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MEMORANDUM

SUBJECT: Acequinocyl in soil - ECM0217S1-S3

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

TO: Hardip Singh
Senior Gatekeeper Team/IO EFED/
Environmental Risk Branch IV (7507C)

The EFED/Environmental Fate and Effects Division has requested an Environmental Chemistry Method Evaluation on Acequinocyl in soil using the method submitted by Arvesta Corporation in accordance with the registration of Acequinocyl MRID No. 461826-02. The method and independent laboratory 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 and those discovered by the independent laboratory. 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

Prothioconazole in soil/ECM0220S1-S4/Bayer CropScience /
ENVIRONMENTAL CHEMISTRY METHOD REVIEW EVALUATION

Data Requirement: PMRA Data Code: NA
EPA DP Barcode: - D3188089
OECD Data Point: NA
EPA Guideline: ECM Method Review

Test material:
Common name: Acequinocyl
Chemical name: 3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
IUPAC: 3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate

Primary Evaluator: Elizabeth Flynt **Date:** 11/02/05
Elizabeth Flynt, Chemist, EPA/OPP/BEAD/ECB

Peer Reviewer: Charles Kennedy **Date:** 11/02/05
Charles Kennedy, Chemist, EPA/OPP/BEAD/ECB

QA Officer: Christian Byrne **Date:** 11/02/05
Dr. Christian Byrne, EPA/OPP/BEAD/ECB

ANALYTICAL METHOD: Westburg, Gary, March 27, 2001. Determination of Acequinocyl, Acequinocyl-OH and AKM-18 in Soil, MRID # Unpublished method 461826-02 created by Morse Laboratories, Inc. and submitted by Arvesta Corporation. Study ID: 1486 Method Effective Date: March 27, 2001.

EXECUTIVE SUMMARY

The method is applicable for the quantitative determination of residues of acequinocyl, acequinocyl–OH, and AKM-18 in soil.

The method was submitted to EPA by Arvesta Corporation to support studies performed to seek registration for acequinocyl. The method was created by Morse Laboratories, Inc. and reviewed by Pyxant Labs Inc. of Colorado Springs, CO in accordance with EPA's Good Laboratory Practice Standards, Title 40 Code of Federal Regulations Part 160. An independent laboratory validation was submitted with this method. It was entitled, "Independent Laboratory Validation for the Determination of Acequinocy. Acequinocyl-OH and AKM-18 in Water and Soil." It was performed by Pyxant Labs Inc. of Colorado Springs, CO.

Method Summary: The three targeted analytes are extracted from soil with acetonitrile:water (90:10, v/v) using a multiple extraction technique. The extracts are combined, then concentrated by rotary evaporation. To the concentrate is added a saturated sodium chloride solution along with solid sodium chloride and hexane. Following vigorous shaking, the layers are separated by centrifugation with the upper hexane-acetonitrile layers containing all three targeted analytes. The lower aqueous layer is extracted one additional time with a fresh aliquot of hexane to insure adequate recovery

of the analytes from the aqueous phase. The organic extracts are combined, concentrated, then purified by means of silica liquid chromatographic column cleanup. Two different eluants are employed to recover all analytes , the eluates being combined upon collection. The resulting solution is evaporated to dryness, reconstituted in acetone:acetonitrile:0.4% aqueous formic acid (2:2:1, v/v), then submitted to LC/MS/MS analysis.

The limit of quantitation (LOQ) in soil for all three analytes is $\geq$ 0.01 ppm.

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

There are several problems/deficiencies with this method. During the ILV several critical steps were discovered which had not been discussed in the registrant method. The test compounds are volatile and have a high affinity for adsorption to glass, especially AKM-18. Moreover, acequinocyl and acequinocyl-OH are light sensitive. It was necessary for the independent laboratory to institute several procedures for dealing with these compound characteristics including modifications to evaporation steps, silanization of the extraction glassware, and steps to ensure protection from light. Also the IL did not filter the samples as suggested in the registrant method.

A major deficiency was that no registrant data was submitted with the registrant method. Only ILV data was presented. Additionally, ECB was unable to verify the ILV data because there were discrepancies in the data presented for the calibration curves. It was impossible to recreate the curves from the data presented.

ECB recommends that the registrant add the modifications made by the IL in an addendum to the original registrant method. ECB also recommends that the registrant provide the agency with the original registrant validation data and contact the IL to correct (explain) the calibration data it submitted. 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) ECB finds this method unacceptable as submitted.

COMPLIANCE

Signed and dated statements that this method was conducted in accordance with 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

Acequinocyl (3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate) is an insecticide in the naphthoquinone family under development by Arvesta Corporation in San Francisco, CA. It is used in the control of insects in apple, cherry, citrus, melon, peach, pear, cucumber, ornamentals, and vegetables.

TABLE A.1. Test Compound Nomenclature

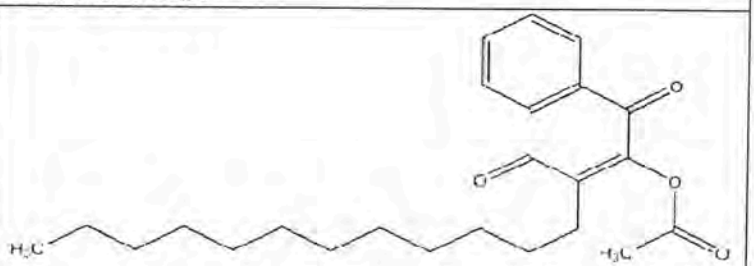
Compound	 Chemical Structure
Common name	Acequinocyl
Company experimental name	Kanemite 15 SC, AKD-2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS Name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7

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

Parameter	Value	
Melting point/range	59.6°C	
pH	6.94	
Density	1.13 at 20°C	
Water solubility (20°C)	6.69	
Solvent solubility (mg/L at 20°C)	Solvent	Solubility (g/L)
	n-Heptane	36.0
	n-octanol	29.2
	acetone	>250
	xylene	>250
	1,2-dichloroethane	>250
Vapour pressure at ___ °C	1.69 X10(-6) Pa at 25°C	
Dissociation constant (pK_a)	Could not be measured, low solubility in water	
Octanol/water partition coefficient	Log Pow ≥6.2	
UV/visible absorption spectrum	pH	λ_{max}(nm)
	neutral MeOH	242, 248, 262, 270, 335
	basic	232, 245, 255, 275, 362
	acidic	242, 248, 265, 270, 330

B. MATERIALS AND METHODS

B.1. Principle of Method

The three targeted analytes are extracted from soil by solvent, evaporated, reconstituted and cleaned up by silica column chromatography. The eluates are combined, evaporated and reconstituted in an acidified acetone:acetonitrile mixture then submitted to LC/MS/MS.

TABLE B.1.1.	**Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied**
Method ID	ECM0217S1-S3
Analyte(s)	Acequinocyl
Extraction solvent/technique	Extraction from soil with acetonitrile:water is followed with addition of NaCl and hexane. The hexane layer is separated, and concentrated.
Cleanup strategies	Silica column chromatography followed by evaporation and reconstitution in mobile phase.
Instrument/Detector	PE Sciex API 2000 LC/MS/MS system with a Perkin Elmer series 200 autosampler, an integrated Shimadzu chromatograph . The system is controlled and data processed by PE Sciex Analyst Software.

C. RESULTS AND DISCUSSION

C.1. Recovery Results Summary (See Appendix A for detailed results report.)

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

NONE GIVEN

C.1.2. Method Characteristics

TABLE C.1.2. Method Characteristics	
Analyte	Acequinocyl
Limit of Quantitation	≥ 0.01 ppm
Limit of Detection (LOD)	Not given
Accuracy/Precision at LOQ (0.05 ug/L)	Not given
Reliability of the Method/ [ILV]	An independent laboratory method validation [ILV], (MRID No. 461826-02), was conducted to verify the reliability of method for the determination of acequinocyl and two degradates in soil. The values obtained indicated that the registrant method is acceptable according to *OPPTS 850.7100 Guidelines*.
Linearity	Although graphs of linear curves were presented, no data to support them was found.
Specificity	The control chromatograms appeared to have a peak in very close proximity to the acequinocyl peak area but it was impossible to confirm this due to lack of data. Peaks were well defined and

TABLE C.1.2. Method Characteristics	
	symmetrical. There appeared to be no carryover to the following chromatograms.

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 Acequinocyl in Soil

Compound	Spiking Level (ug/L)	Average Recoveries Obtained (%)	Relative Standard Deviation
Acequinocyl	0.01 ppm (LOQ) 0.1 ppm (LOQ X 10)	75 96.9	1.6 5.3
Acequinocyl-OH	0.01 ppm (LOQ) 0.1 ppm (LOQ X 10)	75.4 116	7.4 3.7
AKM-18	0.01 ppm (LOQ) 0.1 ppm (LOQ X 10)	93.9 102	8.6 4.3

NOTE: Although the data above meet the acceptability requirements they are based on calibration curves that cannot be verified by ECB.

D. CONCLUSION

From a review of the method "Determination of Acequinocyl, Acequinocyl-OH and AKM-18 in Soil" and the ILV data presented in the "Independent Laboratory Validation for the Determination of Acequinocyl, Acequinocyl-OH and AKM-18 in Water and Soil", a recommendation is made by ECB that modifications made by the ILV be added in an addendum to the original registrant method and that the missing validation data for the registrant method be supplied to the agency. Also new calibration data from the IL should be submitted.