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

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

**February 14, 2002**  
**MEMORANDUM**

**SUBJECT:** Secondary Review of "Validation of GC Method for the Determination of CL-92533 (Pendimethalin) Dislodgeable Foliar Residues from Sugarcane Leaves and Tobacco Leaves." MRID 449699-03. PC Code 108501. DP Barcode D274770.

**FROM:** Christina Jarvis, Environmental Protection Specialist  
Reregistration Branch 2  
Health Effects Division (7509C) *Christina M. Jarvis 2/14/02*

**THROUGH:** Alan Nielsen, Branch Senior Scientist  
Reregistration Branch 2  
Health Effects Division (7509C) *Al Nielsen 2/14/02*

**TO:** Patrick Dobak, Chemical Review Manager  
Reregistration Branch 1  
Special Review and Reregistration Division (7508W)

Attached is a review of the validation of the GC method for determination of dislodgeable foliar residues from sugarcane leaves and tobacco leaves, submitted by American Cyanamid Company in support of the reregistration of pendimethalin. This review was completed by Versar, Inc. on December 29, 2000, under supervision of the Health Effects Division (HED). It has undergone secondary review in HED and has been revised to reflect current Agency policy.

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## MEMORANDUM

**TO:** Christina Jarvis  
**FROM:** Diane Forrest  
Pat Wood  
**DATE:** December 29, 2000  
**SUBJECT:** Review of CL-92553 (*Pendimethalin*): *Validation of GC Method for the Determination of CL-92533 Dislodgeable Foliar Residues from Sugarcane Leaves and Tobacco Leaves*  
- MRID #449699-03

cc: 1000.001.01  
M. Collantes

This report reviews *Validation of GC Method for the Determination of CL-92533 Dislodgeable Foliar Residues from Sugarcane Leaves and Tobacco Leaves*, submitted by American Cyanamid Company, in response to the U.S. Environmental Protection Agency's (US-EPA) Data Call-In (DCI) notice issued October 18, 1995 and March 1, 1993, and in support of the reregistration of pendimethalin [Case #0187]. Requirements for this study are specified by: (1) the U.S. Environmental Protection Agency's (US-EPA) OPPTS Series 875, Occupational and Residential Exposure Test Guidelines, Group C: Quality Assurance/ Quality Control, and OPPTS Series 860.1340 (171-4); (2) 40 CFR 158.240 EPA Residue Chemistry Test Guidelines OPPTS 860.1340 (171-4), and (3) EPA, FIFRA, Good Laboratory Practice Standards (GLP); 40 CFR Part 160 (October, 1989). The following information may be used to identify the study:

<b>Title:</b>	<i>CL-92553 (Pendimethalin): Validation of GC Method for the Determination of CL-92533 Dislodgeable Foliar Residues from Sugarcane Leaves and Tobacco Leaves</i> , 77 pages (i.e., RES-98-213 - 36 pages, and RES-98-212 - 37 pages)
<b>Sponsor:</b>	American Cyanamid Company Agricultural Products Research Division P.O. Box 400 Princeton, NJ 08543-4000
<b>Performing Laboratory:</b>	Maxim Technologies, Inc. 140 Telegraph Road Middleport, NY 14105
<b>Authors:</b>	Toreen A. Bixler, Principal Analyst, Maxim Technologies, Inc. Brion Babbitt, Study Director, American Cyanamid Company
<b>Study Completion Date:</b>	June 18, 1999
<b>Submission Date:</b>	November 11, 1998
<b>I.D. Codes:</b>	MRID #449699-03; Study No. RES 98-213 (Sugarcane) & RES 98-212 (Tobacco); American Cyanamid Protocol Numbers PR98PT07 & PR98PT06; American Cyanamid Company Methods M-3238 and M-3239; Maxim Technologies Inc. Study Numbers: A011.292 & A011.290 Pendimethalin Reregistration Eligibility Decision Document: Case #0187, 2-Year Response

## EXECUTIVE SUMMARY

This report reviews a laboratory validation study for analytical methodology intended to be used in dislodgeable foliar residue (DFR) studies. It quantifies CL-92553 (identified as pendimethalin, formulation unknown) on the leaves of both sugarcane and tobacco. Accordingly, the study report contains two substantially identical sections, addressing findings for sugarcane DFR in "Exhibit 1," and for tobacco DFR in "Exhibit 2."

The studies were conducted in accordance with the American Cyanamid Co. Residue Support Study Protocol Numbers PR98PT07 & PR98PT06, provided in Appendices to each Exhibit in the study report.

Sample preparation, extraction and analytical methods used for both sugarcane and tobacco DFR were basically the same. Individual samples consisted of forty (40) untreated sugarcane or tobacco leaf punches (2.523 cm. in diameter; yielding 400 cm<sup>2</sup> total leaf surface area), which were "dislodged" twice in 100 mLs of 0.01% Aerösol® OT (detergent) solution with 10 minutes mechanical shaking. Control leaf washes were frozen for approximately 3 months before thawing. Samples were then fortified with known amounts of analytical grade pendimethalin in acetone (i.e., corresponding to 0, 10, 20, and 100 ng/cm<sup>2</sup> leaf area), extracted into methylene chloride and analyzed via GC-NPD within 1-2 days. Representative chromatograms provided for review showed good peak separation and reasonably sharp peaks.

Limitations and uncertainties associated with the study are reviewed below:

- The Limit of Quantitation (LOQ) for sugarcane and tobacco was reported to be 10 ng/cm<sup>2</sup> leaf area. The limit of detection (LOD) was reported to be 2 ng/cm<sup>2</sup> for tobacco, but was not defined for sugarcane. The relationship to the LOQ was not discussed.
- The authors tested a very small number of samples (i.e., two samples at each of three fortification levels) and reported an average recovery of 106 ± 2.7 percent for tobacco DFR and 101 ± 4.2 percent for sugarcane DFR. Seven samples per fortification level per matrix are required for method validation experiments.
- The range of fortification levels tested was quite narrow, consisting of 2 samples at the expected LOQ of 10 ng/cm<sup>2</sup>, 2 samples at 20 ng/cm<sup>2</sup>, and 2 samples at 100 ng/cm<sup>2</sup>. It is uncertain whether this range of concentrations truly reflects the entire working range of the method. OPPTS Series 875, Occupational and Residential Exposure Test Guidelines, Group C: Quality Assurance/Quality Control require fortification levels at the method LOQ, an intermediate concentration level (e.g., 10x LOQ), the maximum concentration of the validation range (e.g., 100-1000x LOQ), and blank or control substrate.
- The study did not address whether the analytical methodology could be expected to be specific to the parent compound, or whether it would also detect and quantify breakdown products or metabolites one might expect to find in a DFR field study.
- Only analytical grade pendimethalin of known purity was used in this study. Since formulated products are generally tested in field DFR studies, fortifications with formulated products should be included. The validation study also failed to address whether the method might be subject to

any interferences, for example by breakdown products or ingredients expected to be found in formulated products.

- No information was provided concerning the storage stability of the tested analyte. Prospective users of this method would not necessarily know whether real field samples containing pendimethalin DFR require storage at specific temperatures, how long they might be stored without deterioration of the samples, whether pH of the solutions might require adjustment, etc. The study report does not provide sufficient information to address such concerns.
- The authors did not attempt to define what sensitivity and range either method might be required to have to be useful for the purposes intended (e.g. worker exposure estimates). Therefore, the reviewers cannot be sure whether the methods reviewed in this study report are adequate for real field use.

## STUDY REVIEW

### Study Background

This report reviews a laboratory validation study for analytical methodology intended to be used in dislodgeable foliar residue (DFR) studies. It quantifies CL-92553 (identified as pendimethalin, formulation unknown) on the leaves of both sugarcane and tobacco. Accordingly, the study report contains two substantially identical sections, addressing findings for sugarcane DFR in "Exhibit 1," and for tobacco DFR in "Exhibit 2."

The studies were conducted in accordance with the American Cyanamid Co. Residue Support Study Protocol Numbers PR98PT07 & PR98PT06, provided in Appendices to each Exhibit in the study report.

### Analytical Methodology

On August 11, 1998, Maxim Laboratories received untreated tobacco leaves (under refrigeration) from American Agricultural Services, Inc., Lucama, NC. The tobacco leaves were stored at  $3^{\circ}\text{C} \pm 4^{\circ}\text{C}$ . On August 19, 1998, Aerosol® OT leaf washes were prepared. The samples were stored at less than ( $-$ )  $15^{\circ}\text{C}$ . until November 16, 1998. On that day, they were thawed, fortified with known amounts of analytical grade pendimethalin in acetone (i.e., corresponding to 10, 20, and 100 ng/cm<sup>2</sup> leaf area), and extracted. Samples were analyzed by GC on November 18, 1998.

On August 25, 1998, Maxim Laboratories received untreated sugarcane leaves (under refrigeration) from Nelson Prochaska, R&D Research Farm, Inc., Washington, LA. The sugarcane leaves were stored at  $3^{\circ}\text{C} \pm 4^{\circ}\text{C}$ . On August 28, Aerosol® OT leaf washes were prepared. The samples were stored at less than ( $-$ )  $15^{\circ}\text{C}$ . until November 17, 1998. On that day, they were thawed, fortified with known amounts of analytical grade pendimethalin in acetone (i.e., corresponding to 10, 20, and 100 ng/cm<sup>2</sup> leaf area), and extracted. Samples were analyzed by GC on November 18, 1998.

The methods tested were identified as American Cyanamid Company Methods M-3238 (DFR from tobacco leaves) and M-3239 (DFR from sugarcane leaves). A detailed description of the mechanics of the analytical methods may be found in Exhibit 1, page 27 (sugarcane) and Exhibit 2, page 28 (tobacco) of the study report. A summary is provided below.

Individual samples consisted of forty (40) untreated sugarcane or tobacco leaf punches (2.523 cm. in diameter; yielding 400 cm<sup>2</sup> total leaf surface area) were "dislodged" twice in 100 mLs of 0.01% Aerosol® OT solution with 10 minutes mechanical shaking. The detergent solutions were combined to yield a total volume of 200 mLs. This yielded the "field samples," which were frozen at less than ( $-$ )  $15^{\circ}\text{C}$ .

For method validation, the field samples were thawed, and transferred to glass beakers. Next, the samples were fortified with known amounts of pendimethalin (i.e., 10, 20, and 100 ng/cm<sup>2</sup> leaf area) dissolved in acetone. [Note: The pendimethalin used was analytical grade, of known purity ( $\sim$ 98.4% pure), obtained from American Cyanamid.] The reference standard was stored under refrigeration at  $5^{\circ}\text{C} \pm 4^{\circ}\text{C}$ . Then, 5 mLs saturated NaCl was added, and the solution was extracted twice into 50 mLs methylene chloride, using a separatory funnel. The organic layers were collected, combined, subjected to roto-evaporation, then 5 mLs methanol was added (if residual water was found to exist), and the extract evaporated to dryness. Finally, 8 mLs acetone was added, the sample was sonicated, filtered

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through 0.22 µM nylon filters, and an aliquot injected into a gas chromatograph equipped with a nitrogen phosphorous detector. The rest of the sample was frozen at less than (-)15° C.

Gas chromatography conditions were identical for both tobacco and sugarcane methods. The only difference was sample run time; the run time for tobacco DFR samples was longer (19.2 minutes ) than for sugarcane DFR samples (13.0 minutes). The retention time for pendimethalin was 9.2 minutes whether the sugarcane or the tobacco method was used.

Representative chromatograms of analyte standards were provided in the study report. Representative chromatograms provided for review showed good peak separation and reasonably sharp peaks.

The Limit of Quantitation (LOQ) was reported to be 10 ng/cm<sup>2</sup> leaf area. The limit of detection (LOD) was reported as 2 ng/cm<sup>2</sup> for tobacco. The LOD was not defined for sugarcane. The relationship to the LOQ was not discussed.

## Findings

Limitations and uncertainties associated with the study are reviewed in detail below.

- The authors tested two samples at each of three fortification levels (i.e., six fortified tobacco samples and six fortified sugarcane samples), and reported an average recovery of  $106 \pm 2.7$  percent for tobacco DFR and  $101 \pm 4.2$  percent for sugarcane DFR. Seven samples per fortification level per matrix are required for method validation experiments. Also, the range of fortification levels tested was quite narrow, consisting of 2 samples at the expected LOQ of 10 ng/cm<sup>2</sup>, 2 samples at 20 ng/cm<sup>2</sup>, and 2 samples at 100 ng/cm<sup>2</sup>. OPPTS Series 875, Occupational and Residential Exposure Test Guidelines, Group C: Quality Assurance/Quality Control require fortification levels at the method LOQ, an intermediate concentration level (e.g., 10x LOQ), the maximum concentration of the validation range (e.g., 100-1000x LOQ), and blank or control substrate. It is uncertain whether this range of concentrations truly reflects the entire working range of the method.
- No discussion of the environmental fate or chemical properties of the analyte, pendimethalin, was included in the study report. In addition, the study did not address whether the analytical methodology could be expected to be specific to the parent compound, or whether it would also detect and quantify breakdown products or metabolites one might expect to find in a DFR field study.
- Only analytical grade pendimethalin of known purity was used in this study. Since formulated products are generally tested in field DFR studies, fortifications with formulated products should be included. The validation study also failed to address whether the method might be subject to any interferences, for example by breakdown products or ingredients expected to be found in formulated products.
- No information was provided concerning the storage stability of the tested analyte. The validation study indicates that unfortified "field samples," essentially detergent solutions, were prepared in August, stored frozen until mid-November, thawed, and then fortified 1 to 2 days prior to analysis by GC. From the data presented here, prospective users of this method would not necessarily know whether real field samples containing pendimethalin DFR require storage

at specific temperatures, how long they might be stored without deterioration of the samples, whether pH of the solutions might require adjustment, etc. The study report does not provide sufficient information to address such concerns.

- There is no information presented in this study defining what sensitivity and range such a method might be required to have to be useful for the purposes intended (e.g. worker exposure estimates).

EPA's guidelines (see OPPTS Series 875, Occupational and Residential Exposure Test Guidelines, Group C: Quality Assurance/ Quality Control) strongly recommend that analytical methods be developed with an eye to the Limit of Quantitation (LOQ) required, which depends on the toxicological endpoint of interest. EPA states: "At a very minimum, the LOQ must be sufficient to assess exposures below the No Observable Effect Level (NOEL) based on the toxicological endpoint or its equivalent mg/kg for the appropriate dosing frequency [e.g. acute, subchronic, chronic], which may be daily." EPA further suggests use of transfer coefficients and study design factors to define a useful target LOQ for the DFR analytical methodology. Since this foundation was not laid, the reviewers could not be sure whether the "validated" methods considered here are adequate for real field use.

#### **Authors' Attestations**

1. Method and format requirements were stated to be in accordance with PR Notice 86-5.
2. The authors stated that the study was conducted in accordance with published Good Laboratory Practices (GLP) for tests of substances regulated under FIFRA (40 CFR 160). The authors claimed that there were "no deviations that affect the quality or integrity of the study or the interpretation of this report."