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April 10, 2009

MEMORANDUM DP Barcode: D356962

SUBJECT: AE1170437 in Water - Report No. ECM0245W1-W6

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

TO: Margaret Ervin Gatekeeper
OPP/Environmental Fate and Effects Division
Information and Support Branch (7507C)

The Environmental Fate and Effects Division (EFED) has requested an Environmental Chemistry Method Review of a method for determination of AE1170437 and its metabolites in surface, drinking, and ground waters (MRIDs No. 474432-71, 474432-73, 474432-74). The method was submitted by Bayer CropScience in accordance with the registration of the above mentioned analytes. The method validation data was reviewed and the conclusions included in the attached Environmental Chemistry Method Review Report.

The following report includes an overview of the method and the method completeness, statements of adherence to EPA regulations, a presentation of results and a discussion of problems found in the registrant method. A statement of method acceptability is also included.

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

Elizabeth Flynt
BEAD/Environmental Chemistry Laboratory

AE1170437 in Water/Bayer CropScience/264
ENVIRONMENTAL CHEMISTRY METHOD REVIEW REPORT

Data Requirement: PMRA Data Code: NA
EPA DP Barcode: - D356962
OECD Data Point: NA
EPA Guideline: ECM Method Review

Test material:
Common name: AE 1170347

CAS Name: N-[(1R,2S)-2,3-dihydro-2,6-dimethyl-1H-inden-1-yl]-6-[(1R)-1-fluoro-ethyl]-1,3,5-triazine-2,4-diamine

Primary Evaluator: Charles Kennedy **Date:** 01/05/09
Charles Kennedy, Chemist, EPA/OPP/BEAD/ECB

Peer Reviewer: Elizabeth Flynt **Date:** 03/30/09
Elizabeth Flynt, Chemist, EPA/OPP/BEAD/ECB

QA Officer: Christian Byrne **Date:** 04/06/09
Dr. Christian Byrne, EPA/OPP/BEAD/ECB

ANALYTICAL METHOD: 47443273, 47443271, Tianbo Xu, Ph.D., D., June 27, 2008, *"Method of Analysis for the Determination of Residues of AE1170437 and its Metabolites AE1170437 Carboxylic Acid (AE2158969), AE1170437 Triazine-indanone (AE2158968), AE1170437 Hydroxyethyl (AE2300077), AE1170437 Olefin (BCS-AA10201), and AE1170437 Diaminotriazine (1-Fluoroethyl triazinediamine) in Water Using LC/MS/MS"*. The unpublished study was developed by Bayer CropScience of Stilwell, Kansas and sponsored by Bayer CropScience of Research Triangle Park, North Carolina. Pages 1- 53. Method Number DH-005-W07-02 and Study Number M-302676-02-1.

EXECUTIVE SUMMARY

The method is applicable for the quantitative determination of residues of AE1170437 and its five metabolites in water. The method was created by Bayer CropScience of Stilwell, Kansas and is not considered an experimental study in accordance with EPA's Good Laboratory Practice Standards, Title 40 Code of Federal Regulations Part 160.3. It was independently validated by Bayer CropScience of Monheim am Rhein Germany. The independent laboratory validation that was submitted with this method was entitled, "*Independent Laboratory Validation of Method DH-005-W07-01 for the Determination of Residues of AE1170437 and its Metabolites AE1170437 Carboxylic Acid (AE2158969), AE1170437 Triazine-indanone (AE2158969), AE1170437 Hydroxyethyl (AE2300077), AE1170437 Olefin (BCS-AA10201) and AE1170437 Diaminotriazine (1-Fluoroethy triaziediamine) in Water Using LC-MS/MS*". After completing a method review, ECB finds this method and its validation data acceptable.

AE1170437 in Water/Bayer CropScience/264
ENVIRONMENTAL CHEMISTRY METHOD REVIEW REPORT

Method Summary

This method sets forth the procedure for determining the residues of AE1170437 and its metabolites AE2158969, AE2158968, AE2300077, BCS-AA10201 and 1-Fluoroethyl triazinediamine in water. An aliquot of water containing AE1170437 and its metabolites is fortified with isotopically labeled internal standards of AE1170437 and its metabolites. The sample is directly injected into a liquid chromatograph-tandem mass spectrometer (LC/MS/MS). Quantification is based on the use of internal standards and comparison of peak areas with those of known standards.

The reported limit of quantification (LOQ) was determined to be 0.05 ppb for AE1170437 and its five metabolites. Calculated method detection limits (MDL) was set at 0.02 ppb for all analytes to allow for variation over time and between instruments.

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

ACCEPTIBILITY

The method gave acceptable results and was successfully validated by the registrant and independent laboratory Bayer CropScience AG in Monheim am Rhein Germany. Based on the 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), ECB finds this method acceptable for AE1170437, and its metabolites AE1170437 Carboxylic Acid (AE2158969), AE1170437 triazine-indanone (AE2158968), AE1170437 Hydroxyethyl, AE1170437 Olefin (BCS-AA10201) and AE1170437 Diaminotriazine (1-Fluoroethyl Triazinediamine) as submitted.

COMPLIANCE

A statement was presented by the registrant that according to 40 CFR Part 160.3, this was not an experimental study and therefore, the rules and regulating set forth under 40 CFR 160 do not apply. This is in disagreement with EPA policy as set forth in OPPTS 850.7100 Data Reporting for Environmental Chemistry Guidelines. The guidelines call for adherence to FIFRA GLP as describe in 40 CFR Part 160.3. 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 Certificate of Authenticity dates and signatures.

A. BACKGROUND INFORMATION

AE1170437 is a non-selective herbicide currently being developed by Bayer CropScience.

TABLE A.1. Test Compound Nomenclature	
Compound:	Chemical Structure *(See Appendix A for chemical structure information)
Common Name	AE1170437
Company Name	Indaziflam (ISO proposed)
IUPAC Name	(2S,3R)-3-({4-amino-6-[(1R)-1-fluoroethyl]-1,3,5-trizin-2-yl}amino)-2-methylindane-5-carboxylic acid
CAS Name	N-[(1R,2S)-2,3-dihydro-2,6-dimethyl-1H-inden-1-yl]-6-[(1R)-1-fluoroethyl]-1,3,5-triazine-2,4-diamine
CAS Number	730979-19-8

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

Parameter	Value
Melting point	183-184°C AE1170437 pure substance, 177°C AE1170437 technical substance
pH @ 23°C	pH = 6.5 AE1170437 pure substance, pH = 5.1 AE1170437 technical substance
Density (g/mL @ 20°C)	1.23 AE1170437 pure substance, 1.23 technical substance
Water solubility (20°C)	pH 4: 4.4 mg/L, pH 9: 2.8 mg/L, Distilled water (pH 6.6-6.9): 2.8 mg/L
Solvent solubility (g/L at 20°C)	Acetone: 55 g/L, Acetonitrile: 7.6 g/L, Dichloromethane: 150 g/L, Dimethyl sulfoxide: >250 g/L, Ethanol: 13.0 g/L, Ethyl acetate: 47 g/L, Heptane: 0.032 g/l, Toluene: 4.3 g/L
Vapor pressure	2.5×10^{-8} PA at 20°C, 6.8×10^{-8} PA at 25°C, 6.9×10^{-6} at 50°C
Dissociation constant (pK_a)	3.5
Octanol/water partition coefficient	pH 2: 2.0, pH4, pH7 and pH9: 2.8
UV/visible absortion spectrum	213 nm/Å = 1.428, 268 nm/Å = 0.197, 291 nm/Å = 0.019

B. MATERIALS AND METHODS

B.1. Principle of Method

This method sets forth the procedure for determining the residues of AE1170437 and its metabolites AE2158969, AE2158968, AE2300077, BCS-AA10201 and 1-Fluoroethyl triazinediamine in water.

An aliquot of water containing AE1170437 and its metabolites is fortified with isotopically labeled internal standards of AE1170437 and its metabolites. The samples are filtered if necessary and directly injected into a liquid chromatograph-tandem mass spectrometry (LC/MS/MS). Quantification is based on the use of isotopically labeled internal standards and comparison of peak areas with those of known standards.

TABLE B.1.1.	Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied
Method ID	ECM0245W1-W6
Analyte(s)	AE1170437(Parent), AE2158969, AE2158968, AE2300077, BCS-AA10201, and (1-Fluoroethyl triazinediamine)
Extraction/Fortification technique	An aliquot of water containing AE1170437 and its metabolites is fortified with isotopically labeled internal standards of AE1170437 and its metabolites. The sample is directly injected to liquid chromatograph-tandem mass spectrometry (LC/MS/MS).
Cleanup strategies	Filter with 0.45 µm GF/F, if necessary
Instrument/Detector	Applied Biosystems API 4000 LC with Turbolon Electrospray Interface/ Tandem Mass Spectrometer (LC/MS/MS)

C. RESULTS AND DISCUSSION

C.1. Recovery Results Summary

Table C.1.1
Method Summary Results of AE1170437 and its Metabolites in Water

Analyte	Spike Level, ppb	Mean ± Stdev (RSD)%
AE1170437	0.05	97.7 ± 2.7 (2.7)
	0.5	101 ± 0.9 (0.9)
AE1170437 Diaminotriazine	0.05	93.8 ± 3.1 (3.4)
	0.5	97.4 ± 1.5 (1.6)
AE1170437 Hydroxyethyl	0.05	98.8 ± 2.1 (2.1)
	0.5	103 ± 1.6 (1.6)
AE1170437 Carboxylic Acid	0.05	85.6 ± 2.4 (2.8)
	0.5	96.3 ± 1.9 (2.0)
AE1170437 Triazine-indanone	0.05	91.7 ± 2.4 (2.6)
	0.5	102 ± 1.5 (1.5)
AE1170437 Olefin	0.05	97.5 ± 4.0 (4.1)
	0.5	99.6 ± 1.3 (1.3)

C.1.2. Method Characteristics

TABLE C.1.2. Method Characteristics	
Analytes	AE1170437 and metabolites AE2158969, AE2158968, AE2300077, BCS-AA10201 and 1-Fluoroethyl triazinediamine
Limit of Quantitation	0.05 ppb
Limit of Detection	0.02 ppb

TABLE C.1.2. Method Characteristics	
Accuracy/Precision at LOQ	See chart in Table C.1.1
Reliability of the Method	An independent laboratory method validation [ILV] was conducted to verify the reliability of this method.
Linearity	Detector response was linear within the range of 0.025 – 1.0 ppb for all analytes.
Specificity	The method is highly specific due to the use of tandem mass spectrometry (LC/MS/MS). The control chromatograms generally have no peaks above the chromatographic background. Peaks were well defined and symmetrical.

C.2. Independent Laboratory Validation (ILV)

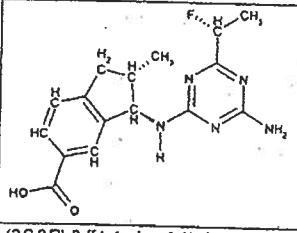
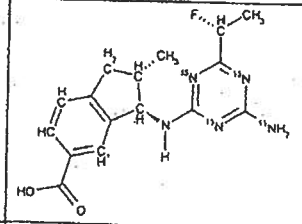
Overall Mean Recovery Rates and Relative Standard Deviations for River Rhine and Tap Water Monheim

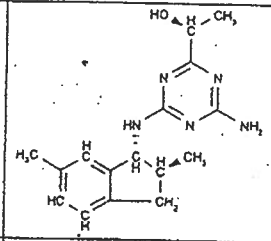
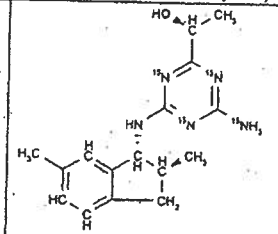
	River Rhine Water		Tap Water Monheim	
Substance	**Overall mean [%]**	**RSD [%]**	**Overall mean [%]**	**RSD [%]**
AE1170437	107	1.4	108	0.6
AE1170437 carboxylic acid (AE2158969)	100	1.7	101	1.7
AE1170437 triazine-indanone (AE2158968)	96	2.1	98	1.5
AE1170437 hydroxyethyl (AE2300077)	99	2.0	100	4.7
AE1170437 Olefin (BCS-AA10201)	105	1.6	104	0.8
AE1170437 diaminotriazine	105	2.3	104	2.3

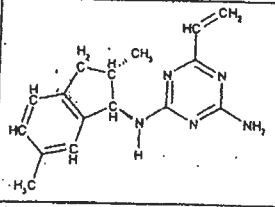
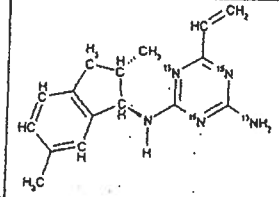
D. CONCLUSION

From a review of the method, "*Method of Analysis for the Determination of Residues of AE1170437 and its Metabolites AE1170437 Carboxylic Acid (AE2158969), AE1170437 Triazine-indanone (AE2158968), AE1170437 Hydroxyethyl (AE2300077), AE1170437 Olefin (BCS-AA10201), and AE1170437 Diaminotriazine (1-Fluoroethyl triazinediamine) in Water Using LC/MS/MS*", ECB concludes that the method is acceptable for determining the residues in water and to support registration studies.

Appendix A: Chemical Structures of AE1170437, its metabolites and associated internal standards

Code Name:	AE2158969 (AE1170437 Carboxylic Acid, metabolite)	AE2158969-triazine-$^{15}N_4$ (Internal standard for AE1170437 Carboxylic Acid)
Structure		
Chemical Name:	(2*S*,3*R*)-3-[[4-Amino-6-[(1*R*)-1-fluoroethyl]-1,3,5-triazin-2-yl]amino]-2,3-dihydro-2-methyl-1*H*-indene-5-carboxylic acid	(2*S*,3*R*)-3-[[4-Amino-6-[(1*R*)-1-fluoroethyl]-1,3,5-triazin-2-yl]amino]-2,3-dihydro-2-methyl-1*H*-Indene-5-carboxylic acid-$^{15}N_4$
Molecular Weight:	331.3, Monoisotopic mass: 331.1444	335.3
Molecular Formula:	$C_{16}H_{16}FN_5O_2$	$C_{16}H_{16}F^{15}N_4N\ O_2$

Code Name:	AE2300077 (AE1170437 Hydroxyethyl, metabolite)	AE2300077-triazine-$^{15}N_4$ (Internal standard for AE1170437 Hydroxyethyl)
Structure		
Chemical Name:	(1S)-1-(4-amino-6-[[(1R,2S)-2,6-dimethyl-2,3-dihydro-1H-inden-1-yl]amino)-1,3,5-triazin-2-yl)ethanol	(1S)-1-(4-amino-6-[[(1R,2S)-2,6-dimethyl-2,3-dihydro-1H-inden-1-yl]amino)-1,3,5-triazin-2-yl)ethanol-$^{15}N_4$
Molecular Weight:	299.4, Monoisotopic mass: 299.1746	303.3
Molecular Formula:	$C_{16}H_{21}N_5O$	$C_{16}H_{21}{}^{15}N_4N\ O$

Code Name:	BCS-AA10201 (AE1170437 Olefin, metabolite)	BCS-AA10201-triazine-$^{15}N_4$ (Internal standard for AE1170437 Olefin)
Structure		
Chemical Name:	N-[(1R,2S)-2,6-dimethyl-2,3-dihydro-1H-inden-1-yl]-6-vinyl-1,3,5-triazine-2,4-diamine	N-[(1R,2S)-2,6-dimethyl-2,3-dihydro-1H-inden-1-yl]-6-vinyl-1,3,5-triazine-2,4-diamine-$^{15}N_4$
Molecular Weight:	281.4, Monoisotopic mass: 281.1640	285.3