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

JUL 12 1993

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM:

SUBJECT: PP#9F3724/9H05575 - Petition Method Validation - New Chemical - Tebuconazole, Fungicide on Peanuts; Results of Plant and Animal Petition Method Validation. CBTS No. 12020. DP Barcode D192254.

FROM: Gary F. Otakie, P.E., Chemist
Tolerance Petition Section II
Chemistry Branch I - Tolerance Support
Health Effects Division (H7509C)

Gary F. Otakie

THRU: Debra F. Edwards, Ph.D., Chief
Chemistry Branch I - Tolerance Support
Health Effects Division (H7509C)

Debra Edwards

TO: Clarence O. Lewis, Acting PM 21
Fungicide - Herbicide Branch
Registration Division (H7505C)

Background

In CBTS's previous review of PP#9F3724 (see G. Otakie review of February 16, 1993) nine Deficiencies remained for the proposed permanent tolerances for tebuconazole in/on peanuts and tebuconazole and its hydroxy metabolite HWG 2061 in/on animal commodities. The current submission responds to deficiencies requiring a successful EPA PMV for peanuts and animal commodities.

Current Submission

EPA Petition Method Validation of the proposed analytical methods for tebuconazole on plants and tebuconazole and the HWG 2061 metabolite have been completed. Attached is a copy of the June 10, 1993 review of E. Hayes ACL/BEAD summarizing the results of plant and animal petition method validations for tebuconazole. The following are the Review Comments:

Peanuts:

ACL was not able to seperate peaks using the suggested DB-5 column and switched to the DB-17 column recommended for liver and milk. This alternate column choice should be noted in the method. The limit of detection from visual inspection of the chromatograms is estimated to be 0.01 ppm for tebuconazole.

Milk and Liver:

While this procedure is clearly written, it is extremely tedious and long. The analysis steps are (1) methanol/water extraction, (2) methanol/hexane/water extraction, (3) hexane/acetonitrile partitioning, (4) acid refluxing for 16 hours, (5) pH=5/acetone/chloroform partitioning, (6) gel permeation chromatography, 7) hexane/acetonitrile partitioning, (8) Mega Bond Elut chromatography, (9) semi-preparative HPLC, (10) MTBSTFA derivatization (90 minutes) and (11) GLC determination. The procedure is extremely time consuming and quantitative loss could occur from the excessive handling of the sample extract.

It is ACL's opinion that the analysis of milk and liver could possibly be shortened by using only GPC and/or HPLC after acid hydrolysis, acetone/chloroform partitioning and selecting an appropriate capillary column in conjunction with the specific NP detector. The length of time for analysis and solvent consumption make the method inappropriate for an enforcement laboratory. The limit of detection from visual inspection of the chromatogram is estimated to be 0.01 ppm for tebuconazole and HWG 2061 in/on liver and milk. Even though recovery data would normally be considered acceptable, it is ACL's opinion that this method does not meet the 40 CFR 158 and EPA's requirement as published in the Pesticide Assessment Guidelines, subdivision "O" for Residue chemistry, Part 171-4(b) as an enforcement method because of total time (laboratory time required for liver or milk was 64 hours) necessary to complete this procedure. This method would have little practical utility in the enforcement laboratory.

Conclusions

1. The EPA PMV for tebuconazole on peanuts has been successfully completed with certain modifications to the proposed analytical method. The limit of detection for tebuconazole in/on peanuts is 0.01 ppm. A revised copy of the analytical method noting the use of the DB-17 column as an alternate column choice is required.

2. The EPA PMV for tebuconazole and its hydroxy metabolite HWG 2061 in animal commodities has indicated that the proposed analytical methodology is unacceptable as an enforcement method. Although acceptable recoveries were obtained the method is too tedious and long (i.e. requiring 64 hours for liver or milk) for approval as an enforcement method. Quantitative analyte loss could result from the excessive handling of the sample extract. Although certain suggestions are included in the attached ACL review for shortening the method, additional modifications may also be required for approval as an enforcement method. A new simplified/effective analytical enforcement method for detecting tebuconazole and its hydroxy metabolite HWG 2061 in animal commodities together with Independent Method Validation and resubmission of the revised analytical methodology for a second EPA PMV are required. Adequate bridging data for the revised analytical method, the original method and the methodology used on C14 residues in the animal metabolism studies are also required.

Recommendations

At this time CBTS recommends against establishing the proposed permanent tolerances for tebuconazole on peanuts and tebuconazole and its t-butyl hydroxy metabolite on animal commodities for the reasons cited in the above conclusions. In addition, other deficiencies concerning product chemistry data, residue chemistry data, and revised Sections B and F as outlined in our 2/16/93 review need to be resolved.

Attachment 1 - June 10, 1993 EPA PMV Review of E. Hayes, ACS/BEAD.

cc: Reviewer-Otakie, RF, Circu, PP#9F3724, E. Hayes (ACS/BEAD), H. Hundley (ACS/BEAD), E. Haeberer.

RDI: EHaeberer:7/1/93 RLoranger:7/7/93

ATTACHMENT 1

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

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

JUN 10 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#9F3724/9F03818. Permanent Tolerance Petition-New Chemical-Tebuconazole, Fungicide on Peanuts; Plant and Animal Petition Method Validation Request

FROM: Elmer H. Hayes, Chemist
Douglas Swineford, Chemist
Analytical Chemistry Section

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
Health Effects Division

The Analytical Chemistry Laboratory was requested by the Chemistry Branch-I, Tolerance Support Health Effects Division to conduct method validation on the new chemical fungicide Tebuconazole(alpha-[2-(4_chlorophenyl)ethyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol) and its metabolite(1-(4-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazole-1-yl-methyl)-pentane-3,5-diol(HWG 2061). Petition Method Validation was requested for Tebuconazole in/on peanut nutmeat as well as Tebuconazole and its metabolite in/on milk and liver. The levels of fortification requested by HED were 0.1 and 0.2 ppm Tebuconazole on/in peanut nutmeat; 0.05 and 0.10 ppm Tebuconazole,0.05 and 0.10 ppm HWG 2061 on/in milk and 0.10 and 0.20 ppm Tebuconazole, 0.10 and 0.20 ppm HWG 2061 on/in liver. The method trial for peanuts followed the method, "Gas Chromatographic Method for the Determination of Residues of Tebuconazole in Crops, Processed Products, Soil and Water" and, "An Analytical Residue Method for the Determination of Tebuconazole and HWG 2061 Residues in Bovine and Poultry Tissue, Milk, and Eggs" for liver and milk.

METHOD SUMMARY

Peanuts:

Peanut nutmeat was fortified with the parent compound in a blender containing 3:1 acetone/water, blended for three minutes,washed several times before being partitioned with dichloromethane. The organic solvent was evaporated to dryness, redissolved in cyclohexane/ethyl acetate and injected onto a Gel Permeation Chromatograph. The sample fraction collected from the GPC that contained the compounds of interest was evaporated to dryness, brought up in toluene and cleaned up using silica gel column chromatography. After collecting the sample eluate from the silica gel column it was further cleaned up using C18 Bond Elut SPE, evaporated to dryness and brought up to volume in ethyl acetate. The sample was subsequently analyzed by a GLC/NP using a Supelco DB-17 megabore column.

Milk and Liver:

Milk or Liver was fortified with combined standards of Tebuconazole and HWG 2061 and blended with 150 mls of methanol for two minutes. The homogenate was washed several times before being partitioned using hexane saturated acetonitrile and acetonitrile saturated hexane. The acetonitrile layer was saved and evaporated until only water remained. Acid was added to the sample and refluxed for 16 hours. The sample was allowed to cool, buffered, and adjusted to pH=5 with either HCL or NaOH. The sample was then partitioned twice using acetone/chloroform solution before the lower organic layer was collected and evaporated to dryness.

The liver residue was solubilized in the flask using methanol/chloroform and transferred to a test tube, before being concentrated to approximately 8 mls using an N-VAP. A 5ml sample aliquot was injected into the GPC and the compounds of interest collected in the appropriate fraction. The collected fraction was evaporated to dryness. The liver or milk residue was redissolved in hexane and partitioned using hexane saturated acetonitrile or acetonitrile saturated hexane, then the acetonitrile layer was evaporated to dryness and redissolved in methanol for cleanup using Bond Elut SPE. After SPE cleanup the residue was further cleaned up using semi-preparative HPLC cleanup. During HPLC cleanup and fraction collection, Tebuconazole and HWG 2061 were collected separately. The collected fraction of Tebuconazole was evaporated to dryness and brought to volume with ethyl acetate before being injected into the GLC. The collected fraction of HWG 2061 was also brought to dryness, but derivatization was extended to 90 minutes before being injected into the GLC. All samples were analyzed by GLC using NP detection employing a 30 meter DB-17 megabore column. The injection temperature was 280°C, detector

temperatures. 210 C and 240 C was ramped up to 280 C at 5 C/min. depending on the compound being analyzed. The higher starting temperature (240 C) was used to shorten the retention time for the analysis of the Tebuconazole metabolite(HWG 2061).

COMMENTS

Peanuts:

ACL was not able to separate interfering peaks using the suggested DB-5 column and switched to the DB-17 column recommended for liver and milk. This alternate column choice should be noted in the method. The limit of detection from visual inspection of the chromatograms is estimated to be 0.01 ppm for Tebuconazole.

The peanut procedure was simple, precise and fell within the time frame and guidelines of 40 CFR 158 and EPA's requirement as published in the Pesticide Assessment Guidelines, Subdivision "O" for residue chemistry, part 171-4(b) as an enforcement method (laboratory time reguired to do six samples of peanut-meat was 24 hours).

Milk and Liver:

While this procedure is clearly written, it is extremely tedious and long. The analysis steps are 1) methanol/water extraction, (2) methanol/hexane/water extraction,(3) hexane/acetonitrile partitioning,(4) acid refluxing for 16 hours,(5) pH=5/acetone/chloroform partitioning,(6) gel permeation chromatography,(7) hexane/acetonitrile partitioning,(8) Mega Bond Elut chromatography,(9) semi-preparative HPLC,(10) MTBSTFA derivatization (90 minutes) and (11) GLC determination. The procedure is extremely time consuming and quantitative loss could result from the excessive handling of the sample extract.

It is ACL's opinion that the analysis of milk and liver could possibly be shortened by using only GPC and/or HPLC after acid hydrolysis, acetone/chloroform partitioning and selecting an appropriate capillary column in conjunction with the specific NP detector. The length of time for analysis and solvent consumption make the method inappropriate for an enforcement laboratory. The limit of detection from visual inspection of the chromatogram is estimated to be 0.01 ppm for Tebuconazole and HWG 2061 in/on liver and milk. Even though recovery data would normally be considered acceptable, it is ACL's opinion that this method does not meet the 40 CFR 158 and EPA's requirement as published in the Pesticide Assessment Guidelines, subdivision"O" for Residue chemistry, Part 171-4(b) as an enforcement because of the total time (laboratory time required for liver or milk was 64 hours)necessary to complete this procedure. This method would have little practical utility in an enforcement laboratory.

COMMODITY	CHEMICAL ADDED	PPM ADDED	PPM FOUND	RECOVERY
Peanuts	None	Control	N.D.	
	None	Control	N.D.	
	Tebuconazole	0.0998	0.0773	77.5
	Tebuconazole	0.0998	0.0669	67.0
	Tebuconazole	0.1999	0.1497	74.9
	Tebuconazole	0.1999	0.1615	80.8

N.D.=indicates not detected at limit of less than <0.01 ppm

MILK	None	Control	N.D.	
	None	Control	N.D.	
	Tebuconazole	0.0499	0.0516	103.5
	Tebuconazole	0.0499	0.0386	77.4
	HWG 2061	0.0499	0.0437	87.5
	HWG 2061	0.0499	0.0369	74.0
	Tebuconazole	0.0998	0.1212	121.4
	Tebuconazole	0.0998	0.0809	81.0
	HWG 2061	0.0998	0.0717	71.8
	HWG 2061	0.0998	0.0856	85.7

N.D.=indicates not detected at limit of less than <0.01 ppm

LIVER

None	Control	N.D.	
None	Control	N.D.	
Tebuconazole	0.0998	0.0677	67.8
Tebuconazole	0.0998	0.0631	63.3
HWG 2061	0.0998	0.0666	66.7
HWG 2061	0.0998	0.0661	66.2
Tebuconazole	0.1999	0.2040	102.0
Tebuconazole	0.1999	0.1606	80.3
HWG 2061	0.1999	0.197	98.7
HWG 2061	0.1999	0.2333	116.9

*N.D.= indicates not detected at limit of less than < 0.01 ppm

Modifications made to method (major or minor):

See attached report

Special precautions to be taken:

None

Source of analytical reference standards:

Standards used for this TMV were from the petitioner. Standards are also available from EPA, RTP.

If derivatized standards used, give source:

In house (derivatized at times of sample derivatization)

Instrumentation for confirmation:

None

Instrument for Quantitation:

HP 5890 - NP Detector

If instrumentation parameters differ from the method as written, list parameters actually used:

See attached report

Commercial source for any special chemicals or apparatus:

None

Comments:

See attached report

Chromatograms:

See attachments