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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

DATE: June 29, 2005

SUBJECT: PP#4F6830. New Chemical - Prothioconazole in/on Plant and Livestock Commodities. **Request for Petition Method Validation.** MRIDs 46246204, 46246206, 46246207, and 46246209. DP Number 318440. Chemical # 113961. Decision Number: 341716.

FROM: William D. Wassell, Chemist
HED/RAB3

THROUGH: Leung Cheng, Team Leader
HED/RAB3

TO: Frederic Siegelman, Chief
Analytical Chemistry Branch/BEAD (7503C)

Bayer CropScience has submitted a petition (PP#4F6830) for the establishment of permanent tolerances for combined residues of the fungicide prothioconazole [2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3*H*-1,2,4-triazole-3-thione] and its desthio metabolite in/on the following agricultural commodities:

Barley, grain	0.2 ppm
Barley, hay	7.0 ppm
Barley, straw	2.0 ppm
Barley, pearled barley	0.2 ppm
Barley, bran	0.4 ppm
Black mustard, seed	0.1 ppm
Borage, seed	0.1 ppm
Canola, seed	0.1 ppm
Crambe, seed	0.1 ppm
Field mustard, seed	0.1 ppm
Flax, seed	0.1 ppm
Grain, aspirated fractions	13.0 ppm
Indian mustard, seed	0.1 ppm
Indian rapeseed	0.1 ppm
Pea and bean, dried, shelled, except soybean, subgroup	0.8 ppm

Peanut, nutmeat	0.02 ppm
Peanut, hay	5.0 ppm
Peanut, meal	0.3 ppm
Rapeseed, seed	0.1 ppm
Rice, grain	0.25 ppm
Rice, straw	1.5 ppm
Rice, hulls	1.0 ppm
Wheat, grain	0.06 ppm
Wheat, forage	7.0 ppm
Wheat, hay	4.0 ppm
Wheat, straw	2.3 ppm
Wheat, bran	1.5 ppm
Wheat, germ	0.15 ppm

Bayer is also proposing the establishment of permanent tolerances for residues of prothioconazole, its desthio and 4-hydroxy metabolites, and conjugates that can be converted to these three compounds by acid hydrolysis in/on the following livestock commodities:

Milk	0.006 ppm
Cattle, fat	0.1 ppm
Cattle, meat	0.01 ppm
Cattle, meat byproducts	1.2 ppm

Bayer CropScience has proposed an LC/MS/MS method, RPA JA/03/01, for the enforcement of tolerances for residues of prothioconazole and its desthio metabolite in/on plant commodities (MRID 46246206). A second LC/MS/MS method, in Bayer Report No. 200537, is proposed for the enforcement of tolerances for residues of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy in ruminant tissues and milk (MRID 46246204). Independent laboratory validations (ILVs) of the methods have also been submitted (MRIDs 46246207 and 46246209). These volumes are appended to this memorandum as Attachments 3 through 6:

46246204 Moore, S.; Harbin, A. (2003) An Analytical Method for the Determination of JAU 6476, JAU 6476-Desthio and JAU 6476-4-Hydroxy Residues in Various Bovine Matrices by LC-MS/MS. Project Number: 200537. Unpublished study prepared by Bayer Corp. 131 p.

46246206 Gould, T.; Timberlanke, B.; Krolski, M.; et. al. (2004) Validation of Bayer CropScience Method RPA JA/03/01: JAU6476: An Analytical Method for the Determination of Total Residues of JAU6476 in Plant Matrices Using LC/MS-MS. Project Number: J6111401, 200799, RPA/JA/03/01. Unpublished study prepared by Bayer Corp. 238 p.

46246207 Reed, D. (2004) Independent Laboratory Validation for the Determination of JAU6476, JAU6476-Desthio, and JAU6476-4-Hydroxy in Bovine Milk and Liver: Final Report. Project Number: RAYAY004, BAYER/1517. Unpublished study prepared by

Pyxant Labs Inc. 298 p.

46246209 Clark, J. (2004) Independent Laboratory Validation of "JAU6476: An Analytical Method for the Determination of Residues of JAU6476 and desthio-JAU6476 in Plant Matrices by LC-MS/MS". Project Number: RAJAY020, 48663. Unpublished study prepared by Analytical Bio-Chemistry Labs., Inc. 77 p.

Additionally, the unedited DERs for the methods and independent laboratory validations (as submitted to HED from Dynamac Corporation) are included as Attachments 7 and 8.

HED has conducted a preliminary review of the ILVs of the proposed enforcement methods. Acceptable recoveries for prothioconazole and prothioconazole-desthio in or on peanut nutmeat and wheat forage were obtained using method RPA JA/03/01, and acceptable recoveries of prothioconazole, prothioconazole-desthio, and prothioconazole-4-hydroxy in or on samples of cattle milk and liver were obtained using the proposed LC/MS/MS enforcement method. The results of the ILVs may be found on pages 29-30 of MRID 46246209 (peanut nutmeat and wheat forage) and pages 31-32 of MRID 46246207 (cattle milk and liver).

In the method for plant commodities (Method RPA/JA/03/01, MRID 46246206), crop matrices are extracted with a mixture of methanol, 30% hydrogen peroxide, and 5% aqueous sodium bicarbonate at 65 °C for 2 hours. The oxidative extraction procedure converts prothioconazole residues to a mixture of prothioconazole-desthio and prothioconazole sulfonic acid; residues of prothioconazole-desthio in the crop matrix are not changed by the extraction procedures. The cooled extract is fortified with an isotopically labeled internal standard, cleaned up by C18 solid-phase extraction (SPE), and mixed with 1% aqueous acetic acid for analysis by LC/MS/MS. The results for prothioconazole sulfonic acid and prothioconazole-desthio are reported in prothioconazole equivalents and then totaled to yield "total prothioconazole derived residues." The validated limits of quantitation (LOQs) reported in the method are 0.02 ppm for residues in/on canola seed, peanut nutmeat, and wheat grain, and 0.05 ppm for residues in/on dried peas and wheat forage, hay, and straw. The calculated limits of detection (LODs) ranged from 2 to 5 ppb for prothioconazole and prothioconazole-desthio in canola seed, dried peas, peanut nutmeat, and wheat forage, hay, straw, and grain, and from 2 to 7 ppb for prothioconazole sulfonic acid in these commodities.

In the method for livestock commodities (Bayer Report No. 200537, MRID 46246204), samples of liver, kidney, and muscle are extracted with acetonitrile (ACN) and 25% aqueous L-cysteine HCl; internal standard solution is added to the extract. The internal standard solution consists of a mixture of [triazole-$^{15}N_2$-$^{13}C_2$]prothioconazole, [triazole-$^{15}N_2$-$^{13}C_2$]prothioconazole-desthio, and [triazole-$^{15}N_2$-$^{13}C_2$]prothioconazole-4-hydroxy in ACN containing 50 µg/mL L-cysteine HCl. Fat samples are extracted with n-hexane and then with a mixture of ACN, 25% aqueous L-cysteine HCl, and acetone; the combined extracts are allowed to separate, and internal standard solution is added to the aqueous phase. Samples of milk and cream are mixed with internal standard solution. For all matrices, the extract/sample is hydrolyzed using aqueous HCl, and the hydrolysate is partitioned with methylene chloride and acetone. The organic phase is concentrated to aqueous, mixed with ACN and water, and analyzed by LC/MS/MS. Samples are analyzed for residues of prothioconazole, prothioconazole-desthio, and prothioconazole-4-

hydroxy, and all results are reported in prothioconazole equivalents. The validated LOQs are 0.005 ppm for each analyte in milk; 0.010 ppm for each analyte in skim milk, cream, muscle, liver, and kidney; and 0.050 ppm for each analyte in fat. The calculated LODs ranged 0.7-2.1 ppb for milk, 1.0-1.9 ppb for skim milk, 2.1-3.5 ppb for cream, 0.6-1.0 ppb for muscle, 0.5-2.9 ppb for liver, 2.1-2.5 ppb for kidney, and 4.1-11.5 ppb for fat.

HED requests that ACB conduct a petition method validation (PMV) of Bayer's proposed tolerance enforcement methods per the experimental design specified in **Attachments 1 and 2.** All samples should be run in duplicate. Please complete and return these attachments as part of your report. Also, please include in your report all relevant information and supporting documentation concerning the method validation, including modifications which were made, and indicate the suitability of the analytical method for enforcement purposes. Please include the Repository ordering code(s) for the reference standards.

We note that for both methods, the petitioner concluded that the specificity of the LC/MS/MS method precludes the need for confirmatory procedures. Please comment on the need for confirmatory procedures for the methods.

Should you find that the necessary analytical reference standards, internal standards, and/or MSDSs are not available to you, please contact Melvin Tolliver (Registration Product Manager, Regulatory Affairs, Bayer CropScience, Research Triangle Park, NC, 919-549-2631) to request that they be provided.

Since one of the purposes of conducting an in-house PMV is to determine whether all necessary instructions are included in the submitted proposed enforcement methods, your laboratory staff scientists should have minimal contact with the petitioner during the conduct of this trial. Any problems encountered in the method as written should be documented and included in your report. The petitioner will be informed of any deficiencies in the method and asked to resolve them.

The RD Product Manager for prothioconazole is Lana Coppolino, and she should be contacted directly (703-305-0086) if you require guidance concerning the priority for initiation/completion of this PMV.

Please address and send your report to W. Wassell, RAB3/HED, 7509C. If you need any further information from me my telephone number is 703-305-6135.

Attachment 1- Experimental Design for PMV of LC/MS/MS method RPA JA/03/01
Attachment 2- Experimental Design for PMV of LC/MS/MS method for Livestock Commodities (Bayer Report No. 200537)
Attachment 3- Proposed LC/MS/MS Enforcement Method for Plant Commodities, MRID 46246206
Attachment 4- ILV of Enforcement Method for Plant Commodities, MRID 46246209
Attachment 5- Proposed LC/MS/MS Enforcement Method for Livestock Commodities, MRID 46246204
Attachment 6- ILV of Enforcement Method for Livestock Commodities, MRID 46246207
Attachment 7- Unedited DER for Residue Analytical Method - Plant Commodities (MRID Nos. 46246206, 46246208, 4624209).
Attachment 8- Unedited DER for Residue Analytical Method - Livestock Commodities (MRID Nos. 46246204, 46246205, 4624207)

cc (with Attachments 1-2 only): L. Coppolino (RD, 7505C), L. Croteau (PMRA/Health Canada), W.D. Wassell

RDI: RAB3 Chem. Review Team: 6/14/2005; L. Croteau (PMRA/Health Canada): 06/23/2005; S. Dapson: 06/24/2005

ATTACHMENT 1

METHOD: 46246206 Gould, T.; Timberlanke, B.; Krolski, M.; et. al. (2004) Validation of Bayer CropScience Method RPA JA/03/01: JAU6476: An Analytical Method for the Determination of Total Residues of JAU6476 in Plant Matrices Using LC/MS-MS. Project Number: J6111401, 200799, RPA/JA/03/01. Unpublished study prepared by Bayer Corp. 238 p.

Please: (i) indicate the limit of detection and quantitation; (ii) do not use control values for recovery calculations; and (iii) do not report control values as zero; if less than the limit of detection, report as such.

Commodity	Chemical Added	ppm Added	ppm Found	Percent Recovery
Peanut nutmeat	Prothioconazole	0		
		0.02 (LOQ)		
	Prothioconazole-desthio	0		
		0.02 (LOQ)		
Wheat forage	Prothioconazole	0		
		0.05 (LOQ)		
		7		
	Prothioconazole-desthio	0		
		0.05 (LOQ)		
		7		

ATTACHMENT 2

METHOD: 46246204 Moore, S.; Harbin, A. (2003) An Analytical Method for the Determination of JAU 6476, JAU 6476-Desthio and JAU 6476-4-Hydroxy Residues in Various Bovine Matrices by LC-MS/MS. Project Number: 200537. Unpublished study prepared by Bayer Corp. 131 p.

Please: (i) indicate the limit of detection and quantitation; (ii) do not use control values for recovery calculations; and (iii) do not report control values as zero; if less than the limit of detection, report as such.

Commodity	Chemical Added	ppm Added	ppm Found	Percent Recovery
Cattle liver	Prothioconazole	0		
		0.01		
		0.5		
	Prothioconazole-desthio	0		
		0.01		
		1.2		
	Prothioconazole-4-hydroxy	0		
		0.01		
		0.5		
Milk	Prothioconazole	0		
		0.005 (LOQ)		
		0.01		
	Prothioconazole-desthio	0		
		0.005 (LOQ)		
		0.01		
	Prothioconazole-4-hydroxy	0		
		0.005 (LOQ)		
		0.01		

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Chemical:	**Prothioconazole**
PC Code:	**113961**
HED File Code	**11500 Petition Files Chemistry**
Memo Date:	**06/29/2005**
File ID:	**DPD318440**
Accession Number:	**412-05-0098**

HED Records Reference Center
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