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July 24, 2006

MEMORANDUM

DP Barcode: ~~D329594~~ D330001/

SUBJECT: Flubendiamide in Soil/Sediment-Report No. ECM0224S1-S5

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 soil using the method submitted by Bayer CropScience AG in accordance with the registration of Flubendiamide, MRID No. 468169-24. 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

Charles D. Kennedy  
BEAD/Environmental Chemistry Laboratory

Flubendiamide in Soil/Sediment/ECM0224S1-S5/Bayer CropScience AG.  
**ENVIRONMENTAL CHEMISTRY METHOD REVIEW EVALUAION**

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**Data Requirement:** PMRA Data Code: NA  
EPA DP Barcode: - 329594  
OECD Data Point: NA  
EPA Guideline: ECM Method Review

**Test material:**

Common name: Flubendiamide

Chemical name: N2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide

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

**Primary Evaluator:**

*Charles Kennedy*  
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Charles Kennedy, Chemist, EPA/OPP/BEAD/ECB

**Date:** *July 21, 2006*

**Peer Reviewer:**

*Shanda Bennett*  
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Shanda Bennett, Chemist, EPA/OPP/BEAD/ECB

**Date:** *July 24, 2006*

**QA Officer:**

*Christian Byrne*  
\_\_\_\_\_  
Dr. Christian Byrne, EPA/OPP/BEAD/ECB

**Date:** *July 24, 2006*

**ANALYTICAL METHOD:** B. Brumhard, August 2004, Analytical Method 00849 for the Determination of Residues of NNI-0001, and its Metabolites NNI-0001-des-iodo, NNI-0001-3-OH, NNI-0001-3-OH-hydroxyperfluoroalkyl and NNI-0001-benzoic acid in Soil and Sediment by HPLC-MS/MS. Unpublished method created by Bayer CropScience AG, Development-Residues, Operator and Consumer Safety, 40789 Monheim am Rhein, Germany, Method Effective Date: August 02, 2004.

**EXECUTIVE SUMMARY**

This method is applicable for the quantitative determination of residues of NNI-0001 and its metabolites NNI-0001 des-iodo and NNI-0001-3-OH, NNI-0001-3-OH-hydroxyperfluoroalkyl and NNI-0001-benzoic acid in soil and sediment. The method was submitted to EPA to support studies performed to seek registration for Flubendiamide. The method was created by Bayer CropScience AG, BCA-D-ROCS, Rhein, Germany and independently validated by Bayer CropScience, Stilwell, KS in the spirit of EPA's Good Laboratory Practice Standards, Title 40, Code of the Federal Regulations Part 160. The independent laboratory validation was submitted with this

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method. 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:** An analytical method was developed to quantify NNI-0001 and its metabolites, NNI-0001-des-iodo, NNI-0001-3-OH, NNI-001-3-OH-hydroxyperfluoroalkyl and NNI-0001-benzoic acid in soil/sediment using high-performance liquid chromatography electrospray tandem mass spectrometry (LC-MS/MS). The method was performed using control soil and sediment from the dissipation study sites Höfchen, Laacher Hof, Nespén in Germany. Soil and sediment (20-g) from these sites were spiked with NNI-0001 and its metabolites at 0.5 µg/kg and 100 µg/kg. Control and fortified samples were extracted by a microwave technique utilizing 40 mL of a solvent mixture of acetonitrile/water/acetic acid (500/500/1, v/v/v), stirring and heating for three minutes at 250 watts at a maximum temperature of 50°C. After extraction, an appropriate amount of labeled internal standard solution was added to each extract. The extract was centrifuged to remove fine particles. Then a portion of each supernatant was then transferred into an HPLC vials for analysis by electrospray LC/MS/MS.

The average recoveries of NNI-0001 and its metabolites fortified at the LOQ (0.5 µg/kg and the 200 x LOQ (100 µg/kg) were all within the acceptable range.

### **METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS**

ECB recommends, for completeness, that the registrant add the modifications made by the IL in an addendum to the original registrant method. This would address the issue of consistent microwave energy absorption required to obtain consistent recoveries. 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 all analytes.

### **COMPLIANCE**

A signed and dated statement was given that this method was conducted in accordance with the requirements for Good Laboratory Practice, 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**

Flubendiamide is an experimental insecticide being developed by Bayer CropScience with potential uses in several crops including vegetables and orchards.

<b>TABLE A.1. Test Compound Nomenclature</b>	
Compound : Flubendiamide ( NNI-0001) plus metabolites NNI-0001-des-iodo, NNI-0001-3-OH, NNI-3-OH-hydroxyperfluoroalkyl, NNI-0001-benzoic acid.	
Common name	Flubendiamide
Company experimental names	NNI-0001 and metabolites NNI-0001-des-iodo, NNI-0001-3-OH, NNI-0001-3-OH-hydroxyperfluoroalkyl, NNI-0001-benzoic acid
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	N2-[1,1-dimethyl-2-(methylsulfonyl)ethyl]-3-iodo-N1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide
CAS #	272451-65-7

<b>TABLE A.2. NNI-0001 Physicochemical Properties of the Technical Grade Test Compound</b>	
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	Not available

Solubility in water	0.00003
Bulk Density	Not available
Molecular Weight	Not available

## MATERIALS AND METHODS

### B.1. Principle of Method

The final analytical method was developed for determination of the total extractable residues of NNI-0001, NNI-0001-des-iodo, NNI-0001-3-OH, NNI-0001-03-OH-hydroxyperfluoroalkyl and NNI-0001-benzoic acid in soil and sediment. The LOQ for each analyte has been set a 0.5 µg/kg (0.5 ppb).

Residues of NNI-0001 and its metabolites in soil are extracted from soil or sediments using a temperature-controlled microwave oven. A 20 gram sample aliquot is extracted with 40 mL of (500/500/1, v/v/v) acetonitrile/water/acetic acid. After centrifugation the final extract is analyzed by LC/MS/MS. Quantification of residues is based on the use on isotopically labeled internal standards and comparison of peak areas with those of known standards.

The ILV method is based on the original registrant method which incorporates minor modifications into their validation. Minor modifications include the use of disposable glassware and minor changes to the microwave extraction time to provide better temperature control independent of the type of glassware and stir bars used. The data generated during the method validation study demonstrated that the limit of quantitation (LOQ) were at or below the target LOQ's of 0.5 µg/kg (0.5 ppb) for NNI-0001, NNI-0001-des-iodo, NNI-0001-3-OH, NNI-0001-03-OH-hydroxyperfluoroalkyl and NNI-0001-benzoic acid, and that the MDL's were at or below 0.15 µg/kg (0.15 ppb).

<b>TABLE B.1.1.</b>	<b>Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied</b>
Method ID	ECM0224S1-S5
Analyte(s)	NNI-0001, NNI-0001-des-iodo, NNI-0001-3-OH, NNI-0001-3-OH-hydroxyperfluoroalkyl, and NNI-0001-benzoic acid
Extraction solvent/technique	Microwave extraction w/50/50 acetonitrile /1% acetic acid for six minutes at 350 watts at 50°C.
Cleanup strategies	Approximately 10 mL of sample extract was centrifuged to separate out solids. The supernatant was transferred into an HPLC vial for analysis.
Instrument/Detector	Mass spectrometer: Perkin Elmer Sciex, API 365 with turbo-ionspray interface mass selective detector (MS/MS), Liquid chromatograph: HP

TABLE B.1.1.	Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied
	1100 column Compartment G1316A, HP 1100 Binary Pump Column: Superspher 100 RP 18, Length 75 mm, i.d. 4 mm, Injection volume: 50 µL, Oven temperature: 40°C, Mobile phase A: water/acetonitrile/acetic acid (900/100/1; v/v/v), Mobile phase B: acetonitrile/acetic acid (1000/1; v/v), Run time: 13 min, Flow rate (column): 1.0 mL/min, Flow rate (interface): 0.15 mL/min, G1312A, HP 1100 Isocratic Pump G1310A mobile phase: water/acetonitrile/acetic acid (500/500/1; v/v/v), HP 1100 Degasser G1322A, Autosampler: HTC PAL System, Microwave Extractor: MLS-Ethos.

## C. RESULTS AND DISCUSSION

### C.1. Recovery Results Summary

TABLE C.1.1. Recovery Results from Method Validation of [matrices]			
Matrix Hofchen-silt Laacher Hof-sandy loam Nespen-silt loam	Spiking Level µg/kg (ppb)	Average Recovery Obtained (%)	Relative Standard Deviation (%)
NNI-0001	0.5		
	Höfchen (silt)	104	11
	Laacher Hof (sandy loam)	102	12
	Nespen (silt loam)	93.5	12
	100		
	Höfchen	98.3	1.7
NNI-0001-des-iodo	0.5		
	Höfchen	113	9.4
	Laacher Hof	101	12
	Nespen	99.6	15
	100		
	Höfchen	99.8	3.3
NNI-0001-3-OH	0.5		
	Höfchen	103	12
	Laacher Hof	109	7.3
	Nespen	91.3	18
	100		
Höfchen	99.8	3.3	



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	Laacher Hof	97.7	4.6
	Nespen	99.1	4.1
NNI-0001-3-OH-hydroxyperfluoroalkyl	0.5		
	Höfchen	94.1	8.1
	Laacher Hof	105	9.7
	Nespen	96.3	13
	100		
	Höfchen	95.9	2.2
NNI-0001-benzoic acid	Laacher Hof	96.4	2.1
	Nespen	93.9	2.0
	0.5		
	Höfchen	100	7.6
NNI-0001-benzoic acid	Laacher Hof	101	7.8
	Nespen	98.2	13
	100		
	Höfchen	101	1.9
	Laacher Hof	100	1.4
	Nespen	101	1.6

### C.1.2. Method Characteristics

<b>TABLE C.1.2. Method Characteristics</b>	
Analytes	NNI-0001, NNI-0001-des-iodo, NNI-0001-OH, NNI-3-OH-hydroxyperfluoroalkyl, NNI-0001-benzoic acid
Limit of Quantitation	0.5 µg/kg (0.5 ppb)
Limit of Detection (LOD)	0.15 µg/kg (0.15 ppb)
Accuracy/Precision at LOQ	See above chart
Reliability of the Method/ [ILV]	ILV available
Linearity	Standard solutions containing NNI-0001, NNI-0001-des-iodo, NNI-0001-3-OH, NNI-0001-3-OH-hydroxyperfluoroalkyl and NNI-0001-benzoic acid were measured in a concentration range of about 0.074 to 100 µg/L corresponding to a concentration in soil of 0.15-200 µg/kg. In this concentration range the mass spectrometric detector showed linear correlation between concentration and peak area. Correlation coefficient r: (NNI-0001 = 0.9999), (NNI-0001-des-iodo = 0.9998), (NNI-0001-3-OH = 0.9992), (NNI-0001-3-OH-hydroxyperfluoroalkyl = 0.9996), (NNI-0001-benzoic acid = 0.9999)
Specificity	The method is specific for the determination of NNI-0001 and its metabolites 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 Residues of NNI-001, NNI-0001-des-iodo, NNI-0001-3-OH, NNI-0001-3-OH-hydroxyperfluoroalkyl and NNI-0001-benzoic acid in Soil and Sediment by HPLC-MS/MS**

Matrix	Spiking Level (ng/g)	Average Recoveries Obtained (%)	Relative Standard Deviation
Mississippi Soil NNI-0001	0.5 5.0	92.9 96.3	11.4 3.1
Mississippi Soil NNI-0001-des-iodo	0.5 5.0	94.7 99.5	11.3 3.4
Mississippi Soil NNI-0001-3-OH	0.5 5.0	84.6 93.1	10.9 3.5
Mississippi Soil NNI-0001-3-OH- hydroxyperfluoroalkyl	0.5 5.0	100.8 96.5	9.8 3.0
Mississippi Soil NNI-0001-benzoic-acid	0.5 5.0	99.9 99.6	9.8 3.3

#### **D. CONCLUSION**

From a review of the method, B. Brumhard “Analytical Method 00849 for the Determination of Residues of NNI-0001, and its Metabolites NNI-0001-des-iodo NNI-0001-3-OH, NNI-0001-3-OH-hydroxyperfluoroalkyl and NNI-0001-benzoic acid in Soil and Sediment by HPLC-MS/MS”, and an incorporation of the minor modification of the ILV, ECB concludes that the final method appears scientifically sound and capable of determining the residues of Flubendiamide (NNI-0001) and its degradates.