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

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

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

Date: 6-NOV-2001

SUBJECT: PP# 7F4848. Diflufenzopyr in/on Field Corn. **Results of Petition Method Validation (PMV).** MRID# 44497402. Barcode D278522. PC Code 005107. Case 293208. Submission S590359.

FROM: Jennifer R. Tyler, Chemist
Registration Action Branch 1 (RAB1)
Health Effects Division (HED) (7509C)

THROUGH: G. Jeffrey Herndon, Branch Senior Scientist
George F. Kramer, Ph.D., Chemist
RAB1/HED (7509C)

TO: Robert Forrest/Shaja Brothers, PM Team 05
Joanne Miller, PM Team 23
Registration Division (RD) (7505C)

BASF previously submitted an application for tolerances for residues of the herbicide diflufenzopyr [2-(1-[([3,5-difluorophenylamino]carbonyl)hydrazono] ethyl)-3-pyridinecarboxylic acid] in/on field corn. Section F of the petition proposed the establishment of permanent tolerances for residues of diflufenzopyr and its two metabolites M1 (8-methylpyrido[2,3-d]pyridazin-5(6H)-one) and M5 (6-((3,5-difluorophenylcarbamoyl-8-methyl-pyrido[2,3-d]-5-pyridazinone), all as the M1 component in/on field corn grain, forage and fodder (stover). Permanent tolerances have been established for the combined residues of diflufenzopyr and its metabolites convertible to M1 in/on field corn forage, grain, and stover at 0.05 ppm [40 CFR §180.549(a)].

In a memo dated 10/2/98, the Chemical and Exposure Branch 1 (CEB1) requested that the Analytical Chemistry Branch (ACB) perform a PMV on the following GC/MS method (Memo, L. Cheng, D250036):

> Determination of Diflufenzopyr and Phthalazinone [M1] Residues in Corn by GC-MS, MRID# 44497402.

The results of the PMV review are appended to this memorandum as Attachment 1 (Memo, J. Negron, 11/24/98).

Results

Acceptable recovery values were obtained at all fortification levels for all commodities tested. ACB agrees with the limit of quantitation (LOQ) of 0.05 ppm reported by the petitioner. ACB estimated the limit of detection (LOD) to be 0.02 ppm. Table 1 summarizes ACB's results.

Table 1. Results of Diflufenzopyr Method Validation Obtained by ACB Using Modified Method.

Commodity	PPM Added	PPM Found	Avg. Percent Recovery
Corn grain	control	<0.00022, <0.00022	-
	0.0515	0.0526, 0.0482, 0.0401	91.2±12.2
	0.103	0.0619, 0.0863, 0.0804	74.0±12.4
Corn forage	control	<0.00022, <0.00022, <0.00022	-
	0.0206	0.0172, 0.0165, 0.019	85.3±6.24
	0.0515	0.0592, 0.0589, 0.0555	112±3.79
	0.103	0.0999, 0.0107	100±3.61
Corn fodder (stover)	control	<0.00022, <0.00022, <0.00022	-
	0.0206	0.0187, 0.0235, 0.0194	99.7±12.5
	0.0515	0.0509, 0.0527, 0.0519	101±1.64
	0.103	0.01050, 0.0880	97.1±10.2

One analyst can prepare a set of 12 samples for analysis in ~ 14 hours plus 30 minutes for each instrumental analysis.

Conclusions/Recommendations

The method was successfully validated by the ACB. The changes recommended in the EPA addendum should be incorporated into the method prior to its use. The enforcement method and the EPA addendum will be forwarded to FDA for inclusion in PAM II. Any copy of the method that is distributed prior to publication in PAM II should include the EPA addendum.

Attachment 1- Memo, P. Schermerhorn 11/24/98 (not available electronically).

cc (w/ Attachments): J. Tyler; F. Griffith (7503W); C. Furlow (7502C)
RDI: G. Kramer (10/25/01); RAB1 Chemists (10/25/01); G. Herndon (10/31/01)
J.Tyler:806W:CM#2:(703)305-5564: 7509C:RAB1

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ATTACHMENT 1

(not available electronically)

-DO READ 11/24/9

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

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: PP#7F4848. Validation of the Diflufenzopyr Enforcement Method In Field Corn Commodities.
(MRID #s 444974-02 and -03){DP Barcode D250036}
B98-(73-75).

From: Juan F. Negron, Chemist
Alexander G. Krymitsky, Chemist
Analytical Chemistry Branch

Thru: Francis D. Griffith, Jr., Chief
Analytical Chemistry Branch

Thru: Donald A. Marlow, Laboratory Coordinator
Biological and Economic Analysis Division (7503C)

To: Beth Doyle, Chief
Chemistry and Exposure Branch 1
Health Effects Division (7509C)
And
Donald Stubbs, Chief
Herbicide Branch
Registration Division (7505C)

INTRODUCTION

On September 3, 1998 the Analytical Chemistry Branch (ACB) was requested by the Chemistry and Exposure Branch 1(CEB)/HED to validate the residue analytical method for Diflufenzopyr. Three commodities of field corn need to be validated at 0.02, 0.05, and 0.1ppm. The proposed tolerances is 0.05 ppm for all three commodities. On October 2,1998 CEB/HED revised the earlier request to exclude the 0.02 ppm. ACB tested the analytical method at three different levels and all samples were run in triplicate. Diflufenzopyr is a herbicide with the following chemical name 2-[Methyl[{(3,5-ifluorophenylamino)carbonyl}hydrazono]3-pyridinecarboxylic acid.

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RECOMMENDATION

ACB finds this method suitable for food tolerance enforcement of Diflufenzopyr in field corn commodities, provided the comments in our addendum are followed. ACB recommends that the residue analytical method be made available to federal and state enforcement laboratories along with our addendum. The changes suggested in the addendum **should** be incorporated into the method prior to its use.

METHOD SUMMARY

The proposed enforcement method submitted by the registrant, BASF, is entitled "Method for Determination of Diflufenzopyr and Phthalazinone (M1) Residues in Corn by GC/MS", by Samy Abdel-Bakey and David Broadwell, BASF Code 654-H (**MRID # 444974-02 and -03**).

In summary, residues of Diflufenzopyr (654 H) and Phthalazinone (M1) are extracted from corn matrices by shaking with dilute aqueous sodium bicarbonate and ammoniated acetone. The extract is filtered, and an aliquot is acidified with concentrated hydrochloric acid and evaporated to dryness. The residue is dissolved in methanol/ethyl acetate and refluxed for 2 hours in presence of concentrated hydrochloric acid to convert Diflufenzopyr to Phthalazinone. The solution is evaporated to dryness and purified by mini-Oasis HLB™ chromatography column; the eluant is evaporated to dryness and the residue is dissolved in MeOH and analyzed by GC-MS. Quantitation is via SIM using m/z 161 which is the molecular weight for Pthalazinone.

Independent Laboratory Validation (ILV) Data

The independent laboratory validation was conducted by the *ADPEN* Laboratories (ADPEN Study No.: ADPEN-97-903-97229) using the method titled "Independent Method Validation of BASF Analytical Method D9709 for Determination of Diflufenzopyr (BAS 654 H), and Phthalazinone Residues in Corn by GC-MS", validated the method in corn fodder. The independent laboratory made a minor modification in the method which the registrant elected to incorporate. The fortification levels for both Diflufenzopyr (654 H) and Phthalazinone (M1) in corn fodder were each at 0.05 and 0.1 ppm level. Overall average recoveries found in corn fodder for Diflufenzopyr and Phthalazinone were 79.5 ± 7.5% and 88.9%±6.3% respectively.

Review of the external laboratory validation supporting chromatograms show no detectable levels of Diflufenzopyr or Phthalazinone in untreated controls. While there were unidentified analytical responses present in these chromatograms ACB concludes that there is no interference problem. The registrant's chromatograms match favorably with those generated by ACB during our method validation.

CONCLUSIONS

1) ACB concludes that this method meets the requirements for an enforcement method as defined in the Pesticide Test Guideline 860.1340, once our suggestions in the attached EPA Addendum are incorporated into the method.

2a) The method states a limit of quantitation (LOQ) of 0.05 ppm in corn grain, corn forage, and in corn fodder for Diflufenzopyr. ACB agrees with the registrant's LOQ.

2b) ACB concludes the method to be suitable to gather residue data from the LOQ of 0.05 ppm to at least 0.1ppm.

2c) ACB gathered recovery data at the 0.02 ppm level (LOD). ACB results confirm the method's lower limit capabilities. They indicate that the residues reported below 0.02 ppm are probably suspect and should be used, with caution, in a dietary exposure estimate for risk analysis.

3) A set of 12 samples can be processed by one analyst in approximately 14 hours plus 30 minutes for each instrumental analysis.

4a) The standards used were provided by BASF. The purity for all of the standards was greater than 90%.

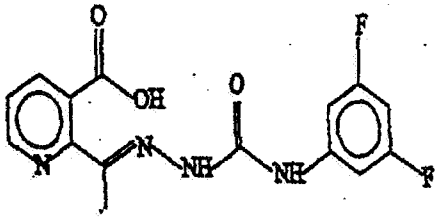
4b) The unused portion of the standards were retained in the ACB standards repository. The Diflufenzopyr (BAS 654 H) RTP ordering code #31128, and Pthalazinone (M1) RTP ordering code #31129 were both available at RTP.

5) The corn commodities, for the recovery studies, were supplied by BASF.

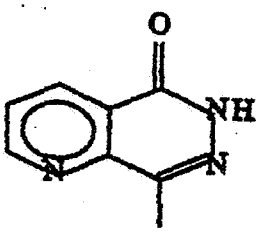
Note to HED Reviewer: Please note item 4 in our addendum when reviewing crop field trial data. This critical step may impact judgements on acceptability of crop field trial data.

Chemical Structure

Chemical Name; 2-[Methyl[{(3,5-ifluorophenylamino)carbonyl}hydrazono]3-pyridinecarboxylic acid
Common Name; Diflufenzopyr
Empirical Formula; $C_{15}H_{12}F_2N_4O_3$
Molecular Weight; 334.28 g/mole



Chemical Name; B-Methyl-6(6H)pyrido[2,3-d]pyridazinone
Common Name; Phthalazinone (M1)
Empirical Formula; $C_8H_7N_3O_1$
Molecular Weight; 161.16 g/mole



ATTACHMENTS: Detector Response
Method Validation Results
EPA Addendum

cc: ACB File B98-(73-75); ACB Analysts-JNegron/AKrynitsky
7503C:ACB: BARC-E/306.JNegron/AKrynitsky:11/5/98:(301)-504-8232:edit:JNegron/AKrynitsky:11/17/98.
RDI:QAPanel:11/17/98:TL: MLaw;11/9/98:BrCh: FDGriffith:11/18/98

Table I

ANALYTICAL CHEMISTRY BRANCH METHOD VALIDATION RESULTS
Method for Determination of Diflufenzopyr and Phthalazinone (M1) Residues in Corn by GC-MS
METHOD AS WRITTEN

Commodity	Chemical Name		PPM Added ug/g	PPM Found ug/g	Percent Recovery %	Percent Avg. Rec. %	Std. Dev. %	RSD %
Corn Grain	1. Diflufenzopyr							
			control	< 0.00022				
			control	< 0.00022				
			control	< 0.00022				
			control	< 0.00022				
			control	< 0.00022				
			control	< 0.00022				
			0.0206	0.0176	85.4			
			0.0206	0.0306	149			
			0.0206	0.0340	165	133	42.1	31.6
		1	0.0515	0.0423	82.1			
		1	0.0515	0.0261	50.7			
		1	0.0515	0.0238	46.2			
			0.0515	0.0258	50.1			
			0.0515	0.0252	48.9			
			0.0515	0.0409	79.4			
			0.0515	0.0260	50.5			
			0.0515	0.0291	56.5	57.1	12.8	22.5
			0.103	0.0511	49.6			
			0.103	0.0508	49.3			
			0.103	0.0581	56.4			
			0.103	0.0389	37.8			
			0.103	0.0482	46.8			
			0.103	0.0507	49.2	48.0	6.03	12.6

Notes : 1. Value not included in final % average recovery calculation due to use of diluted HCl instead of concentrated HCl.

$$\% \text{ Recovery} = \frac{\text{PPM found X 100}}{\text{PPM added}} \qquad \text{RSD} = \frac{\text{STD Dev. X 100}}{\text{Avg. found}}$$

Table II

ANALYTICAL CHEMISTRY BRANCH METHOD VALIDATION RESULTS
Method for Determination of Diflufenzopyr and Phthalazinone (M1) Residues in Corn by GC-MS
MODIFIED METHOD (SEE ADDENDUM)

Commodity	Chemical Name		PPM Added ug/g	PPM Found ug/g	Percent Recovery %	Percent Avg. Rec. %	Std. Dev. %	RSD %
Corn Grain	1. Diflufenzopyr							
		1	control	< 0.00022				
		1	control	< 0.00022				
		1	0.0515	0.0526	102			
		1	0.0515	0.0482	93.6			
		1	0.0515	0.0401	77.9	91.2	12.2	13.4
		1	0.103	0.0619	60.1			
		1	0.103	0.0863	83.8			
		1	0.103	0.0804	78.1	74.0	12.4	16.8

Notes : 1. Aliquots from remainder of same sample extracts in Table 1

$$\% \text{ Recovery} = \frac{\text{PPM found X 100}}{\text{PPM added}} \qquad \text{RSD} = \frac{\text{STD Dev. X 100}}{\text{Avg. found}}$$

Table III

ANALYTICAL CHEMISTRY BRANCH METHOD VALIDATION RESULTS
Method for Determination of Diflufenzopyr and Phthalazinone (M1) Residues in Corn by GC-MS
MODIFIED METHOD (SEE ADDENDUM)

Commodity	Chemical Name		PPM Added ug/g	PPM Found ug/g	Percent Recovery %	Percent Avg. Rec. %	Std. Dev. %	RSD %
Corn Forage	1. Diflufenzopyr							
			control	< 0.00022				
			control	< 0.00022				
			control	< 0.00022				
			0.0206	0.0172	83.5			
			0.0206	0.0165	80.1			
			0.0206	0.019	92.2	85.3	6.24	7.32
			0.0515	0.0592	115			
			0.0515	0.0589	114			
			0.0515	0.0555	108	112	3.79	3.38
		1	0.103	0.0441	42.8			
		1	0.103	0.066	64.1			
			0.103	0.0999	97.0			
		2	0.103	0.1020	99.0			
			0.103	0.107	104	100	3.61	3.61

Notes :

1. Values not included in final % average recovery calculation due to sample loss. Sample was repeated in duplicate using remainder of sample extract.

2. Repeated Analysis: Duplicate aliquots from same sample extract. (0.112 & 0.0922) PPM found of aliquots average of 0.102ppm.

$$\% \text{ Recovery} = \frac{\text{PPM found X 100}}{\text{PPM added}} \qquad \text{RSD} = \frac{\text{STD Dev. X 100}}{\text{Avg. found}}$$

Table IV

ANALYTICAL CHEMISTRY BRANCH METHOD VALIDATION RESULTS
Method for Determination of Diflufenzopyr and Phthalazinone (M1) Residues in Corn by GC-MS
MODIFIED METHOD (SEE ADDENDUM)

Commodity	Chemical Name		PPM Added ug/g	PPM Found ug/g	Percent Recovery %	Percent Avg. Rec. %	Std. Dev. %	RSD %
Corn Fodder	1. Diflufenzopyr							
			control	< 0.00022				
			control	< 0.00022				
			control	< 0.00022				
			0.0206	0.0187	90.8			
			0.0206	0.0235	114			
			0.0206	0.0194	94.2	99.7	12.5	12.5
			0.0515	0.0509	98.8			
			0.0515	0.0527	102			
			0.0515	0.0519	101	101	1.64	1.62
			0.103	0.1050	102			
		1	0.103	0.0402	39.0			
			0.103	0.0880	85.4			
		2	0.103	0.107	104	97.1	10.2	10.5

Notes :

1. Values not included in final % average recovery calculation due to sample loss. Sample was repeated in duplicate using remainder of sample extract.

2. Repeated Analysis: Duplicate aliquots from same sample extract. (0.107 & 0.106) PPM found of aliquots average of 0.107ppm.

$$\% \text{ Recovery} = \frac{\text{PPM found X 100}}{\text{PPM added}} \qquad \text{RSD} = \frac{\text{STD Dev. X 100}}{\text{Avg. found}}$$

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PP#7F4848
ACB # B98-(73-75)

Diflufenzopyr in Corn Commodities

ADDENDUM

1) ACB made only slight modifications to the GC/MSD instrument parameters.

The parameters for the GC/MSD were:
Gas Chromatograph: HP 5972 Mass Selective Detector.
Column: Restek Stabilwax^{R}-DA 30m x 0.25 mm x 0.25 um film thickness
Carrier gas: Helium, 0.78/min.
Oven Temperature: Initial 120°C for 0.50 min.
70°C/min to 250°C hold for 27 min.
Injector temperature: 250°C
MS mode: SIM total ion current of m/z 161.
Transfer line temperature: 280°C
Injection volume: 4 uL

2) The petitioner used control values to correct for recoveries. This practice is not permitted in our guidelines.

3) ACB found that additional reflux time was needed to convert the Diflufenzopyr to Phthalazinone. When using a neat Diflufenzopyr standard ACB found that the 2 hour reflux time (As written in registrant's procedure) only yielded a 67% conversion of Diflufenzopyr to Pthalazinone. However, a 4 hour reflux time yielded an 85% conversion. In this method trial ACB used the 4 hour reflux time.

4) ACB found that **sonicating the samples** and **thoroughly rinsing the vessels, when transferring the sample extracts, was a critical step** as stated in the method. The occasional low recoveries, as shown in the recovery data, was attributed to the analyte adhering to the walls of the glassware.

5) The part number for the HLB solid phase extraction (SPE) material should be corrected to 106068. The specifications for the HLB SPE material are:

Average per diameter(A^0)	= 82
Specific surface area (m^2/g)	= 832
Mean particle diameter (μm)	= 31.4
Mesh(μm)	= 30