UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS
ENVIRONMENTAL CHEMISTRY LABORATORY
Mail Code 7503ECB – Building 1105 – JOHN C. STENNIS SPACE CENTER
STENNIS SPACE CENTER, MISSISSIPPI 39529-6000
TELEPHONE (228) 688-3216 FACSIMILE (228) 688-3536

August 15, 2006

D330004

MEMORANDUM DP Barcode: ~~D329594~~

SUBJECT: Flubendiamide in Drinking and Surface Water-Report No. ECM0224W1-W2

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

TO: Hardip Singh (7507C)
OPP/Environmental Fate and Effects Division
Environmental Risk Branch

The Environmental Fate and Effects Division (EFED) has requested an Environmental Chemistry Method Evaluation on Flubendiamide in Drinking and Surface Water using the method submitted by Bayer CropScience AG in accordance with the registration of Flubendiamide, MRID No. 468169-27. The method validation data was reviewed and the conclusions included in the attached Environmental Chemistry Method Review Evaluation.

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

Data Requirement: PMRA Data Code: NA
EPA DP Barcode: - D329594
OECD Data Point: NA
EPA Guideline: ECM Method Review

Test material:
Common name: Flubendiamide (NNI-0001)

Chemical name: N^2-[1,1-dimethyl-2- (methylsulfonyl)ethyl]-3-iodo-N^1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedibarboxamide

IUPAC: 3-iodo-N'-(2-mesyl-1,1-dimethylethyl)-N-{4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]-o-toyl}phthalamide

Primary Evaluator: Charles Kennedy **Date:** 08/04/2006
Charles Kennedy, Chemist, EPA/OPP/BEAD/ECB

Peer Reviewer: Shanda Bennett **Date:** 07/31/2006
Shanda Bennett, Chemist, EPA/OPP/BEAD/ECB

QA Officer: Christian Byrne **Date:** 08/15/2006
Dr. Christian Byrne, EPA/OPP/BEAD/ECB

ANALYTICAL METHOD: Analytical Method 00838 (MR-134/03) for the Determination of NNI-0001 and NNI-0001-des-iodo in Drinking and Surface Water by HPLC-MS/MS

EXECUTIVE SUMMARY

The method is applicable for the quantitative determination of residues of Flubendiamide (NNI-0001) and its metabolite, NNI-0001-des-iodo in drinking and surface water by LC/MS/MS.

The method was submitted to EPA by Bayer CropScience to support studies performed to seek registration for Flubendiamide. The method was created by Bayer CropScience AG in Rhein, Germany and independently validated by Bayer CropScience in Stilwell, Kansas in accordance with EPA's Good Laboratory Practice Standards, Title 40 Code of Federal Regulations Part 160. The independent laboratory validation that was submitted with this method was entitled, "Independent Laboratory Validation of Method 00838 (MR-134/03) for the Determination of NNI-0001 and NNI-0001-des-iodo in Drinking and Surface Water by HPLC-MS/MS". Based on the information and data which accompanied the method and an incorporation of minor modification of the ILV, ECB found this method to be acceptable.

Method Summary

Twenty milliliters of the water sample was pipetted into a 50-ml disposable centrifuge tube. Fortified samples were prepared by adding 1.0 ml of a mixed standard for each fortification level: 0.05 and 0.50 ng/ml. Samples were then diluted to 24.0 ml by pipetting 3.0 ml of acidified acetonitrile with 0.08% acetic acid into each centrifuge tube. After mixing well, an aliquot of the sample was transferred into an HPLC vial for analysis by electrospray LC/MS/MS.

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

ECB recommends that the registrant add the suggestions made by the IL in an addendum to the original registrant method. As written the method gave acceptable results using both matrix-matched calibration solutions and calibration solutions prepared in deionized water. In order for the method to meet the criteria outlined in OPPTS 860.1340, it is recommended that the original method be revised to include the preparation of calibration solutions in deionized water. This would address the issue of both the preparation of calibration solutions and a more sustain recovery values at MDL and LOQ by reducing matrix interference. Also, the use of normalization (100 %) to the mean is not appropriate for the determination of recovery accuracy needed in the validation of the method. ECB recommends a separate regression of the individual recovery values which would provide a more realistic picture of actual recoveries between the acceptable 70% and 120% range. 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), this method was acceptable for both analytes.

COMPLIANCE

Signed and dated statements that this method fulfills the requirements for Good Laboratory Practice Standards, 40 CFR 160 were present in the method. 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

Nihon Nohyaku Co., Ltd., based in Japan, and Bayer CropScience AG in Germany have jointly developed Flubendiamide, the first example of the phthalic acid diamides, a novel group of insecticides that activate the ryanodine receptor. Flubendiamide acts through a new biochemical mode of action in lepidopterous insect pests.

After foliar application, Flubendiamide has excellent fast-acting and residual activity against a broad spectrum of lepidopterous insect pests. Flubendiamide has a very low toxicity against beneficial arthropods.

TABLE A.1. Test Compound Nomenclature

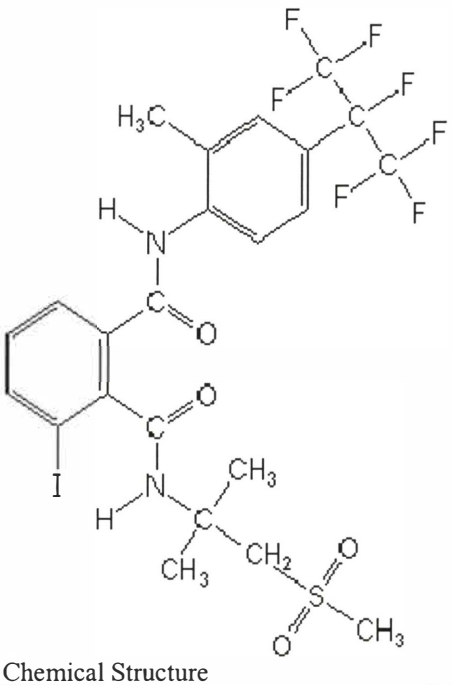
Compound :Flubendiamide	 Chemical Structure
Common name	Flubendiamide
Company experimental name	NNI-0001, NNI-0001-des-iodo
IUPAC name	3-iodo-*N'*-(2-mesyl-1,1-dimethylethyl)-*N*-{4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]-o-tolyl}phthalamide
CAS Name	*N*^{2}-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-*N*^{1}-{2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl}-1,2-benzenedicarboxamide
CAS #	Reg. No. 272451-65-7

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

Parameter	Value
Melting point/range	259°C
Color and physical state	Not available
Volatility	Not available
Freezing Point	Not available
Specific Gravity	Not available
Vapor pressure at ___°C	Not available
Solubility in water	0.00003
Bulk Density	Not available
Molecular Weight	Not available

B. MATERIALS AND METHODS

B.1. Principle of Method

Water samples are diluted with acidified acetonitrile, and analyzed by LC/MS/MS. The LC/MS/MS technique allows quantitation of all analytical targets with a high inherent specificity and without the need of derivatization for the more polar analytes.

TABLE B.1.1.	**Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied**
Method ID	ECM0224W1-W2
Analyte(s)	Flubendiamide
Extraction solvent/technique	Water samples are diluted with acidified acetonitrile.
Cleanup strategies	None
Detector	PE Sciex 4000 LC/MS/MS

C. RESULTS AND DISCUSSION

C.1. Recovery Results Summary

TABLE C.1.1. Recovery Regression Analysis Results from Method Validation of [matrices]

Surface Water NNI-0001 (Separate Regression Analyses)
LOQ - 0.0513 µg/L, Mean values - 114.3 %, RSD – 3.8 %
10 x LOQ – 0.522 µg/L, Mean values – 115.1 %, RSD – 3.4 %

Surface Water NNI—des-indo (Separate Regression Analyses)
LOQ – 0.513 µg/L, Mean values – 108.6 %, RSD – 2.3 %
10 x LOQ – 0.513 µg/L, Mean values – 106.7 %, RSD – 1.9 %

C.1.2. Method Characteristics

TABLE C.1.2. Method Characteristics	
Analyte	Flubendiamide (NNI-0001, NNI-0001-des-iodo)
Limit of Quantitation	0.05 ng/mL
Limit of Detection (LOD calculated)	0.02 µg/L [($t_{0.99}$ x 3) + average apparent residue in the untreated control]
Accuracy/Precision at LOQ (0.05 ng/mL)	See chart in Table C.2.1.
Reliability of the Method/ [ILV]	An independent laboratory method validation [ILV], (MRID No. 461826-28), was conducted to verify the reliability of method for the determination of Flubendiamide and a degradate in water. The values obtained indicated that the registrant method is acceptable according to *OPPT* and *S 850.7100 Guidelines*.

TABLE C.1.2. Method Characteristics	
Linearity	There were eight standard levels ranging from 0.04 µg/L to 12.0 µg/mL for each analyte. The calibration points were weighted 1/x, and the intercept forced to zero to provide better fit near the limit of detection. NNI-0001 Correlation Values: 0.9995, NNI-0001-des-iodo Correlation Values: 0.9997.
Specificity	The method is specific for the determination of NNI-0001 and its metabolite NNI-0001-des-iodo 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. Recovery Results Obtained by an Independent Laboratory Validation of the Method for the Determination of NNI-0001 and NNI-0001-des-iodo in Drinking and Surface Water by HPLC-MS/MS. *Individual Recovery Values For NNI-0001 and NNI-0001-des-iodo in Surface Water with Both Matrix Matched and Deionized Water Calibration Curve. **Individual Recovery Values For NNI-0001 and NNI-0001-des-iodo in Drinking Water with Both Matrix Matched and Deionized Water Calibration.

Compound	Spiking Level (ug/L)	Average Recoveries Obtained (%)	± Standard Deviation (RSD)
Matrix matched			
*NNI-0001	0.05 ng/mL	115	± 4% (4%)
*NNI-0001	0.50 ng/mL	103	± 5% (4%)
*NNI-0001-des-iodo	0.05 ng/mL	105	± 5% (5%)
*NNI-0001-des-iodo	0.50 ng/mL	103	± 3% (3%)
Deionized water			
*NNI-0001	0.05 ng/mL	86	± 2% (3%)
*NNI-0001	0.50 ng/mL	82	± 4% (5%)
*NNI-0001-des-iodo	0.05 ng/mL	77	± 3% (5%)
*NNI-0001-des-iodo	0.50 ng/mL	80	± 4% (5%)
Matrix matched			
**NNI-0001	0.05 ng/mL	111	± 2% (2%)
**NNI-0001	0.50 ng/mL	105	± 3% (3%)
**NNI-0001-des-iodo	0.05 ng/mL	103	± 3% (3%)
**NNI-0001-des-iodo	0.50 ng/mL	104	± 2% (2%)
Deionized water			
**NNI-0001	0.05 ng/mL	110	± 3% (2%)
**NNI-0001	0.50 ng/mL	100	± 6% (6%)
**NNI-0001-des-iodo	0.05 ng/mL	107	± 5% (5%)
**NNI-0001-des-iodo	0.50 ng/mL	105	± 4% (4%)

D. CONCLUSION

From a review of the method, B. Brumhard "Analytical Method 00838 (MR-134/03) for the Determination of NNI-0001 and NNI-0001-des-iodo in Drinking and Surface Water by HPLC-MS/MS", and an incorporation of minor modification of the ILV, ECB concludes that the final method appears scientifically sound and capable of determining the residues of Flubendiamide (NNI-0001, NNI-0001-des-iodo) in drinking and surface water.