



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

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EXPEDITE

APR 12 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 310-84-13, as amended by
Addendum TR 310-86-09, for Determining Residues of
Myclobutanil Per Se in Beef Liver and Milk.

RCB No.: None.

MRID No.: N/A.

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Residue Chemistry Branch
Hazard Evaluation Division (TS-769C)

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 only, in beef liver and milk. (Memo of R. Loranger, PP#7G3479.)

The analytical procedure to be validated was Rohm and Haas Company's, "Analytical Method for the Measure of RH-3866 (myclobutanil) and RH-9090 Residues in Various Crops, Soil, Meat, Milk, and Eggs", by C. K. Brackett, Technical Report No. TR 310-84-13, 6/18/84, as amended by Technical Report No. 310-86-09, "Addendum to Technical Report No. 310-84-13, Analytical Method for RH-3866 and RH-9090", by C. K. Brackett, et al., 2/12/86.

The PMV was for the recoverability of myclobutanil, following fortification to beef liver (at levels of 0, 0.2, and 0.4 ppm) and milk (at levels of 0, 0.02, and 0.04 ppm).

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 310-84-13/ TR 310-86-09 from BUD. (Memo of Ronald P. Jackson, Analytical Chemistry Section, COB, BUD, 3/14/88.) Those results, and their significance, are the subject of this memorandum.

METHOD SUMMARY

A 10 gram sample is homogenized in methanol and filtered. The filtrate is combined with 2% aqueous sodium chloride and the resulting solution is washed with hexane. The hexane layer is discarded and additional 2% aqueous sodium chloride is introduced to obtain a 50:50, v/v, water to methanol ratio. The aqueous layer is extracted with methylene chloride and the organic layer is evaporated to dryness under diminished pressure. The remaining residue is dissolved in toluene for Florisil column chromatography. Myclobutanil is eluted from the Florisil column by 1% methanol/toluene (v/v). The eluant is evaporated to dryness under diminished pressure and the residue is dissolved in 3% methanol/toluene, v/v, for quantitation via electron-capture gas-liquid chromatography (EC-GLC) versus a known standard concentration of RH-3866.

Instrument and Conditions Used - RH-3866

GC:	Tracor 540 with Ni ECD
Column:	Coiled glass; 6 ft x 2 mm I.D.
Packing:	2% OV-17/1% OV-210 on 100/120 mesh Supelcoport
Carrier Gas:	P-10 (10% methane/argon)
Flow Rate:	40 ml/min.
Injector Temp.:	230°C
Oven Temp.:	220°C
ECD Temp.:	350°C

RESULTS

COB reported that unacceptable recoveries from spiked liver and milk samples were initially obtained, which resulted in a review of the entire procedure.

COB decided to repeat the PMV, collecting the 1% methanol/toluene eluant from the Florisil column (as the analytical procedure specified), and to modify the procedure by using and collecting a 3% methanol/toluene eluant as well.

Specifically, the modified analytical procedure entailed elution with 60 ml of 1% methanol/toluene and collection of that fraction, followed by elution with 50 ml of 3% methanol/toluene and separate collection of that fraction.

The 1% methanol/toluene eluant fraction and the 3% methanol/toluene eluant fraction were then analyzed separately via EC-GLC for recovery of myclobutanil residue.

This was done to confirm low recoveries using 1% methanol/toluene, and to substantiate the reason for low recoveries.

The following recoveries of myclobutanil were obtained:

Sample Matrix	Spike Level (ppm)	% Recovery From:		Total Recovery ¹
		1% MeOH/Toluene	3% MeOH/Toluene	
Beef	0.2	30, 35	75, 70	105, 105
Liver	0.4	13, 38	-- --2	-- --2
	0.0	NDR ³	NDR ³	
Milk	0.02	NDR ⁴	85, 80	85, 80
	0.04	13, 8	90, 98	103, 106
	0.0	NDR ⁴	NDR ⁴	

¹ Combined recovery from 1% plus 3% MeOH/toluene fractions.

² Not attempted by COB during PMV.

³ Not detected at or above 0.005 ppm, the limit of detection.

⁴ Not detected at or above 0.003 ppm, the limit of detection.

COB's COMMENTS

- The analytical method as written is unacceptable because of low recoveries when using a 1% methanol/toluene solvent mixture. It is recommended that Rohm and Haas submit new recovery data using a 3% methanol/toluene Florisil column elution solvent for all RCB requested spike levels in both liver and milk.
- A set of four samples requires 4-6 hours for analysis.
- No special safety precautions were required.
- The limit of detection for confirmatory analysis using a nitrogen-phosphorus detector was 0.2 ppm.

RCB's CONCLUSIONS

1. Rohm and Haas analytical procedure TR 310-84-13 (with the Addendum, TR 310-86-09) has been subjected to PMV by COB for its suitability as an enforcement method for determining residues of myclobutanil per se in animal commodities (meat, milk, eggs).

The PMV was conducted with beef liver and milk at fortification levels of 0, 0.2, 0.4 and 0, 0.02, 0.04 ppm, respectively. Low recoveries of myclobutanil resulted from use of this analytical procedure, as written.

2. RCB concurs with COB's assessment that analytical procedure TR 310-84-13 (with Addendum, TR 310-86-09), as presently written, is unacceptable for enforcement or monitoring purposes for myclobutanil residues in animal commodities, owing to the low recoveries encountered.

COB has demonstrated that low recoveries arise from using 1% methanol/toluene to elute the myclobutanil residue from the Florisil column.

3. The petitioner will need to modify this analytical procedure in such a manner that acceptable recovery data for myclobutanil are obtained from milk, eggs, and animal tissues.

We suggest use of a 3% methanol/toluene eluant from the Florisil column as a viable exploratory approach, since COB obtained satisfactory recoveries of myclobutanil from beef liver and milk by this means.

We also suggest the petitioner investigate whether the procedure for activating the Florisil (e.g., moisture content) is adequately standardized to yield reproducible results in actual use.

4. The petitioner will need to submit a rewritten analytical procedure for determining myclobutanil residues in animal commodities, incorporating any modification(s) made to improve recoveries, etc., and also incorporating Addendum TR 310-86-09 into the body of the revised procedure.
5. The petitioner will also need to submit supporting validation data for the revised analytical procedure.

The validation data should consist of recovery data from milk, egg, and animal tissue matrices at several levels of fortification.

Fortification levels chosen should be suitable to support the existing residue data base and the tolerance levels under consideration in PP#7F3476/FAP#7H5524.

Values for controls should also be submitted, and limits of detection (sensitivity) determined. Supporting chromatograms and standard curves should also be provided.

Additionally, the petitioner should demonstrate the reproducibility and reliability of the analytical procedure to yield adequate recoveries on a regular basis (i.e., ruggedness testing).

6. The revised analytical procedure for determining residues of myclobutanil per se in animal commodities may be subject to additional PMV by COB.
7. In future, in developing analytical methods which may be used for enforcement purposes, RCB suggests the petitioner have them subjected to a second lab validation prior to submitting them to the Agency. This requirement is the subject of an Agency PR Notice, currently under development.

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RECOMMENDATION

The PM should notify the petitioner of RCB's comments #1-7, as given above.

NOTE TO PM:

The results of PMV testing by COB with apples for recoverability of myclobutanil and its RH-9090 (free) metabolite will be the subject of a separate RCB memorandum.

That PMV involved testing of the petitioner's analytical procedure TR 310-84-27, as amended by TR 31H-86-15 and Lab Memo 31S-87-46.

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 yet to undergo PMV testing by COB.

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):4/11/88.

RDI:SectionHead:RSQuick:4/12/88:DeputyChief:RDSchmitt:4/12/88.