PC 129131

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June 29, 2007

MEMORANDUM

DP Barcode: D330158

SUBJECT: Fenpyroximate in Freshwater-Report No. ECM032W1

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

TO: Cara Dzubow
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 on Fenpyroximate in Freshwater using the method submitted by Wildlife International, Ltd. in accordance with the registration of MRID No. 468472-01. 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

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Fenpyroximate in Water/ECM0232W1/Nichino America, Inc.
ENVIRONMENTAL CHEMISTRY METHOD REVIEW REPORT

Data Requirement: PMRA Data Code: NA
EPA DP Barcode: - DP330158
OECD Data Point: NA
EPA Guideline: ECM Method Review

Test material:
Common name: Fenpyroximate

IUPAC: tert-butyl (E)-α-(1,3-dimethyl-5-phenoxypyrazol-4-ylmethyl-eneamino-oxy)-p-toluate

CAS: 1,1-dimethylethyl (E)-4-[[[[(1,3-dimethyl-5-phenoxy-1H-pyrazol-4-yl)methylene]amino]oxy]methyl]benzoate

Primary Evaluator: Charles Kennedy **Date:** 6/20/2007
Charles Kennedy, Chemist, EPA/OPP/BEAD/ECB

Peer Reviewer: Elizabeth Flynt **Date:** 06/22/2007
Elizabeth Flynt, Chemist, EPA/OPP/BEAD/ECB

QA Officer: Christian Byrne **Date:** 06/22/07
Dr. Christian Byrne, EPA/OPP/BEAD/ECB

ANALYTICAL METHOD: 468472-01, Timothy Z. Kendall, M.S. and Willard B. Nixon, Ph.D., January 16, 2006, *"Analytical Method Verification for the Determination of Fenpyroximate TGAI in Freshwater"*. The unpublished study was conducted by Wildlife International, Ltd. of Easton, Maryland and sponsored by Nichino America, Inc. at 4550 New Linden Hill Road, Wilmington, DE. The document study is Wildlife International, Ltd. Project Number 397C-101.

EXECUTIVE SUMMARY

The method is applicable for the quantitative determination of residues of Fenpyroximate in freshwater by HPLC/Variable Wavelength Detection.

The method was sponsored by Nichino America, Inc. in Wilmington, Delaware and reviewed by Wildlife International, Ltd. of Easton, Maryland in compliance with EPA's Good Laboratory Practice Standards, Title 40 Code of Federal Regulations, Part 160. An independent laboratory validation was not submitted with this method. The need for an independent laboratory validation was waived for this environmental chemistry method by the Ecological Fate and Effects Division. Nevertheless, the Environmental Chemistry Branch (ECB) finds the method unacceptable due to several critical data omissions and suggests that the registrant present an amended method which includes the missing data.

Method Summary

Residues of Fenpyroxymate in freshwater were detected by HPLC/UV analysis after extraction, clean up, and concentration. Quantification was derived from the instrumental responses of test standards of known concentration using regression analysis. The concentrations of fortified samples were determined by substituting the respective instrumental response into the regression equation.

Freshwater was fortified at 0.180, 1.00, and 3.00 µg/L using stock solutions containing Fenpyroxymate TGAI in acetone. Samples fortified at 0.180, 1.00, and 3.00 µg/L yielded mean recoveries of 119, 107 and 99.7%, respectively. The method was evaluated by determining the average recovery values, standard deviation and relative standard deviation at the spiking concentrations above the claimed LOQ of 0.122 µ/L (ppb).

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

Although the registrant failed to provide an independent laboratory validation (ILV), the Environmental Fate and Effects Division (EFED) of the Office of Pesticide Programs waived the ILV requirement for this ECM. Nevertheless, ECB found several deficiencies and data omissions which were judged sufficient to render the method unacceptable.

A review of the document indicated that several pages are missing (Pages 9, 39, 54, 56, 60, 61, and 62).

There was a lack of specifics in the extraction, isolation, and concentration of the freshwater samples used in the validation study. There was no defined volume of freshwater to be used in the extraction (e.g., 1 liter, 500 mL, etc.). There was no description of the sources of the glassware, solvents, and reagents used in the study. Although there was a silica gel chromatographic clean-up, there was no description of the size, dimensions, silica gel activity, or source in the preparation of this step of the procedure. Many of the steps were very general in nature.

There was a method for the calculation of the limit of quantitation (LOQ) which was not scientifically sound. The registrant claimed that the LOQ of 0.122 µg/L was "based upon the product of the concentration of the lowest calibration standard (10.0 µg/L) and the dilution factor of the matrix blank samples (0.0120), corrected for purity (98.8%)." There is no explanation of the determination of the dilution factor of the matrix blanks. Figure 11, which was to present the reagent blank [397C-101-VREB-1], was missing (Page 39). An examination of Figure 12 [freshwater matrix blank sample -397C-101-VMAB-1] revealed a peak height of ~ 8 mm at a retention time of 9.491 min and an examination of Figure 13 [freshwater verification sample – 397C101-VMAS-1 (0.180 µg/L)] revealed a peak height of ~ 5 mm at a retention time of 9.491. That would indicate a significant interference that would not enable the LOQ to be determined at the

claimed concentration of 0.180 µg/L. The rule of thumb is that the limit of detection (LOD) has a 3:1 ratio of sample response to blank response and that the LOQ has a 10:1 ratio.

There is no representative sample calculation from the validation study to confirm any stated validation results. There were no calibration standards presented in association with the validation study to confirm the calibration regression curve of Figure 10. There were no response values (peak height or area counts) given for any standards or validation samples which would enable direct calculations of the stated recovery values.

Although the review by ECB suggests that the method itself might be able to be used in the validation study of Fenpyroximate in freshwater, the fact remains that critical data are missing from the report, the claimed LOQ was unsubstantiated, and the method calculations cannot be verified due to the failure of the registrant to provide area counts.

ECB finds this method unacceptable at this time and suggests that the registrant be requested to amend the method by providing missing data.

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

Fenpyroximate is a phenoxypyrazole acaricide for application to leaves of citrus, fruits, and nut trees. It is very active against phytophagous mites, relatively less active against predacious mites, and inactive against animal parasitic and soil mites. Fenpyroximate inhibits mitochondrial NADH-coenzyme Q reductase on the electron transport chain in spotted spider mite and in rats.

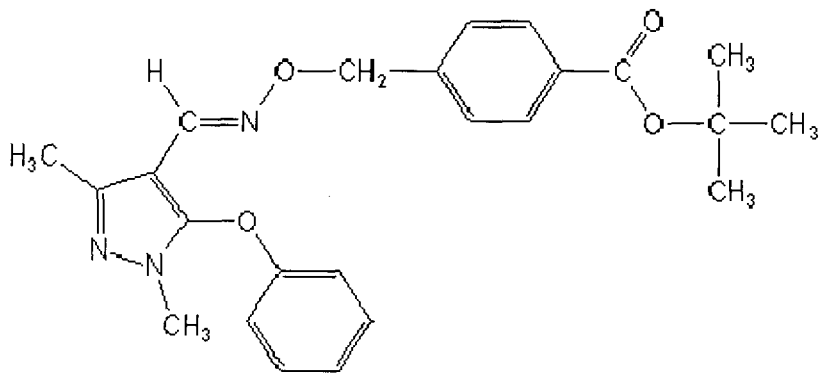
TABLE A.1. Test Compound Nomenclature	
Compound: $C_{24}H_{27}N_3O_4$ (Fenpyroximate)	**STRUCTURE:** 
Common name	Fenpyroximate
Molecular formula	$C_{24}H_{27}N_3O_4$
IUPAC name	tert-butyl (E)-α-(1,3-dimethyl-5-phenoxypyrazol-4-ylmethyl-eneamino-oxy)-p-toluate
CAS Number	111812-58-9
CAS Name	1,1-dimethylethyl (E)-4-[[[[(1,3-dimethyl-5-phenoxy-1H-pyrazol-4-yl)methylene]amino]oxy]methyl]benzoate

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

Parameter	Value
Melting point/range	99.3 - 101.7°C
Color and physical state	Off-white to pale yellowish
Physical state	Solid.
Odour	Practically odorless
Purity	95.9 – 99.8%
Density	1.237 – 1.257 g/cm^3
Solubility in water at 25°C	pH 5 = 0.021 mg/l, pH 7 = 0.023 mg/l, pH 9 = 0.030 mg/l
Storage stability	Stable for more than 1 year
Hydrolysis	Half-life (25°C) at pH 5: 180 days, pH 7: 226 days, pH 9: 221 days.
Photolysis	Half-life at pH 7: 1.5 hours (in aqueous solution irradiated with xenon lamp of 603 watts, 290-800 nm)

B. MATERIALS AND METHODS

B.1. Principle of Method

The method validation used for the analysis of Fenpyroximate in freshwater was based upon methodology developed by Wildlife International, Ltd. The analytical method consisted of adding 100 mL of dichloromethane (DCM) to each sample and allowing the organic and aqueous layers to separate after shaking for approximately one minute. The lower layer (DCM) was drained into a round bottom flask. To the aqueous fraction, a second 100 mL of DCM was added and shaken. Each extract was combined in into the same round bottom flask. Each sample was then rotary evaporated using a water bath at 40°C. A silica gel column was conditioned with two column volumes of 50:50 (v:v) of dichloromethane/hexane. The column was drained to the top of the silica bed and the sample was loaded into the respective column. The round bottom flask was rinsed three times with 2 mL of 50:50 (v:v) of dichloromethane/hexane and the rinses were added to the column and drained to dryness under a slight vacuum. Full vacuum was applied for 30 minutes to further dry the column. The sample was eluted with one column volume of 30:70 (v:v) acetone/hexane into a graduated centrifuge tube and evaporated to dryness under nitrogen. The requisite volume of 70:30 (v:v) of acetonitrile/HPLC water was added and vortexed to dissolve all residues. An aliquot of each extract was transferred to an autosampler vial and samples were submitted for analysis. Concentrations of Fenpyroximate in the samples were determined by an Agilent Series 1100 High Performance Liquid Chromatographic (HPLC) equipped with an Agilent Series 1100 Variable Wavelength Detector.

TABLE B.1.1.	**Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied**
Method ID	ECM0232W1
Analyte(s)	Fenpyroximate
Extraction solvent/technique	Add 100 mL dichloromethane (DCM) to each sample and allowing the organic and aqueous layers to separate by shaking for one minute. The lower layer (DCM) was drained into a round bottom flask. Repeat 1x and combine the two DCM extracts. Each sample was then rotary evaporated using a water bath at 40°C.
Cleanup strategies	A silica gel column was conditioned and the sample was loaded onto a column. The sample was eluted into a graduated centrifuge tube and evaporated to dryness under nitrogen. A volume of 70% acetonitrile:30% HPLC-grade water was added and vortexed to dissolve all residues before analysis.
Detector	Variable Wavelength Detection (220 nm)

C. RESULTS AND DISCUSSION

C.1. Recovery Results Summary

TABLE C.1.1. Recovery Regression Analysis Results from Method Validation of Freshwater.

Method Verification Results of Fenpyroximate for Five Freshwater Samples Fortified at 0.180 µg/L.

Fenpyroximate – Mean = 119 %, SD = 12.8, RSD = 10.8 %

Method Verification Results of Fenpyroximate for Five Freshwater Samples Fortified at 1.00 ng/L.

Fenpyroximate – Mean = 107 %, SD = 7.8, RSD = 7.3 %

Method Verification Results of Fenpyroximate for Five Freshwater Samples Fortified at 3.00 ng/L.

Fenpyroximate – Mean = 99.7 %, SD = 2.5, RSD = 2.5 %

*The LOQ was calculated to be 0.122 µg/L based upon the product of the concentration of the lowest calibration standard (10.0 µg/L) and the dilution factor of the matrix blank samples (0.0120), corrected for test substance purity (98.8%).

C.1.2. Method Characteristics

TABLE C.1.2. Method Characteristics	
Analytes	Fenpyroximate
Limit of Quantitation	0.122 µg/L
Limit of Detection	n/a
Accuracy/Precision at LOQ	See chart in Table C.1.1
Reliability of the Method	An independent laboratory method validation [ILV] was not conducted to verify the reliability of method "Analytical Method Verification for the Determination of Fenproximate TGAI in Freshwater".
Linearity	Calibration standards of Fenpyroximate, ranging in concentration from 10.0 to 100 µg/L, were prepared and analyzed with the appropriate sample set. Five calibration standards were analyzed with each set of samples. The standards were injected at the beginning and end of each run, and one standard was injected, at a minimum, after every five samples. Regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The concentration of Fenpyroximate in the samples was determined by substituting the peak area responses of the sample into the applicable regression equation. The correlation coefficient for Fenpyroximate calibration curve was 0.9993.
Specificity	ECB was unable to determine the specificity of the method for Fenpyroximate in freshwater due to the lack of data provided under this document.

C.2. Independent Laboratory Validation (ILV)

TABLE C.2.1. Mean Recovery Results Obtained by an Independent Laboratory Validation of the Method for the Determination of Fenpyroximate in Freshwater by HPLC/Variable Wavelength.

Compound	Spiking Level (ng/L)	Average Recovery Obtained (%)	Standard Deviation
Fenpyroximate	NA	NA	NA

D. CONCLUSION

From a review of the method, Timothy Z. Kendall and Willard B. Nixon "*Analytical Method Verification for the Determination of Fenpyroximate TGAI in Freshwater*", ECB concludes that the method is unacceptable for determining the residues of Fenpyroximate in freshwater and to support registration studies.