UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS
ENVIRONMENTAL CHEMISTRY LABORATORY
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October 25, 2006

Pc – 114009

MEMORANDUM

DP Barcode: D318310

SUBJECT: O-Desmethyl MKH 6562 [metabolite of MKH 6562 Flucarbazone-sodium)] in Soil-Report No. ECM0222S5

FROM: Joseph B. Ferrario, Branch Chief 10/20/06
OPP/BEAD/Environmental Chemistry Laboratory

TO: Cara Dzubow
OPP/Environmental Fate and Effects Division
Information and Support Branch (7507C)

The EFED Environmental Fate and Effects Division has requested an Environmental Chemistry Method Review on O-Desmethyl MKH 6562 [metabolite of MKH 6562 (Flucarbazone-sodium)] in soil using the method submitted by Bayer CropScience in accordance with the registration of Flucarbazone-sodium, MRID No. 462237-01. Initially a method review was conducted on the method with no Independent Laboratory Validation (ILV) and ECB found the method unacceptable due to that omission. ECB have recently received the ILV, reviewed it, and found that the ILV data does support the method. The method has now been determined to be acceptable. The independent validation of the method was completed successfully on its first trial. The validation recoveries indicates that the method is rugged. The method is suitable for determining residues of O-Desmethyl MKH 6562 to an LOQ of 0.5 ppb.

Please regard this memorandum as the official update of the original ECM Method Review Report which is attached.
(May 25, 2006)

If you have questions concerning this report, please contact Charles Kennedy at (228) 688-2443 or Elizabeth Flynt at (228) 688-2410.

Attachments
cc: Christian Byrne, QA Officer
BEAD/Environmental Chemistry Laboratory

Charles D. Kennedy
BEAD/Environmental Chemistry Laboratory

2053401

Flucarbazone sodium O-Desmethyl MKH 6562 in Soil/ECM0222S5/Bayer Corp.
ENVIRONMENTAL CHEMISTRY METHOD REVIEW EVALUAION

Data Requirement: PMRA Data Code: NA
EPA DP Barcode: - D318310
OECD Data Point: NA
EPA Guideline: ECM Method Review

Test material:
Common name: Flucarbazone sodium O-desmethyl metabolite
Chemical name: 4, 5-dihydro-3-methoxy-5-oxo-N-[[2-(trifluoromethoxy)phenyl] sulfonyl]-H-1,2,4-triazole-1-carboxamide
IUPAC: See above

Primary Evaluator: _____ **Date:**
Charles Kennedy, Chemist, EPA/OPP/BEAD/ECB
Peer Reviewer: _____ **Date:**
Elizabeth Flynt, Chemist, EPA/OPP/BEAD/ECB
QA Officer: _____ **Date:**
Dr. Christian Byrne, EPA/OPP/BEAD/ECB

ANALYTICAL METHOD: C. K. Lam and S. S. Qadri, 2003. Analytical Method for the Determination of O-Desmethyl MKH 6562 (metabolite of MKH 6562) in Soil by High-Performance Liquid Chromatography Tandem Mass Spectrometry. Unpublished method created by Arvesta Corporation, 100 First Street, Suite 1700, San Francisco, CA 94105. Study ID: 200747, Method Effective Date: December 15, 2003.

EXECUTIVE SUMMARY

This method is applicable for the quantitative determination of residues of O-desmethyl MKH 6562 in soil. The method was submitted to EPA to support studies performed to seek registration for Flucarbazone sodium MKH 6562. The method was created by Arvesta Corporation in San Francisco, California and performed by Bayer CropScience of Stilwell, KS in the spirit of EPA's Good Laboratory Practice Standards, Title 40, Code of the Federal Regulations Part 160. Due to the fact that an independent laboratory validation and raw calibration data were not submitted with this method, ECB has found the method to be unacceptable.

Method Summary: An analytical method was developed to quantify O-desmethyl MHK 6562 (metabolite of MKH 6562) in soil using high-performance liquid chromatography electrospray tandem mass spectrometry (LC-MS/MS). The method was

validated using control soil from the dissipation study sites in the states Oklahoma and Washington. Soil samples (25-g) from both sites were spiked with O-desmethyl MKH 6562 at 0.5 ppb and 5 ppb. The soil samples were extracted and derivatized with methanol/water (9:1, v/v) using Accelerated Solvent Extraction (ASE) at 80 °C for 5 minutes. The extracts were evaporated to dryness and reconstituted to 1 mL with HPLC solvent. The concentrated extracts were filtered with 0.45-µm nylon Acrodiscs® and analyzed by LC-MS/MS operated in negative electrospray-ionization mode.

The average recoveries of O-desmethyl MKH 6562 fortified at the LOQ (0.5 ppb) and the 10 x LOQ (5.0 ppb) were all within the acceptable range.

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

There are several problems with this method. First, there was no Independent Laboratory Validation (ILV) completed. It is recommended that an independent lab perform a secondary validation on this data.

Also, there was a lack of raw calibration data provided. This made it impossible to reconstruct the preparation of the calibration curve. Additionally, the quadratic formula provided in the paper as part of the calibration should be removed from the method. The formula in question was the following:

$$\frac{X = -B + \sqrt{B^2 - 4AC}}{2A}$$

An EPA statistician who reviewed the formula agreed with the ECB reviewer that there is no way to use this formula to solve for X since it is a closed formula solution to the quadratic equation. In this case it should not be used, since the quadratic equation is deterministic.

The ECB reviewer speculates that for the validation the mass spectrometer software was used to generate the quadratic equation and automatically calculate the residue concentrations. Apparently, the formulas supplied in the method were the registrant's interpretation of the mass spectrometer calculations that were performed. It is likely that the calculations for the validation of the method were done correctly. Therefore, the incorrect equation should just be removed from the method.

Under the conditions and parameters set in the Ecological Effects Test Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods; "Public Draft." (U.S. Environmental Protection Agency Office of Prevention, Pesticides, and Toxic

Substances (7101). U.S. Government Printing Office: Washington, DC, 1996, EPA-712-C-96-348), this method was unacceptable.

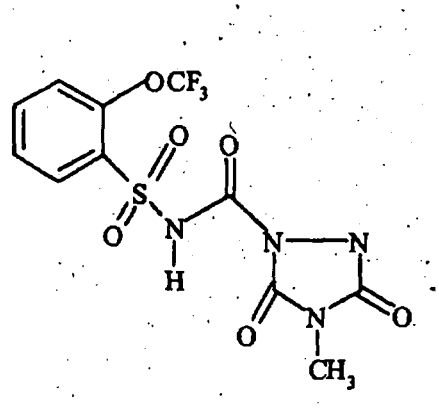
COMPLIANCE

A signed and dated statement was given that this method was not conducted in accordance with the requirements for Good Laboratory Practice, 40 CFR 160, but in the sprit of GLP. Also, a statement of non-confidentiality on the basis of the method falling within the scope of FIFRA Section 10(d)(1)(A)(B) or (C) was signed and dated along with information on the Quality Assurance inspection dates and signatures.

A. BACKGROUND INFORMATION

Flucarbazone-sodium O-desmethyl is an experimental herbicide being developed by Bayer CropScience in conjunction with Arvesta Corporation for use on annual grasses and some broadleaf weeds in wheat.

TABLE A.1. Test Compound Nomenclature

Compound	 O-desmethyl MKH 6562
Common name	Flucarbazone sodium O-desmethyl
Company experimental name	O-desmethyl MKH 6562
IUPAC name	4,5-dihydro-3,4-methyl-3,5-oxo-N-[[2(trifluoromethoxy) phenyl] sulfonyl]-1H-1,2,4-triazole-1-carboxamide, sodium salt)
CAS Name	1 H-1,2,4-triazole-carboxamide,4,5-dihydro-4-methyl-3,5-oxo-N-((2-(trifluoromethoxy)penyl)sulfonyl)-sodium salt

CAS #	NA

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound	
Parameter	Value
Melting point/range	Not Given
Color and physical state	Not Given
Volatility	Nor Given
Freezing Point	Not Given
Specific Gravity	Not Given
Vapor pressure	Not Given
Solubility in water	Not Given
Bulk Density	Not Given
Molecular Weight	Not Given

MATERIALS AND METHODS

B.1. Principle of Method

An analytical method was developed for determination of O-Desmethyl MKH 6562 [metabolite of MKH 6562 (Flucarbazone-sodium)] in 25 grams of control soil. Soils were fortified as appropriate with the internal standard of O-desmethyl MKH 6562. The soil samples were simultaneously extracted and derivatized with methanol/water (9:1, v/v) to MKH 6562 N-methyl carbamate using an Accelerated Solvent Extraction at 80 °C for 5 minutes. The extracts were evaporated to dryness and reconstituted to 1 mL with HPLC solvent. The resultant extracts were filtered and analyzed by LC-MS/MS operated in the negative electrospray-ionization mode. Quantitation of analyte was based on duplicate, five data point solvent calibration curves with a concentration range from 0.2 to 10 ppb. The peak area ratio of native to internal standard of each compound was plotted with its standard concentration. The slope and intercept from a quadratic weighted $1/X^2$ was used for quantitation.

TABLE B.1.1.	Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied
Method ID	ECM0222S5
Analyte(s)	O-desmethy MKH 6562
Extraction solvent/technique	Accelerated Solvent Extraction at 80 °C for 5 min.

TABLE B.1.1.	Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied
Cleanup strategies	0.45-µm nylon Acrodiscs® filter
Instrument/Detector	ESI/MS/MS Conditions: ThermoFinnigan TSQ 7000 LC/Tandem Mass Spectrometer operated in negative electrospray-ionization mode (ESI). Capillary Temp: 300°C, Sheath Gas/pressure (psi): N_2 /90-100, Auxiliary Gas Flow (mL/min): N_2 (15-20), CDI Gas: 2.5 mtorr/argon, Scan Time: 0.3 s (for each ion), Polarity: Negative HPLC Conditions: Column: Luna C18(2), 5 µm, 100 , 100 x 4.6 mm, Flow (column): 0.8 mL/min, Split Ratio: 4:1, Flow (interface): 200 µL/min, Injection Volume: 20-50 µL, Column Temp: 35 °C, Mobile Phase: A: 5 mM NH_4OAc in water, B: 5mM NH_4OAc in MeOH.

C. RESULTS AND DISCUSSION

C.1. Recovery Results Summary

TABLE C.1.1. Recovery Results from Method Validation of [matrices]			
Matrix Soil	Spiking Level ppb	Average Recovery Obtained (%)	Relative Standard Deviation (%)
O-desmethyl MKH 6562 (Washington)	0.5 5.0	83.5 78.7	5.5 5.7
O-desmethyl MKH 6562 (Oklahoma)	0.5 5.0	79.1 79.7	11.1 6.6

C.1.2. Method Characteristics

TABLE C.1.2. Method Characteristics	
Analyte	O-desmethyl MKH 6562
Limit of Quantitation	0.5 ppb.
Limit of Detection (LOD)	0.139 (Oklahoma), 0.072 (Washington)
Accuracy/Precision at LOQ	See above chart
Reliability of the Method/ [ILV]	No ILV available
Linearity	All method responses were non-linear (coefficient of determination for compound was greater than $r^2 = 0.9907$).

TABLE C.1.2. Method Characteristics	
Specificity	The method is specific for the determination of O-desmethyl MKH 6562 by virtue of the chromatographic separation and selective detection system used. According to recently published guidelines, when detection is performed by tandem mass spectrometry methods, confirmation of the presence of the analyte should require the observation of a precursor ion plus one structurally significant product ion observed at the same retention time. Further confirmation is not necessary due to the highly specific nature of the MS/MS transitions monitored.

C.2. Independent Laboratory Validation (ILV)

The ILV was conducted in accordance with the *OPPTS 850.7100 Guidelines.*

TABLE C.2.1. Average Recovery Results Obtained by an Independent Laboratory Validation of the Method for the Determination of O-Desmethy MKH 6562 (Metabolite of MKH 6562) in Soil by High-Performance Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)

Matrix Soil	Spiking Level (ppb)	Average Recoveries Obtained (%)	Relative Standard Deviation
(No ILV)	(No ILV)	(No ILV)	(No ILV)

D. CONCLUSION

From a review of the method, C. K. Lam and S. S. Qadri "Analytical Method for the Determination of O-Desmethyl MKH 6562 (Metabolite of MKH 6562) in Soil by High-Performance Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)", ECB concludes that the registrant have a secondary independent laboratory validation (ILV) conducted on the data. Also, there was a lack of raw calibration data provided which made it impossible to reconstruct the preparation of the calibration curve. In its present form ECB finds this method unacceptable for determining O-Desmethyl MKH 6562 in soil.