UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS
ENVIRONMENTAL CHEMISTRY LABORATORY
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September 17, 2009

MEMORANDUM

SUBJECT: Cyflufenamid - ECM0248W1-W15 DP# 361113

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

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

The EFED/Environmental Fate and Effects Division has requested an Environmental Chemistry Method Review of a method for the determination of Cyflufenamid and its metabolites in soil. The method was submitted by Nippon Soda Co., Ltd. in accordance with the registration of the above mentioned analytes. An independent laboratory validation was done by Morse Laboratories, LLC of Sacramento, California. 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 any questions concerning this report, please contact Elizabeth Flynt at (228) 688-2410 or me at (228) 688-3212.

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

Elizabeth C. Flynt
BEAD/ECL

Cyflufenamid in Soil/555550/Nippon Soda Co., LTD./8033

ENVIRONMENTAL CHEMISTRY METHOD REVIEW REPORT

Data Requirement: PMRA Data Code: NA
EPA DP Barcode: D361113
Data Point: NA
EPA Guideline: ECM Method Review

Test material:
Common name: Cyflufenamid
CAS name: [*N*(*Z*)-*N*-[[cyclopropylmethoxy)amino][2,3-difluoro-6-(trifluoromethyl)phenyl]methylene]benzeneacetamide
IUPAC name: (Z)-*N*-[α-(cyclopropylmethoxyimino)-2,3-difluoro-6-(trifluoromethyl)benzyl]-2-phenylacetamide

Primary Evaluator: Elizabeth Flynt, Chemist — **Date:** 08/17/09

Peer Reviewer: Charles Kennedy, Chemist — **Date:** 08/17/09

QA Officer: Dr. Christian Byrne, QA Officer — **Date:** 08/17/09

ANALYTICAL METHOD: MRID No. 477712-01, May 27, 2009, Westberg, G., "Determination of NF-149 (Cyflufenamid) and Its Metabolites in Soil" pages 1-89. The unpublished method was developed and sponsored by Nippon Soda Co., Ltd., 2-1, 2-Chome Ohtemachi, Chiyoda-ku, Tokyo 100-8165. The method was performed by Morse Laboratory of Sacramento, CA. An independent laboratory validation (ILV) (476208-04) was conducted by PTRL West, Inc. of Hercules, CA. It was entitled, "Independent Laboratory Validation of the Analytical Method for Cyflufenamid and Its Metabolites in Soil."

EXECUTIVE SUMMARY

The method is applicable for the quantitative determination of residues of Cyflufenamid and its metabolites in soil. The method was not created in accordance with EPA's Good Laboratory Practice Standards, Title 40 Code of Federal Regulations Part 160. After a thorough review, the ECB found that this Environmental Chemistry Method (ECM) is acceptable.

Method Summary

Soil samples were extracted with organic solutions and centrifuged. The supernatant was removed and the soil discarded. The supernatant was brought to a set volume and a portion of the aqueous supernatant was removed and concentrated. This concentrate was acidified and extracted with organic solvent. It was then basified and again extracted with organic solvent. The combined extract was evaporated, re-dissolved, and brought to a final set volume for LC/MS/MS analyses.

The Limit of Quantitation (LOQ) was reported as 2.0 ppb for all analytes.

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

Minor deficiencies in the registrant method for the detection of Cyflufenamid and its metabolites in soil are presented below:

Although a Limit of Quantitation was stated (2 ppb) in the registrant method, no explanation of how it or a Method Detection Limit (MDL) was determined was given. In the ILV, the LOQ (2 ppb) was defined as the lowest fortification level successfully tested. The LOD was defined as the detector response from the lowest linearity standard injection with a concentration of 0.25 ng/ml. EPA prefers a statistically based approach.

No characterization of the matrix was given.

No statement of adherence to FIFRA/GLP was made.

As pointed out in the ILV, the ammonia solution labeled as 10% in the registrant method was actually a 3% solution. Although the percentage was stated incorrectly, the instructions for preparation of the ammonium solution are correct in the registrant method.

There was no source given for the standards by the registrant.

The independent laboratory had to make several modifications to the analyses portion of the registrant's method. These included changing the confirmation ion to the quantitation ion for 149-F6 metabolite to eliminate interference and reducing the injection size from 20 µl to 5 µl for the 149-F1 analyses to reduce peak splitting. None of these changes were considered major.

The results from the ILV stated a mean recovery for 149-F1 at the 10 x LOQ (20 ppb) fortification level of 67%. This falls outside the lower limit of 70% for the prescribed recovery range.

After a thorough review, ECB finds this method acceptable as defined in the OPPTS 850.7100 Ecological Effects Test Guidelines.

COMPLIANCE

Signed and dated statements that this method was conducted in accordance with the requirements for Good Laboratory Practice Standards, 40 CFR 160 are not present in this method. The registrant stated that the report is not a guideline study and therefore was not conducted according to the requirements of 40 CFR Part 160. A statement of

non-confidentiality was present on the basis of the method falling within the scope of the FIFRA Section 10 (d)(1)(A), (B), or (C).

A. BACKGROUND INFORMATION

Cyflufenamid is a new amidoxine fungicide used to control powdery mildew of various crops by preventive or curative treatment. It shows long residual and vapor phase activity. The mode of action of cyflufenamid is considered different from that of other existing fungicides.

TABLE A.1. Test Compound Nomenclature for Cyflufenamid and its Metabolites

Compounds	Chemical Structure ***See Appendix A for the chemical structure information**
Common names	Cyflufenamid
Company experimental name	NF-149, 149-F6, 149-F11, 149-F, 149-F1
IUPAC name	(*Cyflufenamid name only*) - (Z)-*N*-[α-(cyclopropylmethoxyimino)-2,3-difluoro-6-(trifluoromethyl)benzyl]-2-phenylacetamide
CAS Name	(*Cyflufenamid name only*) - [*N*(*Z*)-*N*-[[cyclopropylmethoxy)amino][2,3-difluoro-6-(trifluoromethyl)phenyl]methylene]benzeneacetamide *See Appendix A for CAS names for metabolites*
CAS #	180409-60-3

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

Parameter	Value
Melting point/range	61.5-62.5°C
pH	Not available
Density	Not available
Water solubility (25 °C)	0.52 mg/l (20°C)
Solvent solubility (mg/ml at 20 °C)	Not available
Vapour pressure	3.54×10^{-5} Pa (20 °C)
Dissociation constant (pK_a)	Not available
Octanol/water partition coefficient	Not available
UV/visible absorption spectrum	Not available

MATERIALS AND METHODS

B.1. Principle of Method

The soil method is based two acetone extractions followed by a single 2M ammonium chloride/ methanol (1:1; v:v) extraction of analytes from matrix followed by centrifugation to remove the solids. A portion of the combined extract is evaporated to

remove the organic solvent. The aqueous mixture is acidified and partitioned with ethyl acetate. The ethyl acetate layers are removed and the remaining aqueous sample is made basic and partitioned with ethyl acetate. The ethyl acetate layers are removed and combined with the acidified ethyl acetate layers. The combined ethyl acetate sample is concentrated and and brought to a final volume of 10 ml for analysis by LC/MS/MS. For positive ionization, a 3% ammonia solution is added to a portion of the final extract volume. For negative ionization, a methanol water solution is added to a portion of the final extract volume.

TABLE B.1.1.	**Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied**
Method ID	ECM0248S1-S5
Analyte(s)	Cyflufenamid, NF-149, 149-F6, 149-F11, 149-F, 149-F1
Extraction solvent/technique	The soil method is based on two acetone extractions followed by a single 2M ammonium chloride/ methanol (1:1; v:v) extraction of analytes from matrix followed by centrifugation to remove the solids. A portion of the combined extract is evaporated to remove the organic solvent. The aqueous mixture is acidified and partitioned with ethyl acetate. The ethyl acetate layers are removed and the remaining aqueous sample is made basic and partitioned with ethyl acetate. The ethyl acetate layers are removed and combined with the acidified ethyl acetate layers. The combined ethyl acetate sample is concentrated and and brought to a final volume of 10 ml for analysis by LC/MS/MS. For positive ionization, a 3% ammonia solution is added to a portion of the final extract volume. For negative ionization, a methanol water solution is added to a portion of the final extract volume.
Cleanup strategies	NA
Instrument/Detector	Applied Biosystems/MDS Sciex API 4000 LC/MS/MS

C. RESULTS AND DISCUSSION

C.1.Recovery Results Summary

TABLE C.1.1. Cyflufenamid Summary at LOQ (2 ppb) in California, Georgia, and New York Soils		
Compound	**% Recovery**	**RSD**
Cyflufenamid	99.8	7.6
149-F11	100	12.7
149-F6	102	4.6
149-F1	74.1	6.1
149-F	89.3	17.8

TABLE C.1.2. Method Characteristics	
Analyte	Cyflufenamid, NF-149, 149-F6, 149-F11, 149-F, 149-F1
Limit of Quantitation	2 ppb
Limit of Detection (LOD)	Not provided
Accuracy/Precision at LOQ	**See Table C.1.1. above.**
Reliability of the Method/ [ILV]	The ILV was successful.
Linearity	All regression equations had coefficients of determinations above 0.9900
Specificity	This method is specific due to the highly specific nature of LC/MS/MS.

C.2. Independent Laboratory Validation (ILV)

TABLE C.2.1. Recovery Results Obtained by an Independent Laboratory Validation of the Method for the Determination of Cyflufenacil and its Metabolites in Soil at the LOQ (2 ppb)

Analyte	Spiking Level (conc. units)	# repetitions	Mean Recoveries	RSD
NF-149	2	5	107	6.1
149-F6	2	5	87	13
149-F11	2	5	98	8.3
149-F	2	5	90	7.2
149-F1	2	5	74	1.9

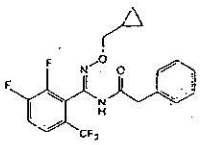
D. CONCLUSION

From a review of the method, "Determination of NF-149 (Cyflufenamid) and Its Metabolites in Soil", ECB concludes that the method appears scientifically sound and capable of determining the residues of Cyflufenamid and its metabolites in soil at the limit of quantitation (2 ppb) and above.

Appendix A – Compound Structure for Cyflufenamid and Its Metabolites

Common name: Cyflufenamid
Code Name: NF-149

Chemical Structure:



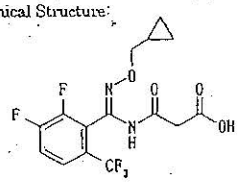
Name: 149-F6

Chemical Structure:

Molecular Formula: $C_8H_4F_5NO$
Molecular Weight: 225.12
Chemical Name: 2,3-difluoro-6-trifluoromethylbenzamide

Name: 149-F11

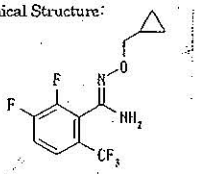
Chemical Structure:



Molecular Formula: $C_{15}H_{13}F_5N_2O_4$
Molecular Weight: 380.27
Chemical Name: (*Z*)-*N*-(α-cyclopropylmethoxyimino)-2,3-difluoro-6-(trifluoromethylbenzyl)malonic acid

Name: 149-F

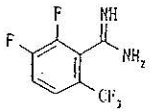
Chemical Structure:



Molecular Formula: $C_{12}H_{11}F_5N_2O$
Molecular Weight: 294.22
Chemical Name: *N*-Cyclopropylmethoxy-2,3-difluoro-6-trifluoromethylbenzamidine

Name: 149-F1

Chemical Structure:



Molecular Formula: $C_8H_5F_5N_2$
Molecular Weight: 224.13
Chemical Name: 2,3-difluoro-6-trifluoromethylbenzamidine