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November 21, 2005

MEMORANDUM

SUBJECT: Acequinocyl in water - ECM0217W1-W2

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 water using the method submitted by Arvesta Corporation in accordance with the registration of Acequinocyl MRID No. 461826-01. 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

Data Requirement: PMRA Data Code: NA
EPA DP Barcode: - D300326
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:** 08/15/05
Elizabeth Flynt, Chemist, EPA/OPP/BEAD/ECB

Peer Reviewer: Charles Kennedy **Date:** 08/18/05
Charles Kennedy, Chemist, EPA/OPP/BEAD/ECB

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

ANALYTICAL METHOD: Anabuki, K. and Tate, S., November 19, 2002. Unpublished method created by Huntingdon Life Sciences Limited and submitted by Pyxant Labs Inc. Study ID: Arvesta-1486. Method Effective Date: November 19, 2002.

EXECUTIVE SUMMARY

The method is applicable for the quantitative determination of residues of acequinocyl (AKD-2023) and metabolite R1 (Hydroxy ACK-2023) in three types of water (drinking, ground and surface).

The method was submitted to EPA by Agro Kanesha-Arvesta Corporation to support studies performed to seek registration for acequinocyl. The method was created by Huntingdon Life Sciences Limited and reviewed by Pyxant Labs Inc. of Colorado, 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:

An aliquot (500 ml) of water was transferred to a separating funnel and fortification solutions were added if required. Aliquots (approx. 20 g and 50 ml) of sodium chloride and hexane were added and the sample was shaken vigorously. To

drinking water it was necessary to add an aliquot (50 ml) of acetonitrile to assist the partition prior to the addition of the hexane. The phases were allowed to separate and the upper (hexane) phase transferred to a round bottom flask (250 ml) through a glass funnel containing anhydrous sodium sulphate. The water was re-extracted with an additional aliquot (50 ml) of hexane, combining the extracts in the round bottom flask. The sodium sulphate was rinsed with an aliquot of hexane (approx. 40 ml) and the rinsate combined with the extracts in the round bottom flask. The volume was reduced to approx. 3 ml by rotary evaporation at <30°C. The residue was reconstituted in an appropriate volume of acetonitrile:water:formic acid (85:15:0.2 v:v:v) with the aid of ultrasonication prior to quantitation by LC-MS-MS.

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

There are several problems/deficiencies with this method. In order to get the registrant method to work, Pyxant Labs Inc, the independent lab, was forced to make several modifications to the registrant method. Most of the modification were minor and made to the extraction portion of the method, but one modification which was made to the mass spectrophotometric and chromatographic procedures was considered major.

The necessity for several minor changes was discovered by the ILV during the extraction portion of the 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.

It was also necessary for the independent laboratory to make a major modification to the mass spectrophotometric and chromatographic procedures in the Huntington (registrant) method in order to get the method to work. In the registrant method, what appears to be an isotopic peak from the molecular ion cluster was chosen to monitor for the parent compound. As stated in the independent lab report, "Such artifactual peaks (M^{+}) can be observed, but are instrument dependent and may not be observed on other instruments, even if they are of the same manufacturer and model." Therefore, the independent lab did not select the (M^{+}) ion but rather monitored for the $(M+H)^{+}$ ion. Additionally, the parent/daughter relationship and the ionization technique selected by the ILV was different from the registrant's method. ECB considers changing the monitored ion, which is the ion used to quantitate the parent/daughter relationship, and the ionization technique as major modifications to the registrant method and, therefore, considers the independent laboratory validation of this method unacceptable.

Although the registrant method as written could possibly have been used to perform the Environmental Fate studies, it was not properly validated by the independent lab. Additionally, ECB has real concerns that in its current written form the registrant

method would not be reproducible by another lab. ECB suggests that the registrant amend their method with the changes made by the independent lab and recognize in the amendment the possibility that method users may opt to monitor for the protonated ion rather than the molecular ion.

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

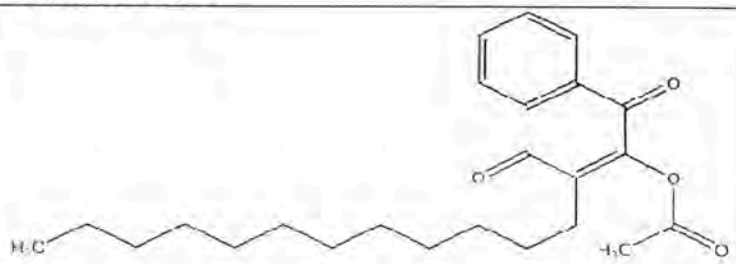
Compound	 Chemical Structure
Common name	Acequinocyl
Company experimental name	Kanemite 15 SC, AKD-2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS Name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7

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

Water samples were extracted with hexane, the extract filtered and the solvent evaporated and reconstituted in acetonitrile:water:formic acid. The residues were quantitated by 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	ECM0217W1-W3
Analyte(s)	Acequinocyl
Extraction solvent/technique	Liquid/liquid extraction of water with hexane.
Cleanup strategies	Filtration with sodium sulphate
Instrument/Detector	Quattro LC with APCI Ionisation

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] (LOQ = 0.1 ug/L)

	AKD-2023			R1 (hydroxyl AKD-2023)		
	RSD	Rec. Range (%)	Mean Rec. (%)	RSD	Rec. Range (%)	Mean Rec. (%)
Surface Water	6.1	93-109	101	5.9	95-110	104
Drinking Water	2.1	95-100	97	3.1	85-92	88
Ground Water	6.2	89-102	95	10.7	85-92	83

C.1.2. Method Characteristics

TABLE C.1.2. Method Characteristics

Analyte	Acequinocyl
Limit of Quantitation	0.1 µg/L
Limit of Detection (LOD)	Not given
Accuracy/Precision at LOQ (0.1 ug/L)	See Table C.1.1
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 acequinocyl OH in water. The values obtained indicated that the registrant method is acceptable but ECB could not recreate the calibration from the data and graph given by the IL.
Linearity	Regression equations and supporting data were provided which established a linear relationship between acequinocyl and acequinocyl OH and instrument response.
Specificity	

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 ground water

Compound	Spiking Level (ug/L)	Average Recoveries Obtained (%)	Relative Standard Deviation
Acequinocyl	0.1 ug/L (LOQ) 1.0 ug/L (LOQ X 10)	82.0 86.7	10 13
Acequinocyl-OH	0.1 ug/L (LOQ) 1.0 ug/L (LOQ X 10)	76.5 91.0	7.4 9.0

D. CONCLUSION

From a review of the method, "Development and Validation of Methodology for the Determination of Residues of AKD-2023 and Its Major Metabolite R1 (Hydroxy AKD-2023) in Drinking Water, Ground Water and Surface Water" and the ILV data presented in the "Independent Laboratory Validation for the Determination of Acequinocyl, Acequinocyl-OH and AKM-18 in Water and Soil", ECB suggests that the registrant amend their method with the changes made by the independent lab and recognize in the amendment the possibility that method users may opt to monitor for the protonated ion rather than the molecular ion.