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

EXPEDITE

APR 14 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#7F3476/FAP#7H5524. Rally™ (Myclobutanil).

Evaluation of the COB Report on the Petition Method Validation of Procedure TR 310-84-27, as amended by Addendums TR 31H-86-15 and 31S-87-46, for Determining Residues of Myclobutanil (RH-3866) and Its Alcohol Metabolite, RH-9090, in Apples.

RCB No.: None. MRID No.: N/A.

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mjn

THRU: Charles L. Trichilo, Ph.D., Chief
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BACKGROUND

On July 1, 1987, Residue Chemistry Branch (RCB) requested the Chemical Operations Branch (COB), Benefits and Use Division (BUD), conduct a method trial (petition method validation (PMV)) for determining residues of myclobutanil (RH-3866) and its alcohol metabolite, RH-9090, in apples or grapes. (Memo of R. Loranger, PP#7G3479.)

The analytical procedure to be validated was Rohm and Haas Company's, "RH-3866 Total Residue Analytical Method for Apple and Grape", by C. K. Brackett, et al., Technical Report No. TR 310-84-27, 11/16/84, as amended by Technical Report No. 31H-86-15, "Addendum to RH-3866 Total Residue Analytical Method for Grape and Apple (TR 310-84-27)", by S. S. Stavinski, et al., 7/8/86, and as further amended by Lab Memo No. 31S-87-46, "Addendum 2 to RH-3866 Total Residue Analytical Method for Grape and Apple (TR 310-84-27)", by R. O. Deakyne, et al., 8/3/87.

4

The PMV was for the recoverability of RH-3866 and free RH-9090 following fortification of apples or grapes at levels of 0, 0.5, and 1.0 ppm. COB chose to work with apples.

Myclobutanil is a new chemical fungicide, and has not previously been tested by PMV in EPA laboratories. The PMV requested was in conjunction with pending petitions (PP#7G3479; PP#7F3476/FAP# 7H5524) proposing tolerances on apples, grapes, their byproducts, meat, milk, and eggs.

In March 1988, RCB received the results of PMV of TR 310-84-27/ TR 31H-86-15/31S-87-46 from BUD. (Memo of Elmer H. Hayes and Everett Greer, Analytical Chemistry Section, COB, BUD, 3/14/88.) Those results, and their significance, are the subject of this memorandum.

METHOD SUMMARY

Apples are soxhlet-extracted with 0.5N HCl/MeOH. Extraction converts RH-9090 conjugates to RH-9090 and any RH-9089 residues are converted to RH-9090 by sodium borohydride reduction. The extract is washed with petroleum ether and partitioned into methylene chloride. Samples are then cleaned-up by Chelex 100-Fe^{+++} affinity chromatography followed by methylene chloride partitioning. Further clean-up is provided by Florisil chromatography. RH-3866 is determined by packed column chromatography and NP-GLC detection. Residues of RH-9090 are analyzed on an EC-GLC equipped with a capillary inlet system.

RESULTS

The following recoveries of myclobutanil (RH-3866) and its alcohol metabolite (RH-9090) were reported:

Commodity	Chemical Added	PPM Added	PPM Found	% Recovery
Apples	Control	0	ND, ND[1]	--, --
	RH-3866	0.504	0.56, 0.54	111, 107
		1.01	1.02, 1.08	101, 107
	RH-9090	0.57	0.44, 0.47	77, 82
		1.05	0.83, 0.77	79, 73

[1] Less than the sensitivity of the method, which was estimated to be 0.1 ppm for RH-3866 and 0.2 ppm for RH-9090 under the instrument operating conditions used for this PMV.

COB'S COMMENTS

- In order to chromatograph RH-9090 on a new DB-17 Megabore column, it was necessary to inject apple control samples for approximately 24 hours. After this conditioning, RH-9090 could be detected, but peak shape was poor due to peak tailing.

- A set of samples can be analyzed by one person in three days provided the GLC is equipped with an auto sampler.

- Due to length of time needed to complete this analysis and the need to condition the GLC column with sample matrix to detect RH-9090, it is our opinion that this method is not suitable for regulatory purposes.

RCB's COMMENTS

On 4/13/88, the RCB reviewer (M. Nelson) discussed this PMV write-up with COB analyst E. Greer.

With regard to conditioning the DB-17 Megabore column, RCB was told COB has no way of knowing if this was necessary because the column being used was new; was faulty; or if considerably less than "approximately 24 hours" [actually, the column was conditioned overnight, ca 18 hours] conditioning would have sufficed.

COB did not attempt to recover RH-9090 from any column other than the "new DB-17 Megabore". Column conditioning adequately resolved the problem. Satisfactory recoveries of RH-9090 were obtained, though peak tailing was observed. COB was able to minimize this using a slow chart speed.

The method, as written, makes no provision for conditioning the DB-17 Megabore column. The petitioner could correct this by a minor rewrite. COB concedes this would be a viable approach, provided the time factor to incorporate this procedure is acceptable from a regulatory standpoint.

It took COB approximately 24 man-hours to complete a set of samples. This included an 8-hour daytime soxhlet extraction step (in lieu of a 16-hour overnight one, as in the method write-up). As we understand it, it also included time spent in calculating/evaluating results.

This 24-hour timeframe falls within the upper limit of acceptability, as stated in the Subdivision O (Residue Chemistry) Guidelines. The Guidelines also permit acceptance, on a case-by-case basis, of regulatory methods which may exceed this timeframe (which analysis of RH-9090 might, with inclusion of a column conditioning step), if "minor metabolites or residues that are not acutely toxic" are involved. RH-9090 qualifies on both counts.

RCB'S CONCLUSIONS

1. Satisfactory recoveries of RH-3866 and RH-9090 were obtained by this PMV.

2. For recovery of RH-9090, COB found it necessary to condition the column (a new DB-17 Megabore) overnight with apple control matrix.

3. COB modified the procedure by using an 8-hour daytime soxhlet extraction, in lieu of the 16-hour (overnight) extraction in the method, as written.

4. The procedure is long, but can be accepted.

5. The petitioner needs to rewrite the method to include the following two statements (appropriately worded):

   a. If difficulties are encountered in recovering RH-9090, the column may require conditioning with apple control matrix.

   b. An 8-hour (or less, if the petitioner knows the minimum time necessary) soxhlet extraction may be used in lieu of a 16-hour (overnight) one, if this is more convenient to the analyst.

6. Provided the revisions described in Conclusion 5 are incorporated into this analytical procedure, RCB can find the PMV run by COB to be acceptable to permit the forwarding of this analytical method to FDA for inclusion in a future updating of the Pesticide Analytical Manual, Volume II.

* * *

RECOMMENDATION

The PM should notify the petitioner of our Conclusion 5.

NOTE TO PM:

The results of PMV testing by COB with beef liver and milk for recoverability of myclobutanil is the subject of a separate RCB memorandum (dated 4/12/88).

That PMV involved testing of the petitioner's analytical procedure TR 310-84-13, as amended by TR 310-86-09.

COB has also found that analytical procedure to be unacceptable for enforcement purposes in its present form.

Two additional analytical procedures [TR 31S-87-02 for the diol metabolite in milk, and TR 31S-87-09 for the RH-9090 (free) metabolite in animal commodities] have just commenced PMV testing by COB. Their projected completion date is June.

cc: Reading File
Circulation
Reviewer (Nelson)
PP#7F3476/FAP#7H5524
PP#7G3479
Myclobutanil Registration Standard File
PMV File
H. Jacoby (SPMS)
D. Marlow (BUD)
ISB/PMSD (Eldredge)

TS-769C:RCB:Reviewer(MJN):CM#2:Rm804:557-7324:typist(mjn):4/14/88.

RDI:SectionHead:RSQuick:4/14/88:DeputyChief:RDSchmitt:(by KHArne):
4/14/88.

8