Ultra LC-MS/MS | | | | | |
| Method Reliability | An independent laboratory validation was conducted with wheat grain and soybean grain. Recoveries ranging from 90% to 115% were obtained. | | | | | |
| Linearity | The detector response for all analytes from 0.005 ppm to 2.50 ppm was linear with a correlation coefficient ($R^2$) of >0.999. | | | | | |
| Specificity | Control samples had residue levels of 0.0012 ppm or below. Peaks for all three analytes were well defined and symmetrical. There appeared to be no carryover to subsequent chromatograms. | | | | | |

## C.2. Enforcement Method

The enforcement method is the same as the data-gathering method. The extraction efficiency of method AI-001-P04-01 for the determination of the total radioactive residue (TRR) of pyrasulfotole was tested using samples obtained from metabolism studies treated with [phenyl-U-$^{14}$C]-pyrasulfotole[10,11]. The analytical residue method effectively extracted 96%, 110% and 104% of the TRR from aged wheat forage, wheat grain, and wheat hay, respectively.

Concurrent recovery data demonstrated that the method can effectively recover pyrasulfotole equivalent residues spiked from 0.01 to 0.250 ppm. The method adequately bracketed the expected residues in wheat, barley, soybean, and corn commodities. The LC-MS/MS was specific for the analysis of analytes of interest. No interferences were noted above the LOD.

**TABLE C.2.1. Distribution of Residues Found in Wheat Forage, Hay and Grain by Residue Method as Compared with Those Found in Metabolism Studies.**

| Matrices | Amount of AE 0317309 equivalents | | | | Extraction Efficiency (%) |
|---|---|---|---|---|---|
| | Analytical Residue Method | | Metabolism Studies | | |
| | % TRR | ppm | % TRR | ppm | |
| Wheat Forage | | 1.472, 1.660, 1.488 | | | |
| Average | 59.9 | 1.540 | 67.1 | 1.61[a] | 95.7 |
| Wheat Hay | | 0.0527, 0.0504, 0.0496 | | | |
| Average | 80.3 | 0.0509 | 80.2 | 0.049[b] | 103.9 |
| Wheat Grain | | 0.1915, 0.1661, 0.1389 | | | |
| Average | 79.8 | 0.1655 | 97.6 | 0.15[a] | 110.3 |

a Value from the wheat metabolism study (phenyl-label) with safener.
b Value from the confined rotational crop study (phenyl-label) with safener.

## C.3. Independent Laboratory Validation

An ILV was performed using wheat grain and soybean grain. The individual recoveries for all analytes were between 70% and 120%, with a relative standard deviation (RSD) below 20%. The laboratory performing the ILV suggested no modifications to the method.

**TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Method for the Determination of AE 0317309, AE B197555, and AE 1073910 in Plant Matrices.**

| Matrix | Analyte | Spiking Level (ppm) | Recoveries (%) | Mean Recovery ± SD (%) |
|---|---|---|---|---|
| **Wheat Grain** | AE B197555 | 0.010 | 115, 104, 114, 100, 108 | 108 ± 5.9 |
| | | 0.200 | 112, 104, 101, 98, 107 | 104 ± 5.2 |
| | | 0.100 | 107, 104, 104, 106, 102 | 105 ± 1.9 |
| | AE 1073910 | 0.010 | 103, 105, 107, 109, 99 | 105 ± 3.7 |
| | | 0.200 | 106, 102, 100, 94, 102 | 101 ± 4.4 |
| | | 0.100 | 100, 102, 100, 101, 100 | 101 ± 0.9 |
| | AE 0317309 | 0.010 | 100, 99, 90, 93, 97 | 96 ± 4.4 |
| | | 0.200 | 96, 98, 96, 98, 94 | 96 ± 1.7 |
| | | 0.100 | 97, 95, 101, 97, 98 | 98 ± 2.2 |
| **Soybean Grain** | AE B197555 | 0.010 | 101, 100, 105, 94, 100 | 100 ± 3.9 |
| | | 0.200 | 103, 104, 97, 101, 104 | 102 ± 2.9 |
| | | 0.100 | 98, 100, 101, 94, 101 | 99 ± 3.0 |
| | AE 1073910 | 0.010 | 99, 97, 97, 96, 93 | 96 ± 2.3 |
| | | 0.200 | 97, 95, 94, 96, 95 | 95 ± 1.2 |
| | | 0.100 | 96, 95, 94, 95, 92 | 94 ± 1.6 |
| | AE 0317309 | 0.010 | 96, 103, 100, 96, 104 | 100 ± 3.8 |
| | | 0.200 | 103, 95, 99, 99, 100 | 99 ± 2.9 |
| | | 0.100 | 102, 98, 98, 99, 100 | 99 ± 1.7 |

## D. CONCLUSION

Adequate method validation data have been submitted for the LC-MS/MS method (AI-001-P04-01) for the determination of residues of pyrasulfotole, pyrasulfotole-desmethyl, and pyrasulfotole-benzoic acid in plant commodities. The validation data are representative of the expected residue levels for the plant commodities. The method is adequate to quantitate incurred residues of pyrasulfotole, pyrasulfotole-desmethyl, and pyrasulfotole-benzoic acid in plant matrices.

## E. REFERENCES

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10. Haas, M. (2004). Metabolism of [phenyl-U-$^{14}$ C] AE 0317309 in Wheat Following Treatment at an Application Rate of 100 g/ha with and without Safener. Unpublished Bayer CropScience Report No. MEF-322/03

11. Beedle, E. C. and Ying, S.L. (2006). The Metabolism of [Phenyl-UL-$^{14}$C] AE 0317309 in Confined Rotational Crops. Unpublished Bayer CropScience Report No. 201152.

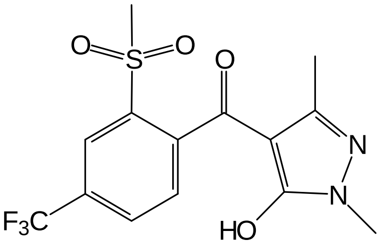
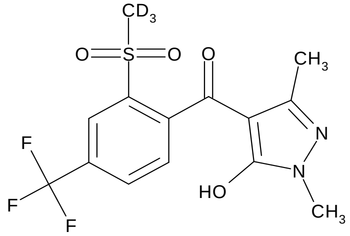
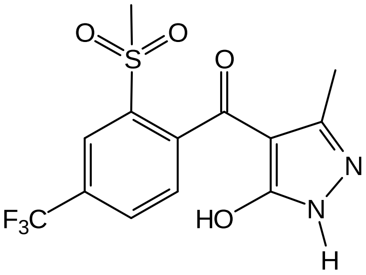
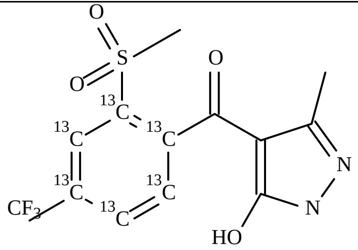
## F. DOCUMENT TRACKING

RDI: Louise G Croteau (6 September 2006); RAB1 Chemists (29 November 2006); George Kramer (29 November 2006)
Petition Number: 6F7059
DP#: 333412
PC Code: 000692

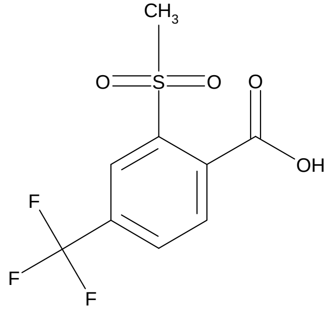
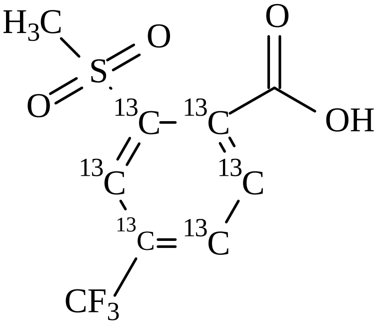
Template Version June 2005.

## APPENDIX 1

Reference standards.

| Common name/code | Chemical name | Chemical structure |
|---|---|---|
| pyrasulfotole<br>AE 0317309 | (5-hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl] methanone |  |
| $d_3$-pyrasulfotole<br>$d_3$-AE 0317309 | (5-Hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-[(methyl-$d_3$)sulfonyl]-4-(trifluoromethyl)phenyl]methanone |  |
| pyrasulfotole-desmethyl<br>AE 1073910 | (5-hydroxy-1*H*-pyrazol-4-yl)[2-mesyl-4-(trifluoromethyl)phenyl]methanone |  |
| [phenyl-$^{13}C_6$]AE 107391<br>AE 1073910-IS | (5-Hydroxy-3-methyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)-phenyl-$^{13}C_6$]methanone |  |

Reference standards continued.

| Common name/code | Chemical name | Chemical structure |
|---|---|---|
| pyrasulfotole-benzoic acid<br>AE B197555 | 2-(Methylsulfonyl)-4-(trifluoromethyl)benzoic acid |  |
| [phenyl-$^{13}C_6$]AE B197555<br>AE B197555-IS | 2-(Methylsulfonyl)-4-(trifluoromethyl)benzoic-*1,2,3,4,5,6-13C6* acid |  |