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

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

FILE

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: 25-FEB-1999

SUBJECT: PP# 6F04772. Fluroxypyr in or on Wheat, Barley, and Oats. **Results of Petition Method Validation (PMV) Request.** Barcode D253504. Chemical #s 128959, 128968. Case 288019. Submission S557140.

FROM: William H. Donovan, Ph.D., Chemist
RAB1/HED (7509C)

THROUGH: Melba Morrow, D.V.M., Branch Senior Scientist
RAB1/HED (7509C)

TO: Joanne Miller/ Daniel Kenny
Registration Division (7505C)

DowElanco submitted a petition requesting a permanent tolerance in support of a Section 3 registration for its herbicide fluroxypyr 1-methylheptyl ester [1-methylheptyl ((4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetate] and its metabolite fluroxypyr [((4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid] in or on wheat, barley, and oats. The proposed tolerances were as follows:

Grain			Forage		
	Barley	0.5 ppm		Barley	10 ppm
	Oats	0.5 ppm		Oats	10 ppm
	Wheat	0.5 ppm		Wheat	10 ppm
Straw			Hay		
	Barley	10 ppm		Barley	20 ppm
	Oats	10 ppm		Oats	20 ppm
	Wheat	10 ppm		Wheat	20 ppm

Aspirated Grain Fractions: 0.5 ppm

Meat, fat, milk and meat byproducts (except kidney) of cattle, goats, hogs, horses and sheep: 0.1 ppm.

Kidney of cattle, goats, hogs, horses, and sheep: 0.5 ppm.

Health Effects Division (HED) requested (D244940, W. Donovan, 25-MAR-1998) that the Analytical Chemistry Laboratory Branch (ACLB) perform a PMV on the following methods:

> "Validation Report for the Determination of Residues of Fluroxypyr and Fluroxypyr 1-methylheptyl Ester as the Acid Equivalent in the Grain, Forage, Straw, and Hay of Wheat, Barley, and Oats by Capillary Gas Chromatography with Mass Selective Detection" (DowElanco study RES95118, 04-JUN-1996, MRID # 44080352).
>
> "Validation Report for the Determination of Residues of Fluroxypyr in Ruminant Tissues and Milk by Capillary Gas Chromatography with Mass Selective Detection" (DowElanco study RES95164, 10-JUN-1996, MRID #44080354).

The results of the PMV review are appended to this memorandum as Attachment 1 (Memo, E.J. Kolbe, D.M. Swineford, and P. Schermerhorn, 16-JUL-1998).

Results

Unacceptably low recovery values were obtained on the first trial for wheat commodities. A second trial (following registrant consultation) gave low but consistent and acceptable recoveries as detailed in Table 1.

Table 1. Results of Fluroxypyr method validation obtained by ACLB.

Raw Agricultural Commodity (RAC)	Fortification Level (ppm)	% Recovery[a]	ACLB LOD (ppm)	ACLB LOQ (ppm)
Wheat Grain	0.01	NA	0.06	0.1
	0.50	69 ± 0		
Wheat Straw	0.05	NA	0.2	0.6
	10	63 ± 4		
Wheat Forage	0.05	NA	0.1	0.3
	10	62 ± 1		
Milk	0.01	69 ± 6	0.006	0.01
	0.10	77 ± 10		
Liver	0.01	61 ± 5	0.003	0.01
	0.10	84 ± 12		

[a] Each listed value is the mean recovery ± the sample standard deviation based on replicated determinations.

A set of six samples can be processed by one analyst in approximately 16 hours. Additional time

is needed for instrumental analysis and data interpretation.

Conclusions

ACLB considers the GC/MSD method suitable for food tolerance enforcement purposes and to gather residue data at the tolerance levels and at the LOQ estimates specified in Table 1. However, the following revisions recommended by ACLB should be incorporated into the method:

1. The GC/MSD method is not completely adequate as an enforcement method as written because it specifies only two major ions (m/z 296 and 298) for confirmation of fluroxypyr PE. ACLB recommends the use of three ions for an enforcement method and suggests that the m/z 211 ion might be suitable for this purpose.

2. The registrant should clarify where the "stopping points" in the method apply.

Recommendations

The registrant should submit a revised version of the proposed analytical enforcement method as specified in conclusions 1 and 2. The requirements for the analytical enforcement methodology will remain unfulfilled until the revised method is received. Also, the registrant should forward fluroxypyr propyl ester and fluroxypyr butyl ester standards to the ACLB standards repository.

Attachment 1 - Memo, E.J. Kolbe, D.M. Swineford, and P. Schermerhorn, ACLB #B98-(28-33).

cc: PP#6F04772, Donovan, F. Griffith (7503W)
RDI: M. Morrow: (25-FEB-1999), RAB1 Chemists (25-FEB-1999)
W. Donovan:806T:CM#2:(703)305-7330:(25-FEB-1999)

ATTACHMENT 1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460
Analytical Chemistry Laboratory Branch
Building 306, BARC-East
Beltsville, Maryland 20705

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Subject: **PP# 6F04772.** Fluroxypyr in/on Wheat,Barley and Oats.
Tolerance Method Validation Request
MRID# 44080352, DP Barcode D232215, B98-(28-33).

From: Elizabeth J. Kolbe, Chemist
Douglas M. Swineford, Chemist
Patricia Schermerhorn, 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 (7503C)

To: Edward Zager, Chief
Registration Action Branch I
Health Effects Division (7509C)
and
Donald Stubbs, Chief
Herbicide Branch
Registration Division (7505C)

INTRODUCTION

The Analytical Chemistry Laboratory Branch (ACLB) was requested by Registration Action Branch I (RAB I) to conduct Tolerance Method Validation (TMV) of the proposed enforcement methods submitted by Dow AgroSciences LLC for the determination of residues of the herbicide fluroxypyr [(((4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid)], formulated as the 1-methylheptyl ester on wheat grain,forage, straw and cow milk, liver and chicken egg. The proposed tolerances are 0.5 ppm for wheat grain; 10 ppm for wheat straw and forage; and 0.1 ppm for cow milk and liver. ACLB was requested by RAB I to fortify each commodity in duplicate at the following levels: wheat grain, 0 ppm, 0.01 ppm, 0.5 ppm; wheat forage, 0 ppm, 0.05 ppm, 10 ppm; wheat straw, 0 ppm, 0.05 ppm, 10 ppm; cow milk, 0 ppm, 0.01 ppm, 0.1 ppm; beef liver, 0 ppm, 0.01 ppm, 0.1 ppm; chicken egg, 0 ppm,0.01 ppm, 0.1 ppm with fluroxypyr 1-methylheptyl ester.

The request to validate the method for eggs was rescinded by Donald Stubbs, Branch Chief, Registration Division. There are no proposed tolerances for fluroxypyr in eggs.

RECOMMENDATION

The GC-MSD method failed due to poor recoveries on first trial for the wheat products. After conferring with the petitioner, (teleconference 6/01/98) the GC-MSD method has been successfully validated at the tolerance levels for wheat grain, forage and straw, and cow milk and liver and is suitable for food tolerance enforcement.

ACLB recommends that the residue analytical method be made available to federal and state enforcement laboratories along with our addendum. The changes recommended in the addendum should be incorporated into the method prior to its use.

ACLB recommends, as a condition of registration, that the petitioner be reminded to supply the appropriate amount of Fluroxypyr, Fluroxypyr 1-MHE, Fluroxypyr PE and Fluroxypyr BE needed for this method to EPA's Pesticide Repository at Research Triangle Park (RTP) in North Carolina, then notify EPA's Product Manager and ACLB of the Repository ordering codes.

METHOD SUMMARY

The proposed enforcement method for grain is titled "Validation Report for the Determination of Residues of Fluroxypyr and Fluroxypyr 1-Methylheptyl Ester as the Acid Equivalent in the Grain, Forage, Straw and Hay of Wheat, Barley and Oats by Capillary Gas Chromatography with Mass Selective Detection", by E.L. Olberding and C.A. Ng, Study ID RES95118, dated 6/4/96, and coded MRID #440803-52.

In summary for the grain products, homogenized samples are extracted with 60% acetone/40% 0.25N hydrochloric acid by blending for 1 minute,then shaking for 1 hour. An aliquot of the extract is basified with sodium hydroxide to hydrolyze any fluroxypyr 1-MHE to the acid form. The sample is concentrated with a nitrogen stream to remove the acetone, acidified with hydrochloric acid, and then hydrolyzed at 90° for 2 hours. Following hydrolysis, the extract is diluted with water and purified using a C_{18} solid-phase extraction column. The eluate is extracted with 1-chlorobutane, which is evaporated to dryness. The residue is reconstituted and derivatized with 1 ml of the 2N sulfuric acid/1-propanol at 90°C for one hour. Following derivatization, the 1-propanol is evaporated and the 1-propyl ester derivative of fluroxypyr is partitioned from an aqueous sodium chloride solution into iso-octane containing fluroxypyr 1-butyl ester as an internal standard. A portion of the iso-octane extract is then analyzed by capillary gas chromatography with mass selective detection.

The petitioner submitted the results of the independant laboratory validation (ILV) in a study titled "Independant Laboratory Validation of Method GRM 96.02- Determination of Residues of Fluroxypyr and Fluroxypyr 1-Methylheptyl Ester as the Acid Equivalent in the Grain, Forage, Straw and Hay of Wheat, Barley and Oats by Capillary Gas Chromatography with Mass Selective Detection", by R. L. McKellar, J. A. MacGregor and B. J. Marley dated 8/02/96 and coded MRID #440803-53 for wheat commodities was performed by Wildlife International Ltd. Grain was fortified at 0.010 and 0.050ug/g, forage, straw and hay were fortified at 0.050 and 0.25ug/g. Average percent recoveries, based on four samples, were 92 ± 14, 94 ± 9, 100 ± 2 and 94 ± 4 for grain, forage, straw and hay, respectively. Review of the ILV data and chromatograms shows detectable levels of fluroxypyr in the reagent blanks, that appears to have been subtracted from sample results in forage, straw and hay.

The method for meat and milk is titled "Validation Report for the Determination of Residues of Fluroxypyr in Ruminant Tissues and Milk by Capillary Gas Chromatography with Mass Selective Detection" by E.L. Olberding and M.A. Huskin Study ID RES95164, dated 6/10/96, and coded MRID #440803-54.

In summary, for the cow liver, homogenized samples are extracted once with 20 ml of 0.5N sulfuric acid, salt is added and partitioned into ethyl ether. Milk is deproteinated with sulfuric acid in the presence of sodium chloride. The extract is partitioned into ethyl ether twice, then, for both liver and milk, an aliquot of the ethyl ether is extracted with dilute sodium hydroxide. The sodium hydroxide is acidified with hydrochloric acid and then purified using C_{18} solid-phase extraction column. It then follows the procedure described for wheat with 1-chlorobutane petitioning through analysis.

CONCLUSIONS

1) The petitioner submitted ILV data for the grain commodities, but none for ruminant tissues and milk. In response to F.D. Griffith's inquiry about the absence of ILV data, a memo for the petitioner, dated 4/20/98, states Dow's rationale for not providing this data. Dow feels the methods are similar enough and that the grain commodity method is more difficult.

2) ACLB concludes that based on the second set of recovery data that are low but consistent, this method meets the requirements for an enforcement method as defined in the Pesticide Test Guideline 860.1390, once our comments are incorporated into a revised methcd. ACLB reiterates that we recommend three ions for an enforcement method and believe, based on our preliminarily data, that the ion m/z 211 could possibly be used for the necessary third ion.

3) The method states a limit of quantitation (LOQ) of 0.01 ppm in wheat grain; 0.05 ppm in wheat forage and straw; 0.01 ppm in milk and liver, and the limit of detection (LOD) of 0.003 ppm for wheat grain, liver and milk; 0.017 ppm for wheat forage and straw. Based on our analysis of background interferences, ACLB estimates that the LOD is 0.06 ppm for wheat grain, 0.2 ppm for wheat straw, 0.1 ppm for wheat forage, 0.006 ppm for milk,and 0.003 ppm for liver. ACLB estimates that the LOQ is 0.1 ppm for wheat grain, 0.6 ppm for wheat straw,0.3 ppm for wheat forage. ACLB agrees that the LOQ for milk and liver is 0.01 ppm.

4) ACLB has included the data from the low spikes that were requested by RAB I even though the level of the spikes is at a level that is below ACLB's estimate of the method LOQ for the wheat commodities. ACLB did not analyze the commodities at the ACLB estimated LOQ levels.

5) A set of six samples can be processed by one analyst in approximately 16 hours plus 30 minutes for each instrumental analysis.

6) ACLB concludes the method to be suitable to gather residue data at the tolerance levels and at the LOQ estimates as determined by ACLB.

7) The fluroxypyr standards were obtained from Dow AgroSciences. The purity of the standards was greater than 90%.

   a) The unused portion of the standard was retained in the ACLB standards repository. The fluroxypyr (acid form),code number 031162 and the fluroxypyr 1-MHE, code number 031163 was available from the RTP repository on 7/08/98. Standards of fluroxypyr propyl ester and fluroxypyr butyl ester were not available as of 7/08/98.

   b) The petitioner should be reminded to forward the fluroxypyr propyl ester and fluroxypyr butyl ester standards to the repository and provide RD and ACLB with the ordering codes.

8) The wheat commodities used for the validation were obtained from Dow AgroSciences. The milk was obtained raw from the dairy at USDA, Beltsville, Maryland. The liver was obtained at a local grocery store.

Attachment: Detector Response
Method Validation Results
Addendum

cc: ACLB # B98-(28-33);
ACLB Analysts- EKolbe/DSwineford/PSchermerhorn;
RDI: QA Panel 7/15/98; BrCh- FGriffith 7/16/98

ADDENDUM

1) ACLB modified instrument operating conditions are as follows.

The parameters for the GC/MSD were:
Gas Chromatograph: HP 5973 Mass Selective Detector.
Column: DB 1701, 15 M x 0.25 mm id, 0.25 um film.
Carrier gas: Helium, 2.2/min.
Oven Temperature: Initial 90°C for 1.0 min.
10°C/min to 255oC, 20oC/min. to 280oC, hold 1.25 min.
Injector temperature: 260°C
MS mode: SIM total ion current of m/z 296, m/z 298, m/z 211 and m/z 310.
Transfer line temperature: 280°C
Injection volume: 3 ul
Quantitation as described in the method.

2) Validation of the milk method requires spiking with fluroxypyr. The initial method validation attempt by ACLB for milk resulted in no recovery. It was determined that spiking with fluroxypyr 1-MHE as indicated in the request for validation (whereas the method states to spike with fluroxypyr.) would result in no recovery. There is no hydrolysis step in the method used for milk and liver in our trials.

3) Method recovery data can be unacceptably low. Method user needs to check the derivatising step and SEP cartridge as sources of loss. In addition, there are several partitioning and concentration steps. ACLB concludes familiarization with the method is key to its successful use.

4) The GC/MS method is not completely adequate as an enforcement method as written, using only two major ions (m/z 296 and 298) for fluroxypyr PE. ACLB recommends three ions for an enforcement method and believes, based on our preliminarily data, that the ion m/z 211 could possibly be used for the necessary third ion.

5) ACLB contacted Dow to clarify the "stopping points" indicated in the method (sec.K.3). Dow confirmed that our interpretation was correct, the "stopping points" are after the indicated step.

Analytical Chemistry Laboratory Branch
Method Validation Results

Commodity	Chemical Added	ppm Added	ppm Found	% Recovery
Wheat Grain	Fluroxypyr	0.00	0.020 1	
1st Trial	1-Methylheptyl ester	0.00	0.014 1	
		0.01	0.010 1	100
		0.01	0.0087 1	87
		0.50	0.26	51
		0.50	0.30	59
2nd Trial		0.00	0.054 1	
		0.00	0.004 1	
		0.01	0.0079 1	79
		0.01	0.0084 1	84
		0.50	0.35	69
		0.50	0.34	69
Wheat Straw	Fluroxypyr	0.00	0.13 2	
1st Trial	1-Methylheptyl ester	0.00	0.29	
		0.05	0.57	1100
		0.05	0.085 2	170
		10	4.9	49
		10	5.1	51
2nd Trial		0.00	0.028 2	
		0.00	0.017 2	
		0.05	0.079 2	160
		0.05	0.045 2	89
		10	6.6	66
		10	6.0	60
Wheat Forage	Fluroxypyr	0.00	0.026 3	
1st Trial	1-Methylheptyl ester	0.00	0.021 3	
		0.05	0.045 3	89
		0.05	0.028 3	56
		10	3.1	31
		10	2.6	26
2nd Trial		0.00	0.012 3	
		0.00	0.023 3	
		0.05	0.063 3	130
		0.05	0.054 3	110
		10	6.1	61
		10	6.2	62

Analytical Chemistry Laboratory Branch
Method Validation Results (continued)

Commodity	Chemical Added	ppm Added	ppm Found	% Recovery
Milk	Fluroxypyr	0.00	0.0036 *4*	
		0.00	0.0026 *4*	
		0.01	0.0065	65
		0.01	0.0073	73
		0.10	0.070	70
		0.10	0.084	84
Liver	Fluroxypyr	0.00	0.0017 *5*	
		0.00	0.0026 *5*	
		0.01	0.0057	57
		0.01	0.0064	64
		0.10	0.075	75
		0.10	0.092	92

1 recovery data based on fortification level below ACLB's LOD of 0.06 ppm for Wheat grain
2 recovery data based on fortification level below ACLB's LOD of 0.2 ppm for Wheat straw
3 recovery data based on fortification level below ACLB's LOD of 0.1 ppm for Wheat forage
4 recovery data based on fortification level below ACLB's LOD of 0.006 ppm for Milk
5 recovery data based on fortification level below ACLB's LOD of 0.003 ppm for Liver