UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS
ENVIRONMENTAL CHEMISTRY LABORATORY
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October 23, 2007

MEMORANDUM

EPA DP Barcode: 340787

SUBJECT: Florasulam in Water Method Review Report No. ECM0234W1-W2

FROM: Joseph B Ferrario, Chief 10/23/07
OPP/BEAD/Environmental Chemistry Laboratory

To: Cara Dzubow, Program Analyst
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 the residues of XDE-520 and its 5-hydroxy metabolite in surface water using Method MRID No. 4680080-11 submitted by Dow Agrosciences, LLC in accordance with the registration of Florasulam. 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 Shanda L Bennett at (228) 688 – 3251 or me at (228) 688-3212.

cc: Dr. Christian Byrne, QA Officer
BEAD/ECL

Elizabeth Flynt, Chemist
BEAD/ECL

Florasulam/129108/Dow AgroSciences LLC/62719

ENVIRONMENTAL CHEMISTRY METHOD REVIEW REPORT

Data Requirement: PMRA Data Code: NA
EPA DP Barcode: - 340787
OECD Data Point: NA
EPA Guideline: ECM 0234W1-W2

Test material:
Common name: Florasulam
Chemical name: *N*-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo[1,5-*c*] pyrimidine-2-sulfonamide
IUPAC Name: 2′,6′,8-trifluoro-5-methoxy[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonanilide
CAS Number: 145701-23-1

Primary Evaluator: Shanda Bennett **Date:** 10/23/2007
Shanda Bennett, Chemist, EPA/OPP/BEAD/ECB

Peer Reviewer: Elizabeth Flynt **Date:** 10/23/07
Elizabeth Flynt, EPA/OPP/BEAD/ECB

QA Officer: Joseph Stone (Joe) **Date:** 10/23/07
Dr. Christian Byrne, EPA/OPP/BEAD/ECB

ANALYTICAL METHOD: 468080-11, Butcher, S., Gibson, R., August 29, 1996. *"Determination of the Residues of XDE-570 and Its 5-hydroxy Metabolite in Surface Water"*. The unpublished study was sponsored by Dow AgroSciences LLC, 9330 Zionville Road 308/2E, Indianapolis, Indiana 46268-1054. The study was performed by DowElanco Limited, Letcombe Laboratory, Letcombe Regis, Wantage, Oxon OX12 9JT UK. Pages 1-23.

EXECUTIVE SUMMARY

The method is applicable for the quantitative determination of the residues Florasulam (XDE-570) and its metabolite, 5-Hydroxy (XDE-570), in surface water.

The method was submitted to EPA by Dow AgroSciences LLC to support the registration of the herbicide - Florasulam. The method was created and reviewed by DowElanco Limited in Oxon OX12 9JT UK in accordance with OECD Principles of Good Laboratory Practice Standards. ECB finds this method unacceptable as submitted.

Method Summary: Florasulam (XDE-570) and its 5-hydroxy XDE-570 metabolite were extracted from surface water using a polystyrene divinylbenzene solid phase extraction cartridge, eluting both analytes from the cartridge with a 50:50 (v/v) acetonitrile/aqueous acid. The eluate was partitioned with methyl-tertiary-butyl ether (MTBE). The ether extract is purified using an aminopropyl solid phase extraction cartridge eluting the analytes with formic acid/acetronitrile/ MTBE mixture. The eluate was evaporated to

dryness. The residue is further purified by using a silica solid phase extraction cartridge eluting the analytes with a formic acid/acetonitrile/toluene mixture. The eluate is evaporated to dryness and the residue is reconstituted into a 20:80:1 (v/v/v) acetonitrile/water/acetic acid solution. XDE-570 and its metabolite are quantified by HPLC using UV absorbance detector set at 260 nm.

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

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) ECL finds this method unacceptable as submitted because of the following reasons:

The registrant did not submit an Independent Laboratory Validation (ILV) with the original method to support the data. The ILV should be performed by an independent laboratory that is not affiliated with the sponsor.

It was proposed that a comparison study entitled, "Determination of XDE-570 Concentrations in Drinking Water Using Both LC/UV and Immunoassay Methods" be used in place of the missing ILV since the LC/UV method used is the same as the method under review. Upon further investigation it was determined that the methods are quite different. The proposed comparison study was numbered R95-142 and was conducted in 1995 and utilized SPE extraction, followed by clean-up with additional SPE, and ion-pairing and derivatization by methylation. The matrix was drinking water. It also did not analyze for the metabolite (5-hydroxy XDE-570). The method under review is MRID #468080-11. It was numbered R96-15 and was conducted in 1996 and utilized SPE extraction, followed by clean-up with additional SPE, without ion-pairing or derivatization by methylation. The matrix was surface water. There are significant differences between the two methods that would preclude a direct comparison between them.

Additionally, although the calibration curves that were submitted in the original registrant's method did meet the acceptability requirements, it could not be verified by ECL due to the omission of the response factors and chromatograms at the LOQ (limit of quantitation), MDL (minimal detection limit) and 10 x LOQ level.

There are insufficient chromatograms and the associated peak height responses to verify the method. On page 13, under Calculations, there are no representative sample calculations to verify any of the values presented on Tables 4 or 6.

In addition, on page 21, the title of the last sample "Control Sample RV96-014-001 (diluted x 4)" is incorrect. The fortification of 5-hydroxy XDE-570 was 2.00 µg/L and not 0.20 µg/L. See Table 6, page 18.

Finally, ECL recommends a minor clarification pertaining to page 11, section 6.6 of the Sample and Fortified Sample Analysis portion of the method. The method states, "include a reagent blank and procedural recovery in each analytical batch". ECL would like for the registrant to clarify by changing the term "procedural recovery" to "procedural recovery control".

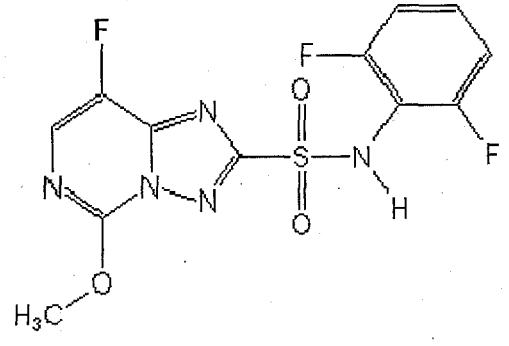
COMPLIANCE

Signed and dated statements that this method was conducted in accordance with the requirements for Good Laboratory Practice Standards, 40 CFR 160 were not presented in the method. However, a statement was noted that this method was conducted in compliance with OECD Principles of Good Laboratory Practice Standards. 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

Florasulam, *N*-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo[1,5-*c*] pyrimidine-2-sulfonamide is a herbicide that is used to control various broadleaf weeds in cereals and corn.

TABLE A.1. Test Compound Nomenclature

Compound	Chemical Structure
	
Common name	Florasulam (XDE-570)
Company experimental name	NA

IUPAC name	2′,6′,8-trifluoro-5-methoxy[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonanilide
CAS Name	*N*-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo[1,5-*c*] pyrimidine-2-sulfonamide
CAS #	145701-23-1

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

Parameter	Value
Melting point/range	Not provided
pH	Not provided
Density	Not provided
Water solubility	6.36g/l @ 20°C
Solvent solubility (mg/ml at 20 °C)	Not provided
Vapor pressure at 25°C	Not provided
Dissociation constant (pK_a)	Not provided
Octanol/water partition coefficient	Not provided
UV/visible absorption spectrum	Not provided

MATERIALS AND METHODS

B.1. Principle of Method

Florasulam (XDE-570) and its 5-hydroxy metabolite is extracted from surface water using a polystyrene divinylbenzene solid phase extraction cartridge, eluting both analytes from the cartridge with a 50:50 (v/v) acetonitrile/aqueous acid. The eluate is partitioned with methyl-tertiary-butyl ether (MTBE). The ether extract is purified using an aminopropyl solid phase extraction cartridge eluting the analytes with a formic acid/acetronitrile/ MTBE mixture. The eluate is evaporated to dryness. The residue is further purified by using a silica solid phase extraction cartridge. The analytes are eluted with a formic acid/acetonitrile/toluene mixture. The eluate is evaporated to dryness and the residue is reconstituted with a 20:80:1 (v/v/v) acetonitrile/water/acetic acid solution. XDE-570 and its metabolite are quantified by HPLC using UV absorbance detector set at 260 nm.

TABLE B.1.1.	**Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied**
Method ID	ECM0234W1-W2
Analyte(s)	Florasulam (XDE-570), 5-Hydroxy (metabolite)
Extraction solvent/technique	The specified volume of fortification solution was added to each 500 mL of surface water, acidified with sulfuric acid, capped and mixed well. Florasulam (XDE-570) and its 5-Hydroxy metabolite were extracted from surface water using a polystyrene divinylbenzene solid phase extraction cartridge, eluating both analytes from the cartridge with a 50:50 (v/v) acetonitrile/aqueous acid. The eluate was partitioned with methyl-tertiary-butyl ether (MTBE).
Cleanup strategies	The ether extract is purified using an aminopropyl solid phase extraction cartridge eluting the analytes with formic acid/acetonitrile/ MTBE mixture. The eluate was evaporated to dryness. The residue is further purified by using a silica solid phase extraction cartridge eluting the analytes with a formic acid/acetonitrile/toluene mixture. The eluate is evaporated to dryness and the residue is reconstituted into a 20:80:1 (v/v/v) acetonitrile/water/acetic acid solution. XDE-570 and its metabolite are quantified by HPLC using UV absorbance detector set at 260 nm.
Instrument/Detector	Milton Roy spectroMonitor 3100 UV detector Varian UK Ltd 9010 solvent delivery system Perkin-Elmer ISS100 autosampler

C. RESULTS AND DISCUSSION

C.1. Recovery Results Summary

TABLE C.1.1. Recovery Results from Method Validation of Water (FLORASULAM AND ITS METABOLITE)

Matrix	Spiking Level (µg/L)	Avg. % Recoveries	Relative Standard Deviation
Florasulam (XDE-570)	0.10	87	10.1
	0.25	94	4.4
	0.50	101	2.0
	1.00	98	2.9
5-Hydroxy XDE-570	0.20	96	10.1
	0.50	86	8.0
	1.00	93	1.9
	2.00	89	4.8

C.1.2. Method Characteristics

TABLE C.1.2. Method Characteristics	
Analyte	Florasulam
Limit of Quantitation (LOQ)	0.10 µg/L for XDE-570 0.20 µg/L for 5-Hydroxy XDE-570
Limit of Detection (LOD)	0.02 µg/L for XDE-570 0.04 µg/L for 5-Hydroxy XDE-570
Accuracy/Precision at LOQ	XDE-570 – 81% to 108% (mean 94%) 5-Hydroxy XDE-570 – 78% - 110% (mean 92%)
Reliability of the Method/ [ILV]	An independent laboratory method validation [ILV] was not submitted with this method.
Linearity	The detector response was linear over the range of 0.025 to 1 µg/mL; r = 0.999 for both compounds.
Specificity	The analytical method employs a highly specific and selective detector; therefore, a confirmatory method is not necessary.

C.2. Independent Laboratory Validation (ILV)

The ILV was not submitted with this method.

TABLE C.2.1. Recovery Results Obtained by an Independent Laboratory Validation of the Method for the Determination of Florasulam (XDE-570) and Its Metabolite 5-Hydroxy XDE-570 in surface water.			
Compound	Spiking Level (µg/L)	Average Recoveries Obtained (%)	Relative Standard Deviation (%)
Not provided.			

D. CONCLUSION

From a review of this method, Butcher, S., Gibson, R., August 29, 1996, *"Determination of the Residues of XDE-570 and Its 5-Hydroxy Metabolite in Surface Water"*, ECL concludes that this method is unacceptable as submitted.