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

Analytical Chemistry Laboratory Branch
Building 306, BARC-East
Beltsville, Maryland 20705

NOV 4 1997

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP# 6F04664. Tolerance Method Validation of Isoxaflutole in/on Field Corn and Animal RACs.

FROM: Mark W. Law, Chemist
Analytical Chemistry Laboratory Branch

THRU: Francis D. Griffith, Jr., Chief
Analytical Chemistry Laboratory Branch

THRU: Donald A. Marlow, Laboratory Coordinator
Biological and Economic Analysis Division

TO: E. Zager, Chief
Registration Action Branch-I
Health Effects Division (7509C)

INTRODUCTION

The Analytical Chemistry Laboratory Branch (ACLB) was requested by the Registration Action Branch - I to conduct a tolerance method validation for Isoxaflutole and one of its metabolites (RPA 203348). Analyses were requested to be run in duplicate on milk control samples, and milk samples fortified at 0.01 ppm,and 0.02 ppm (the proposed tolerance)of each analyte; and on beef kidney control samples, and beef kidney samples fortified at 0.2 ppm, and 0.4 ppm (the proposed tolerance).

RECOMMENDATION

Since the methods for isoxaflutole and its metabolite in beef kidney and milk have successfully completed the TMV, ACLB finds them suitable for food tolerance enforcement, and recommends that they be made available to Federal and State enforcement laboratories along with our addendum.

METHOD SUMMARY

The method submitted for validation from Rhone-Poulenc Ag Company (RPAC) was titled: "Isoxaflutole - Validation of Method of Analysis for Isoxaflutole and Its Metabolite in Animal Tissues.", dated 11/15/96 by Anibal Lopes, MRID # 441690-04.

Residues of isoxaflutole and its metabolite are extracted from milk with a mixture of 0.1% aqueous trifluoroacetic acid and acetonitrile. The extract is purified using a C-8 cartridge column. Isoxaflutole and the metabolite are eluted and collected together in the second fraction.

Beef kidney samples are analyzed by a common moiety technique. Residues of isoxaflutole and its metabolite are extracted from the kidney with 0.1% aqueous trifluoroacetic acid. The samples are treated with 10% sodium hydroxide to convert all isoxaflutole to the RPA 202248 metabolite, and the samples are then cleaned up using a C-18 cartridge.

All residue analysis is accomplished by High Performance Liquid Chromatography on a C-18 column using UV detection (270 nm for Isoxaflutole, and 300 nm for RPA 202248).

CONCLUSIONS

1a. The analytical standards for this method trial were obtained from the EPA Pesticides Repository. The ordering codes are F031080 for the parent and F031081 for the metabolite.

1b. The milk used for controls and samples was obtained from the USDA dairy barn, and the beef kidney used for controls and samples was purchased at Giant Foods.

2a. ACLB concludes that the initial trials on milk failed due to poor recoveries of the parent compound. After completing the successful trial on kidney, the milk trial was repeated using a slightly modified C-8 cleanup step. This trial resulted in improved recoveries of the parent compound, but poor recovery of the metabolite.

2b. ACLB concludes that additional trials on milk using a newer lot number of C-8 SPE cartridges provided by the petitioner provided acceptable results. However, the method should only be run by experienced residue chemists to achieve consistent results.

2c. The ACLB results of the kidney and final milk trials are tabulated on page 4. ACLB concludes that the TMV was successful for kidney and marginally successful for milk.

3a. ACLB's LOD estimate for the RPA 202248 metabolite is 0.004 ppm in milk and 0.024 ppm in kidney. The LOQ estimate on milk is 0.010 ppm and the LOQ estimate on kidney is 0.20 ppm for RPA 202248.

3b. Since the UV response of the parent compound is approximately half that of the RPA 202248 metabolite, ACLB concludes the appropriate LOD and LOQ for the parent (RPA 2171772) in milk should be 0.010 and 0.025 ppm respectively.

4a. ACLB concludes that this method meets the requirements for an enforcement method as defined in the Pesticide Assessment Guidelines, 860.1340.

4b. ACLB concludes that this method is suitable to gather residue data from the validated LOQ to the upper level tested.

5. ACLB suggests that the petitioner consider modifying the milk portion of the method to incorporate the common moiety technique used in the meat (kidney) portion. If the petitioner does not wish to use the common moiety approach,the milk method should be amended to include instructions to test each batch of C-8 cartridges for recovery of the parent and metabolite. ACLB feels that this would also provide the analyst with the necessary practice to obtain good recoveries of both analytes.

6. ACLB concludes that a set of six (6) samples took one chemist approximately 16 hours to work up for HPLC/UV analysis. Unattended, overnight, triplicate injection of samples and standards required ~ 15 hours to analyze including reporting time.

cc: TMV File:Analyst(MWLaw).
ACLB:7503W:BARC-E/306:MW Law:9/25/97:editmwl:10/28/97:(301)505-8230TDD.
RDI:QAPanel:7/21/97,10/31/97:BrCh:FDGriffith:11/03/97.

ANALYTICAL CHEMISTRY LABORATORY BRANCH
METHOD VALIDATION RESULTS

B97-11,12
TMV of Isoxaflutole in/on Beef Kidney and Milk

Commodity	Chemical Added	PPM Added	PPM Found	Percent Recovery
Milk Trial 1	Isoxaflutole	0.0000		
		0.0000		
		0.0101	0.0130	129
		0.0101	0.0100	99.0
		0.0201	0.0180	89.6
		0.0201	0.0210	104
	RPA 202248	0.0000		
		0.0000		
		0.0099	0.0065	65.7
		0.0099	0.0072	72.7
		0.0199	0.0083	41.7
		0.0199	0.0098	49.3
Milk Trial 2	Isoxaflutole	0.0000		
		0.0000		
		0.0101	0.0070	69.3
		0.0101	0.0080	79.2
		0.0201	0.0170	84.6
		0.0201	0.0190	94.5
	RPA 202248	0.0000		
		0.0000		
		0.0099	0.0062	62.6
		0.0099	0.0034	34.3
		0.0199	0.0155	77.9
		0.0199	0.0165	82.9

ANALYTICAL CHEMISTRY LABORATORY BRANCH
METHOD VALIDATION RESULTS

B97-11,12
TMV of Isoxaflutole in/on Beef Kidney and Milk

Commodity	Chemical Added	PPM Added	PPM Found	Percent Recovery
Beef Kidney	Isoxaflutole	0.0000		
		0.0000		
		0.2052	0.2006	97.7
		0.2052	0.1925	93.8
		0.4010	0.3049	76.0
		0.4010	0.3000	74.8
	RPA 202248	0.0000		
		0.0000		
		0.2010	0.2006	99.8
		0.2010	0.1454	72.3
		0.4020	0.3312	82.4
		0.4020	0.3356	83.5
Milk Trial 3	Isoxaflutole	0.0000		
		0.0000		
		0.0100	0.0064	64.0
		0.0100	0.0150	150
		0.0200	0.0508	254
		0.0200	0.0151	75.5
	RPA 202248	0.0000		
		0.0000		
		0.0100	0.0050	50.0
		0.0100	0.0098	98.0
		0.0201	0.0159	79.1
		0.0201	0.0163	81.1

B97-11,12
TMV of Isoxaflutole in/on Beef Kidney and Milk

EPA ADDENDUM

1. ACLB used a Hewlett-Packard 1090 Series HPLC with a Diode Array Detector.

2. There are two corrections needed in the Equipment and Supplies section of the method.

2.a Filter Paper 417 (Whatman No. 28313-0466) does not exist. This is a paper made on a contract with VWR and the part number is a VWR catalog number.

2.b Graphitized carbon, Supelco Cat. No. 5-7210, is no longer available. ACLB used Supelco 5-7230 ENVI-Carb (120-140 mesh) Supelclean Bulk Packing.

3. The method specifies a flow rate of 2 ml/min or less through the SPE cartridges as a critical step. The flow is incorrectly defined as "~2 drops/sec" in Step B17 of Appendix B. The proper definition of 2 ml/min is "1 drop/2 sec" as is indicated in several other sections of the method. ACLB further recommends a flow rate of one drop/3 sec for the milk portion of the method.

4. The method prescribes the use of calibration curves to determine sample concentrations. ACLB determined sample concentrations from a ratio of sample responses to the average of bracketing standard responses. The standard concentrations were equivalent to the expected solution concentration of the samples they bracketed.

5. Isoxaflutole and RPA 202248 standard responses were found to be linear over the range of 0.2 - 8.0 ng injected. All sample analyses were conducted in this range.