PP#4354

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP 18 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: PP#4F04354. Abamectin (Avermectin B_1) for Use in/on the Cucurbit Crop Group (Cucumbers, Melons, and Squash. Results of ACL's Method Validation (Memo of E. Greer, Jr. and D. Wright, Jr. dated 8/4/95).
No MRID#. DP Barcode# D218341. CBTS# 15719.

From: G. Jeffrey Herndon, Chemist
Tolerance Petition Section II
Chemistry Branch I - Tolerance Support
Health Effects Division (7509C)

G. Jeffrey Herndon

Through: Michael Metzger, Chief
Chemistry Branch I - Tolerance Support
Health Effects Division (7509C)

Michael S. Metzger

To: George LaRocca/Adam Heyward, PM# 13
Insecticide-Rodenticide Branch
Registration Division (7505C)

and

William Hazel, Head
Registration Section
Risk Characterization and Analysis Branch
Health Effects Division (7509C)

Merck and Co., Inc. is requesting the establishment of permanent tolerances for abamectin (avermectin B_1) insecticide/miticide and its delta-8,9-isomer in/on the following commodities:

Commodity	Tolerance (ppm)
Cucurbit vegetables (including melons, cucumbers, and squashes)	0.005

In the memo of G.J. Herndon dated 3/29/95, CBTS raised two Deficiencies with PP#4F04354: additional information was needed concerning the conditions under which the field trial samples were held and the method needed to be validated by EPA's Analytical

Chemistry Lab (ACL).

In a memo of G.J. Herndon dated 3/27/95, CBTS requested that ACL validate Merck Method 8920 on cucumbers. CBTS has now received the results of ACL's method validation (see memo of E. Greer, Jr. and D. Wright, Jr. dated 8/4/95 - see Attachment I). The purpose of this memo is to address the comments/conclusions raised in the most recent ACL memo.

Conclusions and Recommendations

CBTS continues to recommend against the issuance of a permanent tolerance on cucurbits since Deficiencies 5a and 6a (from the 3/29/95 memo of G.J. Herndon) remain unresolved. Provided that Merck makes the requested changes to Method 8920 as outlined in Comment 3 of this memo (incorporating additional language to avoid losing the compounds of interest in an emulsion), Deficiency 5a will be resolved.

Detailed Considerations

The following Comments were cited by EPA's Analytical Chemistry Laboratory in Beltsville in the memo of E.S. Greer, Jr. and D. Wright, Jr. dated 8/4/95.

Comment #1

The method states that all standards should be stored in a freezer at -10°C, but the EPA repository at RTP ships this material at ambient temperature. The Certificate of Analysis sheets that the repository includes with the standard states that the material must be kept frozen. ACL feels that the standard as supplied by the repository is not suitable for enforcement purposes because of the potential for degradation. ACL used an analytical standard solution supplied by the registrant that was received packed in dry ice.

CBTS's Comments and Conclusions Concerning Comment #3

This Deficiency is not a fault of the registrant. The RTP repository is not under the purview of the Office of Pesticide Programs. **CBTS considers Comment #1 resolved.**

Comment #2a

A purified analytical standard of avermectin is not available from the registrant. The registrant supplies a dilute glycerol formal solution of both avermectin B_1a/B_1b and the 8,9-Z isomer of B_1a. This issue is addressed in the TMV pre-review included with this report (see Attachment II).

CBTS's Comments and Conclusions Concerning Comment #2a

In a submission of 3/10/93 in response to the analytical method deficiencies cited in the J. Stokes review of 4/16/92 (see memo of G.J. Herndon dated 12/16/93 concerning PP#9F03787 - avermectin on pears), Merck provided the following response to a similar concern raised by ACL.

> Abamectin drug substance (bulk technical or solid state) has two characteristics which make it unsuitable for routine use as a reference standard in laboratory analyses - it is a mixed, non-stoichiometric solvate and chemically unstable.
>
> Abamectin drug substance contains up to 7.0% ethanol and 17.0% water. These solvents are not present in a fixed ratio (arising from defined solvates) and are therefore subject to facile variation (loss of ethanol and/or water, or uptake of water) depending on the environment (temperature and humidity) in which the drug substance is stored and handled. In addition, abamectin is not chemically stable and is subject to solid-state oxidative decomposition.
>
> Both of the unfavorable characteristics have been overcome through the development of an abamectin glycerol formal solution for use as a routine laboratory reference standard. Abamectin, and associated ethanol and water, are completely soluble in glycerol formal at the concentration employed. Glycerol formal is non-volatile and non-hygroscopic, and therefore, solvation variations after dissolution of abamectin are eliminated. In addition, glycerol formal has desirable stabilization properties and inhibits the oxidation degradation of abamectin.
>
> When the abamectin glycerol formal solution was prepared, the B1a and B1b isomer concentrations were accurately determined versus a specially prepared solid reference lot which is no longer available (because of the unfavorable characteristics previously mentioned). The solution was subdivided into individual amber glass containers, each with an amount convenient for multiple analyses, and stored frozen to insure stability. The solution is dilute, permitting the accurate weighing of a convenient amount which does not require excessive dilution to prepare working standards with concentrations appropriate for use in trace residue analyses. Refrigerated, or preferably frozen, shipment and storage is desirable to maintain the standard's integrity. '
>
> The abamectin glycerol formal solution standard is

suitable for its intended use, and has been successfully employed by several Merck laboratories and numerous contract laboratories which conduct residue analyses both in the US and internationally. The glycerol formal standard solution is of defined purity and sufficiently concentrated for all residue determinations, including the method described for pears. Finally, there is no solid abamectin standard that is available or suitable for use.

As might be expected from the similarities in the structure, the avermectin B1a delta 8,9-Z isomer has similar characteristics. Consequently, a solution of avermectin B1a delta 8,9-Z isomer standard in glycerol formal has been prepared and is used. However, we have determined that the avermectin B1a delta 8,9-Z isomer yields the same derivative as is obtained from the parent avermectin B1a so that it is not necessary to use the delta 8,9-Z isomer standard, except during the initial validation of the method

Based on a conversation with Merck (phone conversation with L. Grosso of 9/5/95), Merck is in the process of formulating a neat avermectin standard. However the work has not been completed. Based on the inherent properties (unstable, hygroscopic) of the abamectin standards, the concentration levels of the supplied standards relative to the proposed tolerance level in cucurbits (0.005 ppm), and the process by which abamectin is manufactured [(fermentation process using a strain of Streptomyces avermitilis)], CBTS considers the supplied standard solutions in glycerol to be adequate for enforcement purposes, **until a neat avermectin standard is formulated. CBTS considers Comment #2a resolved.**

Comment #2b

Derivatized standards of B_1b and 8,9 isomer of B_1a were used for quantitating recoveries for these compounds. The method uses standard B_1a for this purpose. This issue is also addressed in the TMV pre-review (see Attachment II).

CBTS's Comments and Conclusions Concerning Comment #2b

In a submission of 3/10/93 in response to the analytical method deficiencies cited in the J. Stokes review of 4/16/92 (see memo of G.J. Herndon dated 12/16/93 concerning PP#9F03787 - avermectin on pears), Merck provided the following response to a similar concern raised by ACL.

> Avermectin B1b is at most 20% and usually less than 10% of the avermectin content in the formulation and in

the incurred residue. Avermectin B1a is at least 80% and usually more than 90% of the avermectin residue. Consequently, the B1b residues are usually not quantifiable and generally not even detectable at the PHI, no matter which calibration curve is used.

B1a and B1b differ by one methylene group connected at the C-25 position. Although B1a and B1b are resolved chromatographically in a reverse phase HPLC system, the quantitation is based on the fluorescent derivative response. The fluorescent part of the molecule is in the extended conjugation associated with the aromatized ring, which is the same for avermectin B1b and B1a. We have demonstrated the equivalence of the response for B1b to B1a and have previously provided documentation (see Attachment II). The pear method uses the same fluorescent derivative as discussed in Attachment II and the matrix does not present any interferences to affect the fluorescence sensitivity, as illustrated in the validation of the method for B1a and B1b.

Although not analytically correct, Merck has provided sufficient data to show that the quantitation of avermectin B1b residues using the B1a curve will accurately measure the contribution of B1b in the total avermectin residue up to approximately 100 ng/g (ppb) total. Since the proposed tolerance level in cucurbits is 5 ppb (i.e. <100 ppb), and the cucurbit matrix has been shown not to present any interferences that would affect the fluorescence sensitivity, CBTS considers Merck Method 8920 to be an adequate method for the enforcement of avermectin residues on cucurbits. **CBTS considers Comment #2b resolved.**

Note: If the need arises to raise the tolerance level on cucurbits above 100 ppb, or if Method No. 8920 is utilized for other commodities (especially other commodities whose tolerance levels exceed 100 ppb or if interferences are seen or expected), Merck will need to provide a revised method and additional validation data.

Comment #3

During the liquid-liquid partitioning (steps Nos. 9 and 10), ACL experienced emulsions in three samples, one control, and two spikes, which caused the clean-up column to become plugged or elute very slowly. The control sample was discarded and the two spiked samples were continued even though the column clean-up step was very slow. These two samples had very low recoveries (20% and 16%). In the remaining samples ACL very gently rocked the tubes during the partitioning step. The emulsions were greatly reduced with no problems in the subsequent column clean-up step.

The ILV report stated that low recoveries were obtained for the 8,9 isomer. After discussions between the independent laboratory and the sponsor, it was determined that the analyte was probably lost in the emulsion formed during the partitioning steps. The report states that very gentle shaking solved this problem. The method should address this issue and caution the analyst to use gentle shaking for these steps.

CBTS's Comments and Conclusions Concerning Comment #3

Additional language should be added to Method 8920 after step# 9 that cautions the analyst that an emulsion can form if the mixture is shaken too vigorously, and if an emulsion forms, the compounds of interest can be lost. Additional guidance on how to clear an emulsion if it forms would also be helpful (additional centrifuge time, etc.). **CBTS does not consider Comment #3 resolved.**

Comment #4

The HPLC mobile phase had to be changed from 10% H_2O/90% MeOH to 7% H_2O/93% MeOH for the analysis of avermectin B_1b in order to separate the analyte from a potentially interfering coextractant.

CBTS's Comments and Conclusions Concerning Comment #4

The HPLC eluent specified in Method 8920 is 7% ultrapure water in methanol (v/v), not 10%. The method mentions that minor changes in the operating conditions may be required to obtain equivalent performance with other equipment. **CBTS considers Comment #4 resolved.**

Comment #5

A set of six samples can be extracted and cleaned up in approximately 12 hours and placed on the autosampler for overnight analysis on the liquid chromatograph.

CBTS's Comments and Conclusions Concerning Comment #5

Comment #5 is not a deficiency.

Comment #6a

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated to be as follows:

	LOD	LOQ
avermectin B_1a/8,9 isomer	0.19 ppb	0.63 ppb
avermectin B_1b	0.3 ppb	1.0 ppb

CBTS's Comments and Conclusions Concerning Comment #6a

Comment #6a is not a deficiency.

Comment #6b

This method meets the requirements in Subdivision O, Section 171.4(b) of the Residue Chemistry guidelines provided the above comments and those included in the attached pre-review are addressed.

CBTS's Comments and Conclusions Concerning Comment #6b

Based on the previous comments, **CBTS considers Comment #6b resolved.**

Attachment I - Tolerance Method Validation of Abamectin on Cucumbers, memo of E. Greer, Jr. and D. Wright, Jr. dated 8/4/95.

Attachment II - TMV Pre-Review of Abamectin on Cucumbers, E. Greer, Jr., 5/18/95.

cc (without Attachments): circu., SF, E. Haeberer (section head), H. Hundley (H7503W).

cc (with Attachments): PP#4F04354, RF, G.J. Herndon.

RDI: TPSII Team: 9/14/95,
Branch Senior Scientist: R.A. Loranger: 9/14/95,
Branch Chief: M. Metzger: 9/18/95.

H7509C: CBTS: G.J. Herndon: 305-6362: CM#2, Rm. 804C: 9/13/95.

Attachment I

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Analytical Chemistry Section
Building 306, BARC-East
Beltsville, Maryland 20705

AUG - 4 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#4F04354. Tolerance Method Validation of Abamectin (Avermectin B_1) on Cucumbers

FROM: Everett S. Greer, Jr., Team Leader
Dallas P. Wright, Jr., Chemist DPW
Analytical Chemistry Section

Harvey K Hundley

THRU: Harvey K. Hundley, Head
Analytical Chemistry Section

THRU: Donald A. Marlow, Chief
Analytical Chemistry Branch

TO: Elizabeth Haeberer, Section Head
Tolerance Petition Section II
Chemistry Branch I-Tolerance Support
Health Effects Division

INTRODUCTION

The Analytical Chemistry Section was requested by the Chemistry Branch I - Tolerance Support to conduct a method validation on the insecticide/miticide Abamectin. Merck & Co.,Inc. Method No. 8920 ("HPLC-Fluorescence Determination for Avermectin B_1 and its 8,9 Isomer in Cucumbers") was used for the analysis of cucumbers spiked with Avermectin B_1a/B_1b and 8,9-Z isomer of B_1a at the 0.005 ppm and 0.01 ppm levels.

METHOD SUMMARY

Cucumber samples are extracted with methanol and the extracts are cleaned up by liquid-liquid partitioning and C-8/aminopropyl solid phase extraction. The eluant from the SPE columns is taken to dryness and derivatized to a fluorescent compound with trifluroacetic anhydride. The reaction mixture is cleaned up on a silica SPE column and the analyte is quantitated by HPLC using a fluorescence detector.

COMMENTS

1. The method states that all standards should be stored in a freezer at -20°C., but previously the EPA repository at RTP shipped avermectin standards at ambient temperature. The Certificate of Analysis sheets that the repository includes with the avermectin standards states that the material must be kept frozen. ACL feels that the analytical standards as supplied by the repository is not suitable for enforcement purposes because of the potential for degradation. ACL used standards supplied by the registrant that were received packed in dry ice.

2. A purified analytical standard of avermectin is not available from the registrant. The registrant supplies a dilute glycerol formal solution of both avermectin B_1a/B_1b and the 8,9-Z isomer of B_1a This issue is addressed in the TMV pre-review included with this report.

Derivatized standards of B_1b and 8,9 isomer of B_1a were used for quantitating recoveries for these compounds. The method uses standard B_1a for this purpose. This issue is also addressed in the TMV pre-review.

3. During the liquid-liquid partitioning (steps Nos. 9 and 10), ACL experienced emulsions in three samples, one control and two spikes, which caused the clean-up column to become plugged or elute very slowly. The control sample was discarded and the two spiked samples were continued even though the column clean-up step was very slow. These two samples had very low recoveries(20% and 16%). In the remaining samples ACL very gently rocked the tubes during the partitioning step. The emulsions were greatly reduced with no problems in the subsequent column clean-up step.

The ILV report stated that low recoveries were obtained for the 8,9 isomer. After discussions between the independent laboratory and the sponsor, it was determined that the analyte was probably lost in the emulsion formed during the partitioning steps. The report states that very gentle shaking solved this problem. The method should address this issue and caution the analyst to use gentle shaking for these steps.

4. The HPLC mobile phase had to be changed from 10% H_2O/90% MeOH to 7% H_2O/93% MeOH for the analysis of avermectin B_1b in order to separate the analyte from a potentially interfering coextractant.

5. A set of six samples can be extracted and cleaned up in approximately 12 hours and placed on the autosampler for overnight analysis on the liquid chromatograph.

6. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated to be as follows:

	LOD	LOQ
Avermectin B_1a/8,9 isomer	0.19 ppb	0.63 ppb
Avermectin B_1b	0.3 ppb	1.0 ppb

6. This method meets the requirements in Subdivision O, Section 171.4(b) of the Residue Chemistry guidelines provided the above comments and those included in the attached pre-review are addressed.

Commodity	Chemical Added	PPM Added	PPM Found	Percent Recovery
Cucumbers	Avermectin B_1a	Control	N.D.	-
		Control	N.D.	-
		0.005	0.0035	70.0
		0.005	0.0041	82.0
		0.005	0.0030	60.0
		0.01	0.0082	82.0
		0.01	0.0083	83.0
		0.01	0.0080	80.0
Cucumbers	Avermectin B_1b	Control	N.D.	-
		Control	N.D.	-
		0.005	0.0045	90.0
		0.005	0.0048	96.0
		0.01	0.0079	79.0
		0.01	*	
Cucumbers	8,9-Z isomer	0.005	0.0047	94.0
		0.005	0.0046	92.0
		0.01	0.0076	76.0
		0.01	0.0078	78.0

* Sample extract was spilled during analysis.

N.D.= Less than the limit of Detection

Modifications to method (major or minor):

See Comments section of report.

Special precautions to be taken:

None

Source of analytical standards:

Merck and Company, Inc.

If derivatized standard is used, give source:

Prepared as per method.

Instrumentation for quantitation:

HPLC/Fluorescence detector

Instrumentation for confirmation:

N/A

If instrument parameters differ from those given in method, list parameters used:

See Comments section in Report

Commercial sources for any special chemicals or apparatus:

N/A

Additional comments:

See report.

Chromatograms
Copies attached

Attachment II

TMV Pre-review of Abamectin on Cucumbers

Reviewed by: Everett S. Greer, Jr. ESG

Date: 5-18-95

Laboratory assignment number: B95-48

Analytes: Avermectin B1a, B1b and 8,9-isomer of B1a

Commodity: Cucumbers

Petitioner: Merck and Company, Inc.

Method: "HPLC-Fluorescence Determination for Avermectin B1 and its 8,9 Isomer in Cucumbers" (Method No. 8920)

IV Method

E. Derivatization

The sample extract is split at this point and. Half of the sample is carried through the reminder of the procedure and the other half is stored at -10c. The stored half is not again referred to in the method, but the flow chart on page 12 indicates that it is to be retained for reassay.

F. Preparation of the standards and quantitation

The analytical standards for this method are supplied by the petitioner in a glycerol formal solution. (See additional comments)

IX Determination

The avermectin B1b is calculated off of a standard curve for avermectin B1a. (See additional comments)

Additional reviewer's comments

1. ACL recently validated Merck method No. 8000, Rev. 4 for pears. That method has a very detailed "Suggestions for the Analyst..." section attached to the procedure. It covers all of the major analytical steps and describes various cautions that the analyst has to observe to properly perform the analysis. This method does not include an addendum addressing individual procedural steps. Method 8000, Rev.4 has a derivatization step that is virtually identical to this method ,and the "Suggestions..." section describes precautions to be observed when performing this step. If the petitioner feels that these

precautions are necessary for Method 8000, Rev.4, they should be included in this method.

2. The standard concentrations described in the method for preparing the standard curve will cover a range of residues up to twice the proposed tolerance level of 0.005 ppm.

3. The issues concerning the need for a purified analytical standard and the problems associated with using the B_1a standard curve for calculating compound B1b are addressed in the following excerpts from the Method 8000, Rev.4 pre-review (Jan. 27, 1995) prepared by Bart J. Puma of ACL.

> The need for purified analytical reference standards is so fundamental in analytical chemistry that it should not be necessary to explain it, especially as applied to a proposed regulatory method! Merck has offered excuses for failing to provide proper reference standards, but has not explained how, in the absence of such standards, EPA (or anyone else, including Merck) can assay the certified abamectin standard solutions that were submitted to ACL and the EPA pesticides repository. Merck says that when the abamectin standard solution in glycerol formal was prepared, the concentrations of B1a and B1b were accurately determined versus a specially prepared reference lot which is no longer available. Does this mean that no other specially prepared reference lot is available? If so, how does Merck retest the standard solutions yearly as indicated in a letter sent to ACL with certificates of analysis for the standard solutions of abamectin and 8,9-Z-B1a? If the EPA repository is to supply regulatory laboratories with dilute standard solutions of abamectin in glycerol formal for use in enforcing tolerances, EPA will need a suitable reference standard and methods for assaying both the pure material and the dilute abamectin standards in order to ensure the integrity of the latter. The fact that the latest abamectin and 8,9-Z-B1a standard solutions currently available from the EPA repository were provided by Merck in 1988 indicates that it would be it would be difficult, if not impossible, to provide legitimate scientific support for any regulatory action based on the use of these standards for residue analysis.
>
> One of the reasons Merck gives for using glycerol formal as solvent for the standard solutions is that it is non-hygroscopic. This is strange because glycerol formal is listed in various suppliers' catalogs as either hygroscopic (Aldrich Chemical; TCI America) or

moisture sensitive (Lancaster Synthesis) and is reported to be hygroscopic in Dictionary of Organic Compounds, 5th Ed., Vol. 2, Item D-07685. What is the evidence to support Merck's claim that glycerol formal is non-hygroscopic?

Using the B1a calibration curve to quantitate B1b residues as proposed in the method is bad science and its acceptance in a regulatory method would establish a bad precedent because it is analytically incorrect and introduces a positive bias of about 10% in the results for B1b residues. Although the effect of this positive B1b bias on the overall result for abamectin is small (because B1b is at most 20% of the incurred residue), using a technique with a known bias toward high results would be difficult to support in an enforcement action against a sample slightly above the tolerance level. Acceptable techniques are readily available for B1b quantitation in the method. Merck should use one of these instead of basing the determination of B1b on the calibration curve for another compound that produces a different analytical response than B1b.

4. Recovery data are included in the method for cucumbers fortified with B1a, B1b and 8,9 B1a isomer at the 0.0059 ppm, 0.0066 ppm and 0.0052 ppm levels respectively. Recoveries at these levels ranged between 71% and 109%. Satisfactory recoveries were also reported at higher levels. The only recovery chromatograms reported with the method were for cucumbers spiked at 0.0059 ppm with B1a and 0.0052 ppm with 8,9 B1a isomer.

5. This method was validated for cucumbers and melons by Hazleton Laboratories America, Inc. in 1990/1991. No references to PR notice 88-5 were made in the validation report. Satisfactory recoveries were reported for cucumbers spiked at 0.005 ppm. 0.020 ppm and 0.07 ppm. Chromatograms for cucumbers spiked at these levels were also included.

6. The validation laboratory initially had difficulty obtaining satisfactory recoveries of the 8,9 B1a isomer. It was determined that some of the analyte was lost in the emulsion formed during the shakeout (steps 9 and 10). A gentle shakeout produced a very small emulsion layer along with acceptable recoveries. If this shakeout step is the source of potential problems, it should be addressed in the method.

ANALYTICAL CHEMISTRY BRANCH
SCREEN FOR RESIDUE METHODS FOR TMV

1. LABORATORY ASSIGNMENT NUMBER: B95-48

2. PP#: 4F04354

3. TECHNICAL REVIEWER: Everett S. Greer, Jr.

4. DATE: 5-18-95

5. ANALYTES/LEVEL: Avermectin B1a, B1b, 8,9-Isomer of B1a /0.005 ppm, 0.01 ppm

6. COMMODITIES: Cucumbers

7. METHOD: HPLC-Fluorescence Determination for Avermectin B1 and its 8,9 Isomer in Cucumbers - Method No. 8920

The Analytical Chemistry Section has been asked to screen the residue chemistry methods submitted by the registrant in order to determine if they contain the essential requirements identified in the Residue Chemistry Guidelines. Full scientific review and laboratory evaluation of those methods will take place after the initial screen. The following items need to be resolved before the analytical method can be evaluated.

	YES	NO
1. Does the method use exotic equipment and/or supplies that are not commercially available in the U.S.?		✓
2. Does the method require any new equipment before the laboratory work begins?		✓
3. Are chromatograms included?	✓	
a. Is (are) peak(s) of interest sufficiently resolved from other peaks?	✓	
b. Has registrant included chromatograms of analyses at or below tolerance on all crop types for which tolerance is requested by HED?	✓	
c. Do the control samples have reasonably low levels of the analyte in relation to the proposed tolerance?	✓	
d. Is the method sufficiently sensitive and specific to measure and identify the residues at levels specified by HED in the TMV request?	✓	