(9/29/93)

MEMORANDUM

Subject: PP# 9F3743 - **CLETHODIM (SELECT®)** IN/ON SOYBEANS, COTTONSEED, AND ANIMAL COMMODITIES.
Evaluation of the Revised Compound Specific Residue Analytical Method, EPA-RM-26D-2, and the New Supporting Independent Laboratory Validation Data.
(No MRID #) [CBTS # 12468] {DP Barcode D194694}

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EXECUTIVE SUMMARY OF CHEMISTRY DEFICIENCIES

- None -

BACKGROUND

In the CBTS review of July 30, 1991, we recommended for total clethodim tolerances with an expiration date not to exceed one year on cottonseed and soybeans, and animal commodities contingent on the petitioner agreeing to do the following:

1) rewrite the clethodim compound specific method, RM-26D-1, per ACB (Analytical Chemistry Branch) recommendations, and

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2) provide additional method validation data for the rewritten method for the same clethodim metabolites at the same fortification levels in the same matrices as were used in the EPA Petition Method Validation (PMV).

In a letter dated July 29, 1993, and signed by E.D. Bruce, Valent requested a meeting with CBTS reviewers and ACB chemists to update the Agency on the status of the method rewrite, and problems encountered in the generation of the new ILV (independent laboratory validation) data. CBTS and ACB agreed to meet with the representatives of Valent on September 22, 1993. The petitioner submitted a data package consisting of a cover letter dated August 30, 1993, and signed by E.D. Bruce; and a supplementary Section D (a revised residue analytical method, EPA-RM-26D-2, dated February 1, 1993; and new ILV data from Colorado Analytical Research and Development Company for the rewritten method).

The deficiencies or contingencies noted in our July 30, 1991, review will be repeated in the body of this review as they appeared in our original review, followed by the petitioner's response, then CBTS comments. Our conclusions and recommendation follow.

CONCLUSIONS

CBTS Conclusions on **Residue Analytical Methods**

1. CBTS concludes that the petitioner has revised and rewritten the compound specific method as ACB has suggested and has included additional modifications from current method development. Deficiency 1 is resolved. Valent's compound specific method, EPA-RM-26D-2, is now suitable to enforce the total clethodim tolerance in crops and animal tissues. It will be forwarded to FDA's Technical Editing Group for publication in PAM-II.

2. CBTS concludes the petitioner has conducted an adequate ILV for the revised/rewritten compound specific method, EPA-RM26D-2. Deficiency 2 is resolved. The method has been shown to be suitable to be a quantitative procedure to enforce the total clethodim tolerances in crops and animal tissues. The ILV confirmed that the method is a qualitative confirmatory method for total clethodim tolerances in milk.

3. The compound specific method, EPA-RM-26D-2, is not quantitative for milk and is not suitable for enforcing the total clethodim tolerance in milk. The common moiety method, RM-26B-2, is quantitative for milk and is the enforcement method for milk.

RECOMMENDATION

There being no further product or residue chemistry deficiencies and TOX considerations permitting CBTS recommends for permanent total clethodim tolerances in cottonseed at 1 ppm, in soybeans at 10 ppm,

0.2 ppm in meat, fat, and meat byproducts of cattle, goats, hogs horses, poultry, sheep, and eggs, 0.05 ppm in milk, 15 ppm in soybean soapstock (oil), and 2 ppm in cottonseed meal.

CBTS recommends that revised residue analytical method, EPA-RM-26D-2 (Valent's compound specific method), serve as the primary tolerance enforcement procedure for cottonseed, soybeans, and animal tissues. Confirmation of total clethodim residues in cottonseed, soybeans, and animal tissues is to be with the common moiety method, RM-26B-2. To enforce the total clethodim tolerance in milk CBTS recommends use of the common moiety method, RM-26B-2. Confirmation of total clethodim residues in milk should be with method EPA-RM-26D-2.

DETAILED CONSIDERATIONS

DEFICIENCIES/CONTINGENCIES

1. Rewrite the clethodim compound specific method, RM-26D-1, per ACB recommendations, and

2. Provide additional method validation data for the rewritten method for the same clethodim metabolites at the same fortification levels in the same matrices as were used in the EPA Petition Method Validation.

PETITIONER'S RESPONSE (No MRID #)

The petitioner has provided a rewritten method titled "Confirmatory Method for the Determination of Clethodim and Clethodim Metabolites in Crops, Animal Tissues, Milk, and Eggs Method: EPA-RM-26D-2." The date is October 16, 1992 with the revision date of February 1, 1993. No authors are listed.

The petitioner has provided new ILV data for the revised/ rewritten compound specific method in a study titled "VP-10256-Independent Method Validation: Method EPA-RM-26D-2, Confirmatory Method for the Determination of Clethodim and Clethodim Metabolites in Crops, Animal Tissues, Milk, and Eggs." The study date is August 12, 1993 and the laboratory project code is Valent - 1177. No authors are listed.

CBTS COMMENTS

The petitioner has presented a revised/rewritten compound specific residue analytical method, EPA-RM-26D-2. In summary, a 50 gram sample is extracted with 150-200 mls of methanol/water (1/4, v/v), filtered through Whatman # 42 paper, and precipitated with 2 grams of calcium hydroxide. The solution is partitioned 3 X 100 mls CH_2Cl_2, dried through a bed of anh. Na_2SO_4, and rotary evaporated to just dry. The derivatized solution is base washed with 10 mls of 0.1N NaOH, then methylated with CH_2N_2 with silica gel catalysis before it is oxidized with 2 mls of a 10% solution of m-chloroperbenzoic acid. The solution is quenched with 10% sodium thiosulfate, washed with sat. sodium

bicarbonate solution, dried through anh. sodium sulfate, and concentrated with a rotary evaporator. Clean-up is through a 10 gram silica-gel column with the clethodim metabolites eluted off in 200 mls of acetone:methylene chloride (3/7, v/v). Determination is by HPLC using a Hewlett-Packard, model 1090 HPLC, equipped with a Hypersil ODS, 3 um, 15 cm X 4.6 mm column. The mobile phase is a water-ACN gradient at a flow rate of 1 ml per min. The detector is UV at 266 mn with 254 mn as the alternate wavelength. The petitioner's data shows the limit of quantitation (LOQ) is 0.05 ppm for crops and tissues, and 0.02 ppm for milk. Quantification is by external standards. The minimum detection limit (MDL) is 0.03 ppm for crops and 0.01 ppm for milk.

ACB's recommendations for major modifications include at the oxidation step increase the amount of oxidizing agent by using 2 mls of 10% m-chloroperbenzoic acid followed by a quench with 30 mls of 10% sodium thiosulfate. This step has been added to Valent's method. ACB recommended use of a 10 gram silica gel clean-up column with elution of the clethodim metabolites with 200 mls of 3/7 acetone:methylene chloride instead of a silica Sep-Pak cartridge. This step has been added to the method. The base wash step has now been added for all matrices.

As part of the minor modifications ACB recommended the rinsing of the Na_2SO_4 after the base wash with 15 mls of CH_2Cl_2. This step has been added to the method. The petitioner also revised the method at this point to proceed to the oxidation step without further concentration per ACB's recommendation. ACB recommended use of a glass fiber filter aid to be certain the alkaline precipitation step is completed within 10 minutes. The petitioner has added this step to the procedure to recover clethodim metabolites from animal tissues, milk, and eggs. The primary wavelength for the UV determination is now 266 nm per ACB's recommendation. The alternate wavelength is 254 nm. The use of cloproxidim sulfoxide as an internal standard has been deleted. The 25 grams of NaCl is added to the round bottom flask before the partition step, thus the problem of a clogged stopcock as noted by ACB is avoided.

As part of this method's continuing evolution the petitioner made several other helpful modifications. The amount of silica gel used for the methylation catalysis is increased to 40-50 mgs from the proposed 5-50 mgs. CBTS agrees with this change. Storage of any methylated and oxidized samples is to be in acetone, not the HPLC mobile phase. CBTS agrees with this minor modification. In the February 1, 1993, revision the petitioner recommends use of sonication for all reconstitution steps. CBTS agrees with this minor modification. CBTS has no objections to the suggestions to optimize the HPLC measurements.

The petitioner has revised and rewritten the compound specific method as ACB has suggested and has included additional modifications from current method development. Deficiency 1 is resolved. Valent's compound specific method, EPA-RM-26D-2, is now suitable to enforce the total clethodim tolerance in crops and animal tissues. It will be forwarded to FDA's Technical Editing Group for publication in PAM-II.

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The petitioner had an additional ILV performed on the revised and rewritten procedure, EPA-RM-26D-2. The analytical work was performed at Colorado Analytical Research and Development Corporation (CARDC) using the method reviewed above. The petitioner supplied CARDC's raw data used for validating the procedure, the standard curves used for quantitating the results, and copies of HPLC chromatograms. An adequate amount of supporting chromatographic data and the raw data have been presented for this ILV.

Liver samples were spiked at 0.2 ppm with clethodim sulfoxide (CSO) and CSO plus sethoxydim sulfoxide (SSO). The control sample had "interference" at the $\leq$ 0.08 ppm for both compounds. For CSO alone the recovery was 92% and from the spike with both compounds the recovery was 86% for CSO and 83% for SSO. There was complete chromatographic separation between CSO and SSO in liver at the 0.02 ppm level.

Soybean samples were fortified with CSO and 5-hydroxy clethodim sulfoxide (5OH-CSO) at 0.05 ppm, 1 ppm, and 5 ppm. The control soybean sample showed an "interference" at 0.025 ppm. CSO recoveries ranged from 93% to 105% and 5OH-CSO recoveries ranged from 104% to 114%. At the 5 ppm level additional recoveries were performed with the addition of SSO and 5-hydroxy sethoxydim sulfoxide (5OH-SSO) alone and in combination. Recovery of CSO at 5 ppm in the presence of 5OH-CSO, SSO, and/or 5OH-SSO ranged from 69 to 85%. Recovery of 5OH-CSO in the presence of CSO, SSO and/or 5OH-SSO ranged from 84 to 99%. SSO recoveries in the presence of CSO and 5OH-CSO ranged from 85 to 99%. Recovery of 5OH-SSO in the presence of CSO and 5OH-CSO ranged from 87 to 112%. The petitioner presented supporting chromatographic data showing complete separation of all 4 compounds in soybeans.

Milk samples were fortified with CSO, SSO and s-methyl clethodim sulfoxide (S-MeCSO) at 0.02 ppm and 0.05 ppm. CSO recoveries ranged from 28 to 46%. SSO recoveries were 32-34% and the S-MeCSO recovery was 13%. The petitioner reported a positive interference in the control milk sample at 0.03 ppm for the 0.05 ppm spike of S-MeCSO. Background in the control milk was <0.01 ppm for CSO and SSO.

CBTS concludes the petitioner has conducted an adequate ILV for the revised/rewritten compound specific method, EPA-RM26D-2. Deficiency 2 is resolved. The method has been shown to be suitable to be a quantitative procedure to enforce the total clethodim tolerances in crops and animal tissues. Because of the wide range of recoveries among the petitioner's recovery data, EPA's petition method validation data, and the ILV data from CARDC, we conclude that the method is a qualitative confirmatory method for total clethodim tolerances in milk.

During the EPA-Valent meeting on September 22, 1993, the petitioner volunteered that a second ILV was being conducted at Ricerca, Inc. for the clethodim-sethoxydim metabolites in milk. Preliminary data from Ricerca confirm that the compound specific method is qualitative, not quantitative for CSO and S-MeCSO in milk. We agreed in the meeting the petitioner needs to submit these data when the ILV is complete, whether it is submitted in this petition, or in the new

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petition for alfalfa and onions/garlic. The second ILV data from Ricerca will not affect our decision that EPA-RM-26D-2 is a qualitative method for CSO and S-MeCSO in milk.

cc:R.F.,Circ.,Reviewer(FDG),PP#9F3743.

H-7509C:CBTS:Reviewer(FDG):CM#2:Rm804Q:305-5836:FDG:9/28/93:edit:fdg:9/29/93.

RDI:SecHd:RSQuick:9/28/93:BrSrSci:RALoranger:9/28/93.